# Proteomics of *Aspergillus nidulans* sexually differentiated cells

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Es leuchtet! Seht! – Nun lässt sich wirklich hoffen, dass, wenn wir aus viel hundert Stoffen durch Mischung – denn auf Mischung kommt es an – den Menschenstoff gemächlich komponieren, in einen Kolben verlutieren und ihn gehörig kohobieren, so ist das Werk im Stillen abgetan.

**Faust II (Johann Wolfgang von Goethe)**

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Affirmation

I declare that my doctoral thesis was written on my own having used only the listed resources and tools.

Erklärung

Die vorgelegte Arbeit wurde von mir selbständig angefertigt und nur die angegebenen Hilfsmittel wurden benutzt. Alle Stellen, die dem Wortlaut oder dem Sinne nach anderen Werken entnommen wurden, sind durch Angabe der Quelle kenntlich gemacht worden

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## Summary

The homothallic mold *Aspergillus nidulans* produces as overwintering structures closed sexual fruiting bodies named cleistothecia, which contain ascospores as products of meiosis. Formation of fruiting bodies is linked to a specific secondary metabolism and includes a number of specialized cells like the globose-thick walled multinuclear Hülle cells. The LaeA methyltransferase represents an epigenetic regulator of numerous secondary metabolite clusters and is required for Hülle cell formation. Abolished Hülle cell formation correlates with a significantly reduced size of the cleistothecia, which suggested a nursing function of these cells for the growing fruiting body.

In this work, proteomes of fungal cells from different developmental programs and from enriched fractions of Hülle cells were compared to colonies grown on surfaces or in liquid medium. In a quantitative proteomics approach these results were compared to vegetative mycelia lacking the methyltransferase LaeA resulting in reduced Hülle cell formation and reduced secondary metabolism linked to the sexual program.

Comparative proteomics in combination with fluorescence microscopic investigations showed that the prenyltransferase XptB and other proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) secondary metabolite gene clusters are found in sexual mycelium as well as in Hülle cells grown on surfaces or in submerged liquid cultures. NptA represents a second prenyltransferase, which could be identified in different fungal cell types including Hülle cells and quantitative proteomics revealed that the protein quantity of NptA is down-regulated in strains lacking LaeA.

Hülle cells grown on surfaces compared to liquid culture shared approximately 72% of the identified proteins in a core proteome. Besides the *mdp*/*xpt* protein, the ankyrin domain protein (AN8434) and the tryrosine domain protein (AN8435) are part of the core proteome and showed in fluorescence microscopic investigations a cellular localization in Hülle cells.

In contrast to Hülle cells derived from liquid culture, surface cells contained increased numbers of glucanase protein levels. Another specific protein, which was only identified in surface and not liquid Hülle cells was the maltose permease-like transporter of Hülle cells MphA. Genetic studies of the corresponding deletion strain revealed that the MphA protein, which is enriched in surface Hülle cells, promotes fungal growth, asexual and sexual development.

## Zusammenfassung

Der homothallische Schimmelpilz *Aspergillus nidulans* produziert als Überwinterungsstrukturen geschlossene Fruchtkörper, welche man als Cleistothecien bezeichnet. Diese enthalten als Produkt der Meiose Ascosporen. Die Entwicklung der Fruchtkörper ist mit einem spezifischen sekundären Stoffwechsel verbunden, der mit der Bildung einer Reihe von spezialisierten Zellen, wie den dickwandigen globosen mehrkernigen Hülle-Zellen, gekoppelt ist. Die Methyltransferase LaeA ist als epigenetischer Regulator von zahlreichen sekundären Metabolit-Gen-Clustern für die Bildung von Hülle-Zellen erforderlich. Das Aussetzen der Bildung von Hülle-Zellen führt zu einer signifikanten Reduktion der Größe der Cleistothecien, woraus auf eine das Wachstum des Fruchtkörpers fördernde Funktion der Hülle-Zellen geschlossen werden kann.

In dieser Arbeit werden Proteome verschiedener Pilz-Zelltypen aus unterschiedlichen Entwicklungsprogrammen und aus angereicherten Fraktionen von Hülle-Zellen mit solchen verglichen, die aus Kolonien an der Oberfläche oder aus Flüssigmedien stammten. In einer quantitativen Proteom-Analyse wurden die Ergebnisse mit vegetativen Mycelien verglichen, denen die Methyltransferase LaeA fehlte. Das führte zu einer signifikanten Reduktion der Hülle-Zellen Bildung sowie des mit dem sexuellen Programm verbundenen Sekundärmetabolismus.

Vergleichende Proteomik in Verbindung mit fluoreszenzmikroskopischen Untersuchungen zeigte, dass zum Beispiel die Prenyltransferase XptB und andere Proteine, kodiert aus den Monodictyphenon (*mdp*) / Xanthon (*xpt*) sekundären Metabolit-Gen-Clustern, in Hülle-Zellen aus Fest- beziehungweise Flüssigmedium sowie aus einem sexuellen Mycel gefunden werden. NptA repräsentiert die zweite Prenyltransferase und konnte in verschiedenen Pilz-Zelltypen sowie in Hülle-Zellen identifiziert werden. Durch quantitative Proteomik-Analysen wurde festgestellt, dass bei Fehlen von LaeA die Prenyltransferase NptA eine reduzierte Proteinmenge aufwies.

Das gemeinsame überlappende (Core-) Proteom der identifizierten Proteine stimmt zu cirka 72% bei an der Oberfläche gewachsenen Hülle-Zellen und jenen, die aus Flüssigmedium stammten, überein. Neben den *mdp*/*xpt* Proteinen zeigte das gemeinsame überlappende (Core-) Proteom noch zwei weitere Proteine, das Ankyrin-Domäne-Protein (AN8434) und das Tryosin-Domäne-Protein (AN8435), die eine

zelluläre Lokalisierung in Hülle-Zellen in fluoreszenzmikroskopischen Analysen aufweisen.

Im Gegensatz zu Hülle-Zellen, welche aus Flüssigmedium stammen, zeigen Hülle-Zellen, welche aus Oberflächen-Kulturen herrühren, eine erhöhte Protein-Anzahl an Glukanasen auf. Ein weiteres spezifisches Protein, das putative Maltose-Transport Protein MphA (Maltose Permease-artiges Protein von Hülle-Zellen) wurde ausschließlich in Hülle-Zellen aus Festmedium und nicht aus Flüssigmedium identifiziert. Genetische Studien zur Deletion von MphA zeigten, dass das MphA Protein, angereichert in Oberflächen-Hülle-Zellen, das Wachstum sowie die asexuelle und die sexuelle Entwicklung des Pilzes unterstützt.

# 1. Introduction

Fungi produce a wide range of secondary metabolites, some of which have properties that protect the fungus against harmful environmental influences. Secondary metabolite gene clusters that encode enzymes for the production of secondary metabolites are often activated under certain environmental conditions. The activation of secondary metabolite gene clusters is often linked to developmental processes. The transition of a rather undifferentiated vegetative growth to multicellular structures such as the cleistothecia activates certain secondary metabolite gene clusters (Bayram et al., 2016). The products of meiosis, the sexual spores, are protected by the cleistothecia. Cleistothecia are embedded in nest-like structures composed mainly of Hülle cells.
In order to protect the cleistothecia against harsh environmental conditions Hülle cells might have a key role in the process of secondary metabolism.

## 1.1. The genus *Aspergillus*

The fungal kingdom contains about eight different phyla. Ascomycetes represent the largest fungal phylum. Phylogenetic data suggest that fungi first appeared during the late Precambrian period and that the Ascomycota diverged from the Basidiomycota approximately 1206 million years ago (Heckman et al., 2001). The Ascomycota are divided into three different subphyla: (i) *Pezizomycotina*, (ii) *Saccharomycotina* and (iii) *Taphrinomycotina*. Genus *Aspergillus* belongs to the class *Eurotiomycetes* within the subphylum *Pezizomycotina.* It is presumed that the genus *Aspergillus* comprises over 350 species and sexual development is known for approximately 70 species (de Vries et al., 2017, Samson et. al., 2014).

### 1.1.1. *Aspergillus* section *Nidulantes*

*Aspergillus* species from section *Nidulantes* include a variety of morphological characteristics such as biseriate conidiophores and if present, sexual fruiting bodies embedded in masses of Hülle cells (Chen et al., 2016). *Aspergillus* species within the section *Nidulantes* are common in nature and play a central role in the decomposition processes in organic material. They colonize different habitates including terrestrial, freshwater and marine environments. *Aspergillus sydowii* is an example that colonizes

corals in marine habitates (Kirkwood et al., 2009, Rypien et al., 2008). Other members of the *Aspergillus* section *Nidulantes* are known to colonize humans and were reported in infectious diseases such as *Aspergillus versicolor* (Veraldi et al., 2010).
It was shown that different species of the section *Nidulantes* produce different mycotoxins (Kim et al., 2017). Secondary metabolites represent organic compounds that are important factors to protect and compete the fungus against harsh environmental conditions (Chang et al., 2017, Zhao et al., 2017, Caballero-Ortiz et al., 2013). Most of the species in the section *Nidulantes* produce the carcinogenic mycotoxin sterigmatocystin. Apart from the production of sterigmatocystin other mycotoxins are produced by different species in the section *Nidulantes* such as emestrin, fumitremorgins, asteltoxins and paxillin. *Aspergillus nidulans* is known to produce more than 100 different secondary metabolites including important pharmaceutically active compounds such as penicillin G (Chen et al., 2016).

### 1.1.2. *Aspergillus nidulans*: A model organism for fungal development and secondary metabolism

*A. nidulans* is a rapidly growing, saprophytic soil organism. The fungus is haploid and therefore, the organism is genetically better tractable. As a result, gene deletions and other mutations are possible. The fungus, therefore, can be used to answer research questions in the field of molecular biology. The presence of a sexual life cycle makes the fungus very attractive to study the molecular differences during various developmental programs.
*A. nidulans* was sequenced in the year 2005 (Galagan et al., 2005). The genome of *A. nidulans* contains 30 million base pairs, with eight haploid chromosomes and encodes 10555 proteins (Uniprot: Proteome ID UP00000056). Molecular biology, bioinformatics and comparative transcriptomics made it possible to study the expression of secondary metabolite gene clusters that are clustered within the genome and are often located near telomers (Clevenger et al., 2017). The well-studied products including important pharmaceuticals of *A. nidulans* like penicillin G made the fungus interesting for researchers (Itoh et al., 2017, Gerke and Braus 2014, Keller et al., 2005). Because *A. nidulans* produces different secondary metabolites in different developmental stages, it is crucial to study the life cycle of the fungus in more detail (Soukup et al., 2017).

### 1.1.3. Economic impact of *Aspergilli*

Some representatives of *Aspergillus* spp. are of significant economic benefit such as *Aspergillus niger* for the production of citric acid (Yu et al., 2018). *Aspergillus oryzae* is used in Asian cuisines for the fermentation of soya beans for the production of soya sauces or other traditional alcoholic beverages (Nishimura et al., 2018). Other representatives of *Aspergillus* spp. are of negative influence for their environment. For instance, *Aspergillus flavus* is an opportunistic, saprophytic fungus that infects maize and other feed crops. This leads to reduced harvest and can cause a substantial economic loss. *Aspergillus flavus* produces carcinogenic secondary metabolites. *Aspergillus flavus* which produces aflatoxins and herewith contaminates maize represents a serious threat to human health (Rajasekaran et al., 2018).

There are other *Aspergilli* with a negative impact on the health of human beings. For instance, *Aspergillus fumigatus* is a widespread fungus typically found in soil and decaying organic matter and represents a serious threat for immunocompromised patients such as organ transplant recipients and people with leukemia. Conidial germination of *Aspergillus fumigatus* into tissue-invasive hyphae can evoke life threating infections (Shlezinger et al., 2017, Jöhnk et al., 2016). This can lead to an acute invasive and chronic pulmonary aspergillosis. *Aspergillus fumigatus* shows a high resistance against higher temperatures, dehydration and even desinfectants being able to survive in extremely harmful environmental conditions, even spores were isolated from the international space station (ISS); (Knox et al., 2016).

## 1.2. Morphological aspects

The filamentous fungus *A. nidulans* develops into a multicellular organism. The transition from undifferentiated growth, such as vegetative growth, to multicellular structures requires environmental and endogenous factors. Asexual and sexual development is linked to the formation of different spore-producing structures.

### 1.2.1. Hyphae

The initial and basic unit of filamentous fungi represents undifferentiated structures named hyphae. The formation of vegetative hyphae facilitates the habitation of various ecological niches. Hyphal growth requires cell surface expansion and cell wall disposition at the hyphal tip. Different types of hypae are known and are formed during developmental processes.

Ascogenous dikaryotic hyphae are formed within cleistothecia. Through the fusion of two haploid nuclei a diploid meiotic nucleus is formed in an ascus mother cell. The ascus mother cell expands through a swelling process into an ascus (Kirk and Morris 1991).

Subtending hyphae are known to be attached to a spore from which the spore develops as a consequence of a swelling process. This is the case in Hülle cells.

### 1.2.2. Hülle cells

Eduard Eidam first described Hülle cells in 1883 where he termed Hülle cells as a “Blasenhülle” or bubble envelope (Eidam 1883). In different species, Hülle cell like structures are known such as in *Candida albicans* which produce at the very end of the hyphae globose blisters named chlamydospores (Navarathna et al., 2016). Eidam suggested that Hülle cells originate from the tip of “secondary hyphae” which in turn emerge from “primary hyphae” and develop as a consequence of a swelling process (Figure 1).

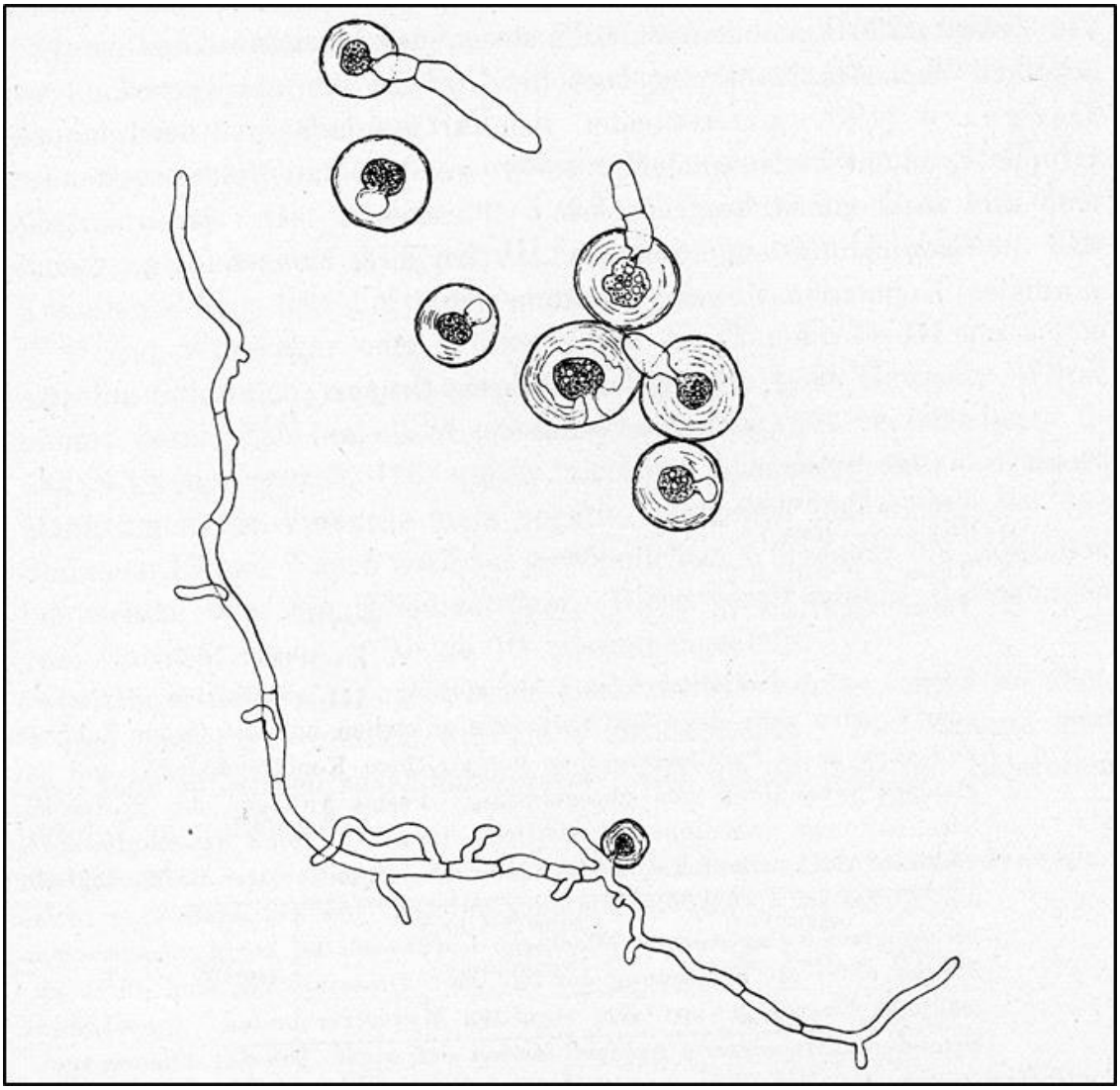


**Figure 1. Adult Hülle cells and the subtending hyphae of Hülle cells.**
Hülle cells are globose in shape with a thick cell wall and are often connected to subtending hyphae. These hyphae are of different size and length between very long and rather short and sometimes show a branch structure. Hyphae also contain subdividing septa. Within the globose structure of Hülle cells, a cytoplasm with metabolic activity is situated. The thick cell wall is ringshaped and open on one side. Two septa are visible in the open part of the ring (Raper and Fennel 1965).

Hülle cells and the subtending hyphae are connected via two distinct types of septa (Figure 2A). The inner one is a single perforate septum where woronin bodies can be observed and represents a typical ascomycetous septum. The second septum which separates Hülle cells from the subtending hyphae is unique and named basal septum (Figure 2B). At the basal septum vesicle fusion is observable. Consequently, to this fusion so called lomasome-like accumulations are visible. These lomasome-like structures are membrane-invaginations (Figure 2C). In Hülle cells several nuclei, mitochondria, lipid bodies and storage products can be observed (Ellis et al., 1973).

During initial Hülle cell formation, it was shown that several nuclei fuse to form a marcronucleus (Carvalho et al., 2002). Different species of the *Aspergillus* genus produce Hülle cells, including *A. nidulans* and *Aspergillus heterothallicus* (Bayram and Braus 2012). Hülle cells have an average size of 12-20 µm, are of globose shape with an unusual thick cell wall and are mainly associated with the sexual developmental program. Hülle cells are known for all species in the section *Nidulantes* (Chen et al., 2016). In different species, Hülle cells vary in shape between the more elongated such as in *Aspergillus ustus* and the globose version like in *A. nidulans*. In *A. nidulans* and *Aspergillus heterothallicus* Hülle cells associate with the cleistothecia, whereas in *Aspergillus protuberus* and *Aspergillus ustus* Hülle cells are not in direct contact with the cleistothecia and are formed in masses (Muntanjola-Cvetkovic and Vukic 1972).

### 1.2.3. Cleistothecia

In *A. nidulans* the products of meiosis, the sexual spores, are situated and produced in the cleistothecia. The function of cleistothecia, therefore, is to protect the sexual spores against harsh environmental conditions. During sexual sporulation, certain hyphae develop to ascogenous hypae. Ascogenous hyphae contain two haploid nuclei of opposite mating type. These hyphae form a hook shaped structure, named crozier, their nuclei divide synchronously. Different septae are formed thereby forming a dikaryotic top cell. After fusion of the end cell and the basal cell of the crozier karyogamy and further ascus development take place (Braus et al., 2002, Busch and Braus 2007). Hülle cells are most probably formed through a swelling process of certain vegetative hyphae. The first morphological manifestation of a visible structure is the appearance of cleistothecial initials, 40 hours after germination (Sohn and Yoon 2002). These structures are coiled lumps of cells, which undergo further coiling and become enlarged to approximately 10 µm. This is the stage where Hülle cells first appear (Sohn and Yoon 2002). *A. nidulans* produces dark reddisch ascospores which are found in globose structures named asci (Dyer and O'Gorman 2012). These asci are protected by dark brown/violet ascomata. The ascomata are named cleistothecia in *Aspergillus nidulans*, due to its closed conformation (Greek: Kleistos = closed, theke = case). Cleistothecia are surrounded by numerous hyaline to pale brown globose Hülle cells.

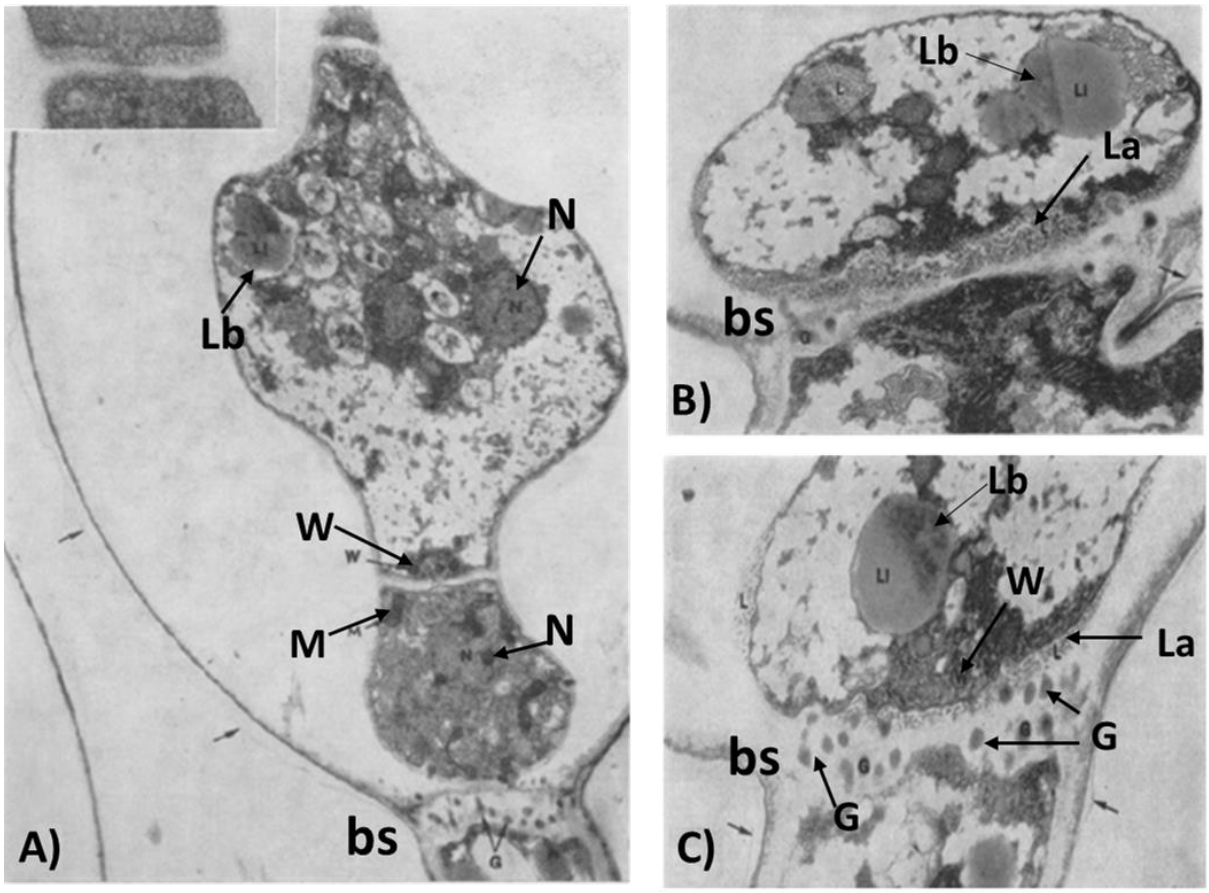



**Figure 2. Electron microscope images of the content and septa found in Hülle cells of *Aspergillus nidulans*.**
A) Overview picture of the content and the septa found in Hülle cells. The basal septum (bs) which separates Hülle cells from the subtending hyphae is unique. A second type of septum inside of Hülle cells is a typical ascomycetous septum where woronin bodies (W) and mitochondria (M) are observed. At the Hülle cell side (cytoplasm) lipid bodies (Lb) and nuclei (N) are visible. Further septa are possible in Hülle cells. B) The unique basal septum (bs) of Hülle cells. At the periphery of this septum (at Hülle cell side) lomasome-like accumulations (La) of the membrane are present (lomasome-like structures are invaginations of the membrane). In between the two septa woronin bodies (W), lipid bodies (Lb) and nuclei (N) are visible. Woronin bodies are close to the basal septum (bs). C) The basal septum (bs) contains globular structures (G). Image modified (Ellis et al., 1973).

## 1.2.4. Conidiophores

The *Aspergillus* species produces first foot cells from vegetative hyphae, which apically extend into a stalk. The very end of this cell swells to a so-called multinucleated vesicle. The vesicle produces a finger like metula as a first layer and a second layer, the phialides are formed by mitosis (Bayram and Braus 2012). These phialides undergo repeatedly asymmetric mitotically processes which lead to the production of haploid airborne conidiospores. After initial formation, conidiospores

undergo the maturation process. The progression of condiospores is a separate process from the initial phase. The development of condiospores contains several changes in cell wall structures and chemical modifications. During the maturation process, four different cell wall layers emerge. This enables impermeability of condiospores, necessary to protect spores from harsh environmental conditions. Chemical modifications include the production of trehalose which serves as an energy source and protects against various environmental conditions such as dehydration, cold and oxidation (Elbein et al., 2003). Matured conidia are formed 15 hours after initial sporulation. The conidiophore represents the complete asexual structure which carries asexual spores called conidia.

## 1.3. Factors influencing fungal development

### 1.3.1. Environmental factors

Various environmental factors are important for *A. nidulans* to develop asexually on the surface (Figure 3A). Exposure to sunlight, in particular, represents a crucial factor (Dasgupta et al., 2016). Other environmental factors that induce the asexual developmental program are high oxygen concentrations, temperature shifts, osmotic stress and high concentrations of reactive oxygen species (ROS) (Bennett and Turgeon 2017, Noble and Andrianopoulos 2013). *A. nidulans* grow preferentially sexually and produce as overwintering structures, closed sexual fruiting bodies called cleistothecia in darkness under the soil (Dyer and O'Gorman 2012). Different environmental signals such as low oxygen concentrations, buffered temperature, humidity and low concentrations of ROS trigger sexual development. Asexual and sexual differentiation of *A. nidulans* is linked to significant changes in the formation of secondary metabolites and the formation of various distinct cell types of the respective developmental programs (Park et al., 2017). Asexual and sexual structures of *A. nidulans* are shown in Figure 3B.

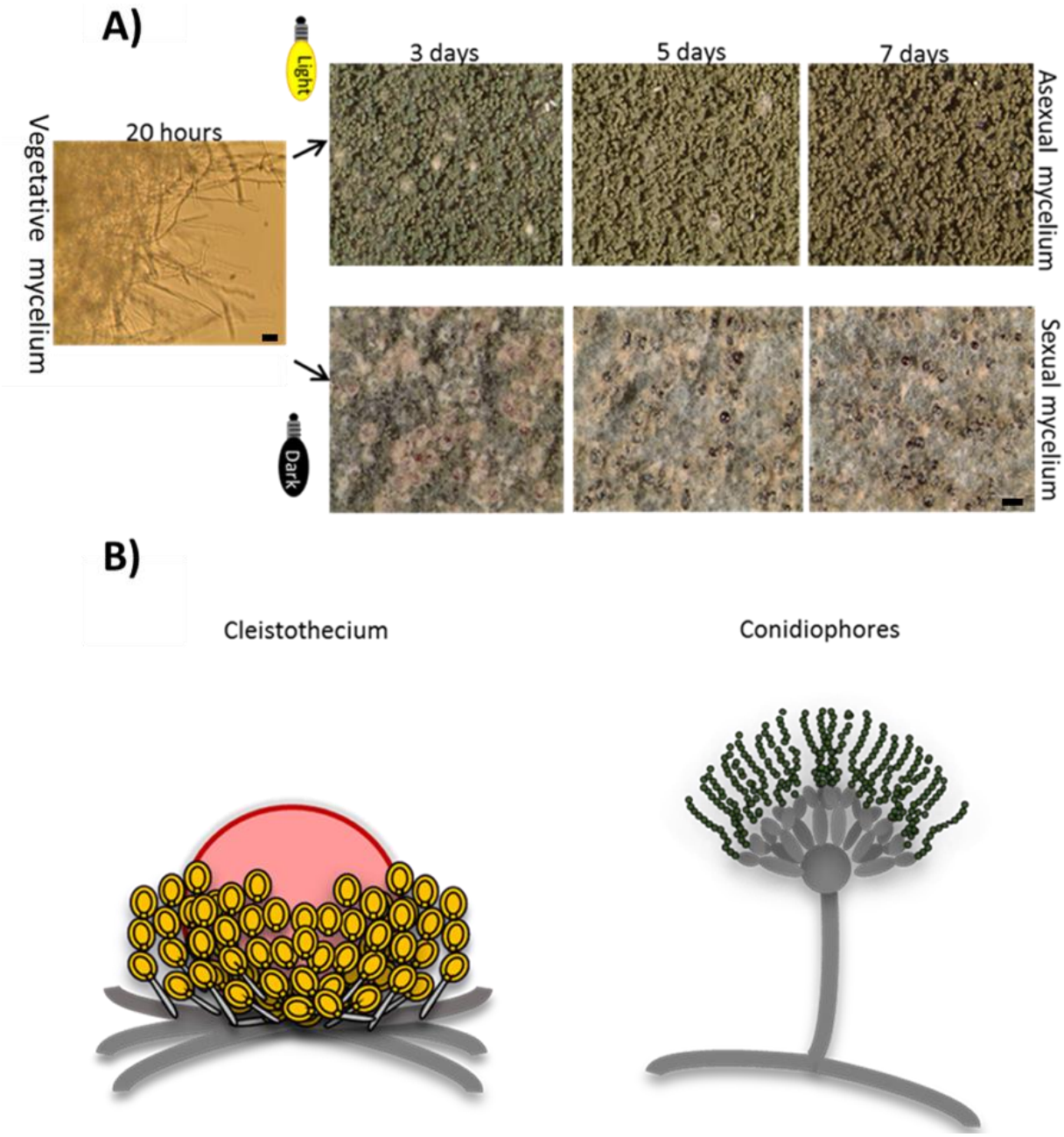



**Figure 3. Asexual and sexual differentation.**
A) The fungus has to develop vegetative hyphae before cellular differentiation. This type of mycelium consists of relatively undifferentiated hyphae without other cell types. After the initial growth phase the fungus reaches a competence state, which defines a barrier to enter a differentiation capability. In light vegetative mycelium develops preferentially to asexual conidiophores, whereas in darkness the sexual developmental program is favored. Vegetative, asexual and sexual mycelia are shown at different time points. B) Different spore-producing structures of *Aspergillus nidulans*. Sexual differentiation increases sexual cell types such as cleistothecia. Hundreds of globose Hülle cells surround each cleistothecium. The cleistothecium contains sexual spores called ascospores. The asexual cell type is named conidiophore that carries the asexual spores called conidia. Scale bar is 200 µm. (Bennett and Turgeon 2017, Noble and Andrianopoulos 2013, Bayram and Braus 2012).

### 1.3.1.1. Light favors asexual differentiation

Light represents an important environmental factor that allows fungi to respond in different ways. Different processes are linked to light sensing in *A. nidulans,* including differentiation or changes in gene expression within primary and secondary metabolism. After the initial growth phase, the fungus reaches a competence state, that defines a barrier to enter a differentiation capability (Jiang et al., 2017, Ruger-Herreros et al., 2011). This allows to respond to different environmental factors. In light, *A. nidulans* preferentially develops asexually. The light-sensing function of fungi require a number of different, wave length specific receptors (Pöggeler et al., 2018). Cryptochromes (Cry) are a class of photolyase-like receptors sensitive to blue light. In *A. nidulans* the cryptochrome/photolyase CryA represses sexual differentitation beside its function in DNA-repair activities. Deletion of the corresponding gene *cryA* stimulated Hülle cell formation in submerged liquid cultures where usually no Hülle cells are formed. Asexual sporulation correlates with the production of few secondary metabolites. It was found that *A. nidulans* produces the antibiotic compound emericellamide (Chiang et al., 2008). The biosynthesis of the secondary metabolites emericellamide A, C and E, is initiated by light (Bayram et al., 2016). These types of antibiotics were shown to be present after 24 hours of growth in light, whereas in darkness, growth is extremely diminished (Bayram et al., 2016).

### 1.3.1.2. Sexual differentiation is promoted by darkness

Illumination mainly inhibits the production of certain secondary metabolites and favors asexual sporulation. Sexual reproduction is favored in darkness and low oxygen concentration. It is accompanied with the accumulation of secondary metabolites. The heterotrimeric velvet complex VelB/VeA/LaeA coordinates light signals with fungal development together with secondary metabolism (Bayram et al., 2008b). VeA bridges VelB to the methyltransferase LaeA. During illumination VeA is mostly situated in the cytoplasm where VelB supports asexual sporulation and LaeA shows low activity. In darkness VeA is imported into the nucleus by KapA (importin).
Additionally, VeA supports the transportation of VelB into the nucleus. VelB/VeA, forms together with laeA the heterotrimeric velvet complex VelB/VeA/LaeA. This complex regulates sexual sporulation and secondary metabolism. It was shown that

additional proteins associate with the heterotrimeric velvet complex and that specific secondary metabolite gene clusters are preferentially activated in darkness that lead to the accumulation of secondary metabolites like monodictyphenone, xanthone, asperthecin and sterigmatocystin (Sarikaya-Bayram et al., 2014, Bayram et al., 2016).

### 1.3.1.3. Nutrients

Sexual and asexual development in *A. nidulans* is strongly affected by nutrients. Han and co-workers showed that the sexual development is favored in well-nourished growth conditions, whereas carbon limitation, light exposure and high concentration of salt promotes asexual development (Han et al., 2003). This suggests that stress conditions provoke asexual development, while well-nourished growth conditions favor sexual development. Chitins, carbohydrates and other compounds of the fungal cell wall are important factors for nutrient supply during development. During initial growth phase of the fungus $\alpha$-1,3-glucans is accumulated in the fungal cell wall. Glucanases such as MutA or AngB degrade glucanes in the cell wall of storage hyphae to yield carbohydrates representing a carbon source (Zonneveld 1973, He et al., 2017, Wei et al., 2001) .

Amino acids are known to influence the growth of cleistothecia in *Aspergillus nidulans*. Amino acid starvation leads to impaired or stalled cleistothecia formation (Eckert et al., 1999, Serlupi et al., 1983). Storage lipids that represent carbon sources are present in lipid bodies and are found in Hülle cells (Ellis et al., 1973). It is known that fruiting body formation in various fungi is increased by addition of fatty acids (Pöggeler et al., 2006, Dyer et al., 1993; Goodrich-Tanrikulu et al., 1999).

## 1.3.2. Endogenous factors

Additional factors are required to allow the differentiation process of the fungus beside the above mentioned environmental factors. Primary as well as secondary metabolism are involved in this process. Primary and secondary metabolites are typically associated with different developmental programs. During initial growth phase primary metabolites are synthesized or obtained from the growth medium.

### 1.3.2.1. Primary metabolism

Primary metabolites such as amino acids, carbohydrates and lipids are molecules essential for growth, development and reproduction. Primary metabolites are mainly accumulated during initial growth phase and are processed during different developmental stages.

The process of sexual differentiation requires massive cell proliferation and as a result, increased carbon sources are required. Zonneveld and co-workers showed that α-1,3-glucans are accumulated during vegetative growth in the cell wall and that these polysaccharides are degraded during sexual development (Zonneveld 1973). The α-1,3-glucanase MutA was shown to be localized mainly in Hülle cells (Wei et al., 2001). The mobilization of α-1,3-glucans was affected in a *mutA*Δ strain although the cleistothecia were still able to grow similar to that of the wild-type (Wei et al., 2001). These results suggest that additional enzymes and carbon sources are most likely required as a nursing resource during the development of the cleistothecia (Yoshimi et al., 2017). It is assumed that Hülle cells comprise cell wall lytic enzymes that release monosaccharides (de Groot et al., 2009, Wei et al., 2001).

Furthermore, it is supposed that high affinity sugar transporters are involved to nurse the developing closed cleistothecia (Pöggeler et al., 2006). Wei and co-workers identified a putative hexose transporter, HxtA, that is expressed in Hülle cells and in ascogenous hyphae after starvation (Wei et al., 2004). The deletion of *hxtA* showed no effect on sexual development. The low affinity glucose transporter HxtB is suggested to be involved in fungal development (Dos Reis et al., 2017). Pantazopoulou and co-workers showed that the purine transporters UapA and AzgA are found in Hülle cells and on the surface of the cleistothecium (Pantazopoulou et al., 2007). The knockout of *uapA* and *azgA* showed no effect on the development of cleistothecia. This suggests that additional transporters are active during sexual development and required to supply nutrients or protective molecules to the maturating cleistothecia.

Amino acids, isopentenyl pyrophosphate and malonyl-CoA are building blocks for the synthesis of secondary metabolites. Malonyl-CoA represents in *A. nidulans* the initial unit for the synthesis of polyketides such as monodictyphenone which is a secondary metabolite (Klejnstrup et al., 2012).

### 1.3.2.2. Secondary metabolism

Primary and secondary metabolism merge into each other and as a result, the border is difficult to define. The morphological border to produce secondary metabolites is mainly determined by entering distinct developmental programs and thereby forming multicellular structures such as cleistothecia (Bayram et al., 2016).

Secondary metabolites are small-molecule metabolites produced by fungi, plants and bacteria. The production of secondary metabolites allows the fungus to colonize a multitude of environmental niches and allows to compete against other bacteria, fungi and insects (Demain and Fang 2000). Additionally, secondary metabolites production is connected to developmental processes of the fungus such as asexual and sexual sporulation.

Genes that encode enzymes for the production of secondary metabolites are often clustered and the expression of these genes are under the regulation of different factors (Hou et al., 2017, Brakhage 2013). Secondary metabolite gene clusters encode typically one of several backbone enzymes: (i) a polyketide synthase (PKS); (ii) a non-ribosomal peptide synthetase (NRPS); (iii) a polyketide synthase/non-ribosomal peptide synthetase hybrid (PKS-NRPS); (iv) a dimethyl-allyl-tryptophane synthase (DMATS) type aromatic prenyltransferase or (v) diterpene synthase (DTS) (Flores-Gallegos et al., 2016, Pan et al., 2017). The actions of the residual tailoring enzymes encoded by the residual genes from the gene cluster diversifies greatly the spectrum of the produced secondary metabolites of the gene cluster (Keller et al., 2005). The genome of *A. nidulans* encodes 29 polyketide synthases, eleven non-ribosomal peptide synthetases and seven prenyltransferases (de Vries et al., 2017, Galagan et al., 2005). Sequencing the genome of *A. nidulans*, proposes more then 40 different secondary metabolite pathways (Galagan et al., 2005).

In recent years, it was demonstrated that the expression of genes from secondary metabolite gene clusters and fungal development are coordinated through environmental factors (Cary et al., 2017, Röhrig et al., 2017). Bayram and co-workers showed that the transcripts of nine different genes from the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters are specifically expressed during darkness in a sexual mycelium (Bayram et al., 2016).

## 1.4. Specialized metabolism in fungal development

Secondary metabolism, also called specialized metabolism, plays an important role in fungal development. Secondary metabolites are produced under special environmental conditions, in competition with other organisms and in special developmental stages.

### 1.4.1. The monodictyphenone (*mdp*) / xanthone (*xpt*) secondary metabolite gene clusters

The monodictyphenone (*mdp*) secondary metabolite gene cluster is found to contain twelve genes that are located near the rather silent telomeric region of chromosome VIII (Figure 4). It is known that chromatin remodeling factors are needed to influence the expression of genes that are responsible for the production of secondary metabolites (Gacek and Strauss 2012).

Bok and co-workers showed that CclA, a member of the histone 3 lysine 4 methylating COMPASS (complex associated with Set1) complex regulates the expression of secondary metabolite gene clusters such as the monodictyphenone (*mdp*) gene cluster (Bok et al., 2009). The deletion of *cclA* alters the expression of genes necessary for the production of monodictyphenone. Monodictyphenone represents a precursor for the synthesis of prenyl-xanthones. The genes *xptA*, *xptB* and *xptC* required for the conversion of monodicytphenone into xanthones are not embedded in the monodictyphenone gene cluster (Pockrandt et al., 2012, Sanchez et al., 2011). Instead, they are localized on two different chromosomes. The genes that encode the two prenyltransferases XptA and XptB are localized on chromosome I and II. The gene *xptC* that encodes an oxidoreductase that is localized on chromosome II and is separated from the gene that encodes the prenyltransferase XptB by the gene *AN7998*. Sanchez and co-workers showed that *mdpE* (encodes a putative C6 zinc finger transcription factor), *mdpI* (encodes a putative AMP-binding CoA ligase) and *AN7998* (encodes a putative oxidoreductase) are not involved in the synthesis of prenyl-xanthones (Sanchez et al., 2011).

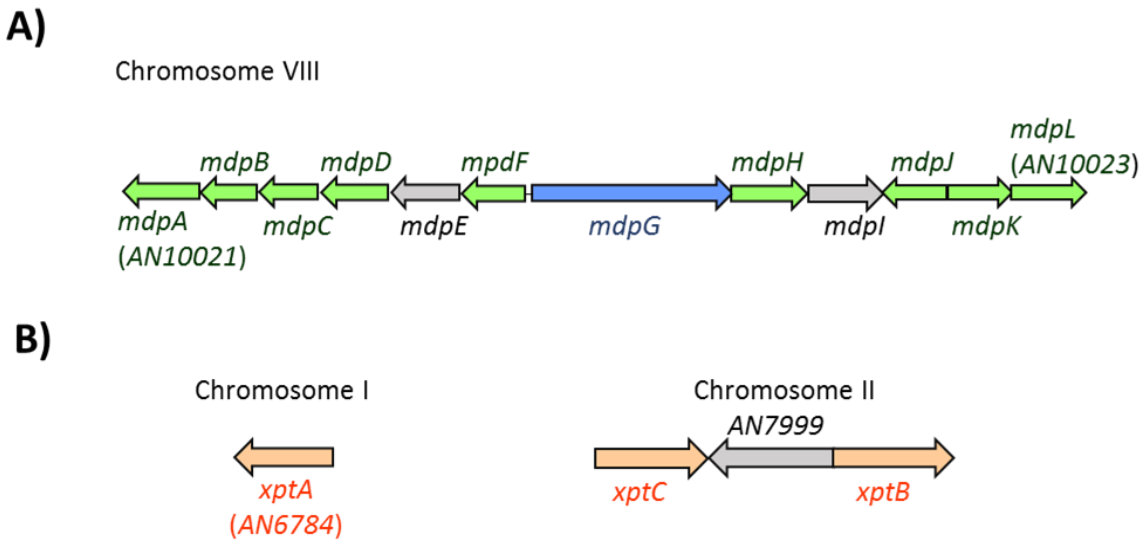



**Figure 4. Secondary metabolite gene clusters which are involved in the production of monodictyphenone and xanthones.**
A) The monodictyphenone (*mdp*) gene cluster is located at the telomeric region of chromosome VIII and contains twelve genes. The gene *mdpG* (blue) encodes a polyketide synthase that is involved in the production of the polyketide backbone core structure of monodictyphenone. The enzymes encoded by the residual genes modify the core structure resulting in the production of emodin and finally in monodictyphenone and prenyl-xanthones. The genes *mdpE* (encodes a C6 zinc finger transcription factor) and *mdpI* (encodes an AMP-binding CoA ligase) in grey are not essential for the production of prenyl-xanthones.
B) Monodictyphenone represents a precursor for the production of prenyl-xanthones. Two prenyltransferases XptA, XptB and an oxidoreductase XptC are involved in the conversion of monodictyphenone into prenyl-xanthones. The genes that encode the prenyltransferases XptA and XptB are localized on chromosome I and II. The oxidoreductase XptC is localized on chromosome II and is situated next to the gene *AN7999* (grey) which encodes an oxidoreductase and is not essential for the production of prenyl-xanthones (Sanchez et al., 2011, Chiang et al., 2010).

### 1.4.1.1. Monodictyphenone is a precursor for the synthesis of xanthones

Xanthones are organic compounds found in different species like the mangosteen tree *Garcinia mangostana* and are responsible for a yellow pigmentation. The chemical building block of xanthones is composed of a core structure named xanthone nucleus (*9H*-Xanthen-9-on) that is an aromatic oxo compound. Different modifications of the xanthone nucleus lead to a variety of different xanthones. A possible modification of the xanthone nucleus can be due to a prenylation event. Xanthones found in *Garcinia mangostana* are known for anticancer activities (Alam and Khan 2018).
The polyketide synthase MdpG encoded by the monodictyphenone (*mdp*) gene cluster is required for the synthesis of anthraquinone emodin, monodictyphenone and related compounds (Klejnstrup et al., 2012). The sequence of the polyketide synthase MdpG contains as many as 1806 amino acids with a predicted molecular mass of 196,8 kDa.

The polyketide synthase MdpG synthesizes the main polyketide backbone core structure. MdpG polyketide synthase consists of several domains with defined functions mentioned in Figure 5. These domains of MdpG are involved in the synthesis of the polyketide backbone.

Emodin and monodictyphenone are precursors for prenyl-xanthone. Sanchez and co-workers deleted *mdpG* and revealed that the product monodictyphenone and other compounds such as prenyl-xanthones are no longer produced (Sanchez et al., 2011). The first step in the production of emodin and monodictyphenone requires MdpG. Malonyl-CoA represents a substrate for MdpG which synthesizes the polyketide backbone. The polyketide synthase MdpG lacks a thioesterase (TE) domain which hydrolyzes the newly formed polyketide backbone off the synthase. The gene *mdpF* that encodes a putative zinc dependent hydrolase, catalyzes most probably the release of the polyketide backbone from MdpG (Chiang et al., 2010). The gene *mdpH* that encodes a decarboxylase, catalyzes the conversion of atrochrysone carboxylic acid into atrochrysone (Klejnstrup et al., 2012). The deletion of *mdpH* results in an inability of the above-mentioned conversion to atrochrysone (Chiang et al., 2010). In order to convert atrochrysone to emodin two unknown dehydrating and modifying enzymes are necessary. The following enzymes finally convert emodin into monodictyphenone, a dehydratase (MdpB), a ketoreductase (MdpC), a glutathione S transferase (MdpJ), an oxidoreductase (MdpK) and a Baeyer-Villiger oxidase (MdpL) (Simpson 2012, Klejnstrup et al., 2012). The monooxygenase MdpD is required for the hydroxylation of monodictyphenone (Bok et al., 2009).

As a next step the hydroxylated monodictyphenone is converted into prenyl-xanthones. Klejnstrup and co-workers demonstrated that the two prenyltransferases XptA and XptB are involved in the prenylation of hydroxylated monodictyphenone (Klejnstrup et al., 2012). The biosynthesis of the stereoisomers shamixanthone and epishamixanthone is finally catalyzed by the oxidoreductase XptC (Sanchez et al., 2011).

It is known that monodictyphenone and xanthones are antimicrobial agents that serve against fungivory and other environmental threats (Bok et al., 2009, Regulin and Kempken 2018). In a transcriptomic and metabolomic profiling study it was observed that after the addition of choline Hülle cell formation occurred in a vegetative mycelium and the secondary metabolite monodictyphenone was present in this liquid culture (Alves et al., 2016).

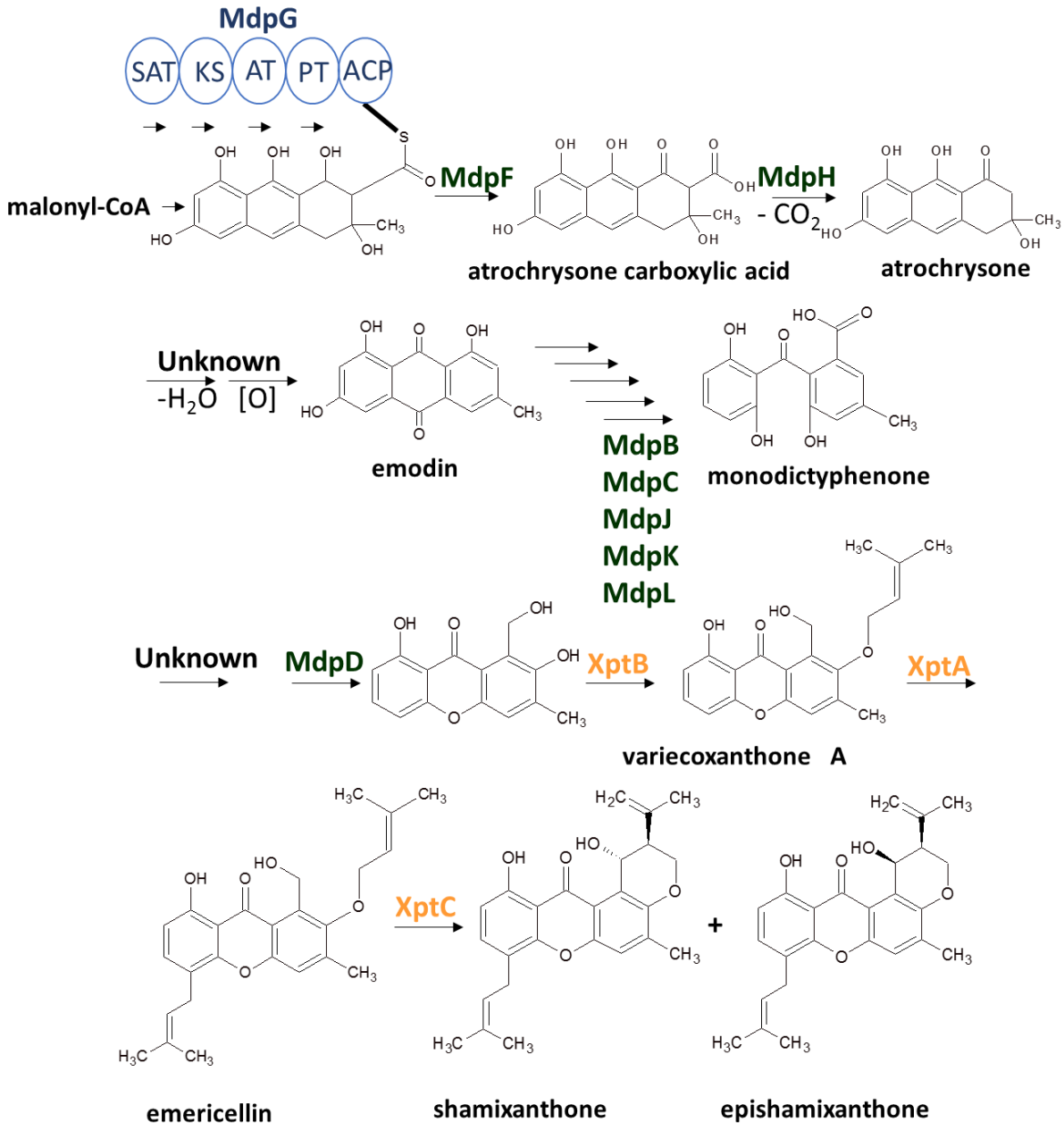



**Figure 5. Biosynthesis of monodictyphenone and xanthones.**
The polyketide synthase MdpG synthesizes the main polyketide backbone core structure and uses malonyl-CoA as a substrate. Several domains of MdpG are involved in the synthesis of the poyketide backbone (SAT domain an ACP-transacylase as a starter unit, KS a ß-ketoacyl synthase domain and AT an acetyltransferase domain. These three domains are involved in the synthesis of a polyketide backbone intermediate. The internal product template (PT) domain is responsible for folding and cyclization of the polyketide backbone intermediate. The ACP is an acryl carrier protein domain and represents a transiently holding domain for the polyketide backbone).
The putative zinc dependent hydrolase MdpF catalyzes most probably the release of the polyketide backbone from MdpG. The decarboxylase MdpH catalyzes the conversion of atrochrysone carboxylic acid to atrochrysone. In the conversion of atrochrysone to emodin unknown enzymes are involved. Several enzymes (MdpB (dehydratase), MdpC (ketoreductase), MdpJ (glutathione S transferase), MdpK (oxidoreductase), MdpL (Baeyer-Villiger oxidase) are required for the further synthesis of emodin to monodictyphenone. The monooxygenase MdpD hydroxylates monodictyphenone. The two prenyltransferases XptB and XptA are required for the prenylation of hydroxylated monodictyphenone. Giving rise to the compounds variecoxanthone A and emericellin. The oxidoreductase XptC converts emericellin into the stereoisomers shamixanthone and epishamixanthone (Simpson 2012; Klejnstrup et al., 2012).

## 1.4.2. LaeA as a factor that coordinates fungal development and secondary metabolism

Secondary metabolism and fungal development is known to be highly coordinated by several protein complexes (Sarikaya-Bayram et al., 2014, Bok et al., 2009). The LaeA methyltransferase protein is a member of the velvet complex that regulates fungal development and secondary metabolism (Bayram et al., 2008b). In *A. nidulans* LaeA regulates not only secondary metabolism but also asexual and sexual sporulation. The coordination of fungal development and secondary metabolism through LaeA is known in different *Aspergilli* (Sarikaya-Bayram et al., 2010, Dhingra et al., 2013, Zhao et al., 2017). In *Aspergillus flavus* it was demonstrated that *laeA* is required for sclerotia formation and crucial for aflatoxin synthesis (Zhao et al., 2017). The correlation between sclerotia formation and aflatoxin production enables *Aspergillus flavus* to grow in harsh enviromental conditions (Chang et al., 2017). This indicates that secondary metabolism and fungal development is correlated in different species.

### 1.4.2.1. LaeA methyltransferase

The LaeA (loss of *aflR* expression of A) methyltransferase domain protein was first identified by a forward mutant screen and it was shown that *laeA*Δ is unable to express the AfIR transcriptional activator that controls the activation of numerous secondary metabolite gene clusters (Bok and Keller 2004). The LaeA protein of *A. nidulans* comprises 374 amino acids with a predicted molecular mass of 43.0 kDa. The protein sequence includes different domain architectures (Figure 6). At the N-terminal region, the protein sequence contains a nuclear localization signal (NLS). LaeA is known to interact with the VeA and VelB proteins in the nucleus (Bayram et al., 2008b). In the center a putative S-adenosylmethione dependent (SAM) metyltransferase domain (MTD) is found and is typical for nuclear protein methyltransferase (Bok and Keller 2004). LaeA together with the velvet family proteins VeA and VelB represent a trimeric complex that is essential for fungal development and secondary metabolism (Bayram et al., 2008b). The deletion of *laeA* leads to a photoinhibition. Therefore *laeA*Δ strains are unable to repress sexual development in light (Sarikaya-Bayram et al., 2010). This results in a phenotype that produces more cleistothecia in light. On the other hand, a *veA*Δ deletion results in total inability to enter the sexual program (Kim et al., 2009).

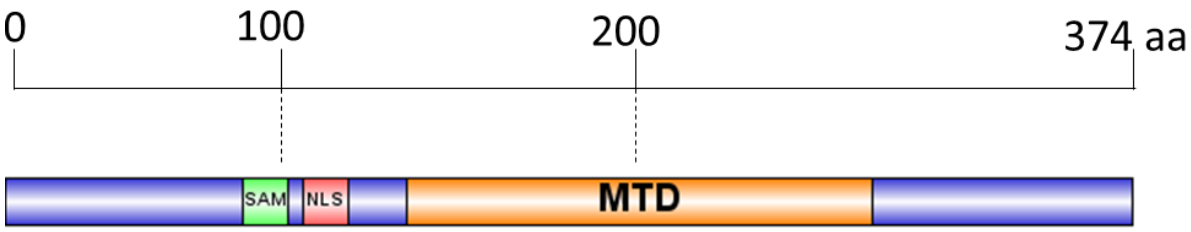
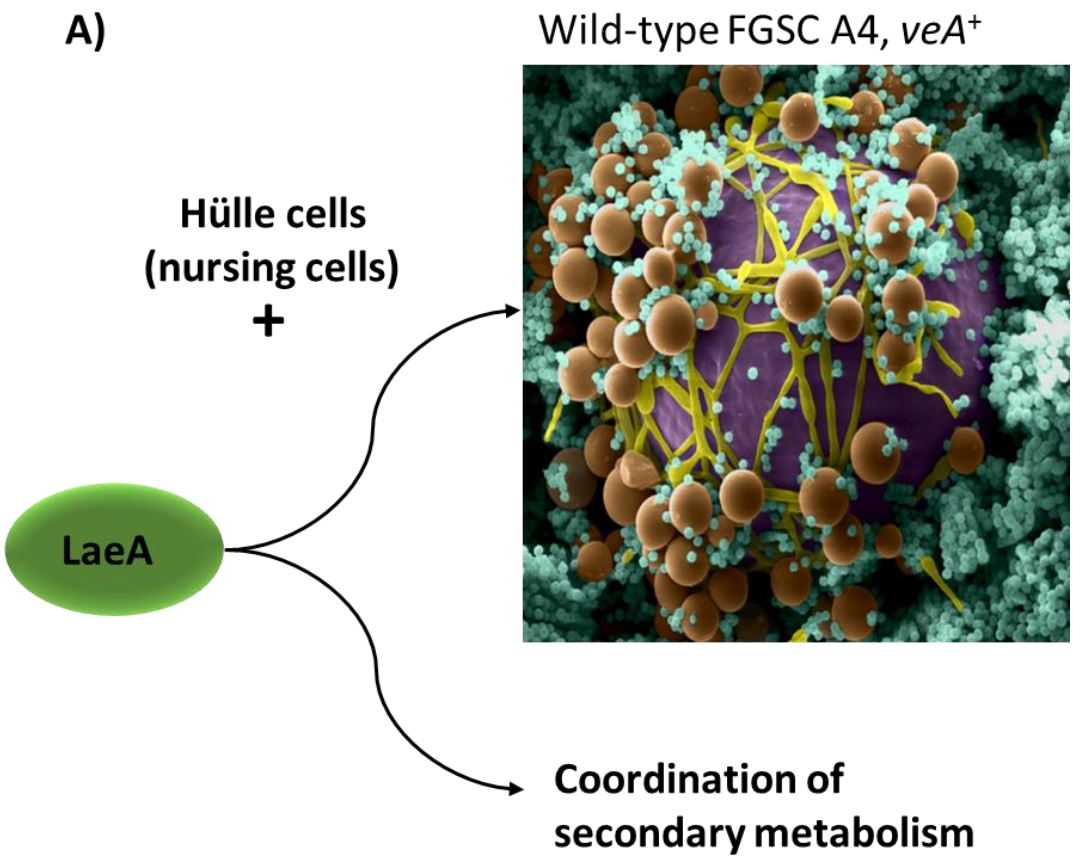



**Figure 6. Domain architecture of the methyltransferase LaeA.**
The sequence of the methyltransferase domain protein LaeA of *A. nidulans* comprises 374 amino acids with a molecular mass of 43.0 kDa and contains different domain architectures (SAM: S-adenosylmethionin- binding site, NLS: nuclear localization signal, MTD: methyltransferase domain).

## 1.4.2.2. LaeA methyltransferase promotes Hülle cell formation

LaeA methyltransferase is required for Hülle cell formation as shown in Figure 7. Abolished Hülle cell formation correlates with a significantly reduced size of the cleistothecia from around 200 µm to 40 µm (Sarikaya-Bayram et al., 2010). This suggests a nursing function of Hülle cells for the growing cleistothecia. The growth of cleistothecia surrounded by Hülle cells are mainly observable under surface conditions since their growth is dependend on surface.Deleting the gene *laeA* led to abolished Hülle cell formation (Sarikaya-Bayram et al., 2010). Genes which are expressed in sexual mycelia and Hülle cells were monitored in a *laeA*Δ strain. The expression of *mutA* that encodes an alpha-1,3-glucanase and which is localized in Hülle cells was monitored during sexual development in a *laeA*Δ strain (Sarikaya-Bayram et al., 2010). The expression of *mutA* was reduced in a *laeA*Δ strain which causes significant reduction in Hülle cell formation. From other ascomycota such as *Aspergillus flavus* it is known that methyltransferases are crucial to coordinate fungal development and secondary metabolism (Satterlee et al., 2016).
Hülle cell formation in liquid media in *A. nidulans* FGSC A4 (Glasgow wild-type, *veA*$^{+}$) is unobservable (Bayram et al., 2008a). Prolonging the growth of the fungus in submerged cultures has no effect on Hülle cell formation. The strain AGB552 (*pabaA1;yA;nkuA*Δ*::argB*) contains a *nkuA*Δ mutation and an additional unknown mutation which results in increased Hülle cell formation in submerged liquid cultures. The gene *nkuA* encodes an ATP-dependent DNA helicase II that is involved in the process of non-homologous end joining (NHEJ) and is required for the repair of double-strand breaks in DNA. For improvement of gene targeting the *nkuA*Δ strain is used (Nayak et al., 2006).



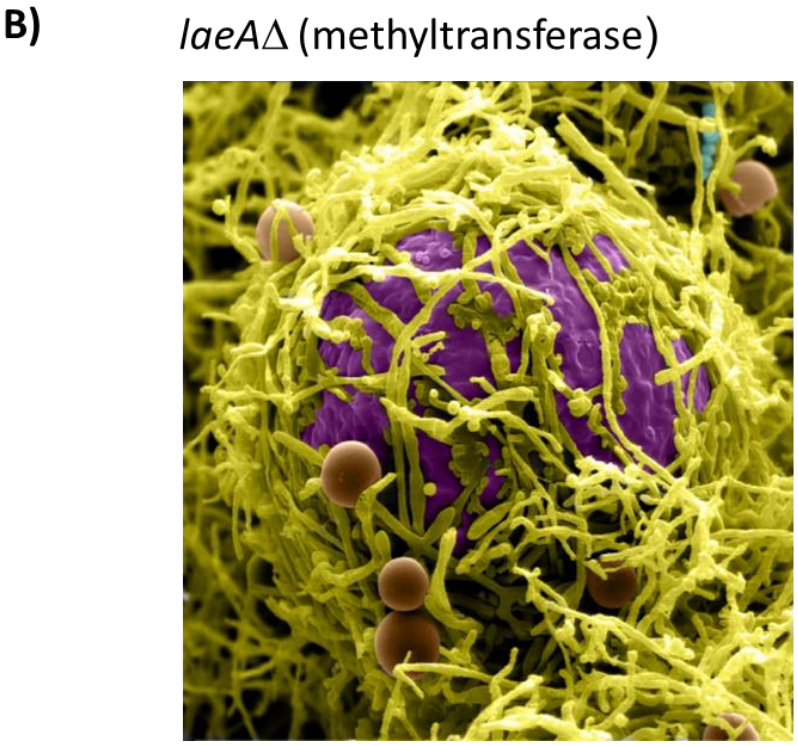




**Figure 7. LaeA methyltransferase promotes Hülle cell formation.**
A) LaeA methyltransferase domain protein coordinates fungal cell type specificity and secondary metabolism. Hundreds of Hülle cells (coloured beige) surround the cleistothecium. B) LaeA is required for Hülle cells formation. A Cleistothecium of a *laeA*Δ mycelium is shown. Abolished Hülle cells are observable and instead filamentous hyphae are covering the cleistotecium. Image modified (Sarikaya-Bayram et al., 2010).

### 1.4.2.3. LaeA regulates the monodictyphenone (*mdp*) and other secondary metabolite gene clusters

It is suggested that LaeA regulates more than 50% of all secondary metabolite gene clusters in *Aspergillus nidulans, Aspergillus fumigatus* and *Aspergillus flavus* (Sarikaya-Bayram et al., 2010, Dhingra et al., 2013, Zhao et al., 2017). Different secondary metabolite gene clusters are regulated by LaeA in *A. nidulans* including monodictyphenone (*mdp*), terrequinone A, penicillin and sterigmatocystin (Bok et al., 2009, Bok et al., 2006, Bok and Keller 2004). Bok and co-workers indicated a role of LaeA in interaction with the CclA (H3K4 methyltransferase) complex which regulates the expression of the monodictyphenone (*mdp*) gene cluster (Bok et al., 2016, Bok et al., 2009). This shows that LaeA regulates the monodictyphenone (*mdp*) gene cluster. The deletion of *laeA* gene leads to more heterochromatin formation with an increased histone 3 (H3) lysine (K9) trimetylation (H3K9me3) in the sterigmatocystin gene cluster (Reyes-Domingues et al., 2010). This suggests that chromatin structures are changed in *laeA*Δ. In order to investigate methylation substrates of LaeA a biochemical study was performed. Patananan and co-workers were not able to find a specific methylation stubstrate and investgated an automethylation reaction of LaeA (Patananan et al., 2013). The methylating function of LaeA is so far unknown.

The LaeA protein regulates several secondary metabolite gene clusters that encode prenyltransferases (Bok et al., 2006, Kale et al., 2008). Seven different prenyltransferases are encoded by the genome of *A. nidulans* (Galagan et al., 2005). Prenyltransferases are involved in the prenylation of aromatic substrates and thereby modifying different secondary metabolites. LaeA regulated prenyltansferase has been characterized in *A. nidulans* (Bok et al., 2006). The terrequinone A biosynthetic pathway involves prenylation events (Bouhired et al., 2007). Bok and co-workers showed that the prenyltansferase *tidB* is not expressed in a *laeA*Δ mycelium in comparison to the wild-type and is up-regulated in a *laeA* overexpression strain (Bok et al., 2006).

### 1.4.3. Relationship between fungal development and secondary metabolism

Secondary metabolite production is commonly associated with the progression of sporulation and fulfills different functions during this process. First, secondary metabolites are needed in order to activate sporulation, such as hydroxylated oleic acid and linoleic acid produced by *Aspergillus nidulans.* Second, the process of sporulation is linked to the production of pigments. Third, mycotoxins and other toxic metabolites are crucial to protect the fungus against fungivory and other environmental threats.

#### 1.4.3.1. Secondary metabolites that activate sporulation

A wide range of secondary metabolites are produced after the fungus has finalized its initial vegetative growth phase (Calvo et al., 2002). In *A. nidulans* endogenous factors such as the oxylipins, hydroxylated oleic acid and linoleic acid also called psi (precocious sexual inducer), are factors that regulate the balance between early sexual and asexual sporulation (Tsitsigiannis et al., 2004b). The oxygenase-like enzymes PpoA, PpoB and PpoC, are involved in the production of psi factors. Localization studies of these enzymes revealed that PpoA::GFP is localized to lipid bodies in Hülle cells and other fungal tissues (Tsitsigiannis and Keller 2004a). Deletion of *ppoA* gene resulted in increased asexual development. Thereby forming less cleistothecia in a sexual mycelium. The number of Hülle cells and ascospores were not affected by the deletion of *ppoA* (Tsitsigiannis and Keller 2004a).

#### 1.4.3.2. Pigments

The production of the pigment melanin is known to be involved in the pigmentation process of fruiting bodies and/or ascospores (Engh et al., 2007). *Aspergilli* that produce no Hülle cells such as *Aspergillus tonophilus* and *Aspergillus fisheri,* also produce non-pigmented cleisthothecia (Hermann et al., 1983). In *Aspergilli* it is known that laccases are involved in the melanin production (Upadhyay et al., 2013). Scherer and co-workers showed that the type II laccase CpeA is highly expressed in Hülle cells (Scherer et al., 2002). The expression of *cpeA* in Hülle cells enables the production of

the cleistin matrix of the cleistothecia, which is possibly a polymeric product of phenolic material. Scanning and transmission electron microscopy reaveled that the cleistin matrix is an electron dense material and forms an outer layer of the cleistothecia which are pigmented (Champe and Simon 1992). Pigmentation protects most likely the cleistothecia against negative environmental influences.

### 1.4.3.3. Secondary metabolites to protect the fungus against fungivory and other environmental threats

A secondary metabolite-based defence mechanism provides a benefit to fungi (Döll et al., 2013, Drott et al., 2017, Caballero-Ortiz et al., 2013). In a recent study it was shown that the production of different secondary metabolites influenced the development of *Drosophila melanogaster* (Regulin and Kempken 2018). Analyzing effects of wild-type (FGSC A4, Glasgow wild-type, *veA*$^{+}$) *A. nidulans* compared to the deletion of the polyketide synthase *mdpG* which is required for the biosynthesis of monodictyphenone showed a 95% higher egg laying activity of *Drosophila melanogaster* on the deletion strain. The egg laying activity of *Drosophila melanogaster* was additionaly analyzed in a *laeA*Δ strain and was compared to *mdpG*Δ *and* wild-type. This resulted in an 57% egg laying activity on a *laeA*Δ mycelium. Döll and co-workers showed that the cleistothecia of *A. nidulans* covered with Hülle cells are more resistant against fungivory when comparing to a vegetative or asexual tissue (Döll et al., 2013). Therefore, secondary metabolites are crucial for the protection against environmental threats and provide resistance against fungivory.

## 1.5. Aim of this work

The central aim of this thesis was to get more insight into the proteomes and their biological functions of specialized fungal cells and during sexual development with a special focus on the accessory Hülle cells. The methyltransferase LaeA is required for Hülle cell formation and normally sized closed fruiting bodies named cleistothecia. Enrichment procedures for Hülle cells were established to gain more specific access to their proteomes. Tissues without and with Hülle cells were compared and analyzed. Identified proteins from an enriched Hülle cell fraction were compared to the identified proteins from a vegetative mycelium, asexual mycelium and sexual mycelium. Two proteomic approaches were performed during this study to investigate which proteins are enriched in Hülle cells. In the first proteomic approach proteins found in surface cultures were analyzed. An enriched Hülle cell fraction was compared to a vegetative, asexual and sexual mycelium. In the second proteomic investigation proteins were analyzed and quantified from submerged liquid cultures. Therefore, a *laeA*Δ strain was used that causes significant reduction in Hülle cells and was compared to a *nkuA*Δ strain with an additional unknown mutation that cause increased numbers of Hülle cells both in submerged liquid cultures. Furthermore, proteins identified in enriched Hülle cells were analyzed and functional gene studies were performed.

# 2. Materials and methods

## 2.1. Materials

### 2.1.1. Strains

*Aspergillus nidulans* strains *nkuA*Δ (AGB552) and *lysA*Δ; *nkuA*Δ (AGB1092) served as transformation host for deletion, complementation and GFP (green fluorescent protein) tagging. Strains used in this study are listed in Table 1.

**Table 1. Strains used in this study.**

*Aspergillus nidulans* strains are denominated AGB or FGSC, p.c. = personal communication.

| Strain | Genotype | Reference |
|---|---|---|
| ***Escherichia coli*** | | |
| DH5α™ | F-, Δ(*arg*F-lac)169, φ80d*lac*Z58(M15), Δ*pho*A8, *gln*X44(AS), λ-, *deo*R481, *rfb*C1, *gyr*A96(NalR), *rec*A1, *end*A1, *thi*E1, *hsd*R17 | Invitrogen, Germany |
| Top10 | F⁻ *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ*ara-leu*) 7697 *gal*U *gal*K *rps*L (Str$^R$) *end*A1 *nup*Gλ⁻ | Invitrogen, Germany |
| Strain | Genotype | Reference |
| ***Aspergillus nidulans*** | | |
| A4 | Glasgow wilde type, *veA*⁺ | FGSC |
| AGB596 | *pgpdA*::*sgfp*::*phleoR;pabaA1;yA;veA*⁺ | Bayram et al., 2012 |
| AGB552 | *pabaA1;yA;nkuA*Δ*::argB* | Bayram et al., 2012 |
| AGB1092 | *lysA*Δ*;pabaA1;yA;nkuA*Δ*::argB* | Meister. p.c. |
| AGB1073 | *laeA*Δ*;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1074 | *laeA*Δ*;lysA*Δ*;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1075 | *laeA*Δ*::laeA;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1076 | *laeA*Δ*::laeA;lysA*Δ*;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1077 | *mphA*Δ*;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1078 | *mphA*Δ*::mphA::gfp;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1079 | *mphA::gfp;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1080 | *mphA*Δ*::mphA;pabaA1;yA;nkuA*Δ*::argB* | This study |
| ABG1081 | *laeA*Δ*;mphA*::*gfp;paba1;yA;nkuA*Δ::*argB* | This study |
| AGB1082 | *lysA*Δ*;nptA::gfp;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1083 | *laeA*Δ*;lysA*Δ*;nptA::gfp;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1084 | *lysA*Δ*;rfeA::gfp;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1085 | *laeA*Δ*;lysA*Δ*;rfeA::gfp;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1086 | *lysA*Δ*;xptB::gfp;pabaA1;yA;nkuA*Δ*::argB* | This study |
| AGB1088 | *xptC::gfp;pabaA1;yA;nkuA*Δ::*argB* | This study |
| AGB1089 | *AN8434::gfp;pabaA1;yA;nkuA*Δ::*argB* | This study |
| AGB1090 | *AN8435*::*gfp;pabaA1;yA;nkuA*Δ::*argB* | This study |

## 2.1.2. Plasmids

Plasmids used in this study are listed in Table 2.

**Table 2. Plasmids used in this study.**
*RM*: recyclable marker, phleoRM = recyclable phleoRM resistance cassette from pME4305 or pJG292, $^{R}$ = resistance, $^{P}$ = promoter, $^{t}$ = terminator, p.c = personal communication.

| Plasmid | Description | Reference |
|---|---|---|
| pBluescript sk + | Cloning plasmid | Fermentas |
| pME4305 | *six*-$^{P}$*xylP*::*B-rec*::*trpc*$^{t}$-*phleo*$^{R}$-*six* | Gerke, p.c. |
| pJG292 | *swa*I::*six*-$^{P}$*xylP*::*B-rec*::*trpc*$^{t}$-*phleo*$^{R}$-*six*::*Eco74*I | Gerke, p.c. |
| pME4292 | Plasmid contains *gfp* | Jöhnk, p.c. |
| pME4636 | AN*laeA*Δ::phleoRM | This study |
| pME4637 | AN*laeA*::phleoRM | This study |
| pME4638 | AN*mphA*Δ::phleoRM | This study |
| pME4639 | $^{P}$*mphA*::AN*mphA*::*gfp*::phleoRM | This study |
| pME4640 | AN*mphA*::phleoRM | This study |
| pME4641 | $^{P}$*nptA*::AN*nptA*::*gfp*::phleoRM | This study |
| pME4642 | $^{P}$*rfeA*::AN*rfeA*::*gfp*::phleoRM | This study |
| pME4643 | $^{P}$*xptB*::AN*xptB*::*gfp*::phleoRM | This study |
| pME4645 | $^{P}$*xptC*::AN*xptC*::*gfp*::phleoRM | This study |
| pME4646 | $^{P}$*AN8434*::*AN8434*::*gfp*::phleoRM | This study |
| pME4647 | $^{P}$*AN8435*::*AN8435*::*gfp*::phleoRM | This study |

## 2.1.3. Primers

Oligonucleotides used in this study are listed in Table 3.

**Table 3. Oligonucleotides utilized for plasmid construction.**

| Designation | Sequence | Basepairs |
|---|---|---|
| BD49 | 5‘-ATC GAT AAG CTT GAT GTT TAA TAA ACA ACC GAG CTG GCC GTT-3‘ | 42 |
| BD50 | 5‘-ACC TAT AGG CCT GAG CTT GTC TCC TTT AAC TTC TCT GC-3‘ | 38 |
| BD51 | 5‘-ATA ATA TGG CCA TCT CTC ATA TCC CAA TTC TCT GAT TTT-3‘ | 39 |
| BD52 | 5‘-CTG CAG GAA TTC GAT GTT TAA ACC ATC TTA ACC ATC TTG GCG-3‘ | 42 |
| BD61 | 5’-ACG GTA TCG ATA AGC TTG ATG TTT AAA CTT CTT TTG TTC CAA ACA-3‘ | 45 |
| BD83 | 5‘-ATC GAT AAG CTT GAT GTT TAA ACA GAT TTG GTA GGA GCT AAC-3' | 42 |
| BD84 | 5'-GAC CTA TAG GCC TGA GGT TGA CGA TCT CTG AGA CGA-3‘ | 36 |

**Table 3. Continued, Oligonucleotides utilized for plasmid construction.**

| | | |
|---|---|---|
| BD85 | 5‘-ATA ATA TGG CCA TCT ATG AAC ATT GTC TTT GGA GAG TC-3‘ | 38 |
| BD86 | 5‘-CTG CAG GAA TTC GAT GTT TAA ACG TGG TGA TTA TCA TCC-3‘ | 39 |
| BD88 | 5‘-TGA GCA TAA TAT GGC CAT CTT TGT GAC TCT GTT GTC GGT TAT C-3‘ | 43 |
| BD89 | 5‘-CCG GGC TGC AGG AAT TCG ATG TTT AAA CCC ATC ACC CAT TCG CTT-3‘ | 45 |
| BD63 | 5‘-ATG GCC GAC GAC TAT CGC GAA GA-3‘ | 23 |
| BD64 | 5‘-TAT TGA CCT ATA AGG CCT GAG CTA GAA CTT CGT CTC AAG TAA CTC CT-3‘ | 47 |
| BD70 | 5‘-TGT GAG GTT ACC TCA GAT CTT GT-3‘ | 23 |
| BD71 | 5‘-TAT TGA CCT ATA GGC CTG AGT TAT CTT AAT GGT TTC CTA GCC TG-3‘ | 44 |
| BD73 | 5‘-ATA AGC TTG ATG TTT AAA CGT ACG TCT TTA TTA TAG TCG AG-3‘ | 41 |
| BD76 | 5‘-ACC TAT AGG CCT GAG CGT GAA CGA ATT CGA TGT G-3‘ | 34 |
| BD87 | 5‘-ATA ATA TGG CCA TCT GGC TGT GCT TCT AGA GAG ACG T-3‘ | 37 |
| BD90 | 5‘-CTG CAG GAA TTC GAT GTT TAA ACA ATG CCA CAA GGA A-3‘ | 37 |
| BD93 | 5‘-TAT TGA CCT ATA GGC CTG AGC TAC TCA TCA TCG CGG ATA T-3‘ | 40 |
| BD96 | 5‘-TTC TCC TTT ACT CAT GTT GAC GAT CTC TGA GAC GAT C-3‘ | 37 |
| BD97 | 5‘-TAC AGG CTG GTG CCA TGA GTA GCG ACA ACA CAG AGA AG-3‘ | 38 |
| BD98 | 5‘-ACC ACC GCT ACC ACC CTC ATC ATC GCG GAT ATC G-3‘ | 34 |
| BD99 | 5‘-GGT GGT AGC GGT GGT GTG AGC AAG GGC GAG GAG-3‘ | 33 |
| BD100 | 5‘-ACC TAT AGG CCT GAG CTA CTT GTA CAG TTC GTC CAT GCC-3‘ | 39 |
| BD104 | 5‘-AAC TAC TCG ACT ATA TCA ATG GA-3‘ | 23 |
| BD105 | 5‘-CTC ACA CCA CCG CTA CCA CCT TTC TCT GGA CAA GGA TGA TT-3‘ | 41 |
| BD106 | 5‘-GGT GGT AGC GGT GGT GTG AG-3‘ | 20 |
| BD107 | 5‘-CTA CTT GTA CAG TTC GTC CAT G-3‘ | 22 |
| BD108 | 5‘-TGC AGT GCC TGA TAA CTC T-3‘ | 19 |
| BD109 | 5‘-AAA CGA AAC CGC CGG TGC CA-3‘ | 20 |
| BD111 | 5‘-CAT ATG TGC AGG CCG CGT-3‘ | 18 |
| BD112 | 5‘-CTC ACA CCA CCG CTA CCA CCG GCC GAA AAT CCA TTA GAC TC-3‘ | 41 |
| BD113 | 5‘-TTC GTG CCG TCT TGG AGA-3‘ | 18 |

**Table 3. Continued, Oligonucleotides utilized for plasmid construction.**

| | | |
|---|---|---|
| BD114 | 5‘-GCT CAC ACC ACC GCT ACC ACC GTT ACC CAG CCA GCC ATG-3‘ | 39 |
| BD113 (*xptC*) | 5‘-AGG CTC AAC CTG ATA CTT ACC-3‘ | 21 |
| BD114 (*xptC*) | 5‘-GCT CAC ACC ACC GCT ACC ACC GTT ACC CAG CCA GCC ATG-3‘ | 39 |
| BD115 | 5‘-ATT AGA TCT ATT AGA CCG CAG G-3‘ | 22 |
| BD116 | 5‘-CAC GTG ATG TGA TAC GGT AC-3‘ | 20 |
| BD119 (*xptB*) | 5‘-AGA TCT ATT AGA CCG CAG GC-3‘ | 20 |
| BD120 (*xptB*) | 5‘-CTC ACA CCA CCG CTA CCA CCC CAC CGA TCA TCC CCC CTC C-3‘ | 40 |
| BD121 (*xptB*) | 5‘-TTC CTC TCT AGA AAC TTC TCA AA-3‘ | 23 |
| BD122 (*xptB*) | 5‘-ACA TGT ACT CGG ACC TGG TTC-3‘ | 21 |
| BD121 | 5‘-TAT TTC AAG GTA ACA GTC TGG-3‘ | 21 |
| BD122 | 5‘-CTC ACA CCA CCG CTA CCA CCG TTT CTG TCC GCG ATA GAC-3‘ | 39 |
| BD123 | 5‘-ACA CCA AAC ATC TGT AGA GAA CA-3‘ | 23 |
| BD124 | 5‘-TGT AGT CAA TTG CGG GGG TA-3‘ | 20 |
| BD125 | 5‘-ATT GAC GAA TTT TGT CGC GA-3‘ | 20 |
| BD127 | 5‘-CTC ACA CCA CCG CTA CCA CCG GTA CTA TGC ACT CCC AGC-3‘ | 39 |
| BD128 | 5‘-TTT CGC TCC TAT AAC TGG ACT-3‘ | 21 |
| BD129 | 5‘-ACT TCT TGA TAT TTA AAG CAA TTT-3‘ | 24 |

## 2.1.4. Chemicals and equipment

Different chemicals and enzymes as well as kits were used for the results of this research.

**Chemicals and the respective suppliers**

The following chemicals were purchased from AppliChem GmbH (Darmstadt, Germany), the order numbers are cited in brackets. Aceton (9R006076), arginine (A3709,0250), ß-glycerophosphat (A2253,0100), benzamidine (A1380,0005), bromophenol blue (A3640,0005), glucose (A3617,1000), isopropanol (A0900,2500GL), l-lysine monohydrochloride (33003468), nonident® P40 (A2239,0025), potassium chloride (A3582,1000), sodium chloride (A3597,1000),

sodium orthovanadate (2196,0005), sucrose (A4734,1000), tween 20® (A4974,0100), xylene cyanol (A4976,0005).

Biozym Scientific GmbH (Hessisch Oldendorf, Germany) provided agarose for agarose gels (840004).
As a subsidiary company of the American multi industry group General Electric GE Healthcare GmbH (Braunschweig, Germany) provided the following chemicals. Amersham™ alkphos direct labelling reagents (11816064), amersham™ cdp-star™ detection reagents (9766233).
Merck KGaA (Darmstadt, Germany), a leading science and technology company in the field of healthcare, life science and performance materials delivered the following chemicals. Di-sodium hydrogen phosphate (1065855000), DMSO (dimethyl sulfoxide) (1029310500), formic acid (1002641000), hydrogen peroxide 30% ($H_2O_2$) (8.22287.2500), magnesium chloride hexahydrate (1.05833.1000), potassium acetate (1.04820.1000), potassium dihydrogen phosphate (1.04873.1000), sodium dihydrogen phosphate monohydrate (1.06346.1000).

**Chemicals and suppliers, continued**

Carl Roth GmbH & Co. KG (Karlsruhe, Germany) is one of the leading companies in Germany for the production of fine chemicals and provided the following chemicals. In brackets the order number is again cited. Acetonitrile (T901.1), acetic acid (3738.2), acrylamide (Rotiphorese® Gel 40 37,5:1) (3029.1), agar-agar (5210.2), ammonium persulfate (APS) (9592.3), β-mercaptoethanol (4227.1), caffeine anhydrous (N815.3), calcium chloride (CN92.1), calcium chloride dihydrate (5239.1), citric acid monohydrate (3958.1), coomassie brilliant blue G-250 (9598.1), coomassie brilliant blue R-250 (3862.1), dimethylformamide (T921.1), DTT (1,4-dithiothreitol) (A1101,0025), EDTA (ethylenediamine tetraaceticacid disodium salt dihydrate) (8043.2), formaldehyde (4979.2), glycine (0079.1), glycerol (3783.1), hydrochloric acid (4625.2), imidazole (5709.3), magnesium sulfate heptahydrate (P027.2), manganese (II) chloride tetrahydrate (T881.1), manganese(II)sulfate monohydrate (4487.1), phosphoric acid (6366.1), ponceau S (5938.2), potassium hydroxide (6751.1), rotiphorese Gel 40 (3030.2), SDS (sodium dodecyl sulfate) (4360.2), sodium acetate (6773.2), sorbitol (6213.1), TEMED (N,N,N',N'-tetramethylethylenediamine) (2367.3),

trichloroacetic acid (7875.4), tris (tris-hydroxymethyl-aminomethane) (AE15.2), tris/HCl (9090.3), urea (2317.3), yeast extract (2904.1).

**Chemicals and the respective suppliers, continued**

SERVA Electrophoresis GmbH (Heidelberg, Germany) acts as a privately-owned company in the field of electrophoresis and also provides a wide portfolio of fine chemicals. They provided agar-agar SERVA high gel-strength, a choice quality for *in vitro* cultures (11396.03).

Sigma-Aldrich (Schnelldorf, Germany) is a leading life science and high technology company, being a subsidiary company of Merck KGaA (Darmstadt, Germany) and provided the following chemicals. Adenine (01830-50G), ampicillin (A9518-25G), ETDA-free protease inhibitor cocktail (48998), ethidium bromide (46065), formamide (47670), maltose monohydrate (112569), PMFS (phenylmethylsulfonylfldauoride) (P-7626), triton X-114 (X114).

The global life science company Thermo Fisher Scentific (Waltham, Massachusetts, United States of America) supports scientists worldwide. Several subsidiary companies are located in Germany (Thermo Fisher Scientific GmbH, Schwerte, Germany) and provided the following chemicals and DNA as well as protein ladders. Gene ruler DNA ladder mix (Thermo Scientific, SM0331), PageRuler$^{TM}$ prestained protein ladder (Thermo Scientific, SM26616), x-gal (5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside) (R0404).

Ethanol (20821.321) and methanol (20864.320) were ordered from VWR International GmbH (Hannover, Germany).

**Enzymes and suppliers**

The following enzymes were purchased from different companies. Natuzym (Schliessmann, Germany, 5090), Phusion® Hot Start high-Fidelity DNA Polymerase (New England Biolabs, Frankfurt am Main, Germany, M0530S), Lysozyme (SERVA Electrophoresis GmbH, Heidelberg, Germany, 28262.03), Phospholipase C (Invitrogen life technologies, Osterode, Germany, P6466), Restriction Endonucleases (Thermo Fisher Scientific GmbH, Schwerte, Germany), RNase A (Darmstadt, Germany, 24690.0), T4 DNA ligase (Thermo Fisher Scientific GmbH, EL0011), Trypsin (SERVA Electrophoresis GmbH , 37286.01), VinoTaste® Pro (Novozymes GmbH, Bagsvaerd, Denmark, 4518)

**Kits and the respective suppliers**

The following different kits were used. Amersham™ Alkphos Direct Labelling Reagents Kit (GE Healthcare, 11816064), Gene Art® Seamless Cloning and Assembly Kit (Invitrogen life technologies, A1328), HiSpeed Plasmid Midi Kit (QIAGEN, Hilden, Germany, 12643), NucleoSpin®Plasmid Kit (Macherey-Nagel, 740615.250), NucleoSpin®Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany, 740609.250), QIAprep Spin Miniprep Kit (QIAGEN, 27106), QIAquick Gel Extraction Kit (QIAGEN, 28704), QIAquick PCR Purification Kit (QIAGEN, 28104).

**Equipment and the respective suppliers**

Further materials were provided by different companies. Standard laboratory equipment including pipets, petri dishes and other plastic consumables were purchased from Gilson, Inc., (Limburg-Offheim, Germany), Sarstedt AG & Co (Nümbrecht, Germany). In order to centrifuge samples abiofuge fresco and biofuge pico (Heraeus Instruments GmbH) were used. To grind mycelia a retsch mill MM400 (Retsch GmbH, Haan, Germany 56789.1) was applied. Measuring the concentration of DNA and proteins a nanodrop ND-1000 photospectrometer (peqlab biotechnologie GmbH, Erlangen, Germany) was used. In order to image gel pictures a gel IX20 imager (Intas Science Imaging Instruments GmbH, Göttingen, Germany) was used. Southern hybridization experiments were performed with x-ray films (Protec GmbH, Oberstenfeld, Germany) and amersham ™ hyperfilm ™ ECL (GE Healthcare, 28906837). For protein work the following instruments were used. Mini-protean®tetra cell (Bio-Rad Laboratories GmbH, Munich, Germany 1658005EDU), mini trans-blot®electrophoretic cell (Bio Rad, 1703930), mini-sub®cell GT (Bio Rad, 1704487EDU), powerpac™ 200 (Bio Rad, 1645050). For western hybridization experiments a nitrocellulose transfer membrane (protran® BA (Sigma-Aldrich, 10401196)) was used. Besides savant speedvac concentrator (Thermo Scientific) and sterile filters 0.45/0.2 µm (Sarstedt, 83.1826/83.1826.001) were used. A liquid chromatography (LC) coupled to an *Orbitrap Velos Pro™ Hybrid Ion Trap-Orbitrap* mass spectrometer (MS) (Thermo Fisher Scientific, Bremen, Germany) was used for the two different proteomics investigations.

## 2.1.5. Solutions and growth media

## 2.1.5.1. Solutions

**Solutions regarding *Aspergillus nidulans***

The trace-element stock solution consisted of 18 µM $FeSO_4$,174 µM ETDA, 76 µM $ZnSO_4$,178 µM $H_3BO_3$, 25 µM $MnCl_2$, 7.1 µM, $CoCl_2$, 6.4 µM $CuSO_4$, 6.2 µM $Na_2MoO_4$. The resulting solution was sterilized with a sterile filter.

In order to extract DNA from *Aspergillus nidulans* the following extraction buffer was used and consisted of 1 M Tris (pH 7.2), 0.5 M ETDA (Ethylenediaminetetraacetic acid), 3 g SDS and 1 ml mercaptoethanol.

In order to perform transformations in *Aspergillus nidulans* the following solutions were used.
As a citrate buffer consisting of 150 mM KCl, 580 mM NaCl, 50 mM sodium citrate, pH 5.5 was used.
Protoplast solution consisted of 20 mg/ml VinoTaste® Pro (Novozymes GmbH, 4518) and 15 mg/ml lysozyme (Serva GmbH, 28262.03).

**Solutions regarding Southern hybridizations:**

Alkphos direct hybridization buffer (GE Healthcare, RPN 36888) was used as a prehybridization buffer. Additionally, 0.5 mM NaCl, 4% blocking solution was added.

Gels were washed in three different washing buffers: Buffer 1: 0.25 M HCl, Buffer 2: 0.5 M NaOH, Buffer 3: 0.5 M Tris, 1.5 M NaOH.

Southern hybridization membranes were washed in two different washing buffers.
Washing buffer 1: 2 M urea, 0.1% SDS, 50 mM $NaH_2PO4x\ H_2O$ pH 7, 150 mM NaCl, 1 mM $MgCl_2$, 0.2% blocking reagent.
Washing buffer 2: 1 M Tris pH 10, 2 M NaCl, 1 mM $MgCl_2$.

**Solutions regarding polyacrylamide gel electrophoresis:**

A 10X SDS running-buffer was used consisting of 0.25 M Tris/base, 1.92 M glycine and 10 g SDS.
A 10X transfer-buffer was used consisting of 0.25 M Tris/base, 1.92 M glycine and 0.2% SDS.

A 10X TBST (Tris buffered saline with tween 20) solution was used consisting of 100 mM Tris/HCl pH 8, 1.5 M NaCl and 0.5% tween 20.

Protease inhibitor solution was used and consisted of 100 mM PMFS (phenylmethylsulfonylfluoride), 0.5 mM benzamidine, 1 M DTT (1,4-dithiothreitol) and 1 M imidazole.

A 100X phosphatase inhibitor solution was used consisting of 100 mM sodium fluoride, 50 mM sodium vanadate and 800 mM ß-glycerophosphat.

A $B^+$ 300 buffer for protein extraction was used and consisted of 100 mM Tris pH 7.5, 300 mM NaCl, 10% glycerol, 1 mM EDTA, 0.1% NP-40, 1 mM DTT (1,4-dithiothreitol), 0.5 M benzamidin, 100 mM PMFS (phenylmethylsulfonylfluoride) and additionally a protease inhibitor and phosphatase inhibitor were added.

Coomassie staining solution was used and consisted of 0.1% (w/v) coomassie brilliant blue G250, 2% (w/v) ortho-phosphoric acid and 10% (w/v) ammonium sulfate.

Ponceau S staining solution was used and consisted of 0.2% ponceau S and 3% trichloroacetic acid.

A destaining solution was used and consisted of 40% (v/v) ethanol and 10% (v/v) acetic acid.

## 2.1.5.2. Growth media

**Growth medium regarding *Aspergillus nidulans*:**

Minimal medium (MM) was used as a growth medium for *Aspergillus nidulans* and consisted of 1% (w/v) glucose, 1% (w/v) AspA, 2 mM $MgSO_4$, 2% (w/v) Agar and trace elements (added after autoclaving), pH 6.5 and 2 mM $MgSO_4$.

**Growth media regarding *Escherichia coli*:**

*Escherichia coli* cultures were cultivated in LB medium and consisted of 1% (w/v) tryptone/peptone, 0.5% (w/v) NaCl, pH 7.2; 0.5% (w/v) yeast extract, 1.5% (w/v) agar-agar for solid medium and ampicillin (100 µg/ml) selection.

*Escherichia coli* cultures were cultivated in SOB medium and consisted of 0.5% (w/v) yeast extract, 2% (w/v) tryptone/peptone, 10 mM NaCl, 2.5 KCl, 10 mM $MgCl_2$ and 10 mM $MgSO_4$.

## 2.2. Methods

### 2.2.1. Cultivation of *Aspergillus nidulans*

Spores of *A. nidulans* wild-type FGSC A4, *veA*$^+$ were inoculated on solid minimal medium (1% glucose, 1% AspA, 2 mM $MgSO_4$, 2% Agar and trace elements (added after autoclaving), pH 6.5; 2 mM $MgSO_4$). Fungus was then cultivated under sexual (incubation in complete darkness and reduced oxygen conditions, therefore the petri dish was sealed with a parafilm) or under asexual conditions (incubation in light). The plates were incubated at 37 °C. The percentage of conidiophores in asexual mycelium were determined as described (Christmann et al., 2013). The diameter of cleistothecia were measured using an Olympus binocular SZX12-ILLB2-200 (Olympus GmbH, Hamburg, Germany). In order to determine the diameter of cleistothecia three biological replicates were considered. For each biological replicate the diameter of five cleistothecia were determined. In order to measure the diameter of cleistothecia Hülle cells were removed from cleistothecia using the cleistothecia-rolling technique.

#### 2.2.1.1. Hülle cells from solid agar plate: cleistothecia-rolling technique

Hülle cells are globose multicellular cells associated with sexual tissue of *Aspergillus nidulans*. In order to perform the cleistothecia-rolling technique, fungus was cultivated on solid agar plates under sexual conditions (incubation in complete darkness and reduced oxygen conditions, therefore the petri dish was sealed with a parafilm). Three, five and seven days after inoculation Hülle cells were enriched and the sexual

mycelium was harvested. In order to enrich Hülle cells, cleistothecium was transferred (with a fine tipped syringe) from sexual mycelium to a fresh petri dish containing 2% agar (Figure 8). This step was performed under a stereo microscope. Cleistothecium was then rolled back and forth on the solid agar plate with the fine tipped syringe and the Hülle cells adhered to the surface of the solid agar plate. In order to collect Hülle cells, they were picked with a fine tipped syringe. Hülle cells were collected into a collection tube containing 40 µl of $dH_2O$. Hülle cells were transferred from the solid agar petri dish to the collection tube with the fine tipped syringe. Hülle cells in the collection tube were centrifuged by 13.000 rpm for one minute and 35 µl of $dH_2O$ was removed. In order to observe enriched Hülle cells, they were visualized under a light microscope. The residual 5 µl $dH_2O$ was resuspended and the Hülle cells were transferred on a microscope slide.

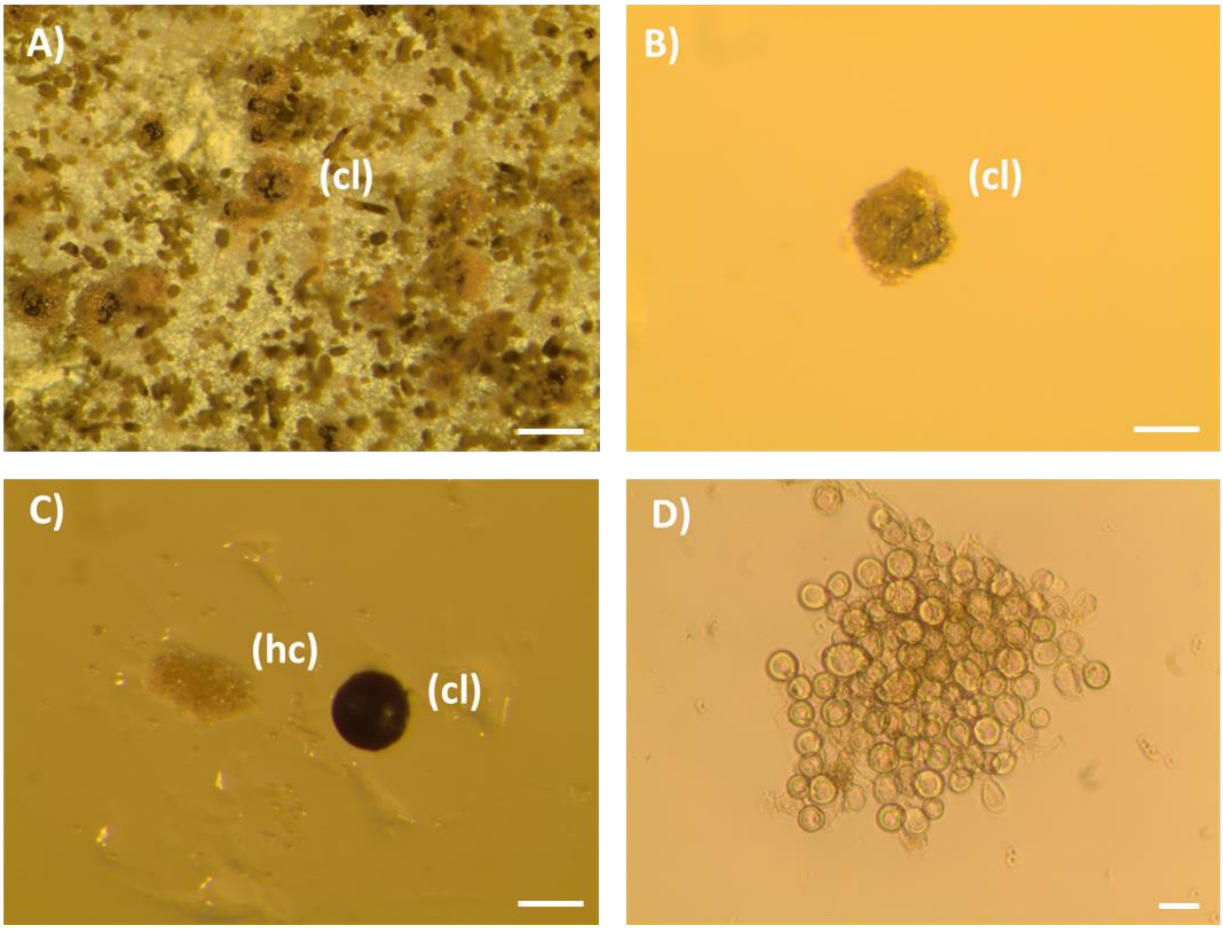



**Figure 8. Illustration of enrichment of Hülle cells from solid agar plates: the cleistothecia-rolling technique.**
A) Sexual mycelium of *A. nidulans* (FGSC A4 Glasgow wild-type, *veA*$^{+}$) grown on solid minimal medium. Scale bar is 200 µm. B) The cleistothecium was transferred with a fine tipped syringe to a new petri dish containing 2% agar. Scale bar is 200 µm. C) The cleistothecium was rolled with the help of the syringe on solid agar plate and Hülle cells adhered to the surface of the solid agar plate. Hülle cells were collected into a collection tube. Scale bar is 200 µm. D) Enriched Hülle cells seen under a light microscope (cl, cleistothecium, hc Hülle cells). Scale bar is 20 µm.

### 2.2.1.2. Hülle cells from submerged cultures

In order to cultivate vegetative mycelium spores were inoculated in 50 ml (250 ml shake flask) minimal medium (1% glucose; 1% AspA; 2 mM $MgSO_4$; and trace elements; pH 6.5; 5.937 $X10^5$ sp/ml) for 20 hours at 37 °C and with constant shaking. Hülle cells were observed under a light microscope. After 20 hours at 37 °C no Hülle cells were observed in wild-type FGSC A4, *veA*$^+$**.** The minimal medium was filtered off and vegetative mycelium was washed with 0.96% NaCl. After 72 hours, vegetative mycelium was harvested and Hülle cells were observed in the hyphal ball of vegetative mycelium of the lysine prototroph *nkuA*Δ (AGB552) strain with an additional unknown mutation and lysine auxotroph *lysA*Δ (AGB1092) strains under a light microscope. The percentage of observable Hülle cells in the hyphal ball was determined within an area of 5 $mm^2$. Hülle cells were counted and their number was put into relation to the parental and the complementation strain with three biological replicates. Additionally, the dry weight of the submerged cultures was measured. Vegetative mycelia were first filtered to remove the liquid media, then they were dried for 12 hours in a drying cabinet (75 °C).

In order to perform a triple SILAC experiment the three lysine auxotroph strains were labeled with different stable isotope lysine and the labels were added from the point on where the strains were inoculated. *lysA*Δ; *nkuA*Δ (AGB1092) was labeled light (L-lysine final concentration: 0.75 mM) *lysA*Δ; *laeA*Δ; *nkuA*Δ (AGB1074) was labeled heavy (L-lysine $^{13}C_6$ $^{15}N_2$ HCl final concentration 0.75 mM) and *lysA*Δ; *laeA*Δ::*laeA*; *nkuA*Δ (AGB1076) was labeled medium (L-lysine D4 labeled 2 HCl final concentration 0.75 mM). For a label swap experiment strains were labeled *lysA*Δ*; nkuA*Δ was labeled heavy*, lysA*Δ*; laeA*Δ*; nkuA*Δ was labeled light and *lysA*Δ*; laeA*Δ*::laeA; nkuA*Δ was labeled medium*.* 300 mg of each mycelium was merged and grinded with a retsch$^{TM}$-mill for 2 min by 30 frequency I/s.

## 2.2.2. DNA methods

### 2.2.2.1. Genetic transformation procedure

Transformations of *Escherichia coli* and *A. nidulans* cells were performed as described (Hanahan et al., 1991, Punt and van den Hondel 1992).

***Escherichia coli:***

For transformation 200 µl of chemo-competent cells were mixed with plasmid DNA and incubated on ice for 20 minutes, followed by an incubation for 1 minute at 42 °C. Next LB – liquid media were added to the cells and the samples were incubated for 30 minutes at 37 °C with 200 rpm. Cells were then inoculated on a solid agar plate containing LB-media with respective antibiotics.

***Aspergillus nidulans:***

For transformation protoplasts were used and prepared. Vegetative mycelium was used. Vegetative mycelium was harvested and washed with citrate buffer. Mycelium was transferred into a 300 ml flask and 25 ml of an enzyme mix was added (VinoTaste® Pro (Novozymes GmbH, 4518)/lysozyme (Serva GmbH, 28262.03)). The flask was incubated for 90 minutes at 30 °C by 70 rpm and protoplasts were filtered. After a washing step with 100 ml STC 1700 protoplasts were mixed with DNA cassettes. Successively 1.5 ml PEG 4000 was carefully added. Protoplasts were incubated for 30 minutes over ice to avoid crystral formation. Protoplasts were then washed with 50 ml STC 1700 buffer and inoculated on sorbitol-solid agar plates at 37 °C.

### 2.2.2.2. Plasmid DNA isolation from *Escherichia coli*

For the isolation of plasmids from *Escherichia coli* the following kits were used, HiSpeed Plasmid Midi Kit (QIAGEN, 12643), NucleoSpin®Plasmid Kit (Macherey-Nagel, 740615.250), QIAprep Spin Miniprep Kit (QIAGEN, 27106). Plasmid DNA isolation was performed according to the manufacturer's instructions. Therefore, *Escherichia coli* was inoculated in LB media with the respective antibiotic. Cultures were centrifuged at 13.000 rpm in a 2 ml reaction tube. The pellet was resuspended

in 250 µl buffer P1. As a next step 250 µl P2 was added and the reaction tube was inverted several times for 5 minutes. Next 350 µl of buffer N3 was added and the reaction tube was centrifuged for 10 minutes at 13.000 rpm. The supernatant was added into a DNA spin column. The DNA spin column was centrifuged for one minute at 13.000 rpm. The column was washed with a washing buffer. The DNA spin column was centrifuged at 13.000 rpm to remove the washing buffer. As a final step the plasmid DNA was eluted in 30 µl of $dH_2O$. The plasmid concentration was measured with a NanoDrop ND-1000 photospectrometer (peqlab biotechnologie, Erlangen, Germany).

### 2.2.2.3. *Aspergillus nidulans* DNA extraction

Vegetative mycelium of *A. nidulans* was used to extract genomic DNA (Lee and Taylor 1990). The mycelium was ground and 500 µl of lysis buffer (1 M Tris (pH 7.2),0.5 M ETDA, 3 g SDS, 1 ml mercaptoethanol) was added and heated at 65 °C for 20 min. 100 µl of 8 M potassium acetate was added and tubes were inverted for 8-10 times. The samples were centrifuged for 15 min at 13000 rpm. This step was repeated. The supernatant was transferred into a new tube and 300 µl isopropanol was added. The samples were centrifuged for 15 min and the pellet was washed with 1000 µl of ethanol and dissolved in 120 µl of water/RNase A and stored at 4 °C.

### 2.2.2.4. Ligation of DNA fragments

Fragments were ligated into the respective vectors using the Gene Art$^R$ Seamless Cloning and Assembly Kit (Invitrogen life technologies, A1328) or the T4 ligase (T4 DNA ligase (Thermo Scientific, EL0011)). Seamless cloning and assembly procedure was performed according to the manufacturer's instructions. The ligation procedure using the T4 ligase was performed as described by (Lehman 1974). The T4 ligase reaction was performed as following: 2-4 µl hydrolyzed vectors, 10-15 µl of the respective amplified DNA fragments and 2 µl T4 ligase buffer were mixed together. The ligation reaction was performed for 3 hours at room temperature. In order to perform the ligation, the hydrolyzed vectors were first dephosphorylated with calf intestine alkaline phosphatase (CIAP, Thermo Scientific). The dephosphorylation was performed as following: 17 µl hydrolyzed vectors, 2 µl calf intestine alkaline

phosphatase buffer, 1 µl CIAP, were mixed together. The samples were incubated for 10 minutes at 37 °C. The enzyme reaction was then inactivated at 75 °C for 10 minutes. The amplified DNA fragments were phosphorylated as following: 15 µl DNA, 1 µl T4 Polynucleotide kinase (T4PNK, Thermo Scientific), 2 µl kinase buffer, 2 µl 10 mM adenosine triphosphate, were mixed together. The samples were incubated for 20 minutes at 37 °C. In order to inactivate the enzyme reaction, the samples were then incubated for 10 minutes at 75 °C.

## 2.2.2.5. PCR (Polymerase chain reactions)

Polymerase chain reactions PCR (Saiki et al., 1988) were performed using either a platinum® *Taq* polymerase (Fermentas GmbH, St. Leon Rot, Germany) or a high-fidelity DNA polymerase Phusion® (Thermo Fisher Scientific GmbH, Schwerte, Germany, 89846). The PCR program which was used in this study is illustrated in Table 4.

**Table 4. PCR program used in this study.**

| Step | Temperature [°C] | Duration [min] | loops |
|---|---|---|---|
| 1 | 98 | 00:30 | |
| 2 | 98 | 00:10 | |
| 3 | 60 | 00:30 | |
| 5 | 72 | 02:00 | repeated 36 times |
| 5 | 72 | 05:00 | |
| 6 | 4 | pause | ∞ |

## 2.2.2.6. Gelelectrophoresis of DNA

DNA was separated on a 1% agarose gel described by (Aaij and Borst 1972). DNA fragments were mixed with 1X loading dye and gelelectrophoresis was performed with 90 V using a horizontal gel chamber. The agarose gel contained ethidium bromide and DNA fragments were visualized in a DNA documentation system, Gel IX20 imager (Intas science instruments, Göttingen, Germany).

### 2.2.2.7. Purification of amplified DNA

For the purification of amplified DNA the following kit was used. QIAquick Gel Extraction Kit (QIAGEN, 28704) or QIAquick PCR Purification Kit (QIAGEN, 28104). This procedure was performed according to the manufacturer's instructions. Excised DNA of agarose gels were transferred into a 2 ml reaction tube. 400 µl QC buffer was added and the reaction tube was put into a 65 °C thermobloc for 10 minutes until the excised agarose gel piece melted. As a next step the melted solution was transferred into a DNA spin column. The DNA spin column was centrifuged by 13.000 rpm for one minute. The procedure was repeated for a second time. 650 µl of washing buffer was added and the DNA spinning column was centrifuged for 13.000 rpm for one minute. This procedure was repeated for a second time and the DNA spin column was centrifuged for 13.000 rpm to remove the washing buffer. As a final step the amplified DNA was eluted in 30 µl with $dH_2O$. The concentration of the amplified DNA was measured with a NanoDrop ND-1000 photospectrometer (peqlab biotechnologie, Erlangen, Germany).

### 2.2.2.8. Southern hybridization

Southern hybridization was performed with a non-radioactive probe (Amersham Bioscience, Buckinghamshire, United Kingdom) as described by (Southern 1975). Primers used for amplification of Southern probes are listed in Table 5. In order to confirm the successful integration of the mutagenesis cassette genomic DNA of the fungal mutant strains was digested with restriction enzymes for 12 hours. The DNA fragments were separated according to size by agarose gel electrophoresis. The agarose gels were washed with three different washing buffers, buffer 1 (0.25 M HCl, 10 minutes), buffer 2 (0.5 M NaOH, 25 minutes), buffer 3 (0.5 M Tris, 1.5 M NaCl, pH 7.4, 30min). All steps were performed at room temperature. DNA was transferred onto an Amersham™ Hybond-N™ Nylon membrane (GE Healthcare, 676409), by dry blotting for 150 minutes at room temperature. Membranes were dried (7 minutes, 75°C) and the transferred DNA was cross linked to the membranes using UV light ($\lambda$ = 254 nm, 3 minutes per each side). Membranes were transferred into a prehybridization glass tube and 10 ml preheated prehybridization buffer was added. The membranes were incubated in a hybridization oven (60 minutes, 55 °C, rotated). After 30 minutes

40 µl DNA southern probes were added. DNA probes were made according to the Amersham™ Alkphos Direct Labelling Reagents Kit (GE Healthcare, 11816064). Labelling of DNA probes were performed according to the manufacturer's instructions. The membrane was incubated with the DNA Southern probes for 12 hours at 55°C and rotated in the hybridization oven. The membrane was washed in 25 ml washing buffer 1 for 10 minutes. This step was performed twice at 55 °C and the membrane was rotated in the hybridization oven. 30 ml washing buffer 2 was added. The washing step was performed at room temperature for 5 minutes, shaking. For detection of DNA the Amersham™ Cdp-star™ Detection reagents (GE Healthcare, 9766233) were applied to the membrane. Finally, the labeled DNA on the membrane was detected with an Amersham ™ Hyperfilm ™ ECL (GE Healthcare, 28906837).

**Table 5. Primers used for amplification of Southern probes.**

| Probe | Primer |
|---|---|
| *laeA* 5' UTR | BD45/BD46 |
| *laeA* 3' UTR | BD47/BD48 |
| *mphA* 3' UTR | BD85/BD86 |
| *nptA* 3' UTR | BD108/BD109 |
| *rfeA* 3' UTR | BD113/BD114 |

## 2.2.2.9. Sequence analysis and oligonucleotides synthesis

In order to analyze DNA sequences the software tool lasergene DNASTAR, Inc., Madison, Wisconsin, United States of America (Durfee and Schwei 2008) was used. Oligonucleotides were ordered from Eurofins MWG GmbH, Ebersberg, Germany. DNA was sequenced at SEQLAB-Sequencing Laboratories GmbH, Göttingen, Germany.

## 2.2.2.10. *Aspergillus nidulans* strain construction

### 2.2.2.10.1. Construction of *laeA*Δ, *laeA* complementation and other strains in *laeA*Δ

DNA sequence informations were obtained from AspGD (Cerqueira et al., 2014). Strains used in this study contain a recyclable marker module (Krappmann et al., 2005). In order to generate the *laeA* deletion construct the 5' UTR region was amplified from wild-type FGSC A4, *veA*$^{+}$ genomic DNA with primers BD45/BD46 and the 3' UTR was amplified with the primers BD47/BD48. The two amplicons were fused with the recyclable *phleo* cassette (the *phleo* cassette contains a bleomycin (Bm) resistance-encoding gene *ble*) using a seamless cloning reaction (Gene Art® seamless cloning and assembly kit, Invitrogen life technologies, A13288). The deletion cassette was integrated into a *pBluescript SK* (+) vector. The linear deletion cassette was excised from the vector using a *Pme*I restriction cutting site and transformed into a *nkuA*Δ (AGBB52) and in a *lysA*Δ; *nkuA*Δ (AGB1092) parental strain. For complementation of *laeA*Δ the *laeA* genomic locus was amplified from wild-type FGSC A4, *veA*$^{+}$ genomic DNA (primers BD45/BD76) fused together with a recyclable *phelo* marker and with the 3' UTR using a seamless cloning reaction.

In order to construct the *nptA*::*gfp* cassette a two-step cloning strategy was used (Mounts et al., 1989). The 5' UTR plus the sequence coding region of *nptA* was amplified with BD104/BD105 and the 3' UTR was amplified with the primers BD108/BD109. The *gfp* (green fluorescent protein) sequence was amplified using BD99 and BD100. The *gfp* sequence was fused with the coding sequence of *nptA* by fusion PCR. The fragment was integrated in the plasmid pJG229 containing a recyclable *phleo* cassette using a *Swa*I restriction cutting site. In the second step, the 3' UTR was integrated into the plasmid using an *Eco*74I restriction cutting site. In order to construct the *rfeA*::*gfp* cassette the 5' UTR plus the gene *rfeA* was amplified with the primers BD111/BD112. The 3' UTR was amplified with the primers BD113/BD114. The amplicons were fused using the two-step cloning strategy explained above. Linear cassettes of *nptA::gfp* and *rfeA::gfp* were transformed into a *lysA*Δ; *nkuA*Δ (AGB1092) and a *laeA*Δ; *lysA*Δ; *nkuA*Δ (AGB1074) strain. All strains were confirmed by Southern hybridization (Figure 9-13).

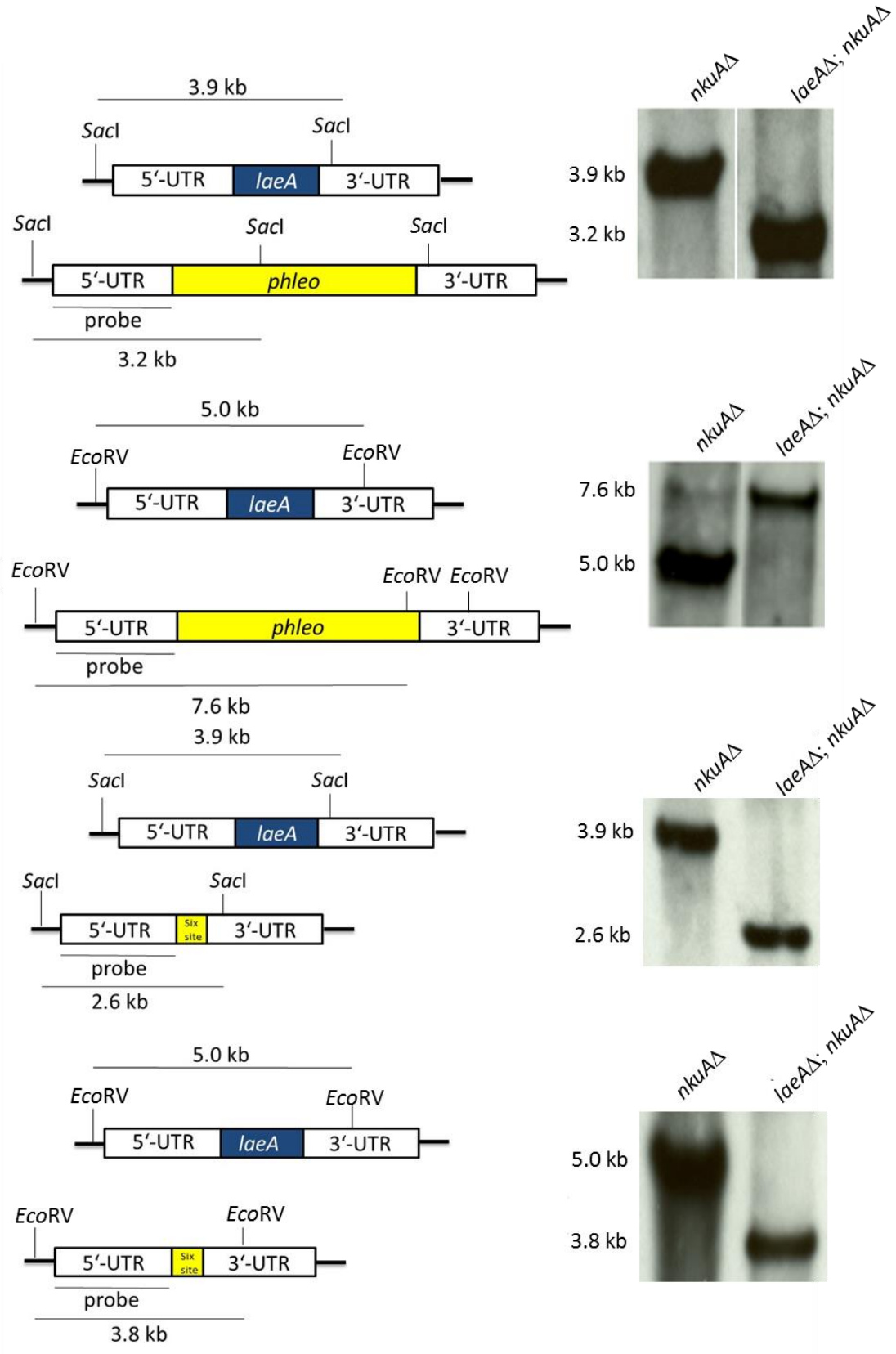



**Figure 9. Restriction map and Southern hybridization before and after the marker was recycled for the deletion strain *laeA*Δ; *nkuA*Δ (AGB1073).**
The following restriction enzymes were used: *Sac*I and *Eco*RV.

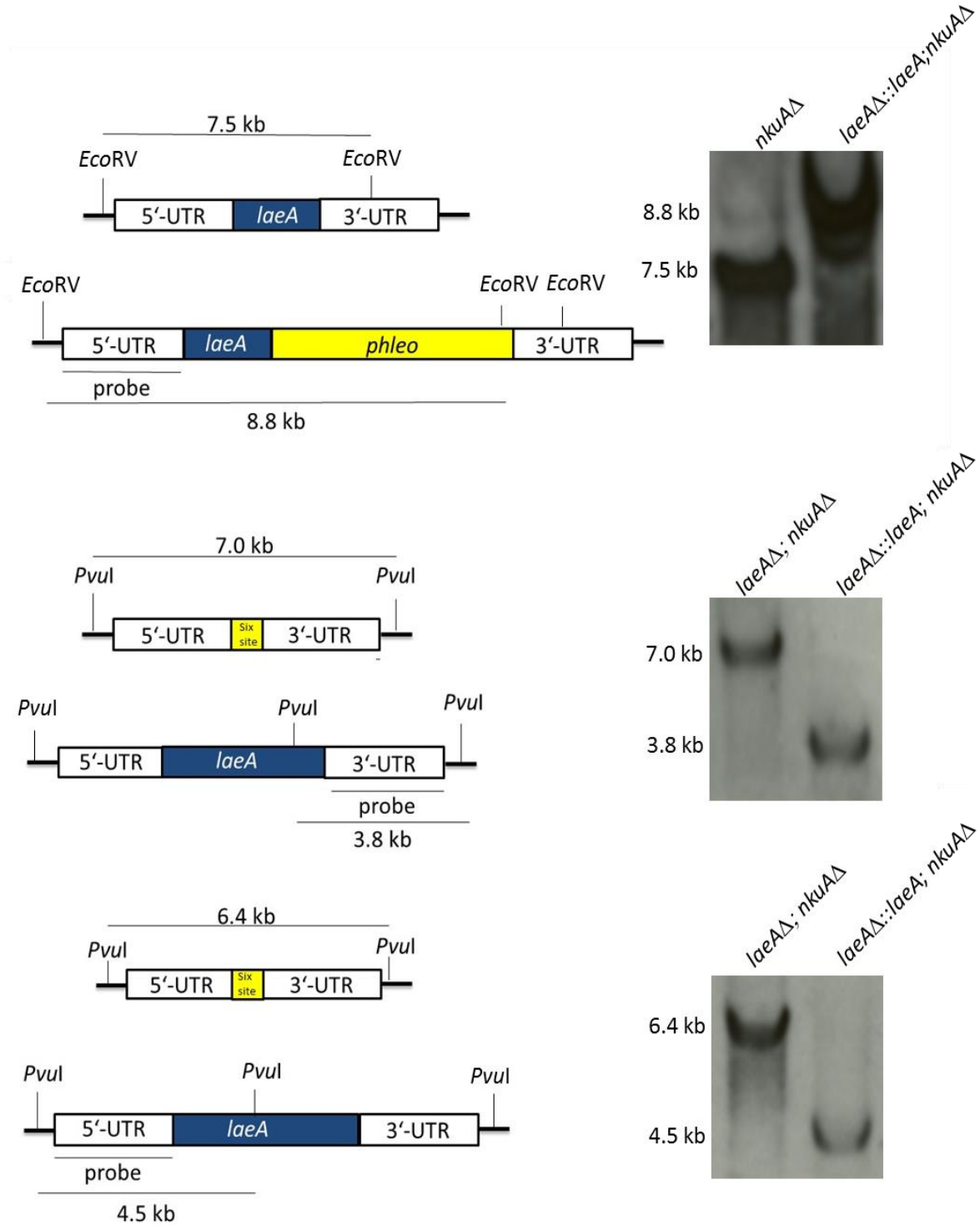



**Figure 10. Restriction map and Southern hybridization before and after the marker was recycled for the complementation strain of *laeA*Δ (*laeA*Δ::*laeA*; *nkuA*Δ, AGB1075).**
The following restriction enzymes were used: *Eco*RV and *Pvu*I.

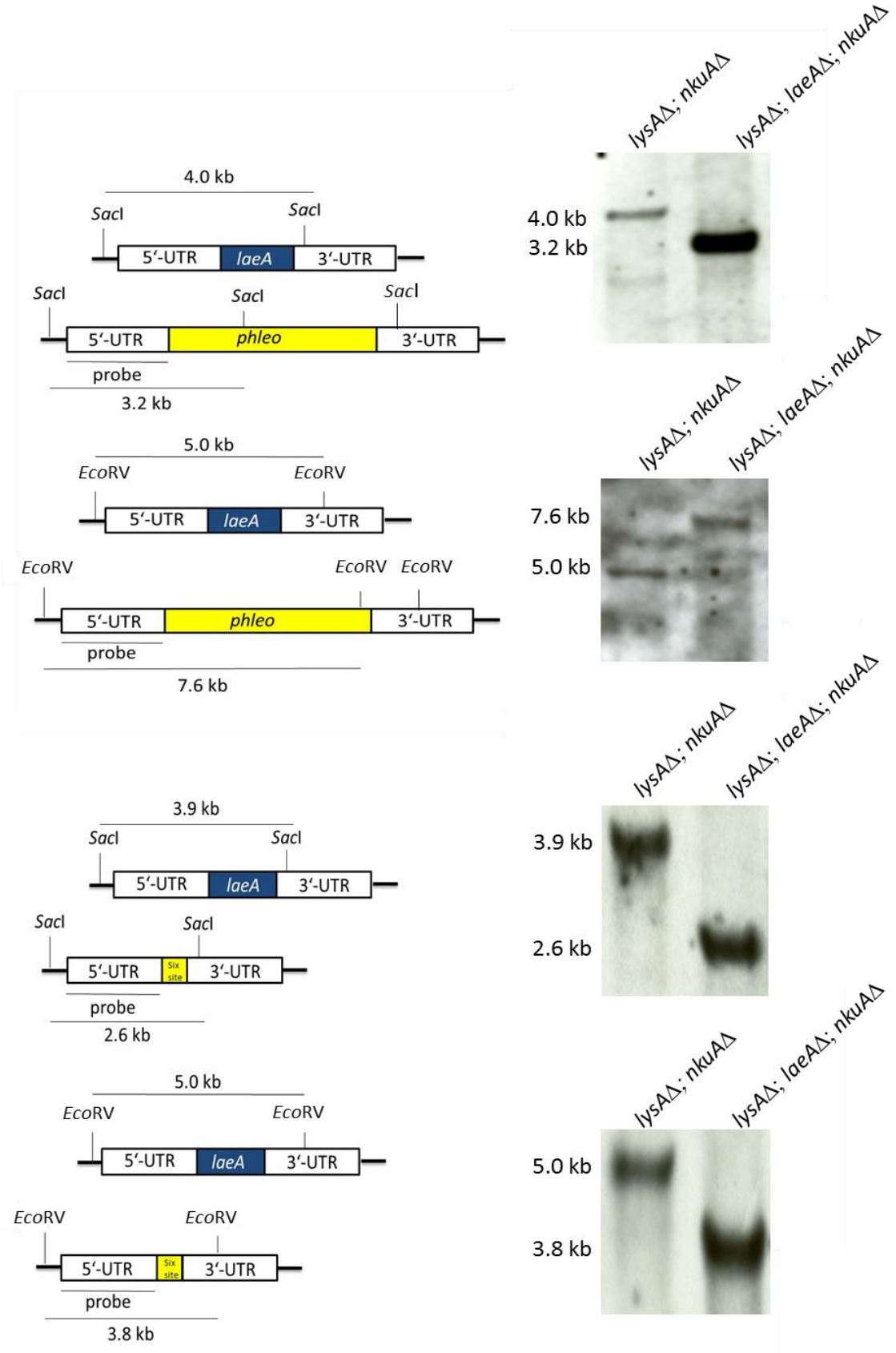



**Figure 11. Restriction map and Southern hybridization before and after the marker was recycled for lysine auxotrophic *laeA*Δ strain (*lysA*Δ; *laeA*Δ; *nkuA*Δ, AGB1074).**
The following restriction enzymes were used: *Sac*I and *Eco*RV.

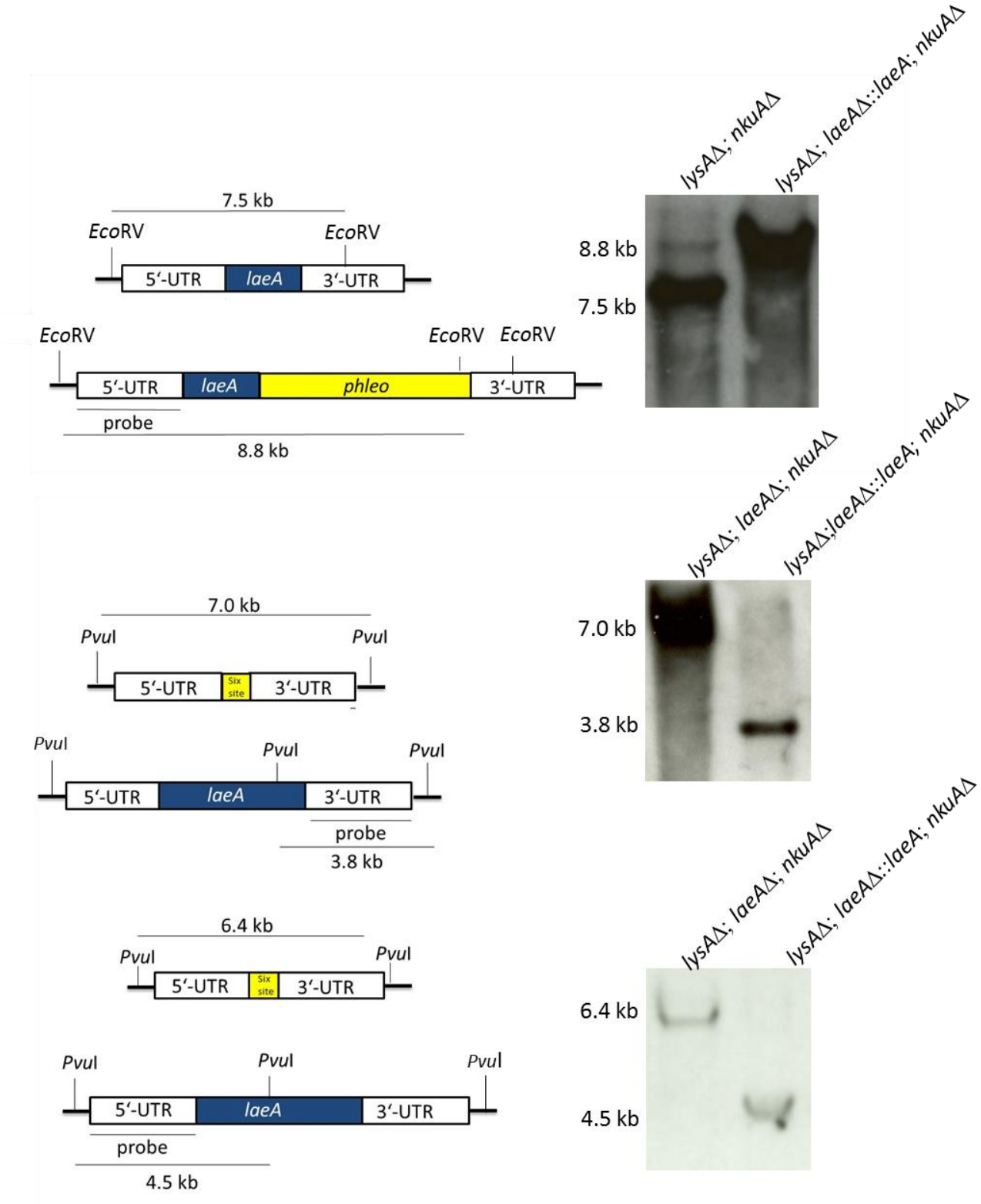



**Figure 12. Restriction map and Southern hybridization before and after the marker was recycled for the complementation strain *lysA*Δ*; laeA*Δ **(*lysA*Δ;*laeA*Δ::*laeA*;*nkuA*Δ, AGB1076).** The following restriction enzymes were used: *Eco*RV and *Pvu*I.

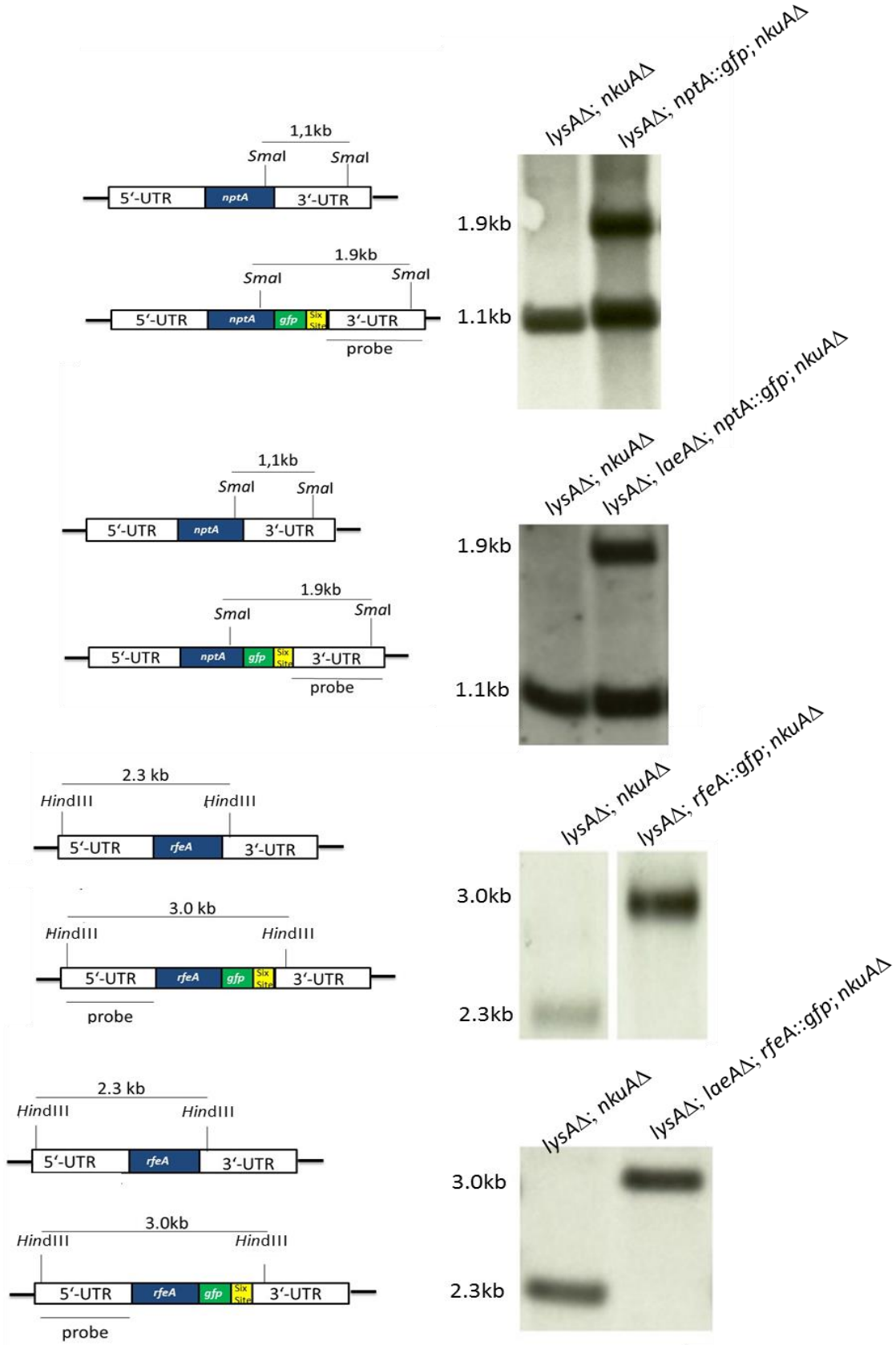



**Figure 13. Restricition map and Southern hybridization to confirm *nptA*::*gfp* and *rfeA*::*gfp* strains**
Strains: *lysA*Δ;*nptA*::*gfp*;*nkuA*Δ (AGB1082), *lysA*Δ;*laeA*Δ;*nptA*::*gfp*;*nkuA*Δ (AGB1083). Strains: *lysA*Δ;*rfeA*::*gfp*;*nkuA*Δ (AGB1084), *lysA*Δ;*laeA*Δ;*nptA*::*gfp*;*nkuA*Δ (AGB1085). The following restriction enzymes were used: *Sma*I and *Hin*dIII. The strains contained a recyclable *phleo* cassette and were already recycled

## 2.2.2.10.2. Construction of *xptB::gfp* strain

The *xptB* (xanthone prenyltransferase) gene was fused with *gfp*. The *xptB::gfp* cassette was constructed using a two-step cloning strategy (Mounts et al., 1989). In order to construct the *xptB*::*gfp* cassette the 5' UTR plus the coding sequence of the gene *xptB* was amplified with the primers BD119/BD120(*xptB*) (Figure 14). The 5' UTR plus the coding sequence of the gene *xptB* were fused with the sequence of *gfp*. This fusion fragment was integrated into the plasmid pJG229 containing a recyclable *phleo* cassette using a *Swa*I restriction cutting site. The 3' UTR was amplified with the primers BD121(*xptB*)/BD122(*xptB*). Next the 3' UTR was integrated into the plasmid pJG299 using an *Eco*74I restriction cutting site.

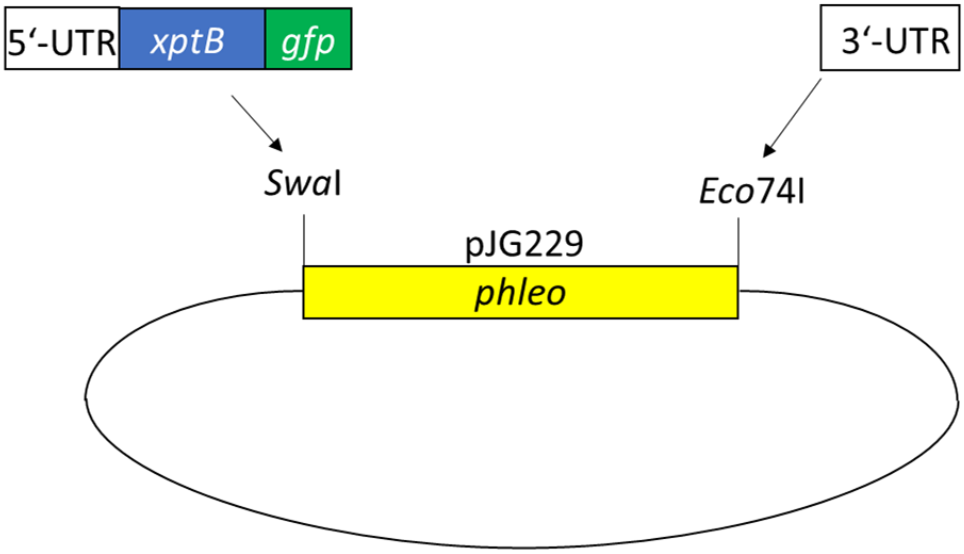



**Figure 14. Schematic illustration of the two-step cloning strategy.**
The sequence of the 5' UTR fused with the coding sequence of the *xptB* and with *gfp* was first integrated into the plasmid pJG229 containing a recyclable *phleo* cassette using a *Swa*I cutting site. Next the 3' UTR was integrated into the plasmid pJG229 using an *Eco*74I restriction cutting site.

The *xptB*::*gfp* cassette was transformed into a *lysA*Δ*; nkuA*Δ (AGB1092) parental strain and integration of the *xptB*::*gfp* cassette was confirmed by diagnostic PCR (Figure 15). In order to perform the diagnostic PCR to confirm the integration of the cassette the 5' UTR plus the coding sequence and the sequence of *gfp* was amplified using genomic DNA with the primers *xptB*::*gfp* BD119/BD107. Additionally, the *gfp* sequence was amplified with the primers BD106/BD107.

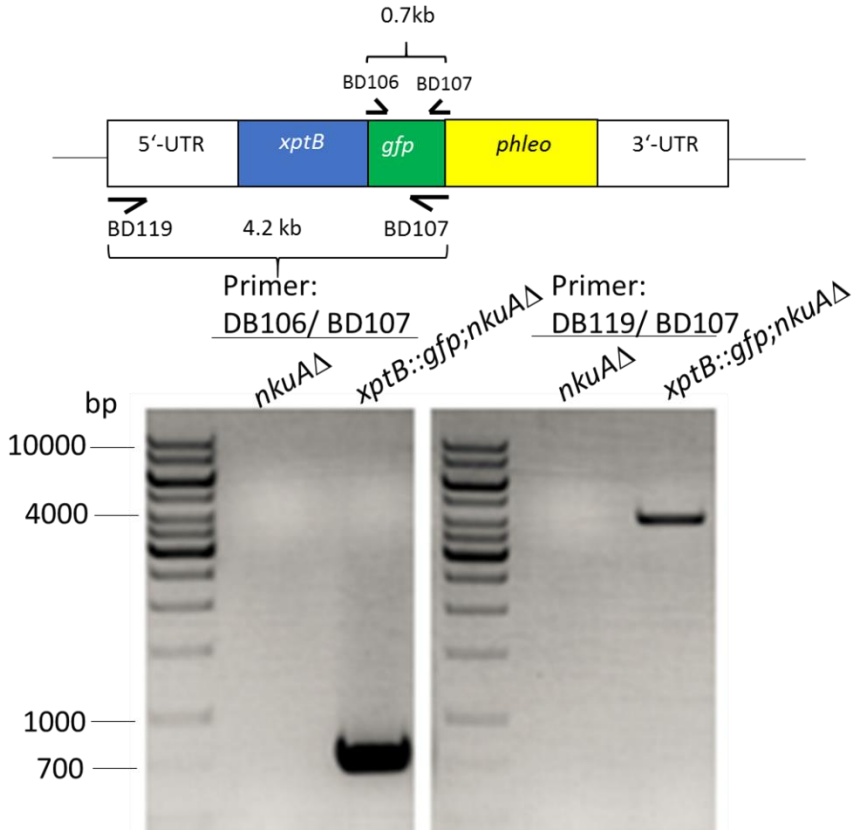



**Figure 15. Diagnostic PCR to confirm the integration of the *xptB::gfp* cassette**.
The 5' UTR plus the coding sequence of *xptB* and the sequence of *gfp* was amplified using genomic DNA (*xptB*::*gfp*; *nkuA*Δ; *lysA*Δ, AGB1086) with the primers BD119/BD107. The amplified product has a size of 4.2 kb. As negative control genomic DNA of *nkuA*Δ; *lysA*Δ was used. In order to amplify the *gfp* sequence the primers BD106 and BD107 were used. The amplified product has a size of 0.7 kb.

## 2.2.2.10.3. Construction of *xptC::gfp* strain

The *xptC* (oxidoreductase) gene was fused with *gfp*. In order to construct the *xptC*::*gfp* cassette the 5' UTR plus the coding sequence of the gene *xptC* was amplified with the primers BD113(*xptC*)/BD114(*xptC*). The sequence of *xptC* was fused with *gfp*. The 3' UTR was amplified with the primers BD115/BD116. The amplicons were fused using the two-step cloning strategy mentioned above. The fragments were integrated into the plasmid pJG229 using a *Swa*I and an *Eco*74I restriction cutting site. The *xptC::gfp* cassette was transformed into a *nkuA*Δ (AGB552) parental strain and integration of the *xptC*::*gfp* cassette was confirmed by diagnostic PCR (Figure 16). In order to perform the diagnostic PCR to confirm the integration of the cassette the 5' UTR plus the coding sequence and the sequence of *gfp* was amplified using genomic DNA with the primers BD114/BD107. Additionally, the *gfp* sequence was amplified with the primers BD106/BD107.

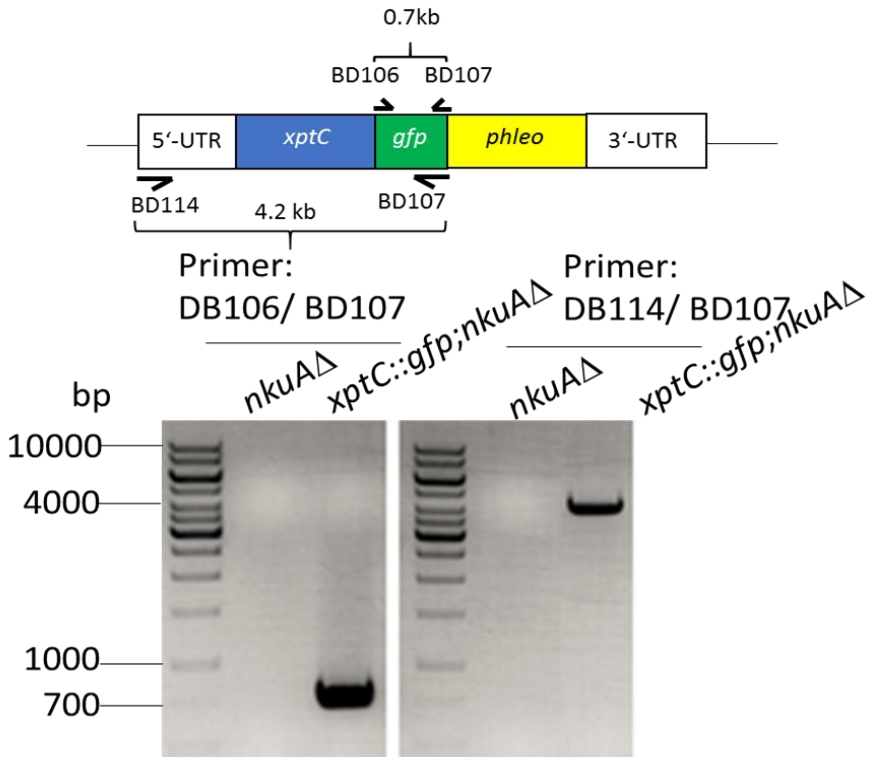



**Figure 16. Diagnostic PCR to confirm the integration of *xptC::gfp* cassette.**
The 5' UTR plus the coding sequence of *xptC* and the sequence of *gfp* was amplified using genomic DNA (*xptC*::*gfp*;*nkuA*Δ, AGB1088) with the primers BD114/BD107. The amplified product has a size of 4.2 kb. In order to amplify the *gfp* sequence the primers BD106 and BD107 were used. The amplified product has a size of 0.7 kb. As negative control genomic DNA of *nkuA*Δ (AGB552) was used and no amplified products were observable.

## 2.2.2.10.4. Construction of *AN8434::gfp* strain

In order to construct the *AN8434::gfp* cassette the 5' UTR plus the gene *AN8434* was amplified with the primers: BD121/BD122 .The 3' UTR was amplified with the primers BD123/BD124. The amplicons were fused using the two-step cloning strategy mentioned above. First the 5' UTR and the coding sequence of *AN8434* was fused with the sequence of *gfp*. The fragment was integrated into the plasmid pJG229 containing a recyclable *phleo* cassette using a *Swa*I restriction cutting site. The 3' UTR was integrated into the plasmid pJG229 using an *Eco*74I restriction cutting site. The *AN8434*::*gfp* cassette was transformed into a *nkuA*Δ (AGB552) parental strain. Integration of the *AN8434*::*gfp* was confirmed by diagnostic PCR (Figure 17). In order to perform the diagnostic PCR to confirm the integration of the cassette the 5' UTR plus the coding sequence and the sequence of *gfp* was amplified using genomic DNA with the primers *AN8434*::*gfp* (BD121/BD107). Additionally, the *gfp* sequence was amplified with the primers BD106/BD107. As negative control genomic DNA of the parental strain *nkuA*Δ was used.

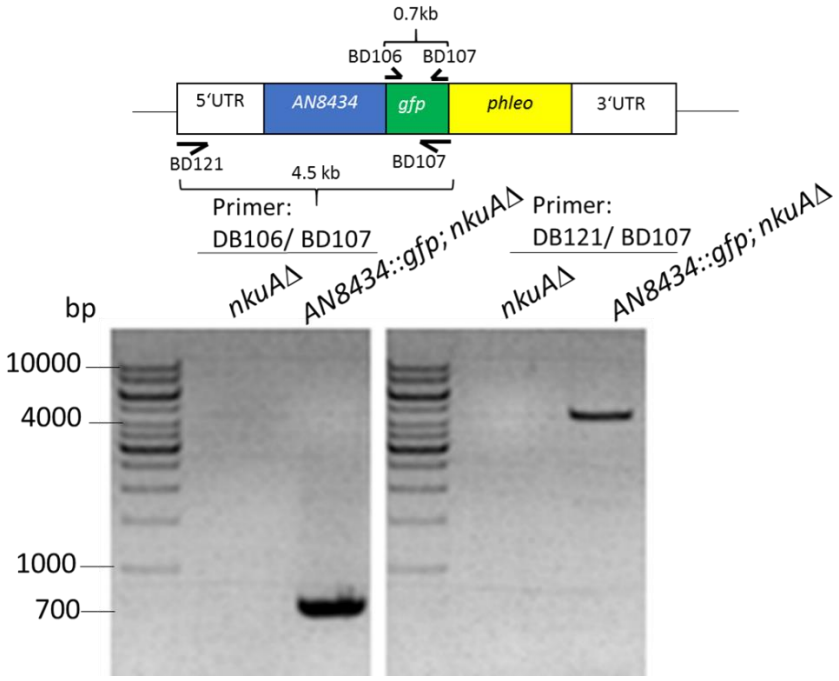



**Figure 17. Diagnostic PCR to confirm the integration of the *AN8434::gfp* cassette.**
The 5' UTR plus the coding sequence *AN8434* and the sequence of *gfp* was amplified using genomic DNA (*AN8434*::*gfp*;*nkuA*Δ, AGB1089) with the primers BD121/BD107. The amplified product has a size of 4.5 kb. The sequence of *gfp* was amplified using the primers BD106/BD107. The amplified product has a size of 0.7 kb. As negative control genomic DNA of *nkuA*Δ (AGB552) was used and no amplified products were observable.

## 2.2.2.10.5. Construction of *AN8435::gfp* strain

In order to construct the *AN8435::gfp* strain cassette the 5' UTR plus the gene *AN8435* was amplified with the primers BD125/BD127. This sequence *AN8435* was fused with the sequence of *gfp*. The 3' UTR was amplified with the primers BD128/BD129. The amplicons were fused using the two-step cloning strategy. The fragments were integrated into the plasmid pJG229 using a *Swa*I and an *Eco*74I restriction cutting site. The *AN8435::gfp* cassette was transformed into a *nkuA*Δ (AGB552) parental strain and integration of *AN8435*::*gfp* was confirmed by diagnostic PCR (Figure 18). In order to perform the diagnostic PCR to confirm the integration of the cassettes the 5' UTR plus the coding sequence and the sequence of *gfp* was amplified using genomic DNA with the primers *AN8435*::*gfp* (BD125/BD107). Additionally, the *gfp* sequence was amplified with the primers BD106/BD107. As negative control genomic DNA of the parental strain *nkuA*Δ was used.

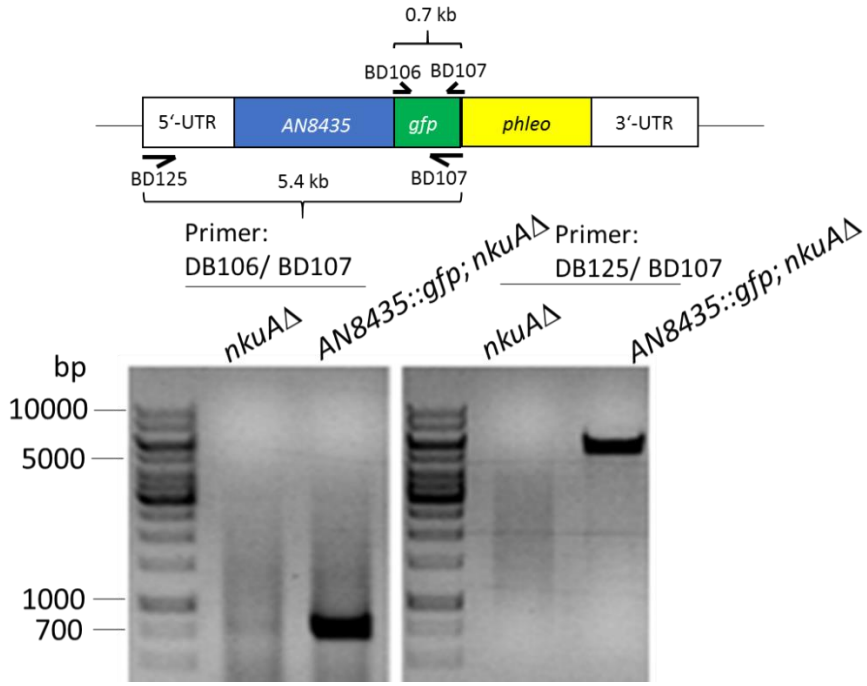



**Figure 18. Diagnostic PCR to confirm the integration of the *AN8435::gfp* cassette.**
The 5' UTR plus the coding sequence of *AN8435* and the sequence of *gfp* was amplified using genomic DNA (*AN8435*::*gfp*;*nkuA*Δ, AGB1090) with the primers BD125/BD107. The amplified product has a size of 5.4 kb. The sequence of *gfp* was amplified using the primers BD106/BD107. The amplified product has a size of 0.7 kb. As negative control genomic DNA of *nkuA*Δ (AGB552) was used and no amplified products were observable.

## 2.2.2.10.6. Construction of *mphA*Δ, *mphA* complementation and other strains in *mphA*Δ

The *mphA* (**m**altose **p**ermease-like protein of **H**ülle cells) knockout plasmid and knockout strain was constructed as mentioned above using the seamless cloning and assembly strategy. The 5' UTR and the 3' UTR region was amplified from the wild-type FGSC A4, *veA*$^{+}$ genomic DNA using the primers BD83/BD84 and BD85/BD86. The linear deletion cassette was excised from the vector using a *Pme*I restriction cutting site and transformed into a *nkuA*Δ (AGBB52) parental strain. In order to construct the *gfp* fusion plasmids the C- terminus of *mphA* was tagged. Therefore, the 5' UTR plus the coding sequence of *mphA* was amplified with the primers BD83/BD98. The *gfp* sequence was amplified using the primers BD99/BD100. As mentioned above 3' UTR was amplified using the primers BD85/BD86. The complementation of *mphA*Δ; *nkuA*Δ was performed with and without *gfp*. The *mphA*::*gfp* cassette was transformed into a *laeA*Δ;*nkuA*Δ (AGB1073) strain. All strains were confirmed by Southern hybridization (Figure 19-21).

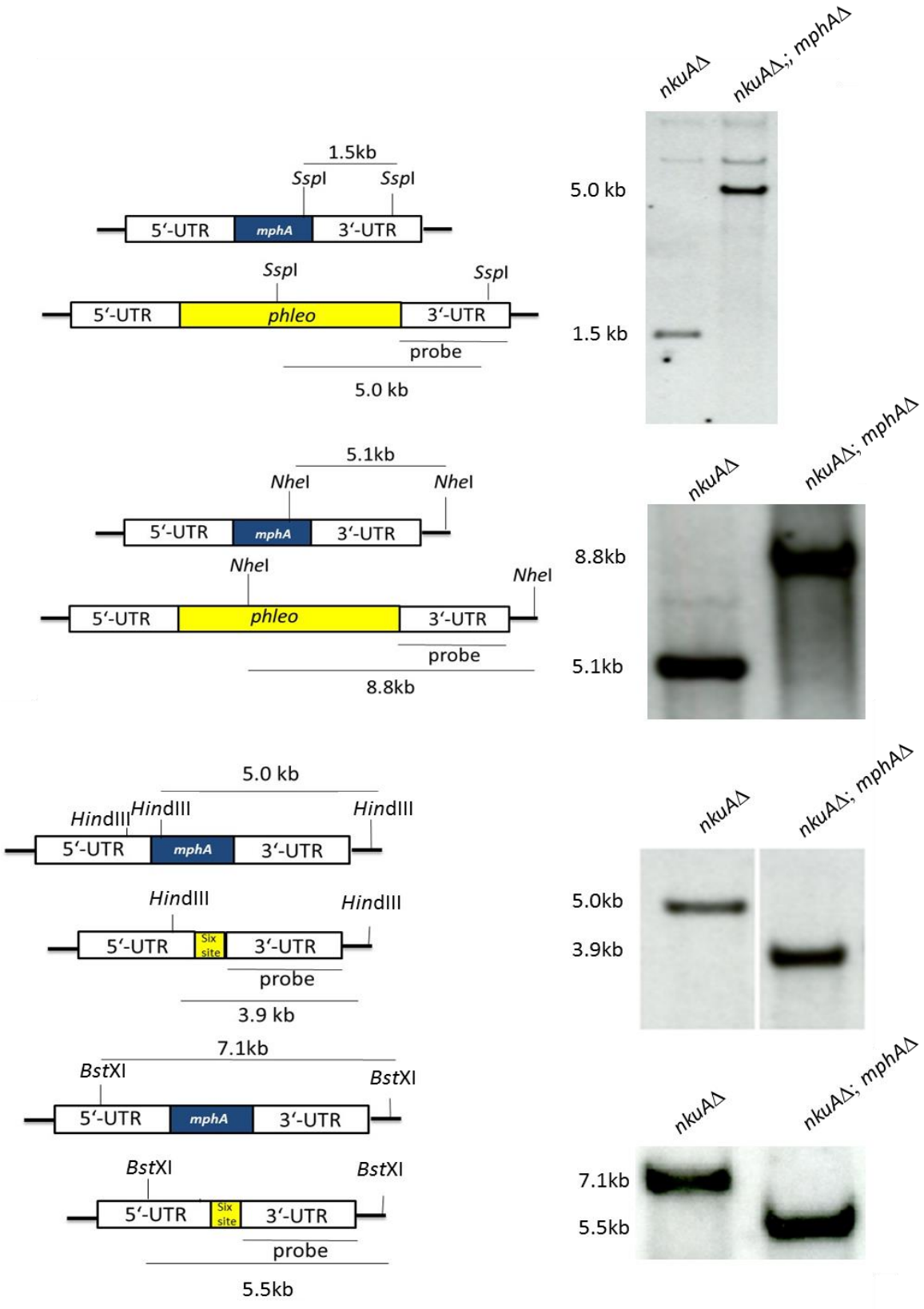



**Figure 19. Restriction map and Southern hybridization before and after the marker was recycled for the *nkuA*Δ; *mphA*Δ (AGB1077) strain.**
The following restriction enzymes were used: *Ssp*I, *Nhe*I, *Hin*dIII and *Bst*XI.

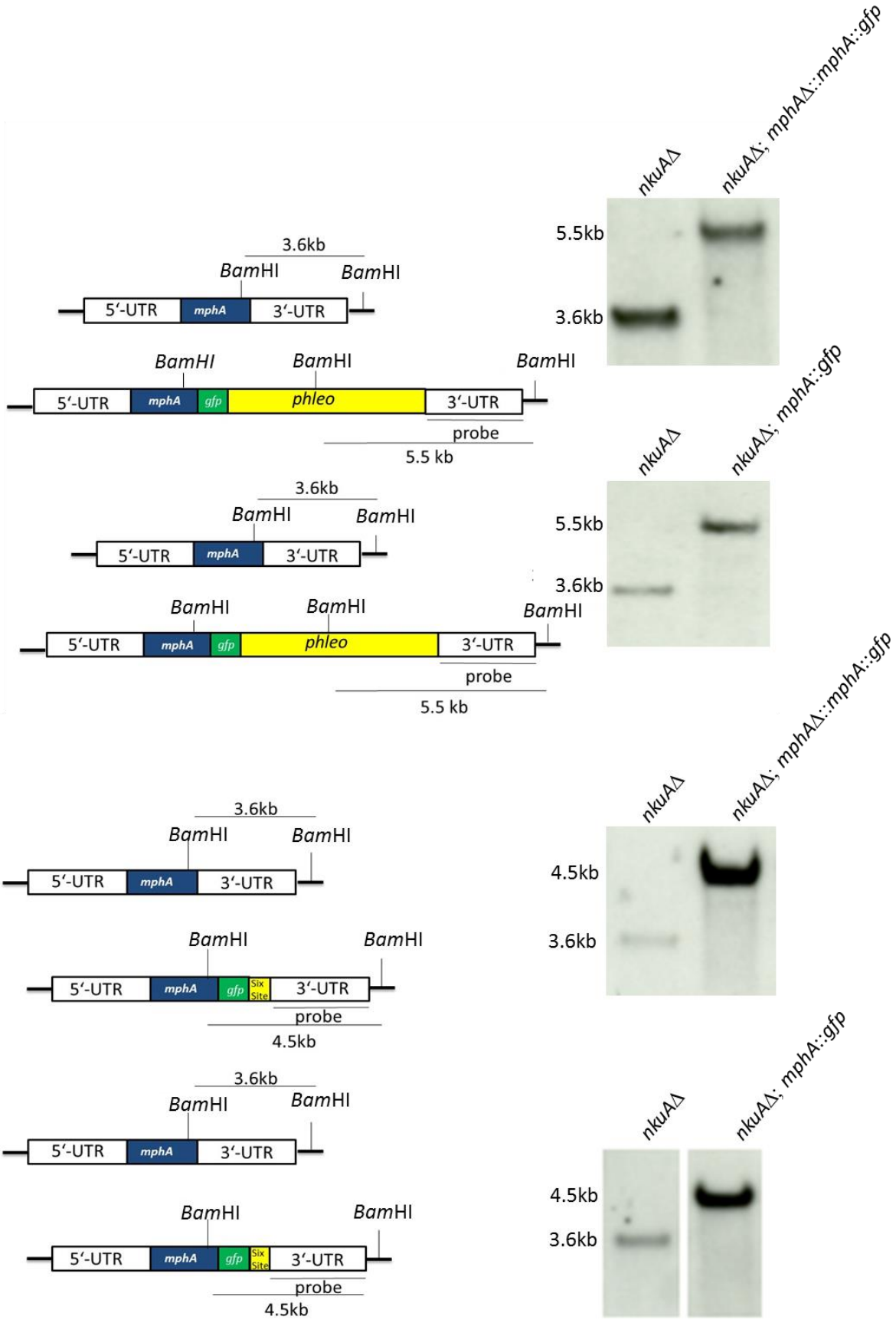



**Figure 20. Restriction map and Southern hybridization before and after the marker was recycled for the complementation strain (*nkuA*Δ; *mphA*Δ::*mphA*::*gfp*, AGB1078) plus the *mphA*::*gfp*;*nkuA*Δ (AGB1079) strain.**

*Bam*HI was used as a restriction enzyme.

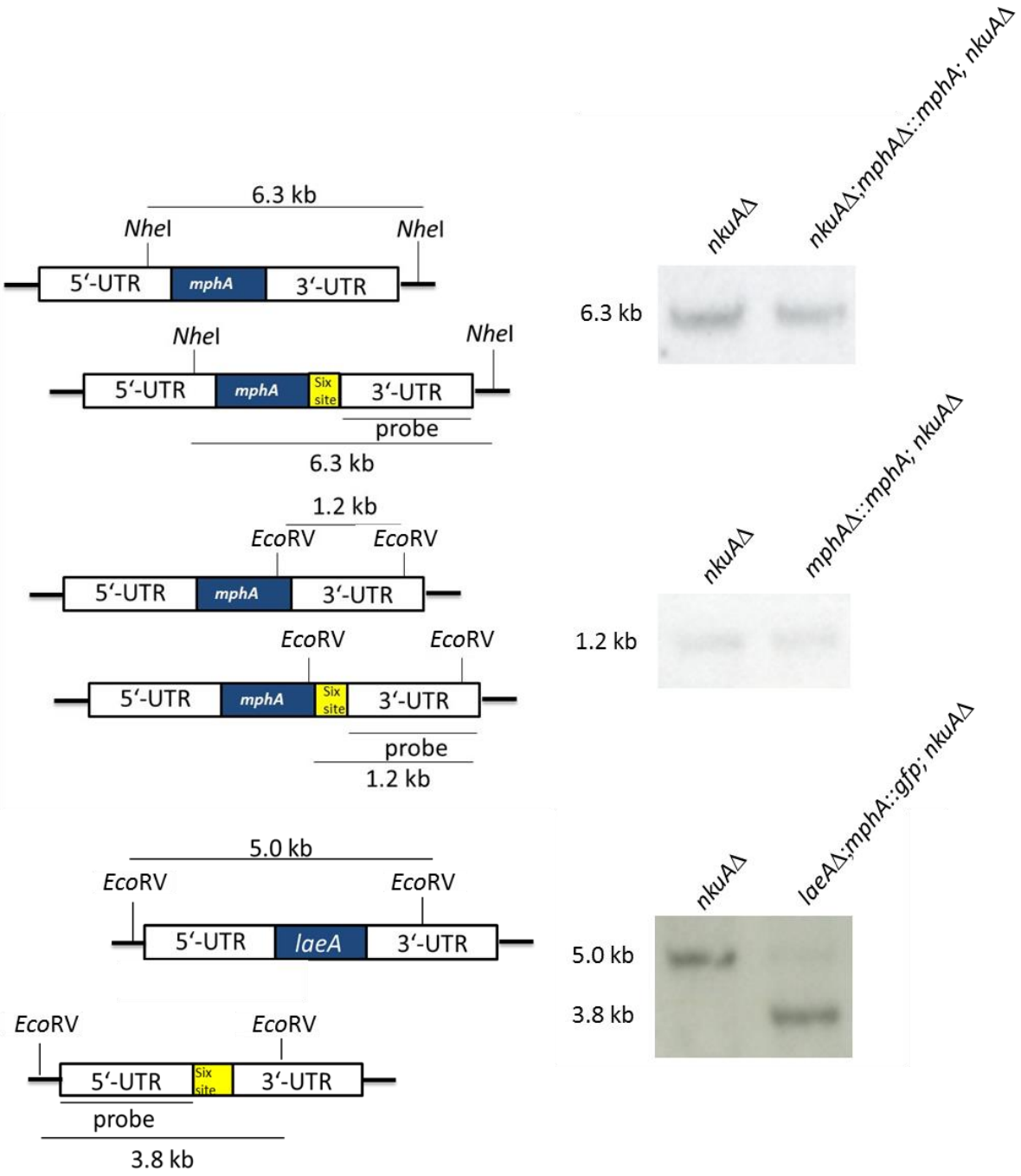



**Figure 21. Restricition map and Southern hybridization to confirm the complementation strain (*mphA*Δ::*mphA*, *nkuA*Δ, AGB1080) and the *laeA*Δ;*mphA*::*gfp*;*nkuA*Δ (AGB1081) strain.** *Nhe*I and *EcoR*V were used as restriction enzymes. The strains contained a recyclable *phleo* cassette and the recyclable *phleo* cassette was already recycled.

## 2.2.3. Protein methods

### 2.2.3.1. Protein extraction of Hülle cells from *Aspergillus nidulans*

In order to extract proteins from enriched Hülle cells the cleistothecia-rolling technique was used as mentioned above. Sexual mycelium of *A. nidulans* wild-type (FGSC A4 Glasgow, *veA*$^{+}$) was grown on solid agar plate cultures. Therefore, spores were inoculated on a solid agar plate and the petri dish was sealed with a parafilm to lower the oxygen condition and was covered with an aluminium foil. After three, five and seven days the cleistothecia were transferred to a new petri dish with the help of a syringe. In order to separate Hülle cells from the cleistothecium they were rolled on the solid agar plate and Hülle cells adhered to the surface. Hülle cells were then transferred with the help of the syringe from the solid agar plate to a 1.5 ml collection tube containing 40 µl extraction buffer B $^{+}$ 300 (100 mM Tris pH 7.5, 300 mM NaCl, 10% glycerol, 1 mM EDTA, 0.1% NP-40, 1 mM DTT, protease inhibitor mix (Roche Pharma AG, Grenzach-Wyhlen, Germany), 0.5 M benzamidine, 100 mM PMSF) with phosphatase inhibitor (100 mM NaF, 50 mM sodium orthovanadate, 50 mM β-glycerolphosphate; sterile filtered). All further steps were performed on ice. 270 – 300 cleistothecia were rolled on the solid agar plate and the resulting Hülle cells were used for further protein extractions. Hülle cells were sonicated (60 seconds, 60% of sonoporation power, in between centrifugation steps for 30 seconds at 4°C, procedure repeated 6 times). The tube was centrifuged for 10 min by 13.000 rpm at 4°C and the supernatant was transferred into a new 1.5 ml tube. The cell disruption was verified by observation of cellular debris. Protein extract was then loaded onto 12% SDS-PAGE.

### 2.2.3.2. Protein extraction of sexual mycelium from *Aspergillus nidulans*

Sexual mycelium of *A. nidulans* wild-type FGSC A4, *veA*$^{+}$ was harvested from the same plate as Hülle cells were collected to be enriched. Sexual mycelium was scraped off the solid agar plate with a spatula and transferred into a 1.5 ml tube. Sexual mycelium was frozen in liquid nitrogen and grinded in a retsch$^{TM}$-mill. 300 µl of

extraction buffer $B^+$ 300 (100 mM Tris pH 7.5, 300 mM NaCl, 10% glycerol, 1 mM EDTA, 0.1% NP-40, 1 mM DTT, protease inhibitor mix (Roche Pharma AG, Grenzach-Wyhlen, Germany), 0.5 M benzamidine, 100 mM PMSF) with phosphatase inhibitor (100 mM NaF, 50 mM sodium orthovanadate, 50 mM β-glycerolphosphate; sterile filtered) was added and a sonication step as described before followed by centrifugation (10 min, 13.000 rpm, 4 °C) was performed. Protein extraction was performed as described (Bayram et al., 2012). 80 µg of protein were loaded onto a 12% SDS-PAGE. All other steps were performed as mentioned above.

### 2.2.3.3. Protein extraction of vegetative mycelium from *Aspergillus nidulans*

In order to cultivate the vegetative mycelium spores were inoculated in 50 ml (250 ml shake flask) minimal medium (1% glucose; 1% AspA; 2 mM $MgSO_4$; and trace elements; pH 6.5; 5.937 $X10^5$ sp/ml) for 20 hours (for Hülle cell formation 72 hours) at 37 °C with constant shaking. The vegetative mycelium was filtered to remove the media and washed twice with 0.96% NaCl. 300 mg of vegetative mycelium was transferred into a 2 ml reaction tube. The vegetative mycelium was grinded with a retsch™-mill for 2 min by 30 frequency I/s. Proteins were extracted as described (Bayram et al., 2012). 80 µg of total protein extract was loaded onto a 12% SDS-PAGE.

### 2.2.3.4. Protein extraction of asexual mycelium from *Aspergillus nidulans*

Protein extraction of an asexual mycelium was performed at the same time points as a sexual mycelium. Harvesting and protein extraction was performed as mentioned above. Asexual mycelium was grown on solid agar plate cultures. Therefore, spore solution was directly inoculated on the solid agar plate. Asexual mycelium was grown in light conditions at 37 °C. The asexual mycelium was scratched off the solid agar plate and proteins were extracted as mentioned above and as described (Bayram et al., 2012).

### 2.2.3.5. Protein concentration measurement

In order to determine the concentration of proteins a Bradford assay was used (Bradford 1976). Additionally, the concentration of proteins was measured with an NanoDrop ND-1000 photospectrometer (peqlab biotechnologie GmbH, Erlangen, Germany).

### 2.2.3.6. SDS-polyacrylamide gel electrophoresis

Protein samples were mixed with 4X loading dye (50 mM Tris pH 6.8, 2% SDS, 10% glycerol, 1% β-mercaptoethanol, 12.5 mM EDTA, 0.02% bromophenol blue). The samples were heated for 5 minutes at 95 °C. The protein samples were loaded onto a 12% SDS page according to (Laemmli 1970). SDS-gels were prepared manually and consisted of a lower running gel (1 M Tris pH 8.8, 0.1% SDS, 12% acrylamide (Rotiphorese® Gel 40 37,5:1) (Roth GmbH, 3029.1), 10% ammonium persulfate (APS), 20 µl TEMED) and an upper stacking gel (1 M Tris pH 6.8, 0.1% SDS, 12% acrylamide¸ 5% ammonium persulfate (APS), 10 µl TEMED). Proteins were separated electrophoretically for 60 minutes at 180-200 V. The PageRuler$^{TM}$ prestained protein ladder (Thermo Scientific, SM26616) was used as size marker.

### 2.2.3.7. Colloidal Coomassie staining of proteins

Protein staining was performed according (Neuhoff et al., 1988). Gels were fixed in 40% (v/v) ethanol, 10% (v/v) acetic acid for 60 min, washed two times in water for 10 minutes and stained for 12 hours in coomassie solution (0.1% (w/v) coomassie brilliant blue G250, 5% (w/v) aluminum sulfate-(14-18)-hydrate, 10% (v/v) methanol, 2% (v/v) ortho-phosphoric acid). The lanes were excised from the polyacrylamide gels. The gel pieces were divided into equal lots into six 1.5 ml peptide low binding reaction tubes, destained in v (methanol):v (water) (40:60).

### 2.2.3.8. In-gel protein digestion with trypsin

The protein lane was excised from the gel and separated into six peptide low binding reaction tubes and tryptic digestion with trypsin was performed as described (Shevchenko et al., 1996). The gel pieces were covered with acetonitrile and shaken

for 10 minutes at room temperature. Acetonitrile was removed and the gel pieces were dried in a speedvac (savant speedvac concentrator, Thermo Scientific). 150 µl 10 mM DTT (in 100 mM $NH_4HCO_3$) was added to the gel pieces. The samples were incubated at 56 °C for 60 minutes. DTT solution was removed and 150 µl 55 mM iodoacetamide (in 100 mM $NH_4HCO_3$) was added and the samples were incubated for 45 minutes at room temperature in dark. Iodoacetamide solution was removed and the gel pieces were washed in 150 µl $NH_4HCO_3$, shaken for 10 minutes at room temperature. The solution was removed and 150 µl acetonitrile was added. The samples were shaken for 10 minutes at room temperature. The washing steps were repeated once. The gel pieces were dried in a speedvac at 50 °C and covered subsequently with trypsin (SERVA GmbH, Heidelberg, Germany, 37286.01). The trypsin buffer was prepared according to the manufacturer's instructions. The samples were incubated for 45 min on ice and the remaining digestion buffer was removed. Next 30 µl 25 mM $NH_4HCO_3$ pH 8.0 was added and the samples were incubated for 12 hours at 37 °C.

The samples were centrifuged (1 minute by 13.000 rpm) and the supernatant was collected in a 1.5 ml peptide low binding reaction tube. The gel pieces were covered with 20 mM $NH_4HCO_3$ and shaken for 10 minutes at room temperature. The samples were centrifuged (1 minute by 13.000 rpm) and the supernatant was collected into the collection tube. Next the gel pieces were covered with 50% acetonitrile / 5% formic acid. After an incubation step of 20 minutes, shaken and at room temperature the samples were centrifuged (1 minute by 13.000 rpm) and the supernatant was collected. This step was repeated twice. The final volume of the combined supernatant was completely dried in a speedvac at 50 °C. The peptides were resupended in 20 µl sample buffer (2% acetonitrile, 0.1% formic acid).

After tryptic digestion peptides were desalted with the use of C18-StageTips as described (Rappsilber et al., 2007). Therefore, three C18 disks were punced out from a solid phase extraction disk (3M, Neuss, Germany, 2215) and placed into a 200 µl tip. The C18 material was equilibrated. The C18-StageTips were placed into a 2 ml reaction tube using an adaptor. For equilibration of the C18-StageTips 100 µl methanol 0.1% formic acid was added to the C18 material. After a centrifugation step (2 minutes, 13.000 rpm) 100 µl 70% acetonitrile 0.1% formic acid was added. Then the C18-StageTips were centrifuged and 100 µl 0.1% formic acid was added. This step was repeated. The peptide solution was added onto the C18 material and the C18-StageTips were centrifuged for 5 minutes by 4.000 rpm. This step was repeated. The

C18-StageTips were washed twice with 100 µl 0.1% formic acid. The peptides were eluted from the C18 material with 70% acetonitrile 0.1% formic acid. The C18-StageTips were centrifuged for 5 minutes at 4.000 rpm. The peptide samples were dried in a speedvac. For LC-MS analysis the peptide samples were resuspended in 20 µl LC-MS sample buffer.

### 2.2.3.9. Liquid chromatography-mass spectrometry (LC-MS) and data analysis

Peptides were analyzed on a Liquid chromatography (LC) coupled to an *Orbitrap Velos Pro™ Hybrid Ion Trap-Orbitrap* mass spectrometer (MS) (Thermo Fisher Scientific, Bremen, Germany). Peptides were dissolved in 2% (v/v) acetonitril, 0.1% (v/v) formic acid and raw data were searched with SEQUEST and Mascot algorithms present in Proteome Discoverer 1.4 (Eng et al., 1994, Koenig et al., 2008). The search parameter for SEQUEST and Mascot algorithm were: (i) precursor ion mass tolerance 10 ppm, (ii) fragment ion mass tolerance 0.6 Da, (iii) maximum of two missed cleavage sites were set, (iv) fixed cysteine static modification by carboxyamidomethylation, (v) variable modification by methionine oxidation. The MS/MS spectra were matched against the *A. nidulans* genome database (UBMG0112_Anidulans_20120325.fasta). Results filter settings of Proteome Discoverer 1.4 were set to: (a) high peptide confidence and (b) minimal number of two peptides per protein. The MS/MS data were furthermore analyzed with the Andromeda search engine operating in MaxQuant 1.5.1.0 software with the program's default parameters using the same genome database as mentioned above (Tyanova et al., 2016).

Identified proteins of enriched Hülle cells from solid agar plate cultures were analyzed within three different time points. For each time point three biological replicates were considered. The proteome of sexual mycelium was analyzed from the same solid agar plates from where Hülle cells were enriched. The proteome of sexual mycelium was also analyzed within three different time points. The same conditions were applied to analyze the proteome of asexual mycelium. For each time point three biological replicates were considered. Only proteins identified in two or more biological replicates and with two or more peptides per protein were considered for the analysis. For visualization of the analysis the accession numbers of the proteins were inserted into

a Venn diagram (Oliveros 2016). The proteome of a vegetative mycelium in submerged liquid cultures was analyzed by three biological replicates. Only proteins identified in two or more biological replicates and with two or more peptides per protein were considered for the analysis. For peptide quantification Lys4 was defined as medium peptide labels and Lys8 as heavy peptide labels. The peptide median ratio distribution was determined using Proteome Discoverer 1.4. In order to correct experimental bias, the protein median was normalized. Only peptide ratios that were determined similar in two or more biological replicates were considered for the analysis. Additionally, normalized ratios were analyzed and processed using the Perseus 1.5.0.15 software. A t-test (p value 0.05) was performed to statistically compare the $log_2$ SILAC ratios across three biological replicates. The $log_2$ SILAC ratio was determined for the strains *laeA*Δ (AGB1074) in comparison to *laeA* (AGB1092). The following threshholds were set. In the range of a $log_2$ SILAC ratio between - 5.0 and - 0.5 the protein quantity of identified proteins was down-regulated. In the range of a $log_2$ SILAC ratio between + 0.5 and + 5.0 the protein quantity of identified proteins was up-regulated. In the range between a -0.5 and + 0.5 $log_2$ SILAC ratio the protein quantity of identified proteins was unchanged. Workflows to process the proteomic data are illustrated in supplementary table 1 and 2.

### 2.2.3.10. Functional annotation of proteins

Functional annotations of identified proteins were performed using the **b**asic **l**ocal **a**lignment **s**earch **t**ool Blast2GO (http://www.Blast2GO.com/b2ghome). BlastP was used to blast against the NCBInr database (NCBI: National Center for Biotechnology Information) with a blast expect value of $1 \times 10^{-3}$. The number of blast hits were set to 20 and the cutoff value for the GO terms was set to 20 (Conesa et al., 2005).

### 2.2.3.11. Fluorescence microscopy of fusion proteins

In order to visualize the localization of fusion proteins (fused to GFP) fluorescence microscopy was used. Hülle cells were enriched in 40 µl of $dH_2O$ using the cleistothecia-rolling technique. After centrifugation (2 minutes at 13.000 rpm) the supernatant was removed. The cellular pellet was resuspended in 5 µl $dH_2O$ and transferred to an object slide. The cells were observed under a reflected-light

microscope (Zeiss Axiolab - Zeiss AG, Jena, Germany) and an Olympus SZX12-ILLB2-200 binocular (Olympus GmbH, Hamburg, Germany). Fluorescence photograph pictures were taken with a confocal light microscope (Zeiss Axiolab - Zeiss AG, Jena, Germany) equipped with a QUAN-TEM: S12SC (Photometrics, Tucson, Arizona, United States of America) digital camera and the SlideBook 6 (Intelligent Imaging Innovations GmbH, Göttingen, Germany) software package. For GFP fusion protein visualization, the following parameters were used; DIC filter 200 ms; GFP filter 300 ms.

### 2.2.3.12. Immunoblotting

Proteins were extracted from different types of mycelia as described above. For immunoblotting experiments, proteins were first separated using a polyarcrylamide gel electrophoresis and transferred onto a nitrocellulose membrane as previously described (Jöhnk et al., 2016, Schinke et al., 2016). Protein concentrations were determined as described above and as reference for equally loaded proteins amount 0.2% Ponceau S (Sigma- Aldrich, Copenhagen, Denmark), 3% TCA was used. Blocking was performed in 5% milk powder (skimmed milk powder (Sucofin, 562570765347)). Membranes were incubated for 12 hours with $\alpha$-GFP antibody (dilution: 1:1000, sc-9996, Santa Cruz Biotechnology Inc., Dallas, Texas, United States of America and Heidelberg, Germany). The nitrocellulose membranes were washed three times in 150 ml 1X TBST (Tris buffered saline with tween 20) for 10 minutes at room temperature. Membranes were incubated for 60 minutes using an $\alpha$-mouse secondary antibody (dilution 1:2000, G21234, Invitrogen AG, Carlsbad, California, United States of America). The nitrocellulose membranes were washed three times in 150 ml 1X TBST (Tris buffered saline with tween 20). For peroxidase reaction 100 µl 2.5 mM luminol, 44 µl 400 µM paracoumarat, 100 mM Tris pH 8.5 added to 9 ml $dH_2O$ and 6.15 µl 30% $H_2O_2$ to 9 ml $dH_2O$. The nitrocellulose membranes were incubated with both solutions for 2 minutes, shaken and in darkness. Detection was performed on a Fusion-SL7 (Vilber Lourmat, Eberhardzell, Germany) system. Western hybridization experiments were performed with three biological replicates and samples were loaded twice on the 12% SDS-PAGE.

# 3. Results

## 3.1. Enrichment of sexual tissue and specialized Hülle cells

### 3.1.1. Hülle cells can be enriched from solid agar plates for comparative proteomics

*Aspergillus nidulans* develops closed sexual fruiting bodies during its sexual life cycle. So-called cleistothecia contain thousands of asci and are surrounded by several layers of thick-walled Hülle cells. It is assumed that Hülle cells have a protective capacity and therefore they could function e.g. in secondary metabolite formation (Alves et al., 2016, Sarikaya-Bayram et al., 2010). In order to identify and compare proteins from enriched Hülle cells from solid agar plates as well as from submerged liquid cultures, a proteome study was performed. To separate Hülle cells from cleistothecia grown on surface cultures, the cleistothecia-rolling technique was performed (Figure 22).

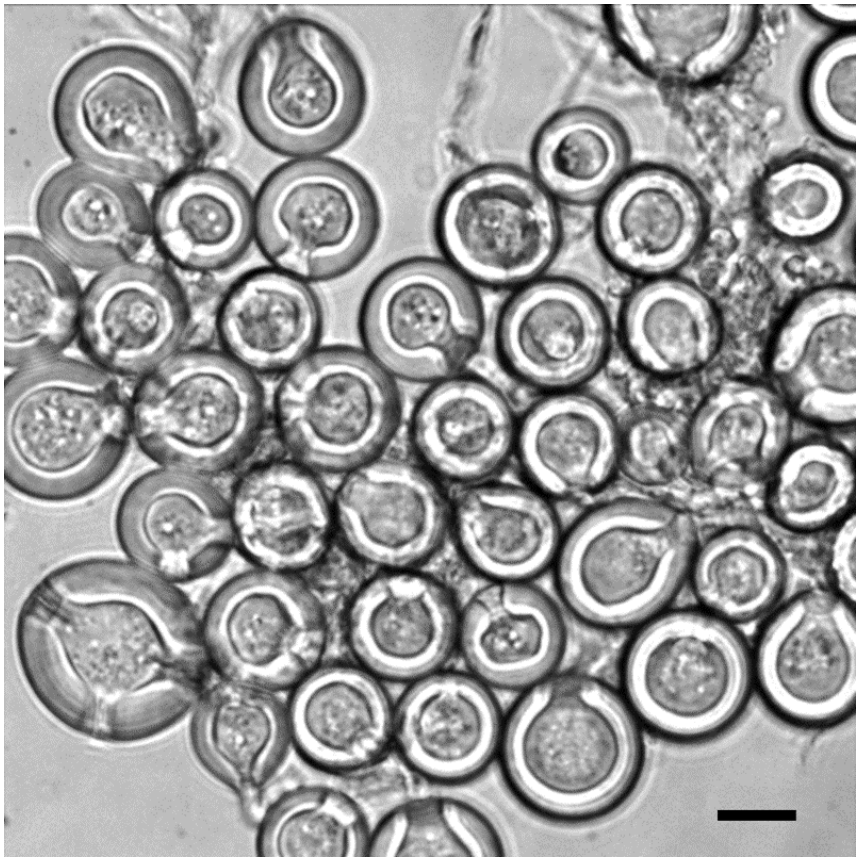


**Figure 22. Enrichment of Hülle cells of cultures from solid agar plates**
The cleistothecia-rolling technique was used for enrichment of Hülle cells from a single cleistothecium. Enriched surface Hülle cells from sexual mycelium using the cleistothecia-rolling technique are shown. Scale bar is 10 µm.

Hülle cells from sexual mycelium grown on solid agar plates were separated and harvested. Cleistothecia of sexual mycelium were transferred with a fine tipped syringe to a fresh solid agar plate. The cleistothecium was then rolled on the solid agar plate with a fine tipped syringe and the Hülle cells adhered to the surface of the solid agar plate. Hülle cells were collected with a fine tipped syringe and transferred into a collection tube.

### 3.1.2. Hülle cells can be enriched from submerged liquid cultures for comparative proteomics

In order to compare the proteome of enriched surface Hülle cells to Hülle cells grown in liquid cultures a second proteomic approach was performed. The proteomic approach from Hülle cells grown in liquid media will be discussed within the next chapter.

Hülle cells have been observed in submerged liquid cultures in different strains (Alkahyyat et al., 2015, Bayram et al., 2009, Bayram et al., 2008a, Vienken et al., 2005, Kim et al., 2002, Han et al., 2001). Vegetative mycelia under submerged liquid conditions usually form no cleistothecia and Hülle cells are, therefore, more randomly distributed throughout the mycelia ball (Bayram et al., 2009, Bayram et al., 2008a). In order to perform a quantitative proteome experiment a lysine auxotroph *lysA*Δ; *nkuA*Δ (AGB1092) strain was compared to a lysine auxotroph *laeA*Δ; *lysA*Δ; *nkuA*Δ (AGB1074) strain that causes significant reduction in formation of Hülle cells (Sarikaya-Bayram et al., 2010). Hülle cells were observed in the lysine auxotroph and in the lysine prototroph parental *nkuA*Δ (AGB552) strain after 72 hours in submerged liquid cultures. The strain AGB552 (*pabaA1;yA;nkuA*Δ*::argB*) contains a *nkuA*Δ mutation with an additional unknown mutation which results in Hülle cell formation in submerged liquid cultures. Deletion of gene *nkuA* in *A. nidulans* leads to an improvement in gene targeting (Nayak et al., 2006). These cultures represent a version of enriched Hülle cells from submerged liquid conditions and were compared to Hülle cells enriched from solid agar plates. In the first two chapters, the results of both proteomic approaches are shown. First of all, the proteome of surface Hülle cells are compared to different mycelia types grown on surface agar plates. The proteome of surface Hülle cells is then compared to the proteome of Hülle cells grown in liquid

media. This finding emphasizes that Hülle cells can be enriched in a vegetative mycelium grown for 72 hours in submerged liquid media.

## 3.2. Enzymes encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) secondary metabolite gene clusters are increased during sexual differentiation: proteome comparison of *Aspergillus nidulans* development grown on surfaces

### 3.2.1. Enrichment of surface Hülle cells enabled to perform a comparative proteome analysis

In the first analysis (proteome experiment 1), proteome of *A. nidulans* wild-type FGSC A4, *veA*$^{+}$ was analyzed to determine differentially expressed proteins in Hülle cells compared to other developmental stages. Vegetative mycelium without Hülle cells was compared to a sexual mycelium with Hülle cells and an asexual mycelium. Proteomes of Hülle cells, sexual and asexual mycelium were analyzed at three different time points, namely three, five and seven days after germination. At each developmental stage three biological replicates were considered. In order to analyze the proteome of surface Hülle cells, they were first enriched from sexual mycelium grown on solid agar plates using the cleistothecia-rolling technique. Therefore, a spore solution was directly inoculated on solid agar plates and the fungus was grown in darkness and reduced oxygen conditions to induce sexual development. Proteins from enriched Hülle cells were extracted, digested with trypsin and peptides were analyzed with a Liquid chromatography (LC) coupled to an *Orbitrap Velos Pro™ Hybrid Ion Trap-Orbitrap* mass spectrometer (MS) (Thermo Fisher Scientific). Different mycelia types were used as controls, namely undifferentiated vegetative mycelium without Hülle cells, sexual mycelium with a high amount of Hülle cells and an asexual mycelium with a low amount of Hülle cells. The total proteome of vegetative mycelium from at least three biological replicates grown under submerged conditions for 20 hours was analyzed. Shaking cultures with submerged mycelia represent undifferentiated filaments without Hülle cells at this time point. In order to harvest vegetative mycelia a spore solution was directly inoculated in liquid media. The amount of vegetative mycelium grown for 20 hours in submerged liquid conditions is significantly higher than that of a vegetative mycelium grown on solid agar plates. Therefore, the vegetative

mycelium of submerged liquid cultures was used for the analysis. Additionally, the proteome of sexual mycelium from where the Hülle cells were enriched was analyzed. The proteome of asexually grown mycelium was also analyzed as outlined in Figure 23.

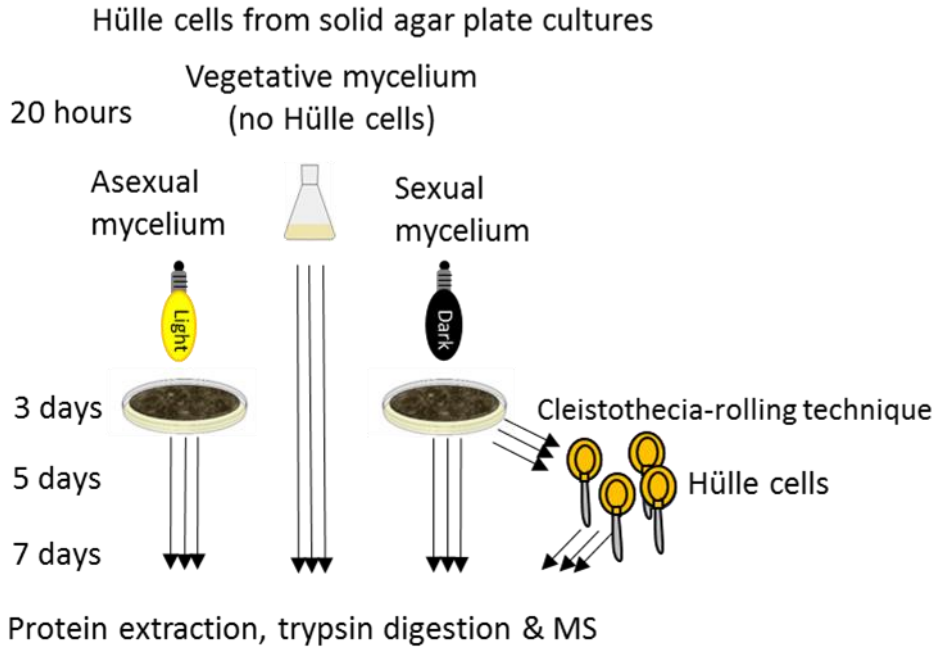



**Figure 23. Proteomics workflow for analyzing enriched surface Hülle cells grown on solid agar plates and different types of mycelia.**
For the first proteome analysis **(Proteome experiment 1)** *A. nidulans* wild-type (FGSC A4, *veA*$^{+}$) was used. Hülle cells were enriched from sexual mycelium using the cleistothecia-rolling technique and from the same solid agar plates sexual mycelium was harvested (all steps were performed on day 3, 5 and 7 after germination). Sexual mycelium contained high amounts of Hülle cells. Vegetative mycelium was cultivated under submerged conditions and was harvested 20 hours after germination. At this time point no Hülle cells were observable in vegetative mycelium. Asexual mycelium was harvested from solid agar plates on day 3, 5 and 7 after germination. For each time point three biological replicates of Hülle cells as well as the other fungal tissues mentioned above were considered and are highlighted with three arrows.

## 3.2.2. Comparative proteomics revealed that the proteome of surface Hülle cells overlaps especially to that of sexual mycelium

Analysis of different mycelia types and Hülle cells at all three time points revealed a total number of 1.525 identified proteins (Figure 24). Detailed evaluation of all LC-MS data by using the MaxQuant, Perseus and Proteome discoverer is described in Materials and Methods. Only proteins identified in two or more biological replicates and with two or more peptides per protein were considered for analysis. In this study 401 proteins were identified from surface Hülle cells enriched from solid agar plates and they are highlighted in the red rectangle (Figure 24); (Supplementary Table 3).

Additionally, the list of identified proteins in sexual, asexual and vegetative mycelium is listed in Supplementary Table 4.

In this study, six proteins were identified in enriched Hülle cells that were unidentified in other fungal tissues (Table 6). Proteins that were common in Hülle cells and different mycelia types were validated. 253 proteins were common in all different cell types. 24 proteins were present in sexual mycelium with high amounts of Hülle cells and overlapped to an enriched Hülle cell fraction from sexual mycelium. The list of proteins identified in surface Hülle cells and sexual mycelium is shown in Table 7. Proteins which are found in sexual mycelium and Hülle cells are potentially crucial during sexual development. The localization of several proteins found in Hülle cells and sexual mycelium, therefore, were studied in detail and functional gene studies were performed. Findings imply that identified proteins of Hülle cells overlap to other fungal tissues, especially to that of sexual mycelium.

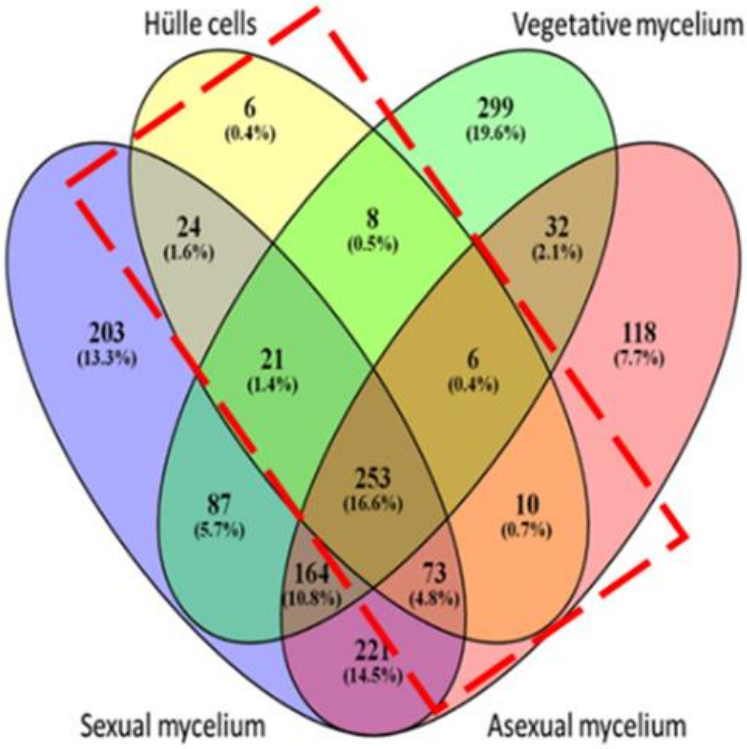



**Figure 24. Comparative analysis of identified proteins from surface cultures.**
*A. nidulans* wild-type (FGSC A4, *veA*$^{+}$) was used for the analysis. The figure shows a Venn diagram of identified proteins of enriched surface Hülle cells, sexual mycelium, asexual mycelium and vegetative mycelium. Vegetative mycelium was grown in submerged liquid cultures and enabled to obtain the sufficient amount of mycelia for the analysis. The Venn diagram contains the 1525 proteins that were identified within the different mycelia types and surface Hülle cells from the first proteomic approach. Proteins that could be identified in two or more biological replicates and with two or more peptides per proteins were used for the analysis. The red rectangle represents the 401 proteins found in surface Hülle cells after 3, 5 and 7 days of inoculation.

### 3.2.3. Comparative proteomics reveal six proteins found in enriched Hülle cells from solid agar plates

Comparing the proteome of surface Hülle cells enriched from solid agar plates to the proteome of different mycelia types mentioned above revealed in this study six proteins which were identified only in surface Hülle cells enriched from solid agar plates. The specific proteins and their putative function are listed in Table 6. Two transmembrane proteins (AN2601 and AN9392) predicted to belong to the major facilitator superfamily (MSF) were identified in surface Hülle cells three, five and seven days after inoculation. A putative peptidase (AN3407) with a predicted serine-type peptidase activity was also found after five and seven days in Hülle cells. The HexA (AN4695) putative woronin body protein was also identified at the same time points. Hülle cells and the subtending hyphae are connected via two distinct types of septa. The inner one is a single perforate septum where woronin bodies often can be observed (Ellis et al.,1973). Woronin bodies were also found to be associated with septal pores of vegetative hyphae (Beck and Ebel 2013). The HexA protein was identified in one biological replicate from a vegetative mycelium and sexual mycelium. Only proteins identified in two or more biological replicates were considered for the analysis. Therefore, the HexA protein was filtered out from vegetative and sexual mycelium. In this study, a protein with an ankyrin repeat containing domain protein (AN8434) and a putative tyrosinase protein (AN8435) was observed in surface Hülle cells after five and seven days.

The biological function of the transporter protein (AN2601) was only identified in Hülle cells from solid agar plate cultures. It was studied in more detail because transporters are considered to be crucial to transport nutrition and other molecules across Hülle cells and thereby, most probably, supporting the cleistothecia (Wei et al., 2004, Pantazopoulou et al., 2007). Additionally, the cellular localization of the ankyrin repeat containing domain protein (AN8434) and the putative tyrosinase protein (AN8435) identified in Hülle cells from surface and liquid media were studied in detail as these two proteins were uniquely found in Hülle cells. This finding shows that surface Hülle cells comprise only few proteins that were not overlapping to other fungal tissues.

**Table 6. Comparative proteomics revealed six proteins that were identified in the enriched Hülle cells.**
Only proteins identified in two or more biological replicates and with two or more peptides per protein were considered for the analysis. Numbers in brackets represent the average of spectral counts from three biological replicates. The yellow colour represents the six proteins found in the Venn diagram (Figure 24). The entire list of proteins identified from solid agar plate cultures is presented in Supplementary table 3.

| Gene ID | Description | Found in 3 days Hülle cells | Found in 5 days Hülle cells | Found in 7 days Hülle cells | Orthologs | Phenotypes and localization in Hülle cells. Literature is cited. |
|---|---|---|---|---|---|---|
| **Transporter** | | | | | | |
| AN2601 (MphA) | Major facilitator superfamily | | X (8) | X (8) | *Saccharomyces cerevisiae* MaL31 maltose permease *Aspergillus fumigatus* putative MFS maltose transporter Afu4g00150 | Knockout effects cleistothecia maturation, growth and development in *Aspergillus nidulans*. Phenotypes in other fungal species are unknown |
| AN9392 | Major facilitator superfamily | X (7) | X (4) | | *Saccharomyces cerevisiae* TnA1 nicotinic acid permease | No literature available |
| **Cellular process** | | | | | | |
| AN3407 | Protein with a predicted serine-type peptidase domain | | X (25) | X (29) | *Neurospora crassa* NCU05016 predicted serine-type endopeptidase acitivity | No literature available |
| AN4695* (HexA) | Woronin body protein | | X (8) | X (7) | *Aspergillus fumigatus* woronin body major protein | Ellis et al., 1973. In Hülle cells one can find woronin bodies |
| AN8434 (AnkG) | Ankyrin repeat domain protein | | X (4) | X (4) | *Neurospora crassa* NCU5316 ankyrin-like protein | Ankyrin repeat domain protein (AN8434) is localized to the subtending hyphae of Hülle cells |
| AN8435 | Tyrosinase domain protein | | X (2) | X (3) | *Aspergillus oryzae* MelB tyrosinase associated with melanization | AN8435 is localized in Hülle cells and most probably in an unstable form |

*The HexA protein was identified in one biological replicate from a vegetative mycelium and sexual mycelium.

### 3.2.4. Surface Hülle cells contain increased enzyme levels for the mobilization of complex sugar molecules

Whithin the next two sections 24 proteins common between Hülle cells and sexual mycelium are discussed. Six of these proteins are enzymes involved in carbohydrate metabolism (Table 7). Glucanase MutA (AN7349) was found in Hülle cells and sexual mycelium at all three time points. This protein is known to be strongly expressed in Hülle cells (Wei et al., 2001). Additionally, another glucanase AgnB (AN3790) was identified. It is thought that additional enzymes for the mobilization of different carbon sources are available during sexual development and are found in Hülle cells (Wei et al., 2001). Putative glucoamylase GlaA (AN11143) and the glucosidase AgdC (AN0941) with a predicted role in the starch and maltose metabolism were identified. This result shows that Hülle cells contain various glucanases that are involved in the mobilization of carbohydrates. Hülle cells are most probably involved in the mobilization of carbohydrates and therefore, enzymes are needed in this process.

### 3.2.5. Enzymes encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters were found in Hülle cells and sexual mycelium from solid agar plates

Six enzymes out of the 24 proteins shown in Table 7 found in sexual mycelium and enriched Hülle cells are encoded by the monodictyphenone (*mdp*) as well as the xanthone (*xpt*) gene cluster (MdpL (AN10023), AN7999 (a putative oxidoreductase), XptC (AN7998), XptB (AN12402), MdpH (AN10022), MdpG (AN0150)). In a previous work it could be demonstrated that the transcripts of 10 genes within the *mdp* and *xpt* gene clusters are specifically expressed during sexual development (Bayram et al., 2016). Five enzymes encoded by the *mdp* and the *xpt* gene clusters were found again in the second approach in vegetative mycelium from submerged liquid conditions where Hülle cell formation occurred (MdpL (AN10023), XptB (AN12402), MdpG (AN0150), XptC (AN7998) and AN7999 (a putative oxidoreductase). The results of the identified proteins in submerged liquid cultures will be presented in the next chapter. This finding shows that enzymes encoded by the *mdp*/*xpt* gene clusters are present in Hülle cells grown on surface and liquid cultures as well as in sexual mycelium.

**Table 7. 24 proteins that were common between sexual mycelia and Hülle cells and were identified from solid agar plate cultures.**
Only proteins identified in two or more biological replicates and with two or more peptides per protein were considered for the analysis. Numbers in brackets represent the average of spectral counts from three biological replicates. The grey colour represents the 24 proteins found in the Venn diagram (Figure 24). The entire list of proteins identified from solid agar plate cultures is presented in Supplementary table 3.

| Gene ID | Description | 3 days sex. mycelium and Hülle cells | 5 days sex. mycelium and Hülle cells | 7 days sex. mycelium and Hülle cells | Reference |
|---|---|---|---|---|---|
| **Carbohydrate metabolism** | | | | | |
| AN7349 (MutA) | Alpha-1,3-glucanase, specifically expressed in Hülle cells | X (3) | X (43) | X (28) | Wei et al., 2001 |
| AN0941 (AgdE) | Alpha-glucosidase activity, predicted role in maltose metabolism | X (6) | X (32) | X (24) | Yuan et al., 2008 |
| AN3790 (AgnB) | Putative alpha-1,3-glucanase | | X (18) | X (17) | He et al., 2017 |
| AN9443 | Predicted catalytic activity and role in carbohydrate metabolic process | | | X (11) | Flipphi et al., 2009 |
| AN7345 (AgdC) | Glucosidase activity, involved in degradation of glucans | X (6) | X (32) | X (24) | Yuan et al., 2008 |
| AN11143 (GlaA) | Putative glucoamylase | | X (4) | | Nekiunaite et al., 2016 |
| **Secondary metabolism** | | | | | |
| AN11049 | NmrA-like; predicted secondary metabolism gene cluster member | | X (35) | X (49) | Andersen et al., 2013 |
| AN10023 (MdpL) | Member of the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters | X (25) | | X (42) | Bayram et al., 2016 |
| AN7999 | Oxidoreductase, member of the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters | | X (8) | X (47) | Bayram et al., 2016 |
| AN7998 (XptC) | GMC oxidoreductase, member of the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters | X (7) | X (6) | | Sanchez et al., 2011 |
| AN12402 (XptB) | Prenyltransferase, member of the monodictphenone (*mdp*) / xanthone (*xpt*) gene clusters | X (5) | | X (8) | Sanchez et al., 2011 |
| AN10022 | Member of the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters | | X (5) | X (6) | Bayram et al., 2016 |
| AN0150 (MdpG) | Polyketide synthase, member of the monodictphenone (*mdp*) / xanthone (*xpt*) gene clusters | X (4) | X (3) | | Sanchez et al., 2011 |
| AN7812 (StcN) | Putative versicolorin B synthase | X (3) | X (4) | | Bok et al., 2009 |
| **Oxidase** | | | | | |
| AN7641 | Putative copper amine oxidase | X (22) | X (31) | | Lenobel et al., 2005 |
| AN6314 | Oxidoreductase | X (5) | X (27) | X (48) | Not available |
| AN8203 | NAD+ synthase (glutamine-hydrolyzing) activity | | X (5) | | Not available |
| AN1142 | Oxidoreductase | | X (4) | | Not available |
| **Celluar process** | | | | | |
| AN11897 | Ribonuclease | | X (6) | | Not available |
| AN5669 | Putative succinyl-CoA:3-ketoacid-coenzyme A transferase | X (8) | | X (2) | Salazar et al., 2009 |
| AN10977 | Ortholog(s) have extracellular region localization | | X (6) | | Not available |
| AN6930 | Predicted transaminase activity | | X (9) | | Not available |
| **Unknown** | | | | | |
| AN5488 | Protein of unknown function | X (2) | X (20) | | Not available |
| AN1863 | Protein of unknown function | X (2) | | X (4) | Not available |

### 3.2.6. Proteins encoded by the xanthone (*xpt*) gene cluster are enriched in the cytoplasm of Hülle cells

Hülle cells might produce specific secondary metabolites to protect the cleistothecia from fungivors. It is known that xanthones are secondary metabolites with antimicrobial activities (Chen et al., 2017, Noordin et al., 2016). To gain more insight into the production of an antimicrobial agent in Hülle cells the localization of enzymes encoded by the xanthone (*xpt*) secondary metabolite gene cluster was further investigated. Monodictyphenone represents a precursor for the production of xanthones. As an example of the 24 proteins that were common between Hülle cells and sexual mycelia the prenyltransferase XptB and the oxidoreductase XptC were found and their localization was investigated (Figure 25). The second prenyltransferase XptA was only found in a sexual mycelium and is listed in Supplementary table 4. The criteria concerning the localization investigation were the presence of the protein in both sexual mycelium and Hülle cells in the proteomics approach. Therefore, the localization of XptA is not shown.

The prenyltransferase XptB and the oxidoreductase XptC are involved in the conversion of monodictyphenone into xanthones, which are antimicrobial agents. Localization of the prenytransferase XptB and the oxidoreductase XptC involved in the conversion of monodictyphenone into xanthones in Hülle cells was investigated and their presence could be confirmed (Figure 25). Hülle cells were enriched from sexual mycelium using the cleistothecia-rolling technique and the localization of XptB::GFP, XptC::GFP was observable in the cytoplasm of Hülle cells. The fusion proteins were mainly observed in the center of Hülle cells. XptB::GFP and XptC::GFP seem to be mainly equally distributed in the cytoplasm of Hülle cells. XptB::GFP and XptC::GFP was not clearly observable in the membrane. This suggests that proteins identified by the applied enrichment of Hülle cells correlate between the proteomic approach and the microscopic data in their fungal localization.

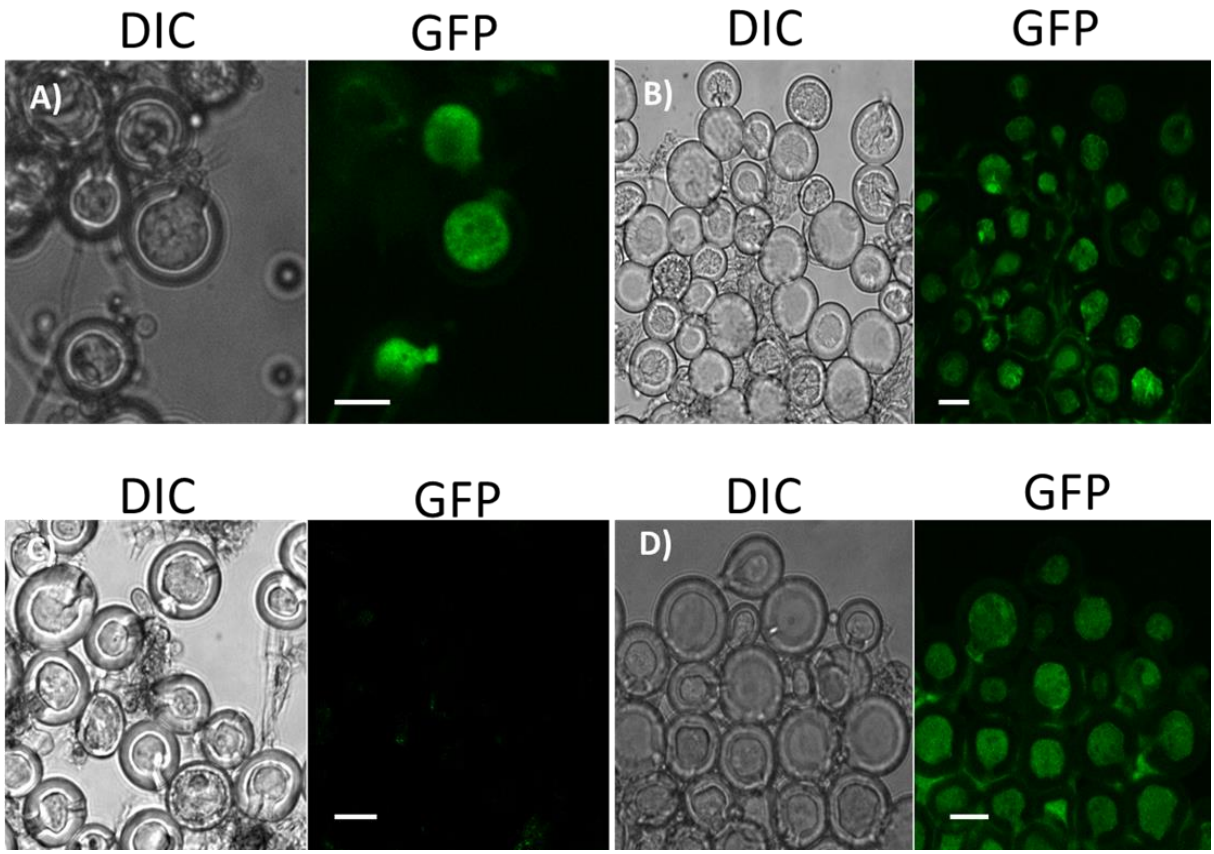



**Figure 25. Enzymes encoded by the xanthone (*xpt*) gene cluster are localized in Hülle cells.** XptB::GFP and XptC::GFP are observable in the cytoplasm of Hülle cells. Fluorescence microscopy of Hülle cells: A) *xptB*::*gfp;lysA*Δ;*nkuA*Δ (AGB1086) B) *xptC*::*gfp; nkuA*Δ (AGB1088) C) *nkuA*Δ (AGB552) parental strain D) A strain expressing GFP constitutively (AGB596). Scale bar is 10 µm.

Additionally, the localization of XptB and XptC was apparent during initial vegetative growth. Therefore, vegetative hyphae in submerged liquid cultures were used to visualize the presence of the fusion proteins. The localization of the fusion proteins XptB::GFP and XptC::GFP could be confirmed mainly in the cytoplasm of vegetative hyphae(Figure 26). This suggests that proteins encoded by the xanthone (*xpt*) gene cluster are found not only in Hülle cells but also in vegetative hyphae suggesting that these proteins are found during initial vegetative growth.

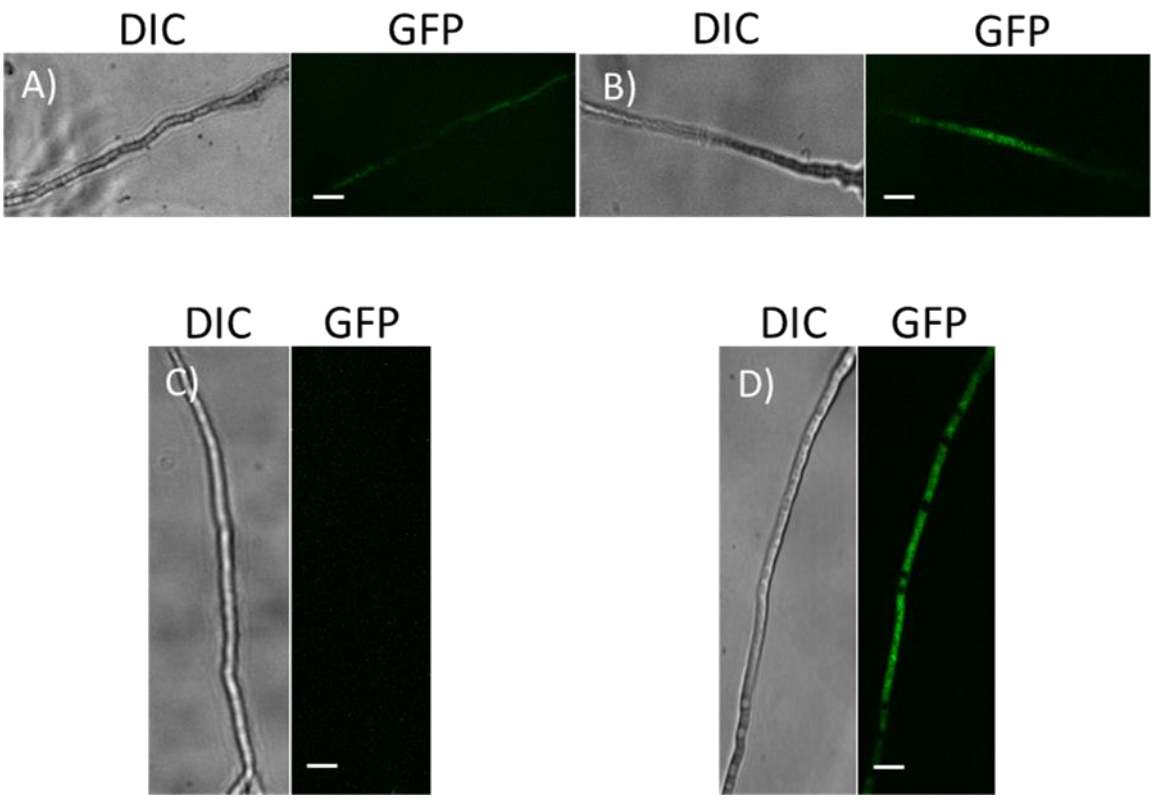



**Figure 26. Enzymes encoded by the xanthone (*xpt*) gene cluster are localized in vegetative mycelium.**
XptB::GFP and XptC::GFP are localized in the cytoplasm of vegetative hyphae. Fluorescence microscopy of vegetative mycelium: A) *xptB*::*gfp;lysA*Δ;*nkuA*Δ B) *xptC*::*gfp;nkuA*Δ. C) *nkuA*Δ E) A strain expressing GFP constitutively (AGB596). Scale bare is 20 µm

The protein sequence of green fluorescent protein (GFP) consists of 239 amino acids with a predicted molecular mass of 26.9 kDa. The XptB protein consists of 463 amino acids with a predicted molecular mass of 52.4 kDa. The XptC protein consists of 622 amino acids with a predicted molecular mass of 67.8 kDa.

Western hybridization experiments showed that XptB::GFP is detectable as expected around 79 kDa and that XptC::GFP is detectable as expected around 95 kDa in three and five day old sexual mycelium with high amounts of Hülle cells plus a three day old vegetative mycelium (Figure 27). A strain expressing GFP constitutively (AGB596) served as a positive control where GFP was detectable around 27 kDa. The parental *nkuA*Δ (ABG552) strain served as negative control where GFP was not observable. A proteolytic cleavage product of XptB::GFP was detectable around 27 kDa. This is most likely a degradation product of the full-length XptB::GFP fusion protein and represents the degraded GFP product. This result implies that the prenytransferase XptB and the oxidoreductase XptC is found in the cytoplasm of Hülle cells and that these proteins are present in vegetative and sexual mycelia.

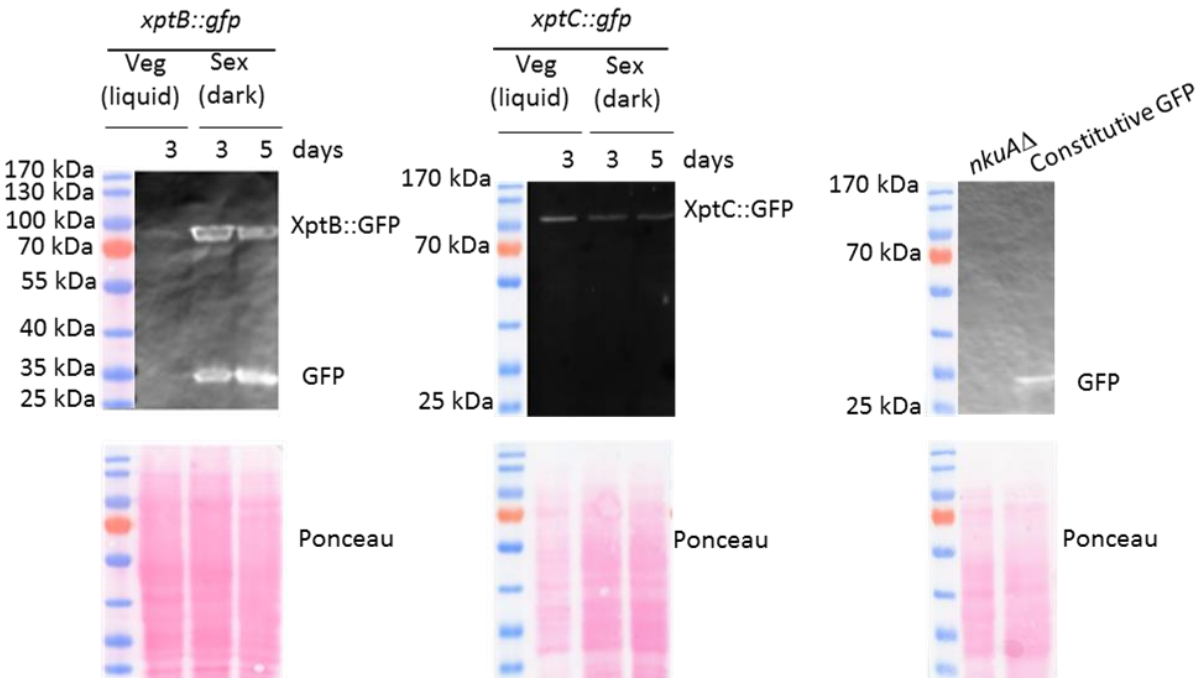



**Figure 27. The fusion proteins XptB::GFP and XptC::GFP are found in sexual mycelium with high amounts of Hülle cells and a vegetative mycelium.**
Western hybridization of vegetative and sexual mycelium of *xptB*::*gfp; lysA*Δ; *nkuA*Δ (AGB1086) and *xptC*::*gfp; nkuA*Δ (AGB1088). Vegetative mycelium was harvested after 3 days. Sexual mycelium with high amounts of Hülle cells was harvested 3 and 5 days after inoculation. The XptB protein consists of 463 amino acids with a predicted molecular mass of 52.4 kDa. The XptC protein contains 622 amino acids with a predicted molecular mass of 67.8 kDa. In order to detect XptB::GFP (*xptB*::*gfp*; *lysA*Δ; *nkuA*Δ, AGB1086) and XptC::GFP (*xptC*::*gfp*; *nkuA*Δ, AGB1088 ) a primary α-GFP antibody (sc-9996; Santa Cruz) was used followed with an incubation of an α-mouse secondary antibody (G21234, Invitrogen).The fusion protein XptB::GFP was detected as expected around 79 kDa and XptC::GFP was detected as expected around 95 kDa. A strain expressing GFP constitutively served as a control where GFP was detectable around 27 kDa. Proteolytic cleavage product of XptB::GFP was detectable around 27 kDa. This is most likely a degradation product of the full-length XptB::GFP fusion protein. The parental strain *nkuA*Δ served as a negative control where GFP and the fusion proteins were not detectable.

## 3.2.7. Comparative proteomics revealed that the proteome of surface Hülle cells overlaps to other fungal tissues besides of a sexual mycelium

As shown in the Venn diagram (Figure 24) the proteome of surface Hülle cells overlapped to other fungal tissues such as asexual and vegetative mycelium besides sexual mycelium. Ten proteins were identified in Hülle cells and asexual mycelium (Table 8). These proteins were unidentified in Hülle cells grown in liquid media. This suggests that these proteins are only present during surface growth. Since these proteins were unidentified in Hülle cells grown in liquid media in the second proteomic

approach the focus was not laid on these proteins. Proteins that were common in Hülle cells and vegetative mycelium are listed in Table 8. These proteins were identified in Hülle cells grown in liquid media and are discussed in the context of core proteome of both types of Hülle cells within the next chapter. Data reveal that the proteome of surface Hülle cells overlaps to that of other fungal tissues besides sexual mycelium.

**Table 8. Overlapping proteins of Hülle cells (HC), asexual (Asex.) and vegetative mycelium (Veg.).** The listed proteins are found in the red rectangle of the Venn diagram of Figure 16. Only proteins identified in two or more biological replicates and with two or more peptides per protein were considered for the analysis. Six proteins are listed and represent the proteins with the highest spectral counts. Numbers in brackets represent the average of spectral counts from three biological replicates. Six proteins out of ten are listed with the highest spectral counts, that were common in Hülle cells and asexual mycelium. The orange colour represents proteins found in Hülle cells and asexual mycelium. Six proteins out of eight are listed with the highest spectral counts, that are common in Hülle cells and vegetative mycelium. The green colour represents the proteins that were found in Hülle cells and vegetative mycelium.

| **Hülle cells & asexual mycelium** | | | | | |
|---|---|---|---|---|---|
| **Gene ID** | **Function** | **3 days HC & Asex.** | **5 days HC & Asex.** | **7 days HC & Asex.** | **Reference** |
| AN5324 (DlpA) | Dehydrin-like protein | X (9) | X (9) | X (6) | Wartenberg et al., 2012 |
| AN5004 | Induced in light | X (2) | X (30) | X (7) | Ruger-Herreros et al., 2011 |
| AN6856 | Up-regulated in *atm*Δ | X (5) | X (6) | | Malavazi et al., 2007 |
| AN5015 (ConJ) | Induced in light | X (2) | X (8) | X (2) | Ruger-Herreros et al., 2011 |
| AN5971 | Induced in light | X (7) | X (4) | | Ruger-Herreros et al., 2011 |
| AN1362 | Cue5 ortholog | | X (4) | | Not available |
| **Hülle cells & vegetative mycelium** | | | | | |
| **Gene ID** | **Function** | **3 days HC & Veg.** | **5 days HC & Veg.** | **7 days HC & Veg.** | **Reference** |
| AN1182 (BenA) | Beta-tubulin | X (5) | X (4) | X (23) | Oakley et al., 2004 |
| AN4236 | Proteasome regulatory particle | X (3) | | X (5) | Not available |
| AN6688 (AspB) | Putative Septin B | X (7) | | X (2) | Hernández-Rodríguez et al.,2012 |
| AN2918 (Cct4) | Chaperonin complex component | X (3) | | X (3) | Malavazi et al., 2007 |
| AN4159 (GlnA) | Glutamate-ammonia ligase | X (4) | | | Margelis et al., 2001 |
| AN2149 (Cct1) | Chaperonin | X (2) | | X (2) | Malavazi et al. 2006 |

## 3.2.8. Functional annotation reveals that surface Hülle cells are highly involved in carbohydrate and amino acid metabolism

In order to perform a gene ontology (GO) annotation based on a multiple NCBI blast search for biological functions of all 401 proteins found in surface Hülle cells the high-quality functional annotation tool Blast2GO was used (Conesa et al., 2005). The largest subsegment of the pie chart with the GO term carbohydrate metabolic process revealed that Hülle cells are highly involved in the carbohydrate metabolism (Figure 28). The presence of enzymes to degrade certain carbohydrates, such as α-1,3-glucanase and other glucanases in Hülle cells most probably represents an additional energy source to support the energy-consuming process of the development of cleistothecia. The second largest subsegment with the GO term amino acid metabolism process together with the GO terms translation plus generation of precursor metabolites and energy highlights that Hülle cells are necessary to promote growth and development. In Hülle cells one can find the laccase type II enzyme CpeA. It is presumed that laccase acts on phenolic compounds in Hülle cells, leading to the generation of reactive oxygen species (ROS), (Scherer et al., 2002). The subsegment with the GO term response to stress reveals that Hülle cells have to deal with reactive oxygen species. The subsegment with the GO term secondary metabolite process shows that Hülle cells are involved in this process. Enzymes encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters were found in the enriched Hülle cell fraction and sexual mycelium. This points out that the proteome of surface Hülle cells comprise high amounts of proteins involved in carbohydrate and amino acid metabolism.

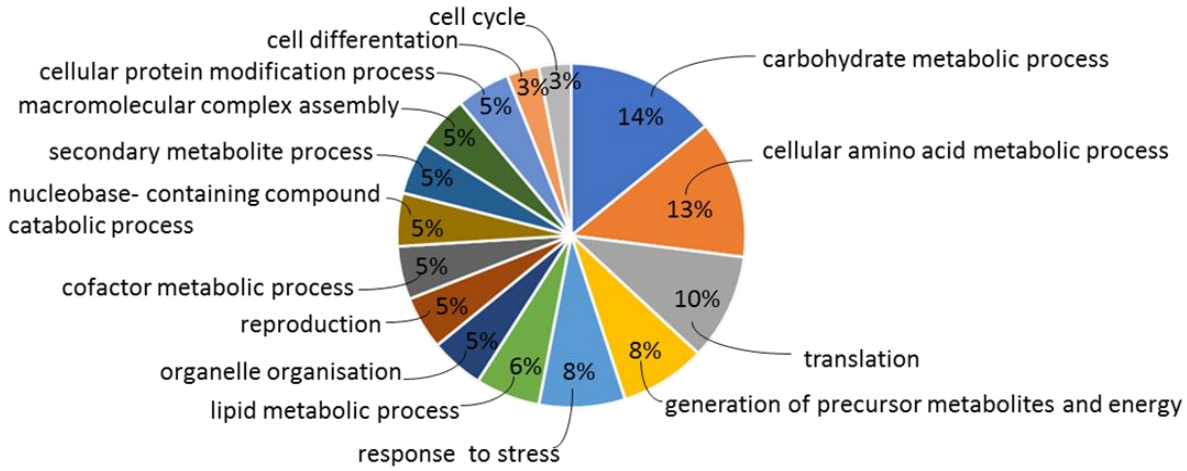



**Figure 28. Functional annotation of identified proteins found in surface Hülle cells.**
Blast2GO was used to perform the functional annotation of all 401 proteins that were found in surface Hülle cells. The gene ontology (GO) annotations for biological functions and the percentages of all GO terms are shown.

## 3.3. Hülle cells from surface growth and liquid media differ in their composition by 28% beside a shared core proteome: Proteomes of *Aspergillus nidulans* development grown on surfaces compared to liquid cultures

### 3.3.1. Hülle cell formation in submerged liquid cultures for comparative proteomics

A second proteomic approach (proteome experiment 2) from submerged cultures was performed to compare proteomes of Hülle cells from liquid cultures versus solid plates (Figure 29). **S**table **i**sotope **l**abeling by **a**mino **a**cids in **c**ell culture (SILAC) is a technique for the relative quantification of changes in protein abundances within complex mixtures (Schmitt et al., 2017). It was shown that the LaeA protein promotes Hülle cell formation and therefore the deletion of *laeA* leads to a significant reduction of Hülle cells (Sarikaya-Bayram et al., 2010).

In order to gain insight into the regulation of proteins involved in secondary metabolism and Hülle cell formation the second proteome approach was performed with a *laeA*Δ strain. In order to identify and quantify proteins in a *laeA*Δ mycelium with high and low amounts of Hülle cells from submerged cultures the gene *laeA* was deleted in a lysine auxotroph strain. The deletion of the gene *lysA* that encodes a putative saccharopine dehydrogenase enabled the efficient incorporation of isotopically labeled lysines into proteins and with that a SILAC study could be performed.

In order to observe Hülle cells in submerged cultures the strains were cultivated for three days in liquid medium and labeled with isotopic lysine variants from the point on where the strains were inoculated. The parental strain *lysA*Δ; *nkuA*Δ (AGB1092) was labeled with light lysine. The knockout strain *lysA*Δ; *laeA*Δ; *nkuA*Δ (AGB1074) was labeled with heavy lysine and the complementation strain *lysA*Δ; *laeA*Δ*::laeA*; *nkuA*Δ (AGB1076) was labeled with medium lysine. For a label swap experiment, the light and the heavy labels were exchanged. This serves as a control that allows to confirm that the isotopically labeled lysines do not influence the analysis.

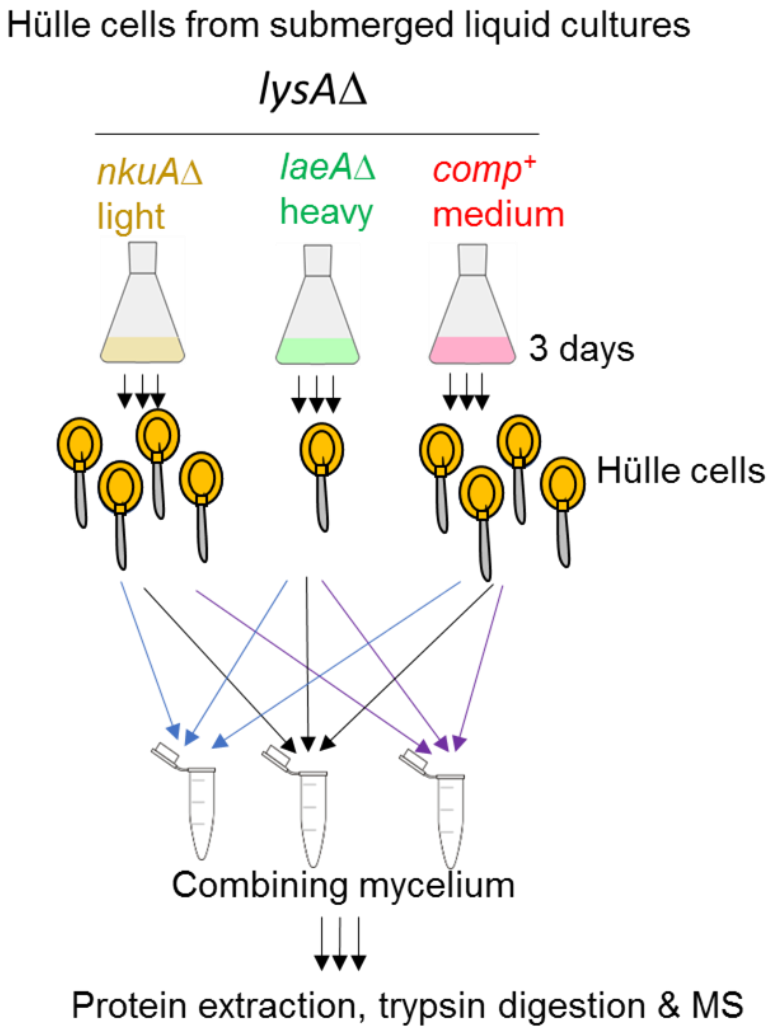



**Figure 29. Proteomics workflow for analyzing enriched Hülle cells from submerged cultures.** For the second proteomic approach **(Proteome experiment 2)** three lysine auxotrophic strains were used: *nkuA*Δ (*lysA*Δ; *nkuA*Δ, AGB1092), *laeA*Δ (*lysA*Δ; *laeA*Δ; *nkuA*Δ, AGB1074), *comp*$^{+}$ (*lysA*Δ; *laeA*Δ::*laeA*; *nkuA*Δ, AGB1076) were inoculated in liquid medium containing light, heavy and medium labeled lysine. The strains were grown for three days and Hülle cells were observed in the hyphal balls of vegetative mycelium. The amount of 300 mg of each mycelium was pooled together and proteins were extracted and digested into peptides. The experiment was performed with three biological replicates and is highlighted with three arrows in this figure.

After growing the three strains in submerged conditions for three days 300 mg mycelia of each individual labeled strain were pooled together and proteins were extracted and digested as described (Shevchenko et al., 1996). Peptide analytics were done with a liquid chromatography (LC) coupled to an *Orbitrap Velos Pro™ Hybrid Ion Trap-Orbitrap* mass spectrometer (MS). The labeled peptides of the samples allowed accurate quantification of peptide ratios relative to each other. The percentage of observable Hülle cells was determined in the hyphal balls of vegetative mycelium and almost no Hülle cells could be observed in the *laeA*Δ strains in contrast to the parental and the complementation strains (Figure 30).

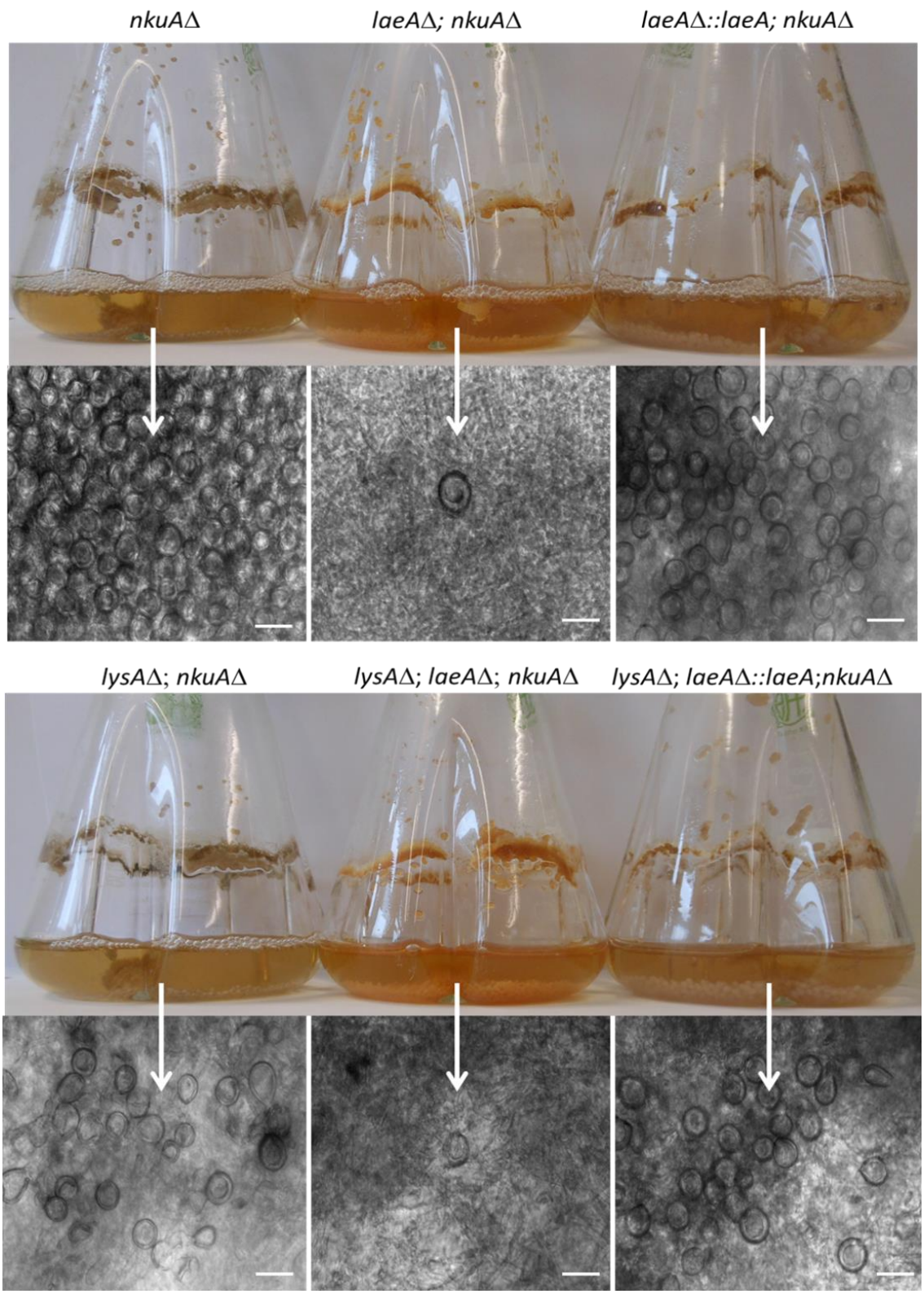



**Figure 30. Lysine prototroph and auxotroph *laeA*Δ strains for the quantitative analysis of Hülle cells under submerged liquid conditions.**
Hülle cells were observed in submerged cultures on day 3 after inoculation. High amounts of Hülle cells were observed in the lysine prototrophic *nkuA*Δ (AGB552) strain with an additional unknown mutation; as well as in the lysine auxotrophic *lysA*Δ; *nkuA*Δ (AGB1092) strain. The deletion of the gene *laeA* (AGB1073, AGB1074) in *nkuA*Δ as well as in *lysA*Δ; *nkuA*Δ strain resulted in a significant reduction in the amount of Hülle cells. The phenotype could be restored in the complementation strains *laeA*Δ::*laeA;nkuA*Δ (AGB1075) and *lysA*Δ;*laeA*Δ::*laeA*; *nkuA*Δ (AGB1076) where high amounts of Hülle cells were present. Scale bar is 20 µm.

The number of observable Hülle cells, determined within an area of 5 mm$^2$ in the hyphal ball were counted and their number was put in relation to the parental and the complementation strain with three biological replicates (Figure 31A). Additionally, the dry weight of the submerged cultures was measured (Figure 31B). Vegetative mycelia were first filtered to remove the liquid media, then dried for 12 hours in a drying cabinet (75 °C). The dry weight was weighed and determined for three biological replicates. A prolongation of the drying time had no significant effect on the dry weight. The dry weight did not change in the *laeA*Δ lysine prototroph and auxotroph strain in comparison to the parental and the complementation strain.

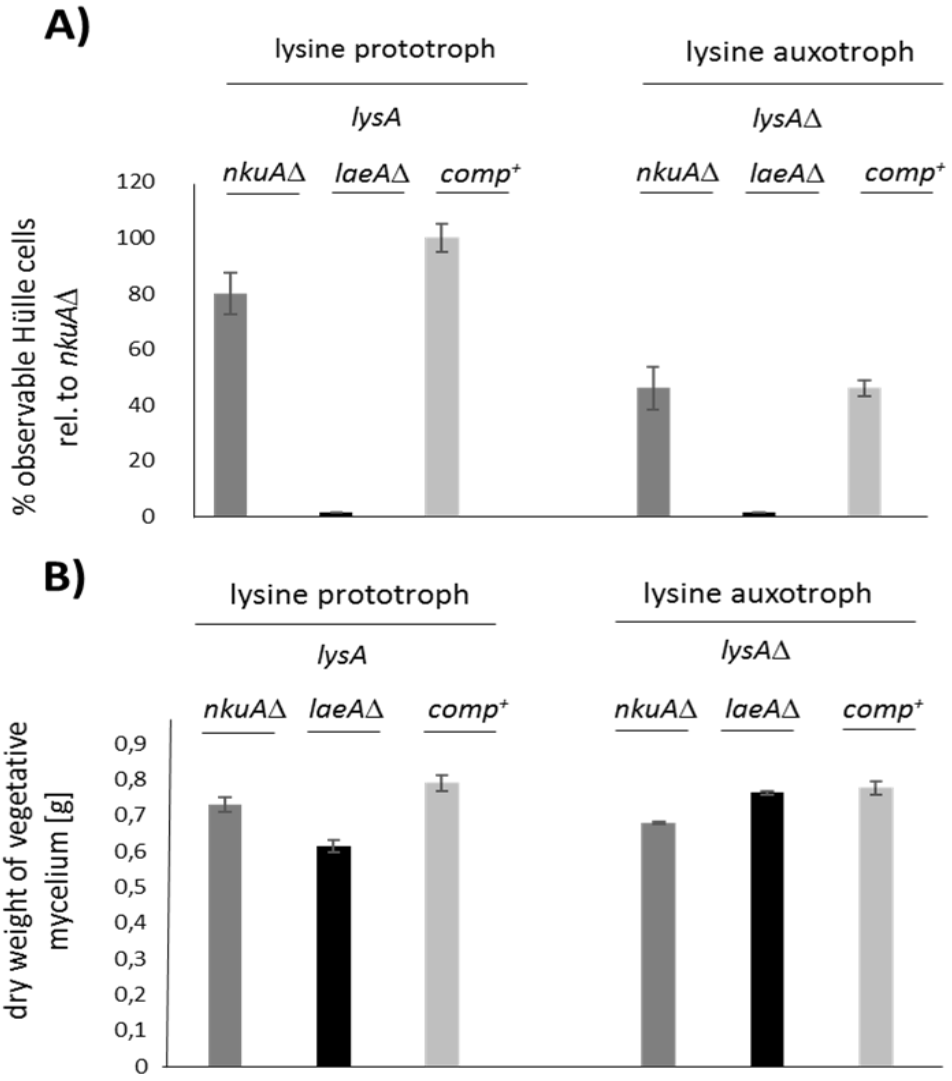



**Figure 31. Determination of the number of Hülle cells in vegetative mycelia and determination of the dry weight of the vegetative mycelia.**
A) The percentage of Hülle cells in the hyphal balls of vegetative mycelium of all strains were determined. The number of observable Hülle cells, determined within an area of 5 mm$^2$ in the hyphal ball were counted and their number was put in relation to the parental and the complementation strain with three biological replicates. B) The dry weight of all strains was measured. Vegetative mycelia were dried for 12 hours in a drying cabinet (75 °C). The dry weight was weighed and determined. Three biological replicates were considered.

### 3.3.1.1. Submerged liquid cultures revealed the proteome for comparative analysis

The proteome analysis of Hülle cells grown in submerged liquid cultures revealed a total number of 974 identified proteins (Supplementary Table 5). Three biological replicates were considered for the analysis. Only proteins identified in two or more biological replicates and with two or more peptides per protein were considered for the analysis. Comparing the proteomes of Hülle cells from solid agar plates versus submerged liquid cultures revealed a common core proteome consisting of 286 proteins that represents 72% of overlapping identified proteins. Therefore, the proteome of Hülle cells from surface growth and liquid media differ in their composition by 28% that corresponds to 115 identified proteins. Two proteins identified in the proteome of a vegetative mycelium in submerged conditions were chosen for further investigation regarding their localization. Prenyltransferase NptA (AN11080) and the serine/threonine kinase RfeA (AN2943) could be identified and quantified in the SILAC approach. These two proteins involved in the process of secondary metabolism showed a significant reduction in quantity in a *laeA*Δ strain compared to the parental strain. A substantial reduction of Hülle cells in a *laeA*Δ strain could correlate with the absence of these two proteins. NptA and RfeA, therefore, were investigated regarding the localization of these two proteins to gain more insight into the secondary metabolism in Hülle cells. 72% as a result shows a high overlapping of proteins in the core proteome of Hülle cells grown in solid agar plates and liquid media.

## 3.3.2. Quantification of proteins in submerged liquid cultures of a *laeA*Δ mycelium revealed two Hülle cells proteins

Protein quantities of the prenyltransferase NptA (AN11080) and the serine/threonine kinase RfeA (AN2943) were reduced in *laeA*Δ (AGB1074) mycelium. These two proteins are involved in the process of secondary metabolism (De Souza et al., 2013, Oakley et al., 2017). It is known that LaeA is a key regulator of secondary metabolism and Hülle cell formation in *A. nidulans* (Bok and Keller 2004, Sarikaya-Bayram et al., 2010).

SILAC, stands for **s**table **i**sotope **l**abeling of **a**mino **a**cids in **c**ell cultures and was applied as a technique for the relative quantification of changes in protein abundance within complex mixtures. The $log_2$ SILAC ratio was determined for the strains *laeA*∆ (AGB1074) in comparison to *laeA* (AGB1092). The following threshholds were set. In the range of a $log_2$ SILAC ratio between -5.0 and -0.5 identified proteins are down-regulated. In the range of a $log_2$ SILAC ratio between + 0.5 and +5.0 identified proteins are up-regulated. In the range between a -0.5 and + 0.5 $log_2$ SILAC ratio identified proteins were unchanged.

The protein quantity of identified proteins that is significantly down-regulated in a *laeA*∆ vegetative mycelium in submerged liquid cultures is shown in Table 9. Proteins that are down-regulated in *laeA*∆ vegetative mycelium are involved in secondary metabolism (2 proteins, NptA and RfeA), amino acid metabolism (6 proteins). In addition, the quantity of tRNA charging enzymes (16 enymes), proteins that are required for protein folding (4), nuclear import protein (1), proteins that are involved in the carbohydrate metabolism (4) and proteins which are involved in metabolic processes (8) are reduced. The prenyltransferase NptA (AN11080) showed the most significant reduction in quantity in a *laeA*∆ mycelium (Table 9). Bok and co-workers showed that the prenyltransferase *tidB* is not expressed in a *laeA*∆ mycelium in comparison to the wild-type (Bok et al., 2006). The serine/threonine kinase RfeA (AN2943) is predicted to be involved in the regulation of secondary metabolism (De Souza et al., 2013). This kinase was significantly reduced in a *laeA*∆ mycelium (Table 9). A substantial reduction of Hülle cells in a *laeA*∆ strain could correlate with the reduction of the two proteins NptA and RfeA. These two proteins were further analyzed in more detail to gain insight into the process of secondary metabolism in Hülle cells.

**Table 9. Proteins that are down-regulated in a *laeA*∆ mycelium**

SILAC is applied as a technique for the relative quantification of changes in protein abundance within complex mixtures. The protein quantity of the following proteins is reduced in a *laeA*∆ vegetative mycelium. These proteins are down-regulated in *laeA*∆ mycelium. The $log_2$ SILAC ratio was determined for the strains *laeA*∆ (AGB1074) in comparison to *laeA* (AGB1092). The following threshholds were set. In the range of a $log_2$ SILAC ratio between -5.0 and -0.5 identified proteins are down-regulated. In the range between a -0.5 and + 0.5 $log_2$ SILAC ratio identified proteins are unchanged. The $log_2$ SILAC ratio for the complementation strain (*laeAcomp*$^+$, AGB1076) in comparison to the parental *laeA* strain (AGB1092) is between -0.5 and + 0.5. The color scales represent $log_2$ SILAC ratios. Ø = median of $log_2$ SILAC ratio, SD = standard deviation, NaN = not a number. Biological replicates are numbered 1-3.


| Gene ID | Description | Spectral counts | | | *laeA*Δ/*laeA* | | | Ø | SD | *laeA*comp+/*laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| **Secondary metabolimsm** | | | | | | | | | | | | | | |
| AN11080 (NptA) | Prenyltransferase | 122 | 79 | 47 | -4.9 | -3.9 | -4.2 | **-4.2** | 0.5 | -0.2 | -0.5 | 0.4 | **-0.2** | 0.5 |
| AN2943 (RfeA) | Kinase | 19 | 33 | 63 | -2.0 | -1.3 | -0.6 | **-1.3** | 0.7 | -0.5 | -0.2 | -0.5 | **-0.5** | 0.2 |
| **Amino acid metabolism** | | | | | | | | | | | | | | |
| AN5591 | Aminotransferase | 21 | 63 | NaN | -3.6 | -3.9 | NaN | **-3.8** | 0.2 | 0.5 | -0.1 | NaN | **0.2** | 0.3 |
| AN2901 (AgaA) | Arginase | 28 | 99 | 173 | -3.0 | -1.9 | -2.3 | **-2.5** | 0.6 | -0.5 | 0.2 | -0.6 | **-0.5** | 0.4 |
| AN4159 (GlnA) | Glutamate-ammonia ligase | 367 | 384 | 481 | -3.1 | -1.0 | -0.5 | **-2.1** | 1.4 | -0.4 | -0.2 | 0.5 | **-0.2** | 0.5 |
| AN5886 (LuA) | Isomerase | 46 | 60 | 218 | -1.9 | -1.0 | -1.7 | **-1.5** | 0.5 | 0.0 | 0.0 | NaN | **0.0** | 0.0 |
| AN0797 | Role in histidine metabolism | 20 | 41 | 71 | -1.7 | -1.1 | -0.7 | **-1.4** | 0.5 | 0.0 | -0.4 | -0.1 | **-0.1** | 0.2 |
| AN4218 | Translation elongation factor | 384 | 845 | 1035 | -1.4 | -0.6 | -1.5 | **-1.0** | 0.5 | 0.2 | 0.1 | -0.1 | **0.1** | 0.1 |
| **tRNA charging enzyme** | | | | | | | | | | | | | | |
| AN0046 | Histidyl-tRNA ligase | 46 | 66 | 100 | -3.6 | -1.8 | -0.8 | **-1.8** | 1.4 | -0.4 | -0.4 | 0.0 | **-0.4** | 0.3 |
| AN0705 | Isoleucine-tRNA ligase | 57 | 113 | NaN | -2.2 | -1.2 | NaN | **-1.7** | 0.7 | -0.5 | -0.1 | NaN | **-0.3** | 0.3 |
| AN11125 | Glycine-tRNA ligase | 117 | 159 | NaN | -2.1 | -0.9 | NaN | **-1.5** | 0.8 | -0.5 | -0.3 | NaN | **-0.4** | 0.3 |
| AN0057 | Tyrosine-tRNA ligase | 33 | 68 | 33 | -1.9 | -0.9 | -1.3 | **-1.3** | 0.5 | -0.5 | -0.3 | NaN | **-0.4** | 0.3 |
| AN10475 | Tryptophan-tRNA ligase | 36 | 40 | NaN | -2.0 | -0.7 | NaN | **-1.3** | 0.9 | -0.4 | -0.1 | NaN | **-0.3** | 0.2 |
| AN10474 | Tryptophan-tRNA ligase | 97 | 113 | 178 | -1.9 | -0.7 | -1.3 | **-1.3** | 0.6 | -0.4 | -0.3 | 0.4 | **-0.3** | 0.4 |
| AN6368 | tRNA charging enzyme | 52 | 117 | 125 | -2.0 | -1.1 | -0.7 | **-1.1** | 0.6 | -0.4 | -0.1 | 0.0 | **-0.1** | 0.2 |
| AN8224 | Glutamate-tRNA ligase | 60 | 101 | 171 | -2.0 | -1.0 | -1.0 | **-1.0** | 0.6 | -0.5 | -0.3 | 0.3 | **-0.3** | 0.4 |
| AN3824 | Phenylalanine-tRNA ligase | 30 | 52 | 103 | -2.0 | -1.0 | -0.9 | **-1.0** | 0.6 | -0.4 | -0.4 | 0.3 | **-0.4** | 0.4 |
| AN8867 | Serine-tRNA ligase | 83 | 104 | 147 | -1.2 | -1.0 | -1.0 | **-1.0** | 0.1 | 0.5 | 0.1 | 0.0 | **0.1** | 0.3 |
| AN3702 | Leucine-tRNA ligase | 73 | 115 | 213 | -2.3 | -0.8 | -0.9 | **-0.9** | 0.8 | -0.4 | -0.2 | 0.2 | **-0.2** | 0.3 |
| AN4086 | Phenylalanine-tRNA ligase | 40 | 67 | 93 | -2.0 | -0.7 | -0.9 | **-0.9** | 0.7 | -0.4 | -0.2 | 0.3 | **-0.2** | 0.4 |
| AN9419 | Alanine-tRNA ligase | 149 | 200 | 374 | -2.0 | -0.8 | -0.9 | **-0.9** | 0.7 | -0.3 | 0.1 | 0.0 | **0.0** | 0.2 |
| AN2150 | Proline-tRNA ligase | 149 | 225 | 329 | -1.8 | -0.7 | -0.9 | **-0.9** | 0.6 | -0.4 | -0.1 | 0.3 | **-0.1** | 0.3 |
| AN1380 | Methionine-tRNA ligase | 60 | 114 | 148 | -1.8 | -0.8 | -0.7 | **-0.8** | 0.6 | -0.3 | -0.3 | 0.2 | **-0.3** | 0.3 |
| AN10195 | Valine-tRNA ligase | 119 | 109 | 280 | -1.9 | -0.7 | -0.6 | **-0.7** | 0.7 | -0.5 | -0.1 | 0.5 | **-0.1** | 0.5 |
| **Protein folding** | | | | | | | | | | | | | | |
| AN10202 (HscA) | Heat shock protein | 89 | 285 | NaN | -1.8 | -0.7 | -1.8 | **-1.8** | 0.7 | -0.3 | 0.0 | NaN | **-0.2** | 0.2 |
| AN1047 | Heat shock protein | 136 | 186 | NaN | -1.7 | -0.7 | -2.1 | **-1.7** | 0.7 | -0.2 | -0.3 | NaN | **-0.2** | 0.1 |
| AN2918 (Cct4) | Chaperonin complex | 46 | 48 | 102 | -1.6 | -0.7 | -1.7 | **-1.6** | 0.6 | -0.1 | -0.2 | -0.5 | **-0.2** | 0.2 |
| AN2062 (BipA) | Chaperonin complex | 177 | 142 | 287 | -1.9 | -1.0 | -1.4 | **-1.4** | 0.5 | 0.0 | 0.0 | -0.2 | **0.0** | 0.1 |
| **Nuclear import** | | | | | | | | | | | | | | |
| AN5482 (Ran) | RAS-related nuclear protein | 54 | 38 | NaN | -1.6 | -0.7 | NaN | **-1.1** | 0.7 | -0.2 | -0.1 | 0.3 | **-0.1** | 0.3 |
| **Carbohydrate metabolism** | | | | | | | | | | | | | | |
| AN6655 (GudC) | Glucose 1-dehydrogenase | 15 | 16 | 7 | -1.5 | -1.3 | -0.5 | **-1.3** | 0.5 | 0.4 | -0.2 | 0.3 | **0.3** | 0.3 |
| AN3223 (pfkA) | 6-phosphofructokinase | 184 | NaN | 431 | -1.9 | -0.7 | NaN | **-1.3** | 0.9 | -0.1 | -0.2 | NaN | **-0.1** | 0.1 |
| AN7459 (HxkA) | Hexokinase | 53 | 121 | 146 | -2.3 | -1.2 | -0.7 | **-1.2** | 0.8 | -0.3 | -0.5 | -0.2 | **-0.3** | 0.2 |
| AN0285 | 6-phosphogluconolactonase | 91 | 100 | 119 | -1.8 | -0.7 | -1 | **-1.1** | 0.6 | -0.4 | -0.4 | -0.4 | **-0.4** | 0.0 |
| **Metabolic process** | | | | | | | | | | | | | | |
| AN4923 | HMG-CoA synthase | 7 | 49 | 212 | -5.0 | -2.6 | -2.3 | **-2.6** | 1.5 | -0.5 | -0.4 | 0.5 | **-0.4** | 0.6 |
| AN0565 | Multifunctional enzyme | 61 | 91 | 458 | -2.0 | -0.9 | -2.2 | **-2.0** | 0.7 | -0.5 | -0.4 | 0.3 | **-0.4** | 0.4 |
| AN3581 (TrxR) | Thioredoxin reductase | 100 | 105 | 184 | -2.1 | -1.1 | -1.7 | **-1.7** | 0.5 | -0.5 | -0.3 | -0.4 | **-0.4** | 0.1 |
| AN9180 | Transketolase | NaN | 113 | 133 | NaN | -1.3 | -1.5 | **-1.4** | 0.8 | NaN | -0.2 | 0.5 | **0.2** | 0.4 |
| AN10220 (Ccp1) | Cytochrome c peroxidase | 25 | 29 | 57 | -2.4 | -1.2 | -0.7 | **-1.2** | 0.9 | -0.5 | -0.5 | -0.4 | **-0.5** | 0.1 |
| AN0641 | TCTP family protein | 76 | 17 | NaN | -1.4 | -0.8 | NaN | **-1.1** | 0.7 | 0.5 | 0.3 | NaN | **0.4** | 0.2 |
| AN0232 (UreD) | Nickel-binding protein | 24 | 45 | 43 | -2.2 | -1.0 | -0.7 | **-1.0** | 0.8 | 0.0 | -0.3 | 0.5 | **0.0** | 0.4 |
| AN7710 | Haloacid dehalogenase | 153 | 336 | 273 | -1.4 | -0.7 | -0.9 | **-0.9** | 0.4 | 0.5 | 0.1 | -0.3 | **0.1** | 0.4 |

The proteins that are up-regulated in a *laeA*Δ vegetative mycelium are shown in Table 10. The following four proteins are up-regulated in a *laeA*Δ mycelium e.g. alcohol oxidase, two alcohol dehydrogenases (AlcA and AlcB) and a choline oxidase. These proteins are most likely not found in Hülle cells since *laeA*Δ causes significant reduction in formation of Hülle cells.

**Table 10. Proteins that are up-regulated in a *laeA*Δ mycelium**
The protein quantity of the following proteins is increased in a *laeA*Δ vegetative mycelium. These proteins are up-regulated in *laeA*Δ mycelium. The $log_2$ SILAC ratio was determined for the strains *laeA*Δ (AGB1074) in comparison to *laeA* (AGB1092). The following threshholds were set. In the range of a $log_2$ SILAC ratio between +5.0 and +0.5 identified proteins are up-regulated. In the range between a -0.5 and + 0.5 $log_2$ SILAC ratio identified proteins are unchanged. The $log_2$ SILAC ratio for the complementation strain (*laeAcomp*$^+$, AGB1076) in comparison to the parental *laeA* strain (AGB1092) is between -0.5 and + 0.5. The color scales represent $log_2$ SILAC ratios. Ø = median of $log_2$ SILAC ratios, SD = standard deviation, NaN = not a number. Biological replicates are numbered 1-3. Spectral counts (PSM) for three biological replicates are shown.

-5 -3.25 -1.5 -1.3 -1 -0.75 -0.5 -0.25 0 0.25 0.5 0.75 1 1.25 1.5 3.25 5

| Gene ID | Description | Spectral counts | | | *laeAΔ/laeA* | | | Ø | SD | *laeAcomp+/laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN0567 | Alcohol oxidase | 149 | 315 | NaN | 4.2 | 4.4 | NaN | 4.3 | 0.1 | -0.5 | -0.3 | NaN | -0.4 | 0.3 |
| AN3741 (AlcB) | Alcohol dehydrogenase II | 32 | 44 | 55 | 4.0 | 4.5 | NaN | 4.2 | 0.4 | 0.3 | 0.4 | NaN | 0.4 | 0.2 |
| AN8979 (AlcA) | Alcohol dehydrogenase | 307 | NaN | 82 | 2.5 | NaN | 3.0 | 2.5 | 0.3 | 0.5 | NaN | -0.4 | 0.5 | 0.6 |
| AN1429 (CodA) | Choline oxidase | 14 | 27 | 93 | 1.8 | 0.8 | 0.2 | 1.3 | 0.8 | 0.3 | -0.4 | 0.2 | -0.1 | 0.4 |

## 3.3.2.1. Quantitative determination of proteins in *laeA*Δ revealed that NptA is present in Hülle cells as well as in other fungal tissues

NptA (nidulanin A prenyltransferase A, AN11080) is involved in the prenylation of the secondary metabolite nidulanin A (Oakley et al., 2017). In order to verify the localization of the prenyltransferase NptA in Hülle cells, a *nptA*::*gfp*; *lysA*Δ; *nkuA*Δ (AGB1082) strain was used. Hülle cells were enriched from sexual mycelium using the cleistothecia-rolling technique and the localization of NptA::GFP was observable in the cytoplasm of Hülle cells (Figure 32). The parental strain *lysA*Δ; *nkuA*Δ (AGB1092) was used as a negative control. The strain *laeA*Δ; *nptA*::*gfp*; *lysA*Δ; *nkuA*Δ (AGB1083) was used to verify the reduced protein abundance of NptA in a *laeA*Δ mycelium. Fluorescence microscopy revealed that almost no NptA::GFP was observable in the remaining Hülle cells except for a weak autofluorescence. A strain expressing GFP constitutively served as a control where GFP was mainly observable in the cytoplasm of Hülle cells.

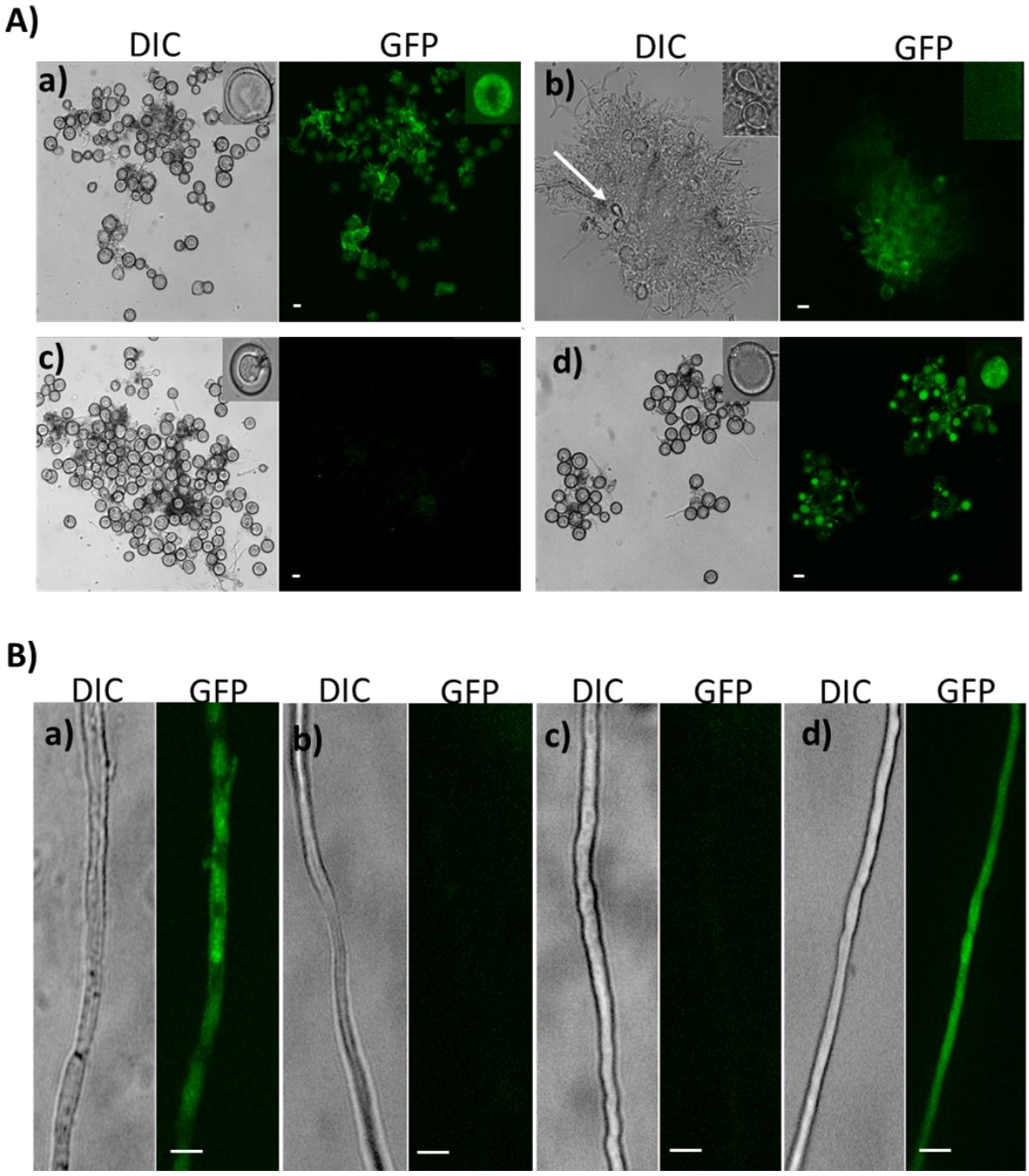



**Figure 32. The prenyltransferase NptA is localized in the cytoplasm of Hülle cells.**
A) Fluorescence microscopy pictures of Hülle cells: a) The picture shows the strain *nptA::gfp; lysA*Δ; *nkuA*Δ (AGB1082). NptA::GFP is localized to the cytoplasm of Hülle cells. b) The picture shows the strain *laeA*Δ*; nptA::gfp; lysA*Δ*; nkuA*Δ (AGB1083). Reduced NptA::GFP was observed in the remaining Hülle cells (white arrow) and in hyphae caused by *laeA*Δ. c) The picture shows the strain *lysA*Δ*; nkuA*Δ (AGB1092) where only autofluorescence could be detected in Hülle cells. d) A strain expressing GFP constitutively (AGB596) where GFP was mainly observable in the cytoplasm of Hülle cells. Scale bar is 10 µm. B) Fluorescence microscopy pictures of a vegetative hypha: a) *nptA::gfp; lysA*Δ; *nkuA*Δ where NptA::GFP was detectable b) *laeA*Δ*; nptA::gfp; lysA*Δ*;nkuA*Δ where NptA::GFP was significantly reduced. c) *lysA*Δ*; nkuA*Δ where only autofluorescence could be detected. d) A strain expressing GFP constitutively where GFP was observable in the cytoplasm of the vegetative hypha. Scale bar is 20 µm.

The protein quantity of NptA was significantly reduced in a *laeA*Δ; *lysA*Δ; *nkuA*Δ strain. Western hybridization experiments revealed that the quantity of NptA::GFP protein is reduced in a *laeA*Δ*; nptA::gfp*; *lysA*Δ; *nkuA*Δ strain in a vegetative, sexual and asexual mycelium (Figure 33). The NptA protein consists of 434 amino acids with a predicted molecular mass of 48.9 kDa. NptA::GFP was detected as expected around 75 kDa. Additionally, degradation products of the NptA::GFP fusion protein were observable. A strain expressing GFP constitutively served as a positive control where GFP was detectable around 27 kDa. The parental *nkuA*Δ (ABG552) strain served as a negative control where GFP and the fusion protein was not observable. This finding shows that prenyltransferase NptA is reduced in a *laeA*Δ mycelium and that this prenyltransferase is localized in Hülle cells and other types of mycelia.

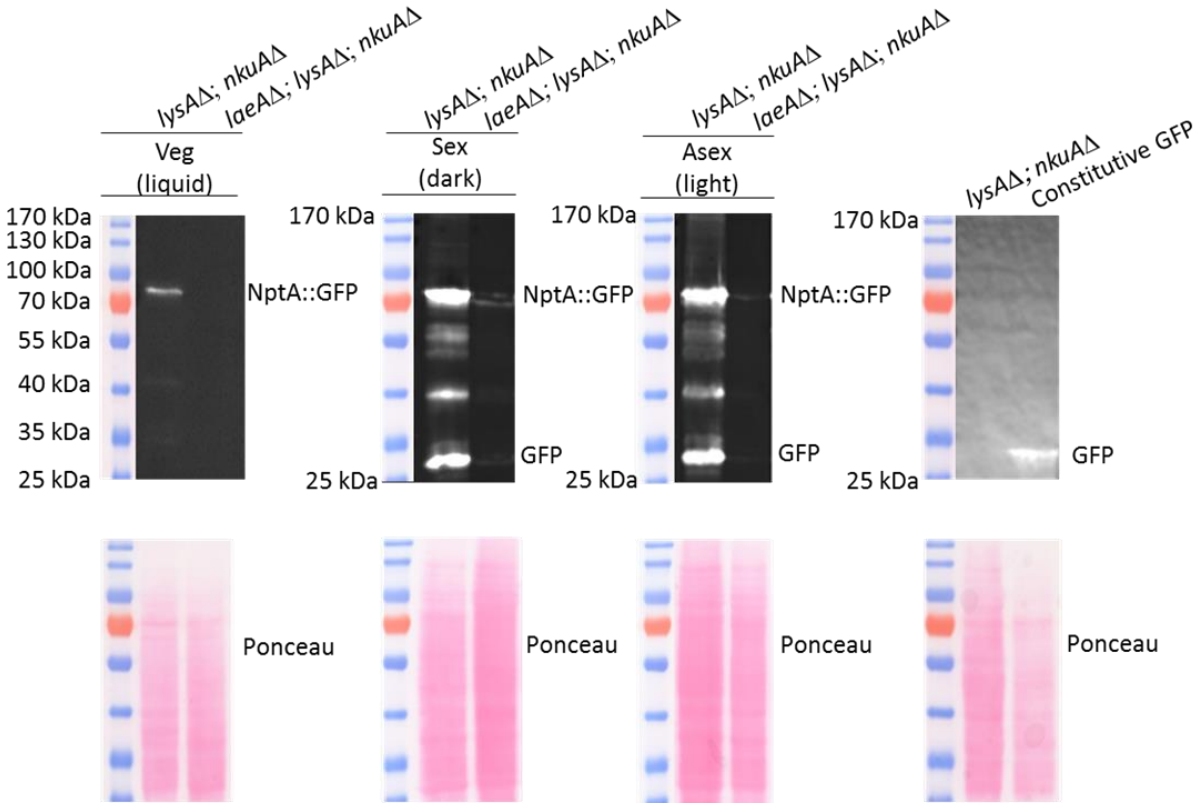



**Figure 33. Reduced protein abundance of the prenyltransferase NptA in a *laeA*Δ mycelium.** Western hybridization to detect NptA::GFP in a *laeA*Δ (AGB1083) strain. NptA::GFP was detected weakly in *laeA*Δ*; nptA::gfp; lysA*Δ*; nkuA*Δ (AGB1083) strain. In order to detect NptA::GFP a primary α– GFP antibody (sc-9996; Santa Cruz) was used followed with an incubation of an α-mouse secondary antibody (G21234, Invitrogen). The NptA protein consists of 434 amino acids with a predicted molecular mass of 48.9 kDa. NptA::GFP was detected as expected around 75 kDa. Additionally, degradation products of the NptA::GFP fusion protein were observable. A strain expressing GFP constitutively served as a positive control where GFP was detectable around 27 kDa. Parental *nkuA*Δ (AGB552) strain served a a negative control where GFP and the fusion protein were not detectable.

### 3.3.2.2. Quantitative protein analysis revealed the kinase RfeA enriched in Hülle cells and other fungal tissues

Besides NptA, a serine/threonine kinase RfeA (AN2943) showed a siginifcant reduction in quantity in a *laeA*Δ strain. To gain more insight into secondary metabolism in Hülle cells RfeA was further investigated. RfeA was identified and quantified in submerged liquid cultures and the localization of RfeA was further analyzed. The localization of RfeA::GFP was mainly observable in the cytoplasm of Hülle cells and vegetative hyphae (Figure 34). RfeA::GFP is possibly also located in other cellular compartments. In order to observe the localization of RfeA::GFP Hülle cells were enriched by the cleistothecia-rolling technique. A *refA*::*gfp*;*lysA*Δ;*nkuA*Δ (AGB1084) strain was used to observe RfeA::GFP in Hülle cells. In a *laeA*Δ;*rfeA*::*gfp*; *lysA*Δ; *nkuA*Δ (AGB1085) strain Hülle cells were observed in a reduced number. In the remaining Hülle cells, RfeA::GFP was used to verify the reduced quantity of RfeA::GFP protein in a *laeA*Δ mycelium.

Additionally, RfeA::GFP was observed in vegetative hyphae grown in submerged liquid cultures. In a *laeA*Δ; *rfeA*::*gfp*; *lysA*Δ; *nkuA*Δ strain, significantly reduced RfeA::GFP was observed in vegetative hyphae. The parental strain *lysA*Δ; *nkuA*Δ (AGB1092) was used as a negative control. Only autofluorescence could be detected in the *lysA*Δ*; nkuA*Δ strain. A strain expressing GFP constitutively served as a control where GFP was mainly observable in the cytoplasm of Hülle cells. The quantity of RfeA::GFP protein is reduced in a *laeA*Δ (AGB1085) strain.

Western hybridization experiments confirmed that without LaeA the amount of RfeA protein is reduced in vegetative, sexual and asexual mycelium (Figure 35). The RfeA protein consists of 448 amino acids with a predicted molecular mass of 50.5 kDa. The RfeA::GFP fusion protein was detected as expected around 76 kDa. A strain expressing GFP constitutively served as a positive control where GFP was detectable around 27 kDa. Parental *nkuA*Δ (ABG552) strain served as a negative control where GFP and the fusion protein were not detectable. The abundance of RfeA::GFP protein was reduced in a *laeA*Δ*; nptA::gfp*; *lysA*Δ; *nkuA*Δ strain in a vegetative, sexual and asexual mycelium. Additionally, degradation products of the RfeA::GFP fusion protein were observable. This suggests that RfeA is unstable in different fungal tissues.

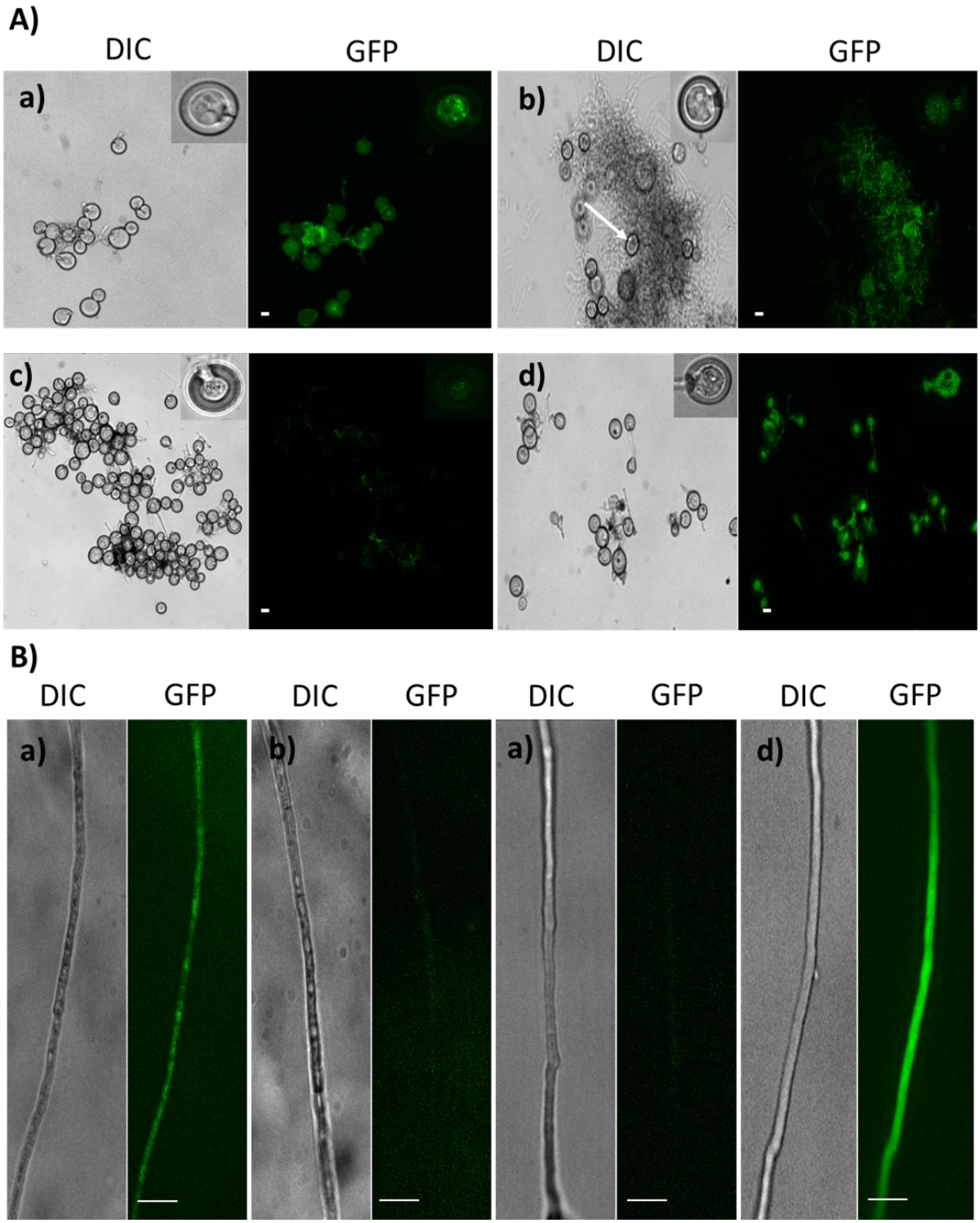



**Figure 34. The serine/threonine kinase RfeA is localized in Hülle cells and is reduced in a *laeA*Δ mycelium.**
A) Fluorescence microscopy pictures of Hülle cells and a vegetative hypha: a) *rfeA::gfp; lysA*Δ*; nkuA*Δ (AGB1084) b) *laeA*Δ*; rfeA::gfp; lysA*Δ*; nkuA*Δ (AGB1085) (white arrow points to one Hülle cell) c) *lysA*Δ; *nkuA*Δ (AGB1092) d) A strain expressing GFP constitutively (AGB596). Scale bar is 10 µm. B) Fluorescence microscopy pictures of a vegetative hypha: a) *rfeA::gfp; lysA*Δ*; nkuA*Δ (AGB1084) b) *laeA*Δ*; rfeA::gfp; lysA*Δ*; nkuA*Δ (AGB1085) c) *lysA*Δ; *nkuA*Δ (AGB1092) d) A strain expressing GFP constitutively (AGB596). RfeA::GFP is localized in Hülle cells and vegetative hyphae and might be in different cellular compartments. RfeA::GFP is reduced in a *laeA*Δ mycelium. Scale bar is 20 µm.

This result indicates that the amount of RfeA is reduced in a *laeA*Δ mycelium and that this kinase is found in Hülle cells and other types of mycelia and that RfeA::GFP is most probably unstable in different fungal cell types including Hülle cells.

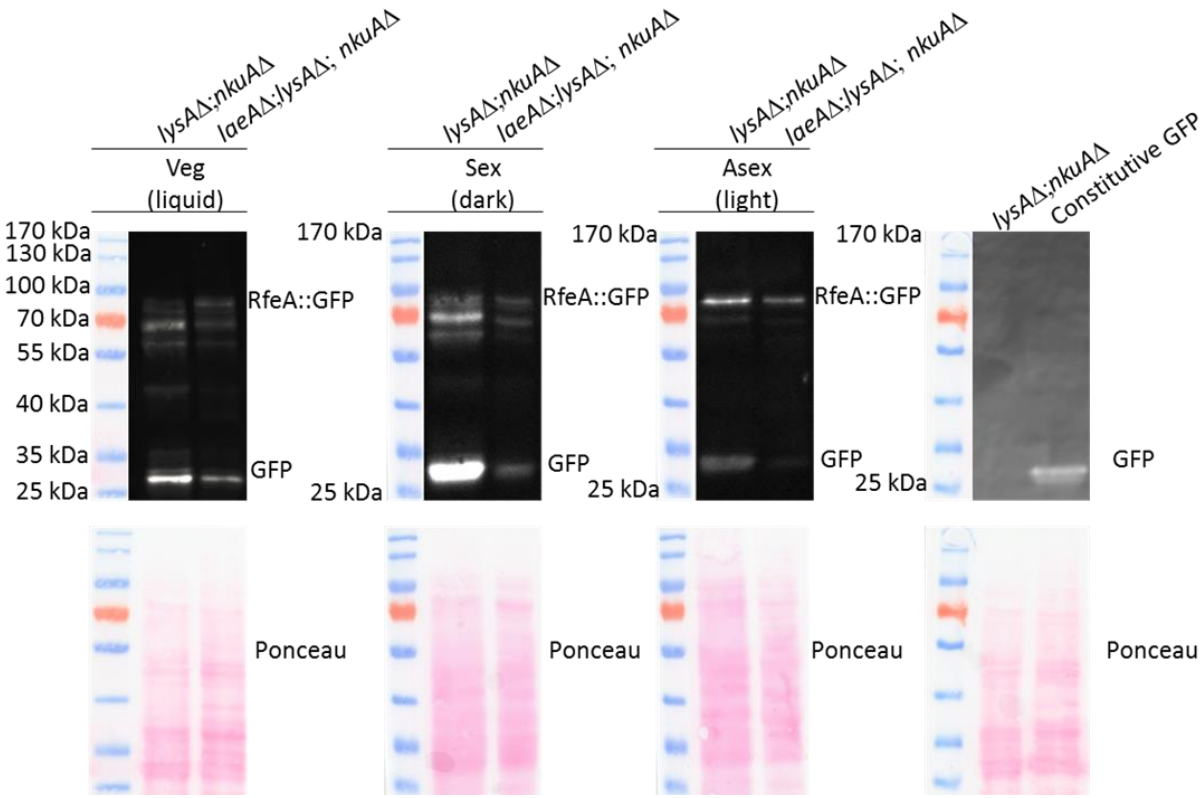



**Figure 35. The serine/threonine kinase RfeA is localized in Hülle cells and the protein quantity is reduced in a *laeA*Δ mycelium.**
Western hybridization of *rfeA::gfp; lysA*Δ*; nkuA*Δ (AGB1084) and *laeA*Δ*; rfeA::gfp; lysA*Δ; *nkuA*Δ (AGB1085). In order to detect RfeA::GFP a primary α-GFP antibody (sc-9996; Santa Cruz) was used followed with an incubation of an α-mouse secondary antibody (G21234, Invitrogen). The RfeA protein consists of 448 amino acids with a predicted molecular mass of 50.5 kDa. RfeA::GFP was detected as expected around 76 kDa. Additionally, degradation products of the RfeA::GFP fusion protein were observable. It seems that RfeA::GFP is unstable in different fungal tissues. A strain expressing GFP constitutively served as a positive control where GFP was detectable around 27 kDa. Parental *nkuA*Δ strain (AGB552) served as negative control where GFP and the fusion proteins were not detectable.

### 3.3.3. The core proteome revealed a 72% overlap between the identified proteins of both types of Hülle cells

Proteomic approaches from solid agar plates and submerged cultures were compared to identify overlapping proteins found in enriched Hülle cells from solid agar plates versus submerged conditions. As such, the 401 proteins identified in Hülle cells from solid agar plates were overlaid with the proteins in submerged liquid cultures where Hülle cell formation occurred. This comparison revealed a 72% overlap between identified proteins from Hülle cells originated from solid agar plates compared to ones that were found in vegetative mycelium in submerged liquid cultures (Figure 36). The core proteome that represents the overlapping identified proteins from Hülle cells grown on solid agar plates and in liquid media consists of 286 proteins which are shortlisted in Table 11. The criterium to select proteins for the shortlist is that these proteins were identified with the highest spectral counts in Hülle cells from surface and liquid media.

The localization of the two proteins (AN8434, AN8435) uniquely found in Hülle cells from solid agar plates, were identified again in vegetative mycelium where Hülle cell formation occur, are discussed within the next chapter. The core proteome of both types of Hülle cells contain five enzymes that are encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters (MdpG (AN0150), MdpL (AN10023), XptC (AN7998), XptB (AN12402) and AN7999 (a putative oxidoreductase). This means that five out of the six enzymes found in Hülle cells from solid agar plates were identified again in Hülle cells from submerged liquid cultures. Alves and co-workers demonstrated in a recent study that after the addition of choline Hülle cell formation occurred in a vegetative mycelium and the secondary metabolite monodictyphenone was present in these cultures (Alves et al., 2016).

These findings imply that Hülle cells from surface and liquid cultures comprise shared proteins encoded by monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters and that these enzymes are also found in a sexual mycelium with high amounts of Hülle cells.

**Table 11. List of overlapping proteins identfied in Hülle cells from solid agar plates and submerged liquid cultures (Hülle cells core proteome).**
Only proteins identified in two or more biological replicates and with two or more peptides per protein were considered for the analysis. 1.2.3: spectral counts of three biological replicates. ***** Identified proteins in two replicates and not in all three biological replicates.

| Gene ID | Function | 1 | 2 | 3 | Reference |
|---|---|---|---|---|---|
| **Uniquely found in both Hülle cell types** | | | | | |
| AN8435 | Tyrosinase domain protein | 58 | 110 | 73 | This study & Bayram et al., 2016 |
| AN8434 | Ankyrin repeat domain protein | 36 | 102 | 62 | This study & Bayram et al., 2016 |
| **Identified in both Hülle cell types & sex. mycelium** | | | | | |
| AN10023 (MdpL) | Member of the *mdp*/*xpt* gene clusters | 14 | 19 | 18 | Bayram et al., 2016 |
| AN12402 (XptB) | Prenyltransferase, member of the *mdp*/*xpt* gene clusters | 9 | 10 | 17 | Sanchez et al., 2011 |
| AN0150 (MdpG) | Polyketide synthase, member of the *mdp*/*xpt* gene clusters | 4 | 17 | 5 | Sanchez et al., 2011 |
| AN7998***** (XptC) | GMC oxidoreductase, member of the *mdp*/*xpt* gene clusters | 3 | 7 | | Sanchez et al., 2011 |
| AN7999 | Oxidoreductase, member of the *mdp*/*xpt* gene clusters | 63 | 64 | 84 | Bayram et al., 2016 |
| AN8203 | Hydolase | 10 | 16 | 35 | Not available |
| AN6314***** | Oxidoreductase | | 15 | 10 | Not available |
| **Identified in both Hülle cell types & asex. mycelium** | | | | | |
| No overlapping proteins were identified | | | | | |
| **In both Hülle cells types & vegetative mycelium (7 proteins were identical, 6 are listed)** | | | | | |
| AN1182 (BenA) | Beta-tubulin | 327 | 352 | 648 | Oakley et al., 2004 |
| AN6688 (AspB) | Putative septin B | 180 | 124 | 8 | Hernández et al., 2012 |
| AN4159 (GlnA) | Glutamate-ammonia ligase | 384 | 367 | 481 | Margelis et al., 2001 |
| AN2918 (Cct4) | Chaperonin complex component | 48 | 46 | 102 | Malavazi et al., 2007 |
| AN2149 (Cct1) | Putative chaperonin complex component | 27 | 12 | 86 | Malavazi et al., 2006 |
| AN4236 (RPT5) | Proteasome regulatory particle | 25 | 32 | 55 | Not available |
| **In both Hülle cells types, sexual & vegetative mycelium (18 proteins were identical, 6 are listed)** | | | | | |
| AN7570 (TubB) | Alpha-tubulin | 236 | 192 | 363 | Kirk et al., 1991 |
| AN8182 (AspC) | Septin | 160 | 139 | 252 | Hernández et al.,2012 |
| AN4997 (AspA) | Phosphatidylinositol transporter | 44 | 85 | 110 | Hernández et al.,2012 |
| AN2142 (KapA) | Karyopherin (importin) alpha | 66 | 43 | 111 | Etxebeste et al., 2013 |
| AN5895 (GdiA) | Rab GDP-dissociation inhibitor | 142 | 112 | 251 | Goody et al., 2017 |
| AN4775 | Cullin deneddylation | 47 | 45 | 101 | Dambacher et al., 2016 |
| **In both Hülle cells types, sexual & asexual mycelium (35 proteins were identical, 6 are listed)** | | | | | |
| AN11080 (NptA) | Prenyltransferase | 79 | 122 | 47 | Oakley et al., 2017 |
| AN7912***** (OrsC) | Tyrosinase | 14 | 24 | | Guerriero et al., 2017 |
| AN7914 | Alcohol dehydrogenase | 203 | 159 | 75 | Sanchez et al., 2010 |
| AN4871 (ChiB) | Chitinase | 44 | 66 | 43 | Shin et al., 2009, Yamazaki et al., 2007 |
| AN1502***** (NagA) | Chitin hydrolysis | 8 | 8 | | Pusztahelyi et al., 2006, Kim et al., 2002 |
| AN2017***** (AgdA) | Alpha-glucosidase | 6 | | 8 | Nakamura et al., 2006 |
| **Identified in all cell types (213 proteins were identical, 6 are listed)** | | | | | |
| AN8041 (GpdA) | Glyceraldehyde-3-phosphate dehydrogenase | 971 | 1104 | 1794 | Punt et al., 1988 |
| AN7388 (CpeA) | Laccase | 319 | 450 | 63 | Scherer et al., 2002 |
| AN9339 (CatB) | Catalase | 637 | 669 | 575 | Calera et al., 2000 |
| AN0359 (SgdA) | Translation initiation factor 3 (eIF3) | 4 | 13 | 217 | Obayashi et al., 2017 |
| AN7105 | Translation initiation factor 3 (eIF3) | 7 | 22 | 237 | Obayashi et al., 2017 |
| AN6542 (ActA) | Actin A | 335 | 425 | 563 | Upadhyay et al., 2008 |

### 3.3.4. The proteome of surface Hülle cells compared to Hülle cells from liquid media differ in composition by 28%

Identified proteins of the proteome from surface Hülle cells differ in 28% composition compared to identified proteins found in vegetative mycelium grown in submerged liquid cultures (Figure 36A). The difference between the proteomes of both types of Hülle cells revealed that surface Hülle cells contain increased numbers of glucanases as shown in Figure 36B. In a recent study it was shown that the transcripts of the α-1,3-glucanase *mutA* and *agnB* are expressed during sexual development on surface agar plates (He et al., 2017).

The MphA (AN2601) maltose permease-like protein was identified from surface Hülle cells within different developmental time points mentioned above. These results show that sexual differentiation increases α-1,3-glucanase levels and a maltose permease-like protein found in surface Hülle cells preferentially surrounding the cleistothecia. Proteins such as the α-1,3-glucanase MutA, reduced in a *laeA*Δ mycelium, are potentially found in Hülle cells (Sarikaya-Bayram et al., 2010). Hülle cell growth in submerged liquid cultures revealed several proteins that were reduced in a *laeA*Δ; *lysA*Δ; *nkuA*Δ (AGB1074) mycelium. Among these proteins, several tRNA ligases were identified in this type of mycelium shown in Figure 36B. The reduction of tRNA ligases in *laeA*Δ vegetative mycelium suggests most probably that protein synthesis is reduced in this type of mycelium. This finding suggests that tRNA ligases are found in a submerged liquid mycelium where Hülle cell formation occurred and that these ligases are reduced in a *laeA*Δ mycelium.

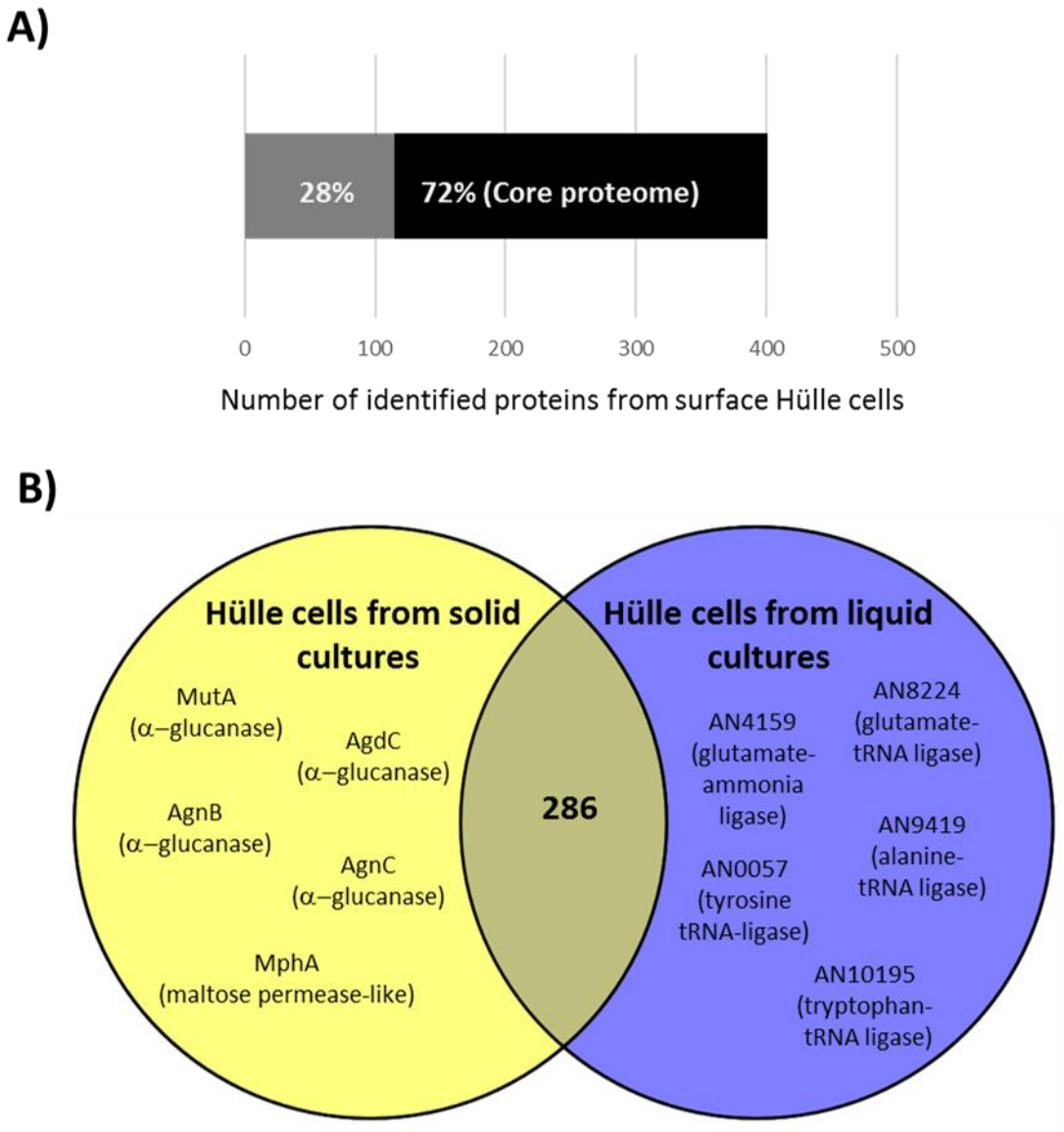



**Figure 36. Hülle cells from surface growth compared to Hülle cells from liquid media differ in composition by 28% beside a shared core proteome.**
A) 401 proteins shown in the graph were identified from surface Hülle cells and were overlaid with the proteins that were identified in Hülle cells grown in submerged liquid cultures. The percentages of overlapping and non-overlapping proteins are shown. The black box represents the overlapping proteins identified from Hülle cells grown on solid agar plates versus Hülle cells grown in liquid media. The grey box represents the non-overlapping proteins identified from Hülle cells grown on solid agar plates versus Hülle cells grown in liquid media. B) Comparison between proteins that were identified either from solid agar plates or from liquid cultures. The core proteome of Hülle cells that represents the identified overlapping proteins from Hülle cells grown on solid agar plates and liquid media contains 286 proteins and are shortlisted in Table 11 (page 95).

## 3.4. Functional analysis of genes for Hülle cell enriched proteins: A maltose permease-like protein of surface Hülle cells supports fungal growth and development

### 3.4.1. Comparative proteomics revealed two overlapping proteins found in Hülle cells from both approaches

Comparative proteomics revealed that two proteins were uniquely found in both types of Hülle cells and these are listed in Table 11. These two proteins encoded by the neighboring genes, *AN8434* and *AN8435,* are predicted to contain an ankyrin repeat and a tyrosinase domain. Bayram and co-workers showed that the transcripts of *AN8434* and *AN8435* are expressed in an asexual and sexual mycelium (Bayram et al., 2016).

In order to verify the localization of the ankyrin repeat domain protein (AN8434) and the putative tyrosinase domain protein (AN8435) in Hülle cells, an *AN8434*::*gfp* (AGB1089) and an *AN8435*::*gfp* (AGB1090) strain were used. Proteins with an ankyrin repeat domain are adaptor proteins bound to the membrane and are known to interact with multiple protein interaction partners for instance with the mammalian transcription factor NF-κB (König et al., 2017). NF-κB comprises structural similarities to the velvet domain protein VosA (Ahmed et al., 2013). It was shown that the *laeA* ortholog of *Trichoderma reesei* (*lae1*) regulates genes encoding ankyrin-proteins (Karimi-Aghcheh et al., 2013). The amino acid shared between AN8434 and their *Homo sapiens* homologs is 36.2% to Ank3 (Cerqueira et al., 2014). The AN8434 protein was named AnkG and stands for ankyrin repeat domain protein G.

In order to study the localization of AnkG::GFP Hülle cells were enriched from sexual mycelium using the cleistothecia-rolling technique and AnkG::GFP was detectable at the membrane of the subtending hyphae of Hülle cells as shown in Figure 37. Additionally, AnkG::GFP was observed in vegetative hyphae grown in submerged liquid cultures and was underrepresented in the membrane of vegetative hyphae and is most likely localized primarily in the endoplasmic reticulum (ER) and in the cytoplasm. The *nkuA*Δ (ABG552) parental strain was used as negative control. Only autofluorescence could be detected in the *nkuA*Δ strain. A strain expressing GFP constitutively revealed that GFP is mainly visible in the cytoplasm of vegetative hyphae.

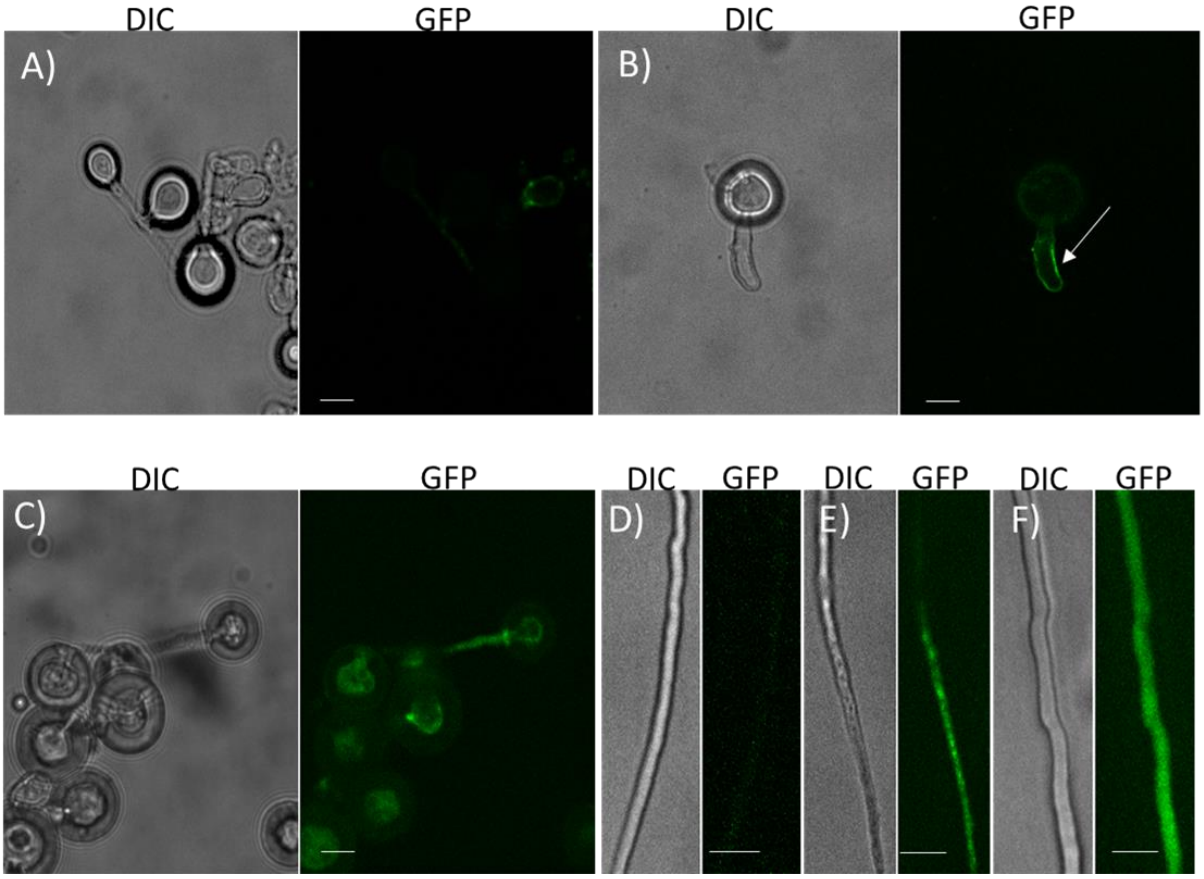



**Figure 37. The AnkG (AN8434) ankyrin repeat domain protein is localized at the membrane of the subtending hyphae of Hülle cells.**
Fluorescence microscopy pictures of Hülle cells A) *nkuA*Δ (ABG552) B) *ankG*::*gfp*; *nkuA*Δ (AGB1089) C) A strain expressing GFP constitutively (AGB596). AnkG::GFP is detectable at the membrane of the subtending hyphae of Hülle cells (white arrow points to one subtending hypha of Hülle cell). Scale bar is 10 µm. Fluorcence microscopy picture of a vegetative hypha D) *nkuA*Δ E) *ankG*::*gfp*; *nkuA*Δ F) A strain expressing GFP constitutively. AnkG::GFP is detectable mainly in the cytoplasm as well as in the endoplasmic reticulum (ER) of a vegetative hypha. Scale bar is 20 µm.

The AnkG protein consists of 477 amino acids with a predicted molecular mass of 52.0 kDa. Western hybridization experiments revealed that AnkG::GFP is detectable as expected around 79 kDa in a three and five day old sexual mycelium with high amounts of Hülle cells plus a three day old vegetative mycelium (Figure 38). Proteolytic cleavage product of AnkG::GFP fusion protein was detectable around 27 kDa. This is most likely the degradation product of the full-length AnkG::GFP fusion protein and represents the degraded GFP product. A strain expressing GFP constitutively served as a positive control where GFP was detectable around 27 kDa. Parental *nkuA*Δ (ABG552) strain served as a negative control where GFP and the fusion protein was not observable.

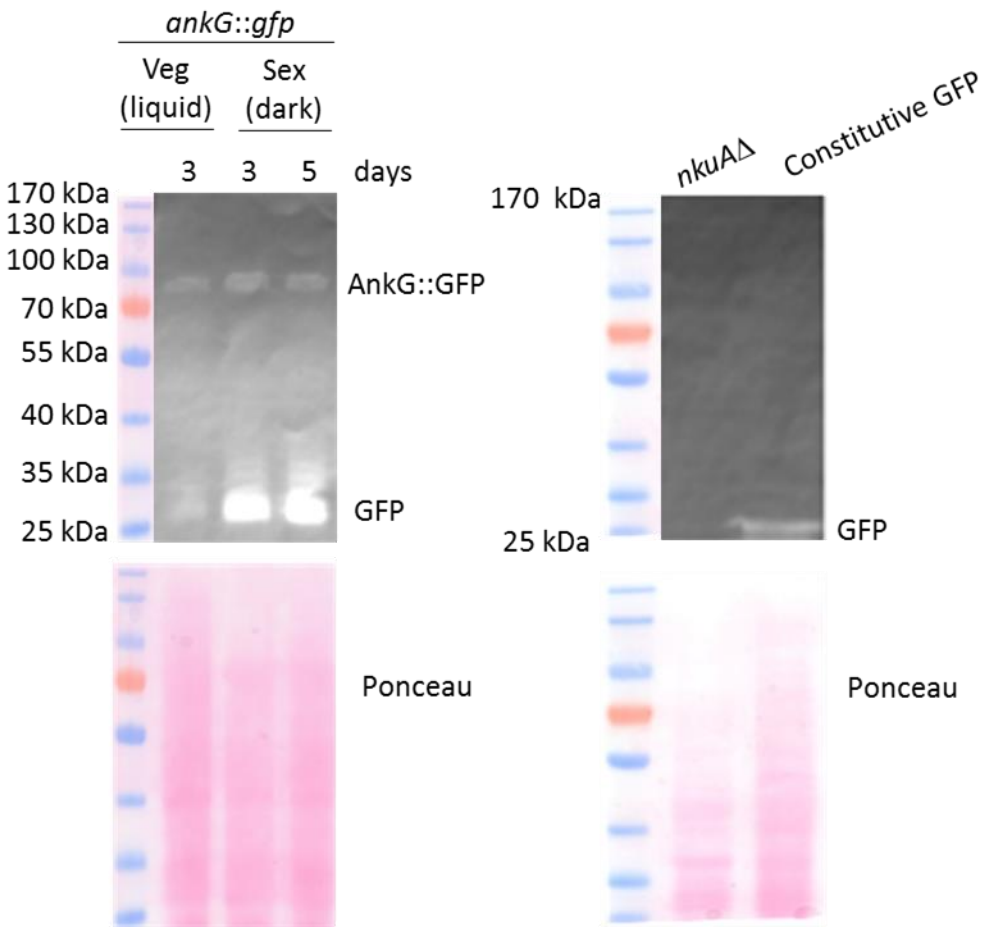



**Figure 38. The AnkG::GFP fusion protein is found in sexual mycelium with high amounts of Hülle cells and a vegetative mycelium.**
Western hybridization of *ankG*::*gfp*; *nkuA*Δ (AGB1089) of a vegetative and a sexual mycelium. To detect AnkG::GFP (*ankG::gfp;nkuA*Δ, AGB1089) a primary α-GFP antibody (sc-9996; Santa Cruz) was used followed with an incubation of an α–mouse secondary antibody (G21234, Invitrogen). The AnkG protein consists of 477 amino acids with a predicted molecular mass of 52.0 kDa. AnkG::GFP was detected as expected at around 79 kDa. A proteolytic cleavage product of AnkG::GFP fusion protein was detectable around 27 kDa and represents most probably the degraded GFP. A strain expressing GFP constitutively served as a positive control where GFP was detectable around 27 kDa. Parental *nkuA*Δ (AGB552) strain served as negative control where GFP and the fusion proteins were not detectable.

In order to study the localization of the putative tyrosinase domain protein (AN8435) Hülle cells were enriched from sexual mycelium using the cleistothecia-rolling technique and AN8435::GFP fusion protein was observable (Figure 39). It is possible that AN8435::GFP is situated in different cellular compartments within Hülle cells such as the cytoplasm. AN8435::GFP was also observable at the thick cell wall ringshaped structure of Hülle cells. AN8435::GFP was observable in vegetative hyphae in submerged liquid cultures. It seems that AN8435::GFP is not stable in different fungal cell types.

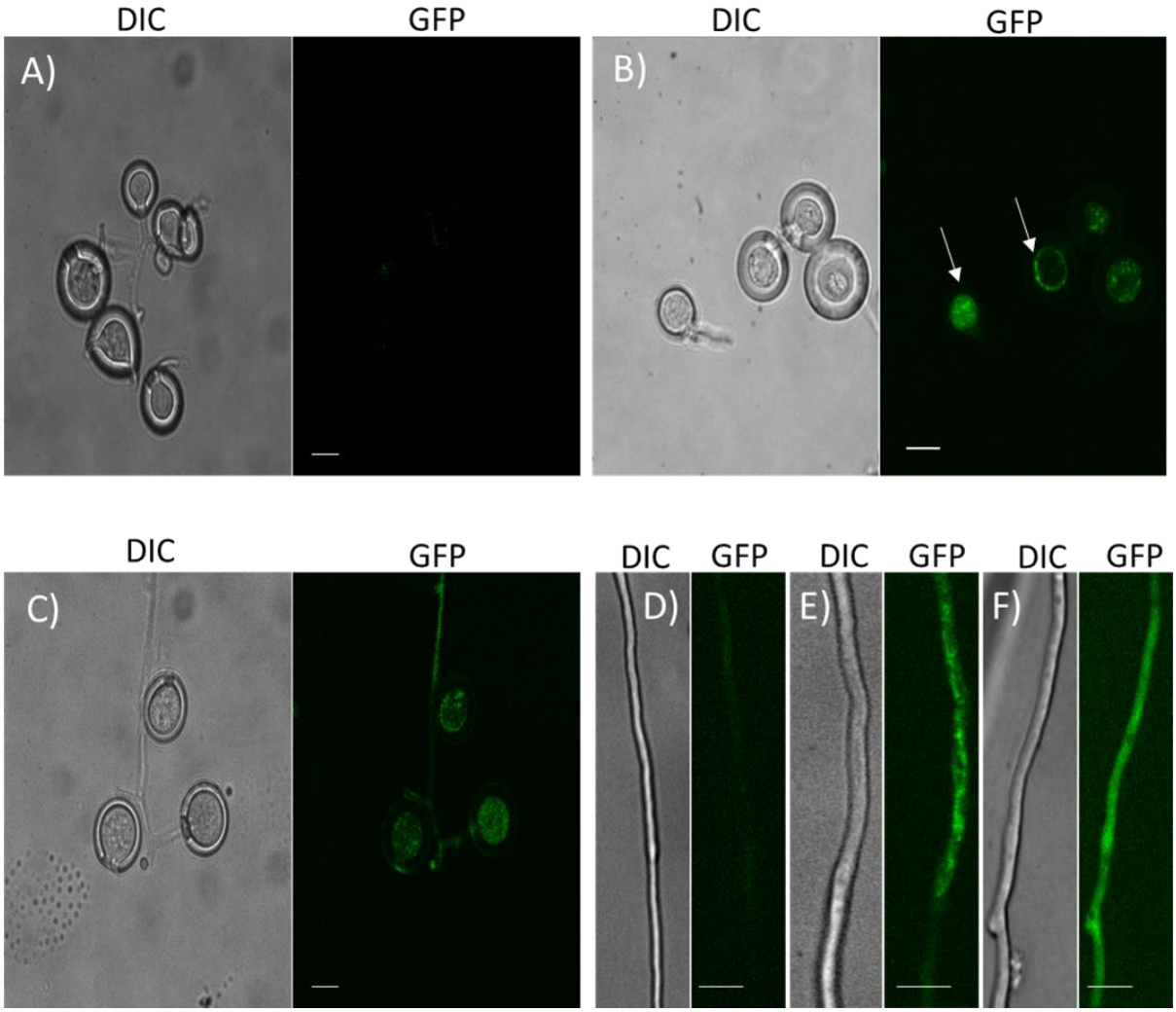



**Figure 39. The localization of the tyrosinase domain protein (AN8435).**
Fluorescence microscopy pictures of Hülle cells A) *nkuA*Δ (AGB552) B) *AN8435*::*gfp*; *nkuA*Δ (AGB1090) C) A strain expressing GFP constitutively (AGB596). AN8435::GFP was observed in different regions in Hülle cells (white arrows point to cytoplasm and ringshaped structures of Hülle cells). Scale bar is 10 µm. Fluorcence microscopy picture of a vegetative hypha D) *nkuA*Δ E) *AN8435*::*gfp*; *nkuA*Δ F) A strain expressing GFP constitutively. AN8435::GFP is detectable mainly in the cytoplasm of a vegetative hypha. Scale bar is 20 µm

The AN8435 protein consists of 846 amino acids with a predicted molecular mass of 95.7 kDa. Western hybridization experiments revealed that AN8435::GFP is probably detectable at the expected size of 123 kDa in a sexual mycelium with high amounts of Hülle cells and vegetative mycelium (Figure 40). Additionally, products with a higher size were observable, suggesting that the tyrosinase domain protein (AN8435) could have been modified. Degradation products of the AN8435::GFP fusion protein were also observable.

A strain expressing GFP constitutively served as a positive control where GFP was detectable around 27 kDa. Parental *nkuA*Δ (ABG552) strain served as a negative control where GFP and the fusion protein were not detectable. It seems that the fusion protein AN8435::GFP is not stable.

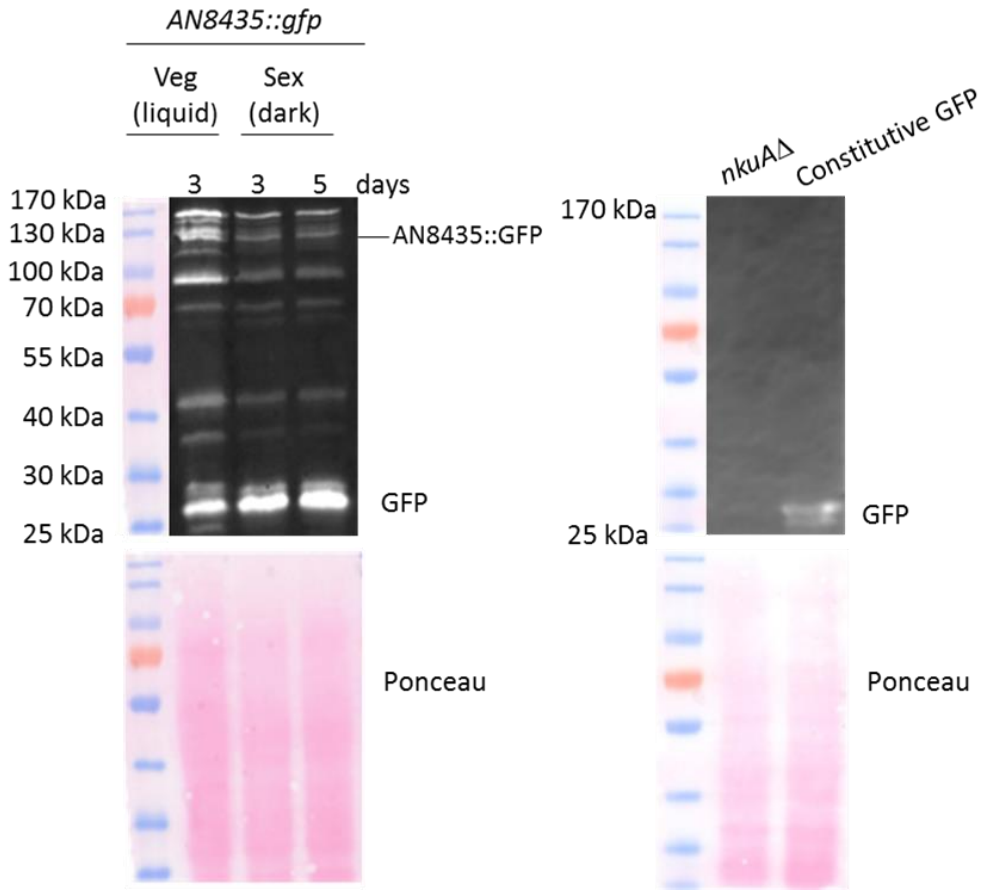



**Figure 40. Western hybridization of AN8435::GFP of a vegetative and a sexual mycelium**.
In order to detect AN8435::GFP (*AN8435*::*gfp*; *nkuA*Δ, AGB1090) a primary α-GFP antibody (sc-9996; Santa Cruz) was used followed with an incubation of an α-mouse secondary antibody (G21234, Invitrogen). The sequence of AN8435 consists of 846 amino acids with a predicted molecular mass of 95.7 kDa. AN8435::GFP was most probably detected at the expected size of 123 kDa. Additionally, products with a higher size were observable. Degradation products of the AN8435::GFP fusion protein were also observable. A strain expressing GFP constitutively served as a positive control where GFP was detectable around 27 kDa. Parental *nkuA*Δ (ABG552) strain served as a negative control where GFP and the fusion protein were not detectable.

These findings reveal that the AnkG (AN8434) ankyrin domain protein is localized to the membrane of the subtending hyphae of Hülle cells. It seems that AnkG is localized in the cytoplasm as well as in endoplasmic reticulum (ER) during vegetative growth. The localization of AN8435::GFP fusion protein was investigated. Probably AN8435 is located in different fungal tissues such as Hülle cells and vegetative mycelium. The fusion protein seems not to be stable.

## 3.4.2. A similar protein to a maltose transporter is enriched in surface Hülle cells and supports growth and fungal development

### 3.4.2.1. The sequence of MphA a maltose permease-like protein enriched in Hülle cells contains a distinctive sugar motif

The putative transmembrane transporter AN2601 was found in enriched Hülle cells of solid agar plates mentioned above. Transporters are thought to be crucial to transport nutritions and other molecules across Hülle cell membranes thereby supporting the cleistothecia (Wei et al., 2004, Pantazopoulou et al., 2007). The biological function of this putative transmembrane transporter was studied in detail. The AN2601 protein was named MphA and stands for **m**altose **p**ermease-like protein of **H**ülle cells. The best hit for an orthologous gene in an *Aspergillus* species for MphA is the putative MFS maltose transporter (Afu4g00150) of *Aspergillus fumigatus* and has a sequence identity of 88.3%. This is shown with other sequences of maltose transporters in the multiple sequence alignment in Figure 41.

The sequence of MphA contains 540 amino acids with a predicted molecular mass of 60.3 kDa. The secondary structure of MphA is predicted to contain twelve transmembrane helices which, in turn, consist of two 6-helix bundles connected by a cytoplasmic loop. The N-terminal tail of the sequence with a predicted length of 64 residues is longer than the C-terminal end with 34 residues. The sequence of MphA contains one conserved distinctive sugar motif (Horák 1997). All four sequences of the multisequence alignment (Figure 41) contain a conserved sequence region after the sixth putative transmembrane region. This region contains a sequence motif (PESP(W)L) which is specific to all sugar transporters (Horák 1997). This points out that the MphA sequence contains similarities with other sugar transporters such as the Mal31 of *Saccharomyces cerevisiae*.

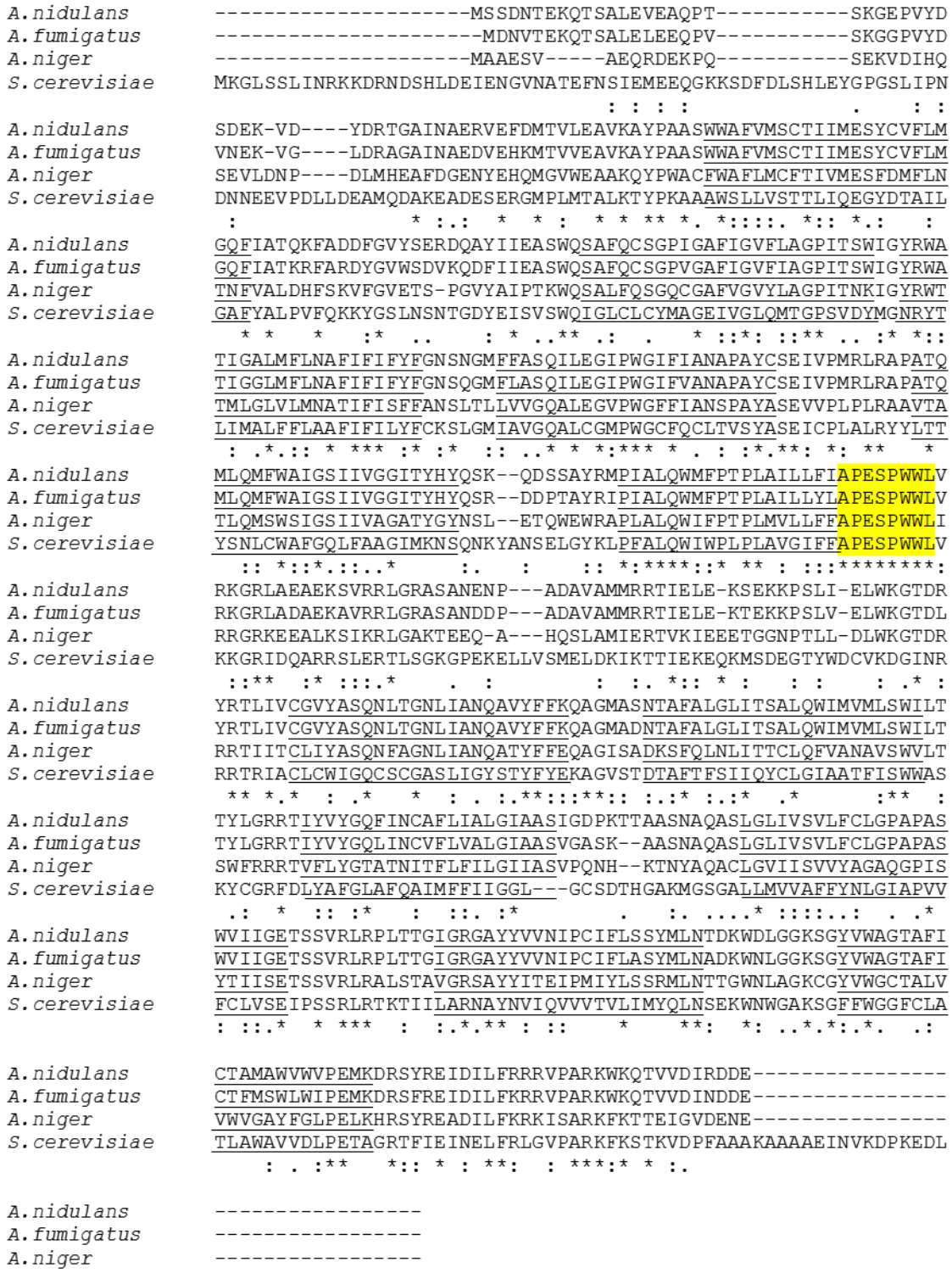



**Figure 41. Multisequence alignment of four putative maltose permease-like protein sequences from MphA (*Aspergillus nidulans*), Afu4g00150 (*Aspergillus fumigatus*), Mal11 (*Aspergillus niger*), Mal31 (*Saccharomyces cerevisiae*).**
The predicted twelve transmembrane regions of the transporters are underlined. The conserved distinctive sugar motif as described by (Horák 1997) is highlighted in yellow.

## 3.4.2.2. MphA protein supports fungal growth and development

A gene deletion of *mphA* was performed in an *nkuA*Δ (AGB552) parental strain. Hülle cells are observable in an *mphA*Δ; *nkuA*Δ (AGB1077) strain. The dry weight of *mphA*Δ; *nkuA*Δ was determined from submerged liquid cultures and was reduced in comparison to the *nkuA*Δ strain and the complementation strain shown in Figure 42.

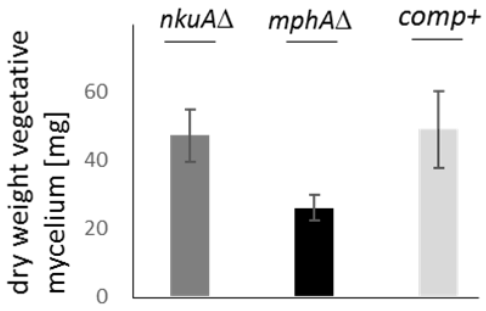



**Figure 42.The MphA maltose permease-like protein supports vegetative growth.**
Dry weight of vegetative mycelium concerning the parental strain (*nkuA*Δ, AGB552), the deletion strain (*mphA*Δ; *nkuA*Δ, AGB1077) and the complementation strain *comp*⁺ (*mphA*Δ::*mphA*::*gfp*; *nkuA*Δ, AGB1078). Vegetative mycelium was harvested 20 hours after inoculation.

The percentage of conidiophores was determined from surface cultures and was reduced in comparison to the *nkuA*Δ strain and the complementation strain as shown at the end of this chapter. The development of cleistothecia was studied after three, five and seven days after germination (Figure 43). After three days, no cleistothecia could be observed in the *mphA*Δ; *nkuA*Δ strain, whereas in the *nkuA*Δ and in the complementation *mphA*Δ::*mphA*::*gfp;nkuA*Δ (AGB1078) strain small non-pigmented primordia could be observed. Cleistothecia of *mphA*Δ; *nkuA*Δ were first visible after five days and at this time point, they were lightly pigmented. In the case of the *nkuA*Δ and the complementation strains, the cleistothecia were pigmented and larger in size. After seven days, the cleistothecia of *mphA*Δ; *nkuA*Δ reached almost the size of the *nkuA*Δ strain but did not fully recover the pigmentation of cleistothecia. After 14 days, all observable cleistothecia of *mphA*Δ; *nkuA*Δ were fully pigmented. After five days, the cleistothecia of *mphA*Δ; *nkuA*Δ contained no dark reddish ascospores in contrast to the *nkuA*Δ and the complementation strain. Dark reddish ascospores could be observed after 14 days in the *mphA*Δ; *nkuA*Δ strain. This finding firmly establishes that MphA supports generally growth, development and also the maturation of the cleistothecia.

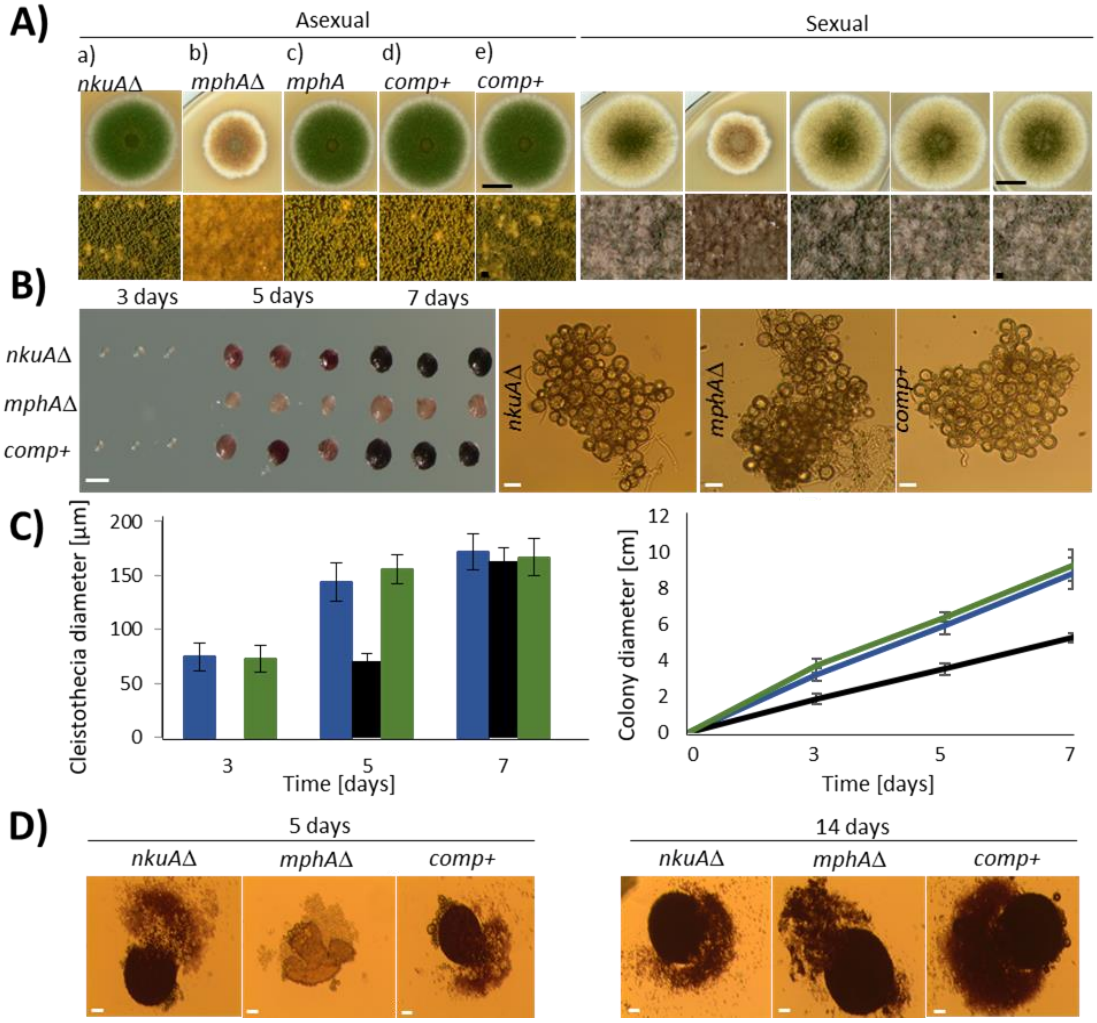



**Figure 43. MphA promotes fungal growth and development.**
(A) The gene *mphA* was knocked out in a a) *nkuA*Δ (AGB552) strain receiving the b) *mphA*Δ; *nkuA*Δ (AGB1077) strain. The phenotype could be restored in complementation strains with and without a tagged version of *gfp*: c) *mphA::gfp; nkuA*Δ (AGB1079) *d*) *mphA*Δ*::mphA::gfp; nkuA*Δ (AGB1078) and e) *mphA*Δ*::mphA nkuA*Δ (AGB1080). Fungal colonies scale bar is 1 cm. Fungal mycelium scale bar is 100 µm. B) Cleistothecia were observed on day 3, 5 and 7 after germination. 3 days after germination no primordia were observable in sexual mycelium of the deletion strain (*mphA*Δ; *nkuA*Δ). After 3 days small non-pigmented undeveloped primordia were observable in *nkuA*Δ and the complementation strain *mphA*Δ*::mphA::gfp; nkuA*Δ (*comp*$^{+}$). 5 and 7 days after germination most of the cleistothecia of the strain *mphA*Δ; *nkuA*Δ were not fully pigmented. Scale bar is 200 µm. Hülle cells are shown and Hülle cells are observable in the deletion strain. Scale bar is 20 µm. C) The diameters of the cleistothecia were measured after 3, 5 and 7 days after germination. After 3 days the size of the primordia of the strains *nkuA*Δ (blue) and *mphA*Δ*::mphA::gfp; nkuA*Δ (green)*;* were around 70 µm in diameter. After 5 days, the cleistothecia of the knockout strain (black) reached the size of around 70 µm. Meanwhile the strains *nkuA*Δ and *mphA*Δ*::mphA::gfp; nkuA*Δ*;* reached the size of around 150 µm. On day 7 all three strains could reach the adult cleistothecia size of around 170 µm. Colony diameter [cm] of the deletion *mphA*Δ; *nkuA*Δ strain (black), the parental strain and the complementation strains were measured after 3, 5 and 7 days. Growth is reduced in the deletion strain in comparison to the *nkuA*Δ strain and the complementation strain. The knockout of *mphA* shows a defect in the development of ascospores. D) The production of dark reddish ascospores were observed in the *nkuA*Δ strain, in the knockout strain *mphA*Δ; *nkuA*Δ and in the complementation strain *mphA*Δ*::mphA::gfp::nkuA*Δ on day 5 and 14 after germination. Most of the cleistothecia of the knockout strain contained no observable ascospores on day 5 after germination. Instead of dark reddish ascospores only non-pigmented spores were observable. In contrast thereto dark reddish ascospores were observable in the strains *nkuA*Δ and the complementation strain *mphA*Δ*::mphA::gfp::nkuA*Δ. On day 14 all three strains were able to produce dark reddish ascospores. Scale bar is 50 µm.

### 3.4.2.3. MphA protein is localized to the envelope of Hülle cells

The localization of MphA::GFP was studied in Hülle cells and in a vegetative mycelium. Therefore, the complementation strain *mphA*Δ*::mphA::gfp;nkuA*Δ (AGB1079) was used. Hülle cells were enriched from sexual mycelium using the cleistothecia-rolling technique and the localization of MphA::GFP was mainly observable in the envelope of Hülle cells as shown in Figure 44A. MphA::GFP was also observable in the center of the Hülle cells. The parental strain *nkuA*Δ (AGB552) without GFP and a strain expressing GFP constitutively (AGB596) were used as controls. Only autofluorescence could be detected in the *nkuA*Δ strain. The strain expressing GFP constitutively revealed that GFP is mainly visible in the cytoplasm of Hülle cells. Therefore, GFP was mainly observable in the center of Hülle cells and was unrepresentative in the envelope of Hülle cells. In a *laeA*Δ; *mphA*::*gfp*; *nkuA*Δ (AGB1081) strain almost no Hülle cells were observed. The remaining Hülle cells of a *laeA*Δ strain displayed almost no MphA::GFP. In the remaining hyphae MphA::GFP was much stronger detectable.

In order to observe MphA::GFP in vegetative hyphae the strains were grown in submerged liquid conditions for 20 hours and only autofluorescence could be detected in the *mphA*Δ*::mphA::gfp;nkuA*Δ and the *laeA*Δ; *mphA*::*gfp*; *nkuA*Δ strains (Figure 44B). This result was comparable to the observation of a *nkuA*Δ strain where only autofluorescence was detectable. The strain expressing GFP constitutively revealed that GFP is mainly visible in the cytoplasm of vegetative hyphae grown for 20 hours. These results show that MphA is most likely enriched in Hülle cells and is located mainly in the envelope of Hülle cells. Growing the complementation strain *mphA*Δ*::mphA::gfp;nkuA*Δ showed that MphA::GFP is not detectable in a vegetative mycelium grown within submerged cultures for 20 hours.

The sequence of MphA contains 540 amino acids with a predicted molecular mass of 60.3 kDa. Western hybridization experiments were performed without success since MphA::GFP, with an expected molecular mass around 87 kDa, was not detectable. This finding reveals that MphA is most probably enriched in sexually differentiated cells such as Hülle cells.

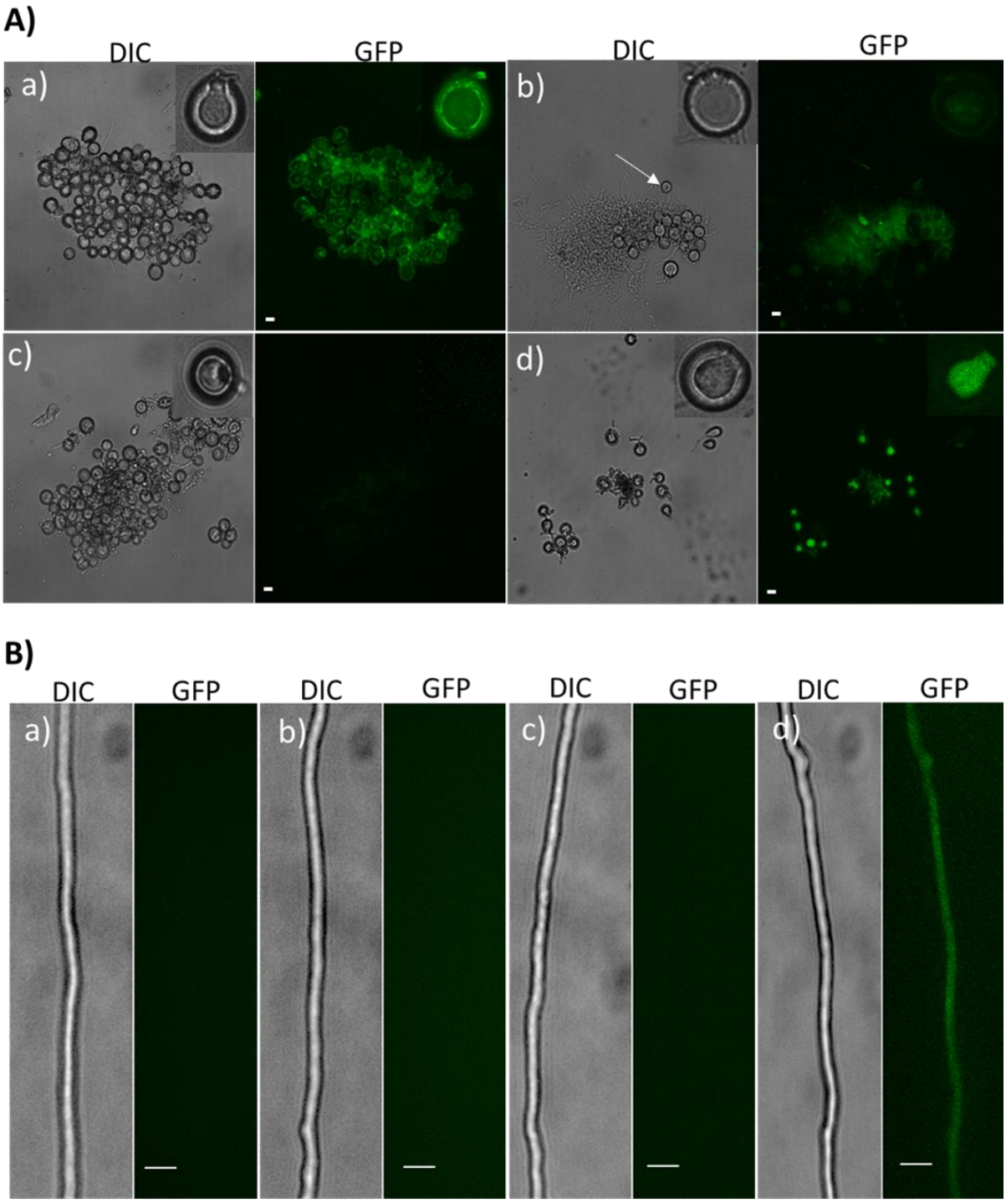



**Figure 44. Localization of the maltose permease-like protein MphA.**
A) Hülle cells were observed: a) The complementation strain (*mphA*Δ*::mphA::gfp; nkuA*Δ, AGB1078). MphA::GFP is localized mainly to the envelope of Hülle cells. MphA::GFP was also detected inside of Hülle cells. b) *laeA*Δ::*mphA*::*gfp*;*nkuA*Δ (AGB1081), where MphA::GFP was not clearly detectable in hyphae (white arrow points to one Hülle cell). c) *nkuA*Δ (AGB552) strain without GFP where mainly autofluorescence was detected. d) A strain expressing GFP constitutively (AGB596) where GFP was mainly detectable in the cytoplasm of Hülle cells. Scale bar is 10 µm. B) Vegetative mycelium was observed: a) In the complementation strain (*mphA*Δ*::mphA::gfp;nkuA*Δ), MphA::GFP was not detectable. b) In the strain *laeA*Δ::*mphA*::*gfp*;*nkuA*Δ, no MphA::GFP was detectable. c) *nkuA*Δ strain where mainly autofluorescence was detected in the vegetative hypha d) A strain expressing GFP constitutively where GFP was mainly detectable in the cytoplasm of the vegetative hypha. Scale bar is 20 µm.

### 3.4.2.4. The deletion of *mphA* prevents mycelia differentiation at higher concentration of carbohydrates in an early developmental time point

In order to investigate the influence of different carbohydrates on the phenotype of the deletion of *mphA,* different disaccharides and monosaccharide with different concentrations were added to the media. The *nkuA*Δ strain and the deletion strain *mphA*Δ; *nkuA*Δ plus the complementation strain were inoculated on solid agar plates containing 1% or 5% of different carbohydrates. Three different carbohydrate sources were used and the influence of the different carbohydrates were observed three days after germination. The phenotype of the *mphA* deletion strain changed significantly in the case of growing the fungus on a medium containing 5% of different carbon sources such as disaccharides or monosaccharide. Hülle cells and primordia could not be observed growing the strain *mphA*Δfor three days on 5% maltose (Figure 45A).

The addition of 5% maltose to the medium influenced the development of the deletion strain negatively. Therefore, Hülle cells and conidiophores were significantly reduced and instead the fungus formed undifferentiated aerial hyphae after 3 days. The percentage of observable conidiophores in mycelia was determined (Figure 45B). The addition of 5% glucose or sucrose to the medium had a weaker effect on the phenotype of the *mphA* deletion strain in contrast to the above mentioned 5% maltose. This shows that the deletion of *mphA*Δ responds negatively to high concentrations of different carbohydrates since the transportation of carbohydrates is affected in this strain. Adding 1% maltose resulted in a differentiation of the mycelium, but with reduced development. This was also the case by adding 1% glucose or sucrose to the media. This supports the observation that with higher concentration of maltose and without the transporter MphA the fungus reacts negatively on developing complex structures such as Hülle cells or conidiophores and instead forms an undifferentiated mycelium.

The key point of this thesis is that the central observations demonstrate that sexual differentiation increases monodictyphenone (*mdp*) and xanthone (*xpt*) biosynthetic proteins localized in different sexual cell types. Genetic studies of the deletion strain *mphA*Δ illustrated that MphA protein promotes fungal growth, asexual and sexual development.

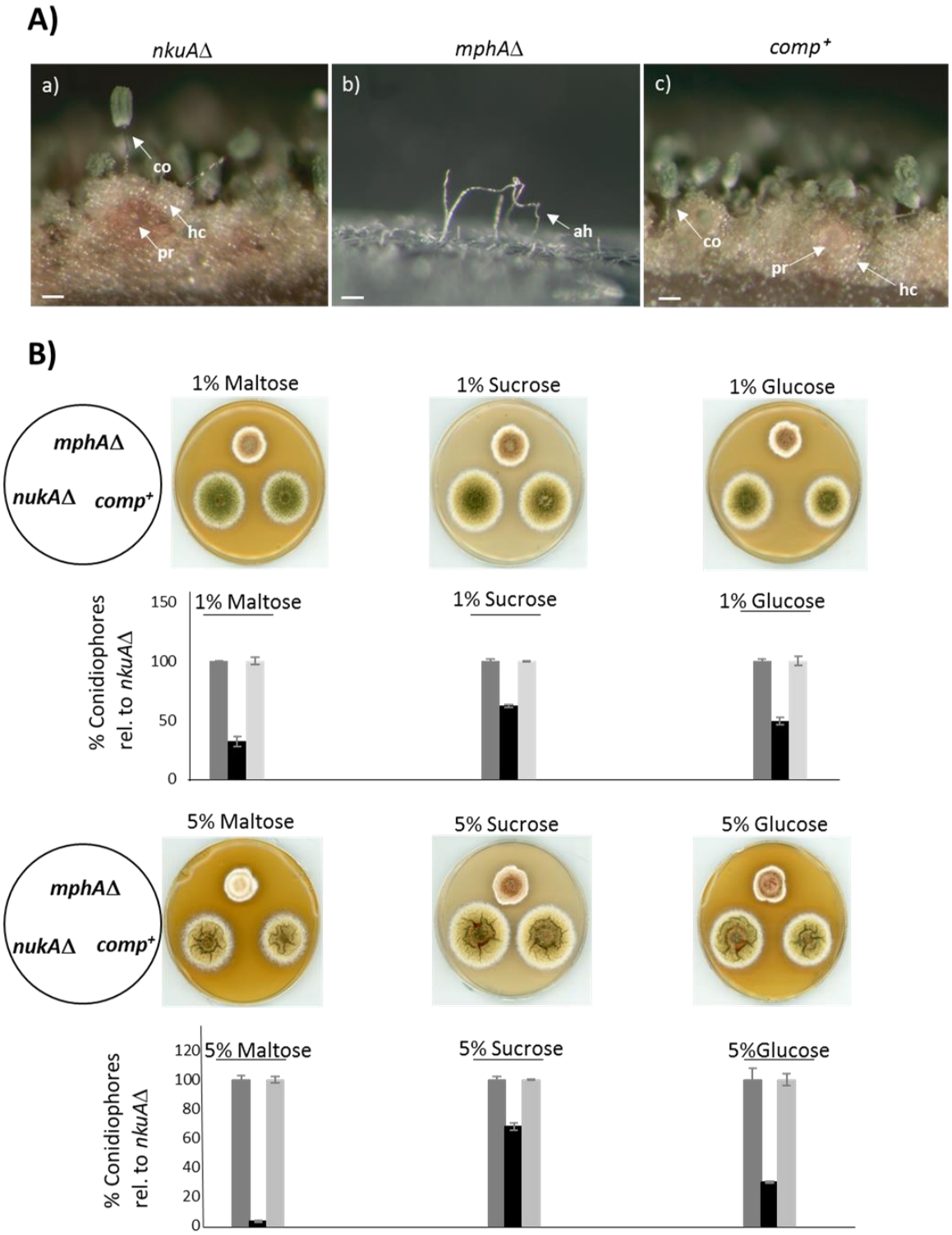



**Figure 45. The maltose permease-like protein MphA prevents mycelia differentiation at higher concentration of carbohydrates.**

A) Cross section of sexual mycelium three days after inoculation. a) The parental strain (*nkuA*Δ, AGB552) b) deletion strain *mphA*Δ (*mphA*Δ; *nkuA*Δ, AGB1077) and c) complementation strain *comp*$^+$(*mphA*Δ*::mphA*; *nkuA*Δ, AGB1080) were inoculated on solid agar plates containing 5% maltose. In the case of the *nkuA*Δ (AGB552) and the complementation strain (AGB1080) sexual (pr: primordium, hc: Hülle cells) and asexual structures (co: conidiophore) were observable. Cleistothecia are surrounded by hundreds of Hülle cells. In the case of *mphA*Δ; *nkuA*Δ (AGB1077) mainly aerial hyphae (ah: aerial hyphae) were observed. Scale bar is 50 µm. B) The percentage of observable conidiophores relative to the parental strain *nkuA*Δ was determined and was significantly reduced in the *mphA*Δ (AGB1077) strain. Three different strains are shown: *nkuA*Δ (AGB552), *mphA*Δ (*mphA*Δ; *nkuA*Δ, AGB1077) and *comp*$^+$(*mphA*Δ*::mphA*; *nkuA*Δ, AGB1080).

# 4. Discussion

Fungal mutant strains, which are unable to produce Hüll cells, form only small cleistothecia. This suggests that Hülle cells might have a nursing function for the cleistothecia (Sarikaya-Bayram et al., 2010, Braus et al., 2002). This thesis aimed to define specific proteins associated to sexual fungal tissue with a specific emphasis on Hülle cells. Comparative proteomic was used to determine proteins from Hülle cell fractions enriched from surface and submerged liquid cultures. Genetic methods were accomplished to investigate the function of the corresponding genes of the proteins that were identified in the comparative proteomic approach. The major finding of this thesis is that sexual differentiation increases monodictyphenone (*mdp*) and xanthone (*xpt*) biosynthetic proteins localized in different sexual cell types. Besides a maltose permease-like protein was identified in Hülle cells that supports fungal growth and development.

## 4.1. Hülle cells and sexual tissue grown on solid agar plates revealed the presence of proteins for the synthesis of an antimicrobial substance

One of the main findings of this thesis is that biosynthetic proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters are enriched in Hülle cells and sexual tissue, in comparison to other fungal tissue types (Figure 46). Bayram and co-workers showed that the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters are specially expressed during sexual development (Bayram et al., 2016). This correlates with the findings of this thesis since expectedly during sexual differentiation biosynthetic proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters are present. These proteins are diminished in other fungal tissues. Furthermore, the present study shows that these proteins are localized in Hülle cells during sexual development. It seems that biosynthetic proteins for the synthesis of monodictyphenone and xanthones are most probably present all over the sexual mycelium, especially within enriched Hülle cells.

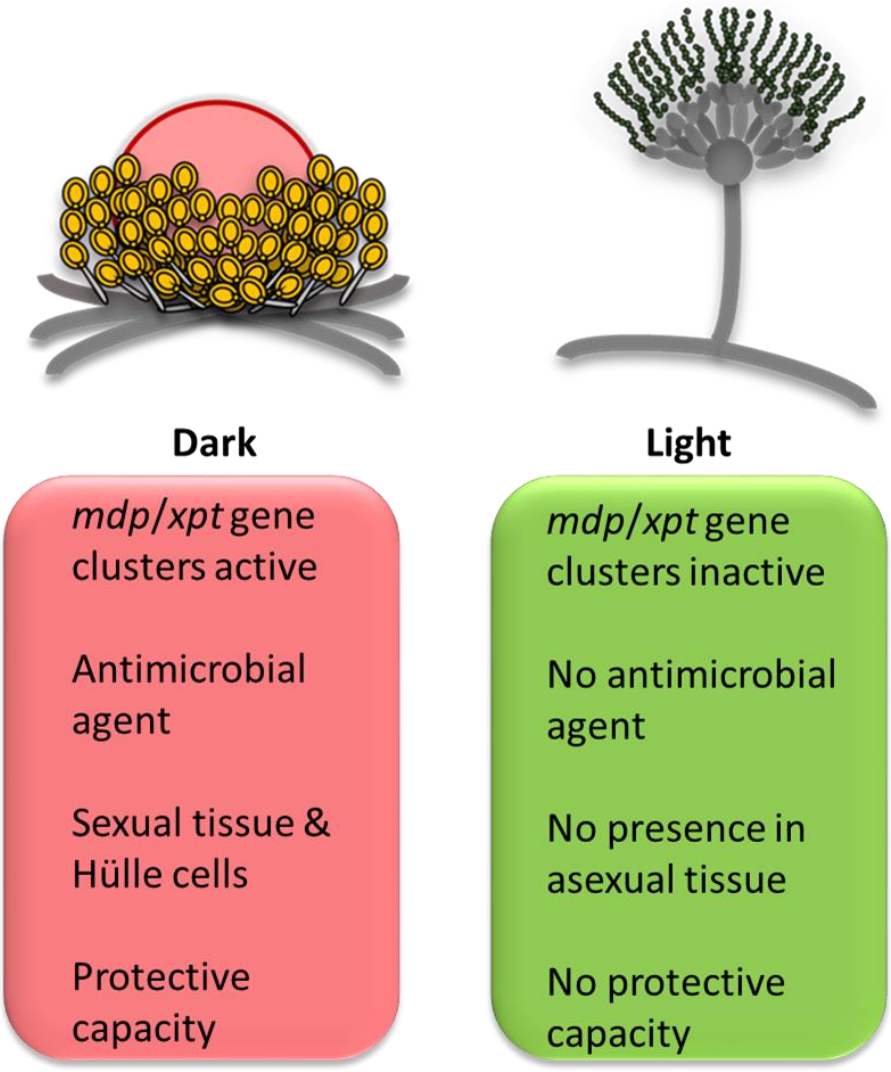



**Figure 46. The monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters are active in sexual tissue including Hülle cells from surface growth.**
Biosynthetic proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters are enriched during sexual development. Monodictyphenone and xanthones are antimicrobial agents. The presence of monodictyphenone and xanthones lead to the assumption that the cleistothecium is protected against various harsh environmental situations like fungivory. In asexual tissue the monodictyphene (*mdp*) / xanthone (*xpt*) gene clusters are inactive. The compounds of monodictyphenone and xanthones are consequently not present. It seems to be that monodictyphenone and xanthones are not protecting the conidiophores (Bayram et al., 2016).

This suggests that the synthesis of an antimicrobial substance is crucial during maturation of the cleistothecia. This finding aligns to the insight that the cleistothecia of *Aspergillus nidulans* covered with Hülle cells are more resistant against fungivory when comparing to a vegetative or asexual tissue (Döll et al., 2013). Furthermore, it was shown that monodictyphenone reduces the egg laying activity of *Drosophila melanogaster* on an *A. nidulans* mycelium (Regulin and Kempken 2018). Both findings validate the assumption that Hülle cells have a protective capacity.

Limitations to the findings are that not all biosynthetic proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters were present in sexual tissue and Hülle cells. This also correlates to other studies, because not all genes of the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters were expressed (Bayram et al., 2016). The monodictyphenone (*mdp*) gene cluster is located at the silent telomeric region of chromosome VIII. This could lead to the assumption that the expression of the monodictyphenone (*mdp*) gene cluster is generally reduced under laboratory conditions. Biosynthetic proteins encoded by the monodictyphenone (*mdp*) gene cluster are required for the production of the precursor monodictyphenone. This compound is converted into prenyl xanthones (Sanchez et al., 2011).

Interestingly genes for the production of xanthones are not embedded in the monodictyphenone (*mdp*) gene cluster. Biosynthetic proteins encoded by both gene clusters are present in sexual mycelium and Hülle cells. This suggests that both gene clusters collaborate, further correlating with other insights. Sanchez and co-workers showed that these two gene clusters collaborate for the synthesis of xanthones (Sanchez et al., 2011).

The findings of this thesis indicate that Hülle cells contain biosynthetic proteins for the synthesis of antimicrobial substances such as xanthones. This leads to the assumption that Hülle cells have a protective capacity during the maturation of cleistothecia.

## 4.2. Submerged liquid cultures revealed the presence of the prenyltransferase NptA and the serine/threonine kinase RfeA in Hülle cells and in other fungal tissues

Proteins from liquid cultures were analyzed and quantified. A *laeA*Δ strain with abolished Hülle cells was used for this analysis. The result was compared to the parental strain which produces Hülle cells (Figure 47). Biosynthetic proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene cluster were present. Besides of that the prenyltransferase NptA and the serine/threonine kinase RfeA which are involved in the process of secondary metabolism are localized to Hülle cells and other fungal tissues.

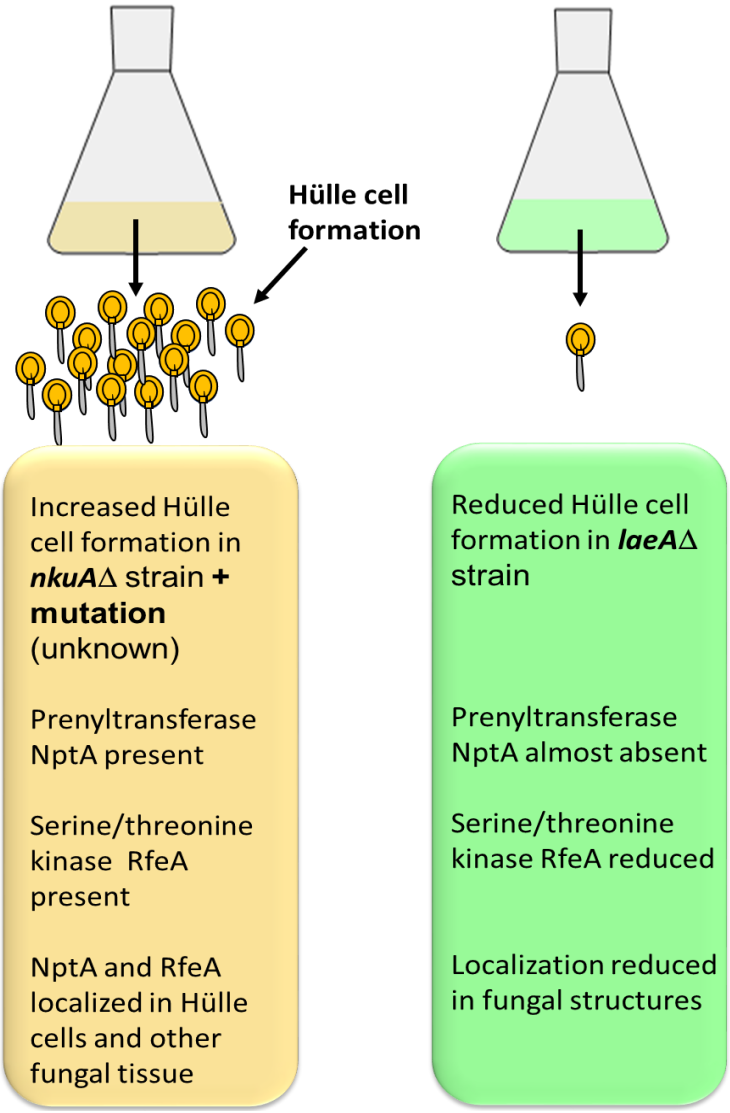



**Figure 47. Hülle cell formation in submerged liquid cultures and comparison of two strains causing different results.**
In a *nkuA*Δ parental strain with an unknown mutation increased Hülle cell formation is observable whereas in a *laeA*Δ strain reduced Hülle cell formation occurs. Both strains are shown under submerged liquid conditions. The protein amount of the prenyltransferase NptA and the serine/threonine kinase RfeA is significantly reduced in *laeA*Δ strain in comparison to the parental strain. The protein quantity of NptA is almost not detectable in a *laeA*Δ strain. NptA and RfeA are localized in Hülle cells and other fungal tissues. The localization of these proteins is reduced in a *laeA*Δ mycelium in different fungal structures.

Alves and co-workers showed that after the addition of the nutrient choline Hülle cell formation occurred in submerged liquid cultures and the compound monodictyphenone was present (Alves et al., 2016). This suggests that a vegetative mycelium forming Hülle cells in submerged liquid cultures has the potential to produce an antimicrobial agent. This aligns to the insights of this thesis since biosynthetic proteins for the production of monodictyphenone and xanthones are observable in submerged liquid cultures.

This could lead to the assumption that Hülle cells, present in these submerged liquid cultures, produce also these proteins. These proteins also appear in hyphal structures. Cleistothecia are normally not formed in submerged liquid cultures. This is why the production of monodictyphenone, that has a protective capacity is noteworthy in submerged liquid cultures. The presence of proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters is unexpected in submerged liquid cultures since the production of monodictyphenone and xanthones are linked to the sexual development of the fungus (Bayram et al., 2016).

In order to analyze proteins from submerged liquid cultures, a *laeA*Δ strain was used. In a *laeA*Δ strain, the formation of Hülle cells is abolished in comparison to a *nkuA*Δ strain with an additional unknown mutation that produce increased numbers of Hülle cells under submerged conditions. As expected, a *laeA*Δ strain correlates with abolished Hülle cells (Sarikaya-Bayram et al., 2010). *A. nidulans* wild-type (FGSC A4 Glasgow, *veA*$^{+}$) usually forms no Hülle cells or cleistothecia in submerged conditions. Prolonging the growth has no effect on the production of Hülle cells (Bayram et al., 2009, Bayram et al., 2008a). Unexpectedly, a *nkuA*Δ (*pabaA1;yA;nkuA*Δ*::argB,* AGB552) parental strain that contains an additional unknown mutation produces enlarged numbers of Hülle cells in submerged liquid cultures. Bayram and co-workers showed that after deleting the *cryA* gene that encodes a cryptochrome/photolyase Hülle cell formation occurred in submerged liquid cultures. Furthermore, it was shown that CryA has a function in DNA-repair (Bayram et al., 2008a). The gene *nkuA* encodes an ATP-dependent DNA helicase II that is involved in the repair process of double-strand breaks in DNA (Nayak et al., 2006). DNA repair activities possibly have an influence on the formation of Hülle cells in submerged liquid conditions. A variety of possible mutations in the *nkuA*Δ (AGB552) strain could most probably cause the production of Hülle cell formation in submerged liquid cultures. This mutation could be caused by single-nucleotide polymorphism (SNP) and this leads to a diverse possibility of mutations that could cause Hülle cell formation in submerged cultures.

Bok and co-workers showed that a prenyltransferase *tidB* was not expressed in a *laeA*Δ mycelium in comparison to the wild-type (Bok et al., 2006). The same applies to the insight of this study regarding the prenyltransferase NptA, since the protein quantity of NptA is significantly reduced in *laeA*Δ.

This is consensual and expected since it is known that LaeA regulates more than 50% of all secondary metabolite gene clusters in various fungi (Sarikaya-Bayram et al., 2010, Dhingra et al., 2013, Zhao et al., 2017). Andersen, Oakley and co-workers predicted that the prenyltransferase NptA is involved in the prenylation of nidulanin A (Andersen et al., 2013, Oakley et al., 2017). The addition of stable isotope labeled amino acids to *A. nidulans* cultures and the incorporation of these labels into nidulanin A, revealed that this compound is a tetracyclopeptide consisting of four different amino acids (Klitgaard et al., 2015).

The finding of this thesis is that the prenyltransferase NptA is localized in the cytoplasm of Hülle cells and other fungal tissues. Therefore, the prenylation of a cyclopeptide such as nidulanin A might also occur in Hülle cells. Besides of NptA which is reduced in a *laeA*Δ strain additional proteins such as tRNA ligases are also reduced. It was shown that cyclopeptides influence the production of chlamydospores in *Candida albicans* (Spraker et al., 2016). This supports the assumption that cyclopeptides may have an influence on Hülle cells.

A serine/threonine kinase RfeA is not yet characterized in detail and *in silico* analyses showed that it is a protein kinase with a predicted role in secondary metabolic regulation (Mogensen et al., 2006). Deletion of *rfeA* in *A. nidulans* had no effects on fungal development (De Souza et al., 2013). Findings of this thesis revealed that the serine/threonine kinase RfeA is localized in different fungal tissues such as Hülle cells and is reduced in a *laeA*Δ mycelium. This shows that beside LaeA as a key regulator in secondary metabolism RfeA might also be involved in the process of secondary metabolism. The amino acid sequence identity shared between RfeA and their *Homo sapiens* homologs is 32% to Chk1 (Stajich et al., 2012). The serine/threonine-specific protein kinase Chk1 encoded by the *chek1* gene coordinates DNA damage response (Kabeche et al., 2018). Again, possible DNA repair activities of RfeA could have an influence on Hülle cells. The finding of this thesis indicates that increased Hülle cell formation in submerged cultures is observable in a *nkuA*Δ parental strain together with an additional mutation. This is in contrast to a *laeA*Δ strain that causes a significant reduction in Hülle cell formation. Moreover, NptA and RfeA are reduced in a *laeA*Δ strain.

## 4.3. Hülle cells from surface and liquid cultures comprise shared proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters

In order to perform a comparative proteomic approach cultures derived from solid agar plates and submerged liquid media were analyzed. Hülle cells from solid agar plates and submerged liquid cultures were enriched and proteins found in both types of cultures were analyzed. In order to perform the investigation in submerged liquid cultures a *nkuA*Δ strain with an additional unknown mutation which forms increased numbers of Hülle cells in submerged liquid cultures was compared to a *laeA*Δ strain which under these conditions forms less Hülle cells. The comparison of Hülle cells from surface and liquid cultures shows that both cultures comprise shared proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters (Figure 48). The ankyrin repeat domain protein AnkG (AN8434) and a the tyrosinase domain protein (AN8435) are also commonly observable in both cultures. The maltose permease-like protein MphA is observable in enriched Hülle cells from surface cultures. In liquid cultures MphA seems not to be clearly present.

Findings of this thesis showed that different approaches to enrich Hülle cells led to different results. In order to increase the enrichment of Hülle cells from solid agar plate cultures the cleistothecia-rolling technique was applied. Enriched Hülle cell fractions from surface agar plate compared to a submerged liquid culture represent a small amount of starting material. In comparison homogenous material in a submerged liquid culture represents optimal conditions for proteomics. This might be the reason why only 401 solid agar plate culture proteins were identified from enriched Hülle cell fractions. From vegetative mycelia 974 proteins could be identified and differed in more than 50% compared to the 401 proteins mentioned above.

Limitation in quantification of protein abundances within the SILAC approach need to be considered. The use of only lysine auxotropic parental strains instead of lysine in combination with arginine auxotrophic parental strains limited the quantification of a complete pattern of fungal proteins. Peptides that contain arginine residues were therefore not considered for the quantification. Peptides that only contain isotopically labeled L-lysine reduce the quantification of protein abundances in complex mixtures.

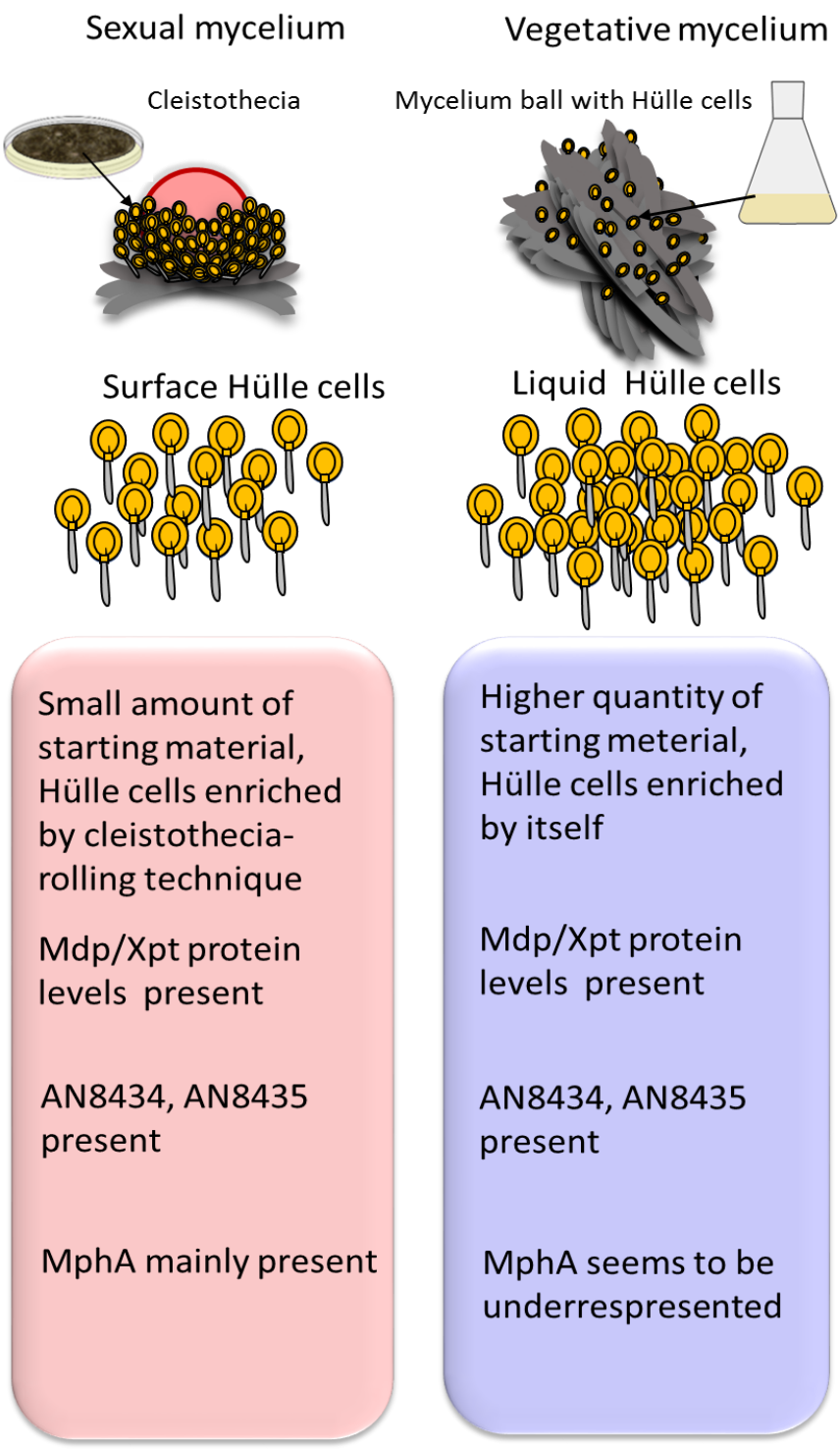



**Figure 48. The comparison of the proteome of both types of Hülle cells.**
The picture shows Hülle cells that are situated in a mycelium grown on surface agar plates or in submerged liquid cultures. On the left-hand side hundreds of Hülle cells surround the cleistothecium grown on solid agar plate and are enriched by the cleistothecia-rolling technique. On the-right hand side Hülle cells are shown in a mycelium ball of a submerged liquid culture. This culture contains thousands of small vegetative mycelium balls with Hülle cells. After 72 hours the undifferentiated vegetative mycelium is capable to produce Hülle cells. These Hülle cells are rather randomly distributed and are not in contact with the cleistothecia since they are not produced in liquid cultures. Hülle cell enrichment is enhanced by itself. Hülle cells contain increased monodictyphenone (*mdp*) / xanthone (*xpt*) biosynthetic protein levels. The ankyrin domain protein (AN8434) and the tyrosinase domain protein (AN8435) are found in both types of Hülle cells. The maltose permease-like protein MphA (AN2601) seems to be mainly present in Hülle cells grown on solid agar plates.

Besides of technical issues, thereare several biological reasons why the different approaches led to different outcomes. Hülle cells in vegetative mycelia in submerged liquid cultures are rather randomly distributed throughout the mycelia ball. In comparison Hülle cells are associated with the cleistothecia on solid agar plate cultures. The development of cleistothecia on surface cultures leads to differences in the composition of the proteome. Formation of cleistothecia on surface cultures most likey requires different carbohydrate transporters (Wei et al., 2004). This could be the reason why MphA is not clearly represented in submerged liquid cultures since cleistothecia are not usually formed under these conditions.

Interestingly biosynthetic proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters are present in both cultures. During initial vegetative growth phase, the fungus produces less secondary metabolites (Calvo et al., 2002). The production of secondary metabolites is specifically linked to the distinct developmental programs of the fungus (Bayram et al., 2016). Martins and co-workers showed in a proteomic study that after the addition of the nutrient choline, Hülle cell formation occurred in submerged liquid cultures and proteins encoded by secondary metabolite clusters were found (Martins et al., 2013). It is interesting that in a vegetative growth where Hülle cell formation occurred biosynthetic proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters are observable. It seems that the formation of Hülle cells in submerged liquid cultures goes along with the production of proteins which are required for biosynthesis of monodictyphenone and xanthones which are antimicrobial substances.

Different outcome from cultures on solid agar plate and under liquid conditions shows that biosynthetic proteins of the monodictyphenone (*mdp*) / xanthone (*xpt*) clusters are present in both cultures. Only the MphA protein does not seem to be clearly represented in submerged liquid cultures. This could lead to the assumption that MphA has a specific function during surface growth. One of these functions could be the nursing of cleistothecia.

## 4.4. Hülle cells from surface growth and liquid media comprise an ankyrin and a tyrosinase protein

The AnkG (AN8434) ankyrin repeat domain protein and the tyrosinase domain protein (AN8435) were found in both approaches. The domain architecture of the AnkG (AN8434) ankyrin repeat domain protein and the tyrosinase domain protein (AN8435) is shown in Figure 49.

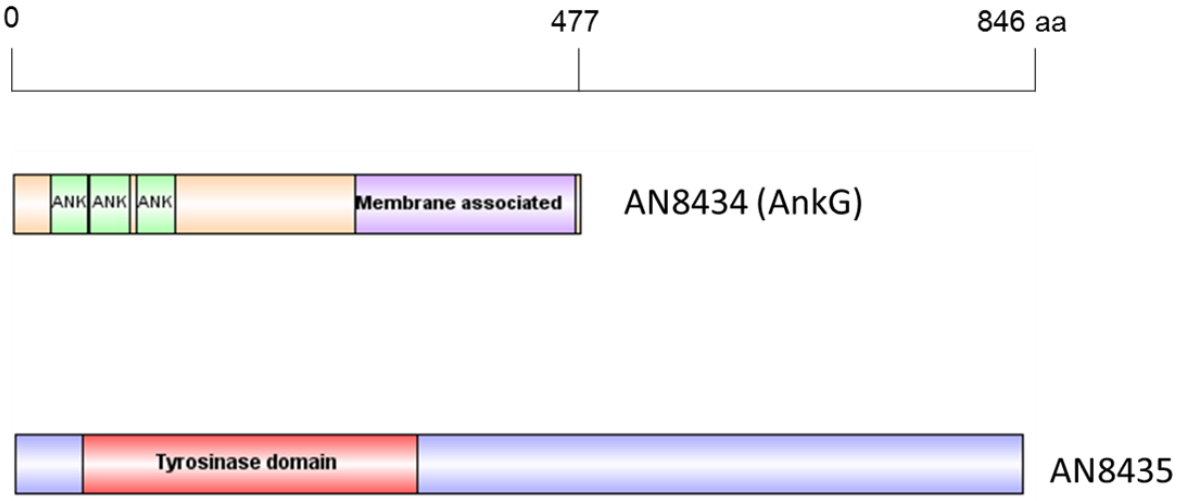



**Figure 49. Domain architecture of the AnkG (AN8434) ankyrin repeat domain protein and the tyrosinase domain protein (AN8435).**
The sequence of the AnkG ankyrin repeat domain protein of *A. nidulans* comprises 477 amino acids contains three ankyrin repeat domains and seems to have a membrane associated region (with a von Willebrand factor typ A-like domain). The sequence or the tyrosinase domain protein (AN8435) comprises 846 amino acids and a tyrosinase domain (Jones et al., 2014).

The present thesis sheds light on the localization of the ankyrin repeat domain protein AnkG to the membrane of the subtending hyphae of Hülle cells. This aligns to the findings that ankyrin proteins are localized to membranes (Islam et al., 2018). Interestingly AnkG was underrepresented in the membrane of vegetative hyphae and therefore AnkG might play a role in the outer membrane of the subtending hyphae of Hülle cells. During initial growth AnkG is most likely situated also in the endoplasmic reticulum (ER) of vegetative hyphae. AnkG is most probably transported by vesicle mediated transportation to the membrane of the subtending hyphae of Hülle cells (Figure 50). During vegetative growth the fusion protein AnkG::GFP is probably situated in the ER as well as in the cytoplasm shown in Figure 37 E). The localization of AnkG::GFP in subtending hyphae is shown in Figure 37 B).

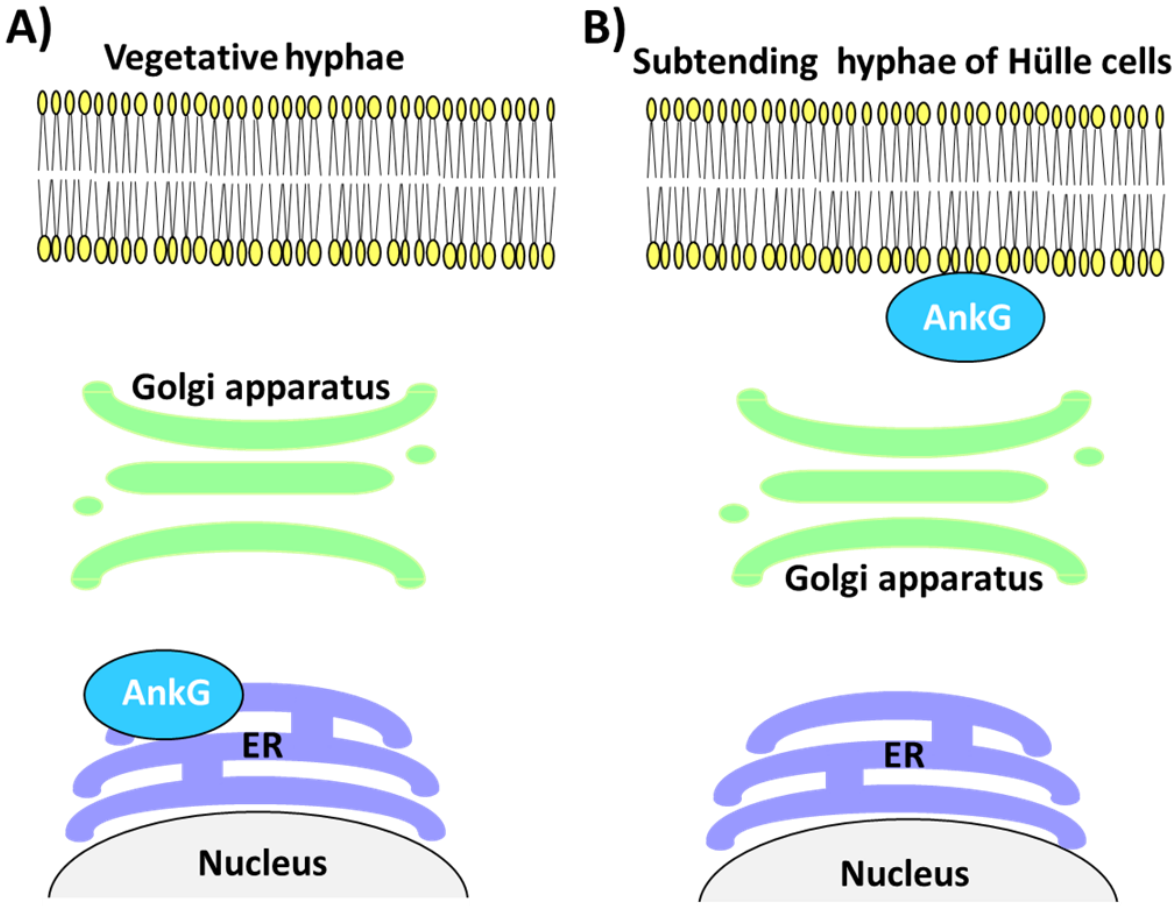



**Figure 50. Protein trafficking of the AnkG ankyrin domain protein**.
A) During initial vegetative growth AnkG is underrepresented in the membrane. It seems that AnkG is localized in the endoplasmic reticulum (ER) as well as in the cytoplasm during initial vegetative growth. B) Subsequently AnkG is transported to the subtending hyphae of Hülle cells.

Ankyrin repeat domain proteins are involved in the process of trafficking tyrosinase into melanocytes (Tamura et al., 2011). Melanocytes are cells which produce melanin for pigmentation. Non-pigmented cleistothecia such as those in *Aspergillus tonophilus* or *Aspergillus fisheri* are accompanied with the lack of Hülle cell formation (Hermann, et al., 1983). This goes along with the absence of the laccase II CpeA that is localized in Hülle cells (Scherer et al., 2002). Tyrosinase and laccase are known to be involved in the regulation and production of melanin pigmentation (Chai et al., 2017, Hsu et al., 2016). This supports the finding that Hülle cells have an auxiliary function in pigmentation during cleistothecia maturation. The possible localization of the tyrosinase domain protein AN8435 in Hülle cells supports the finding that Hülle cells have an auxiliary function in pigmentation of the cleistothecia. This aligns to the finding that in different *Aspergilli* without Hülle cells the cleistothecia are not pigmented.

Tyrosinases are known to undergo a complex post-translational processing before reaching melanocytes. This process includes N-glycosylation in several binding sites, movement from the endoplasmic reticulum (ER) to the golgi apparatus and copper binding (Olivares et al., 2003). As shown in Figure 39 and 40 fluorescence microscope investigation in combination with western hybridization suggest that the tyrosinase domain protein AN8435 found in different cellular compartments within fungal structures like Hülle cells is modified by a post-translational process. This corresponds to the fact that tyrosinase domains are post-translationally modified and are found in different cellular compartments (Olivares et al., 2003). The tyrosinase protein AN8435 seems to be unstable, which limited the observation of a precise localization.

Bayram and co-workers showed that the transcripts of *AN8435* and *AN8434* (*ankG*) are expressed in asexual and sexual mycelia (Bayram et al., 2016). This suggests that AN8434 (AnkG) and AN8435 are found in different mycelia types. This aligns to the findings of this thesis since AN8435 and AN8434 (AnkG) is present in different mycelia types including sexual and vegetative mycelia as well as Hülle cells.

The tyrosinase domain protein (AN8435) and the AnkG (AN8434) ankyrin repeat domain protein are encoded by neighbouring genes. This could be interpreted as a co-regulation of these proteins. Bayram and co-workers also suggested that the transcripts *AN8435* and *AN8434* (*ankG*) are expressed by a gene cluster which is involved in the production of an unknown pigment (Bayram et al., 2016). This supports the assumption that the proteins are co-regulated.

## 4.5. A maltose permease-like protein enriched in surface Hülle cells supports fungal growth and development

A so far unknown maltose permease-like protein MphA was identified and investigated. The corresponding *mphA* gene was deleted in this study. The presence of the MphA maltose permease-like protein in Hülle cells mainly grown on solid agar plates supports the insight that Hülle cells have a function in nutrient supply during fungal growth (Wei et al., 2001). MphA protein was studied in this thesis and the localization of the protein was observed in Hülle cells. The studies revealed that MphA most probably is a membrane protein with difficulties in respect of solubilization. Western hybridization was performed but without success. MphA contains a distinctive sugar motif and comprises high sequence similarities to other carbohydrate transporters (Horák 1997). This suggests that MphA might be active in the transportation of carbohydrates (Figure 51). Since MphA supports growth in general, vegetative, sexual and asexual growth, it is assumed that MphA could also function in other cell types beside Hülle cells. MphA is not essential for Hülle cell formation when Hülle cells are formed on solid agar plates. Adding a high concentration of carbohydrates reduces the formation of early Hülle cells in a *mphA*Δ strain. This is accompanied by a reduced maturation of the cleistothecia and the development of ascospores. Additionally, the development of conidiophores is stalled.

Several nutrient transporters were identified in Hülle cells. Fungal development especially the maturation of the cleistothecia was not effected by deleting the putative hexose *hxtA* and the putative purine transporters *uapA* or *azgA* (Wei et al., 2004, Dos Reis et al., 2017, Pantazopoulou et al., 2007). This suggests that additional nutrient transporters are needed during maturation of the cleistothecia. Cleistothecia formation requires high amounts of energy in the form of carbohydrate initially stored in vegetative hyphae. This fact indicates an existence of effective transportation systems for nutrient uptake into the cells of developing cleistothecia (Pöggeler et al., 2006). As expected, alternative possible nutrient transporters were observed in Hülle cells in this study.

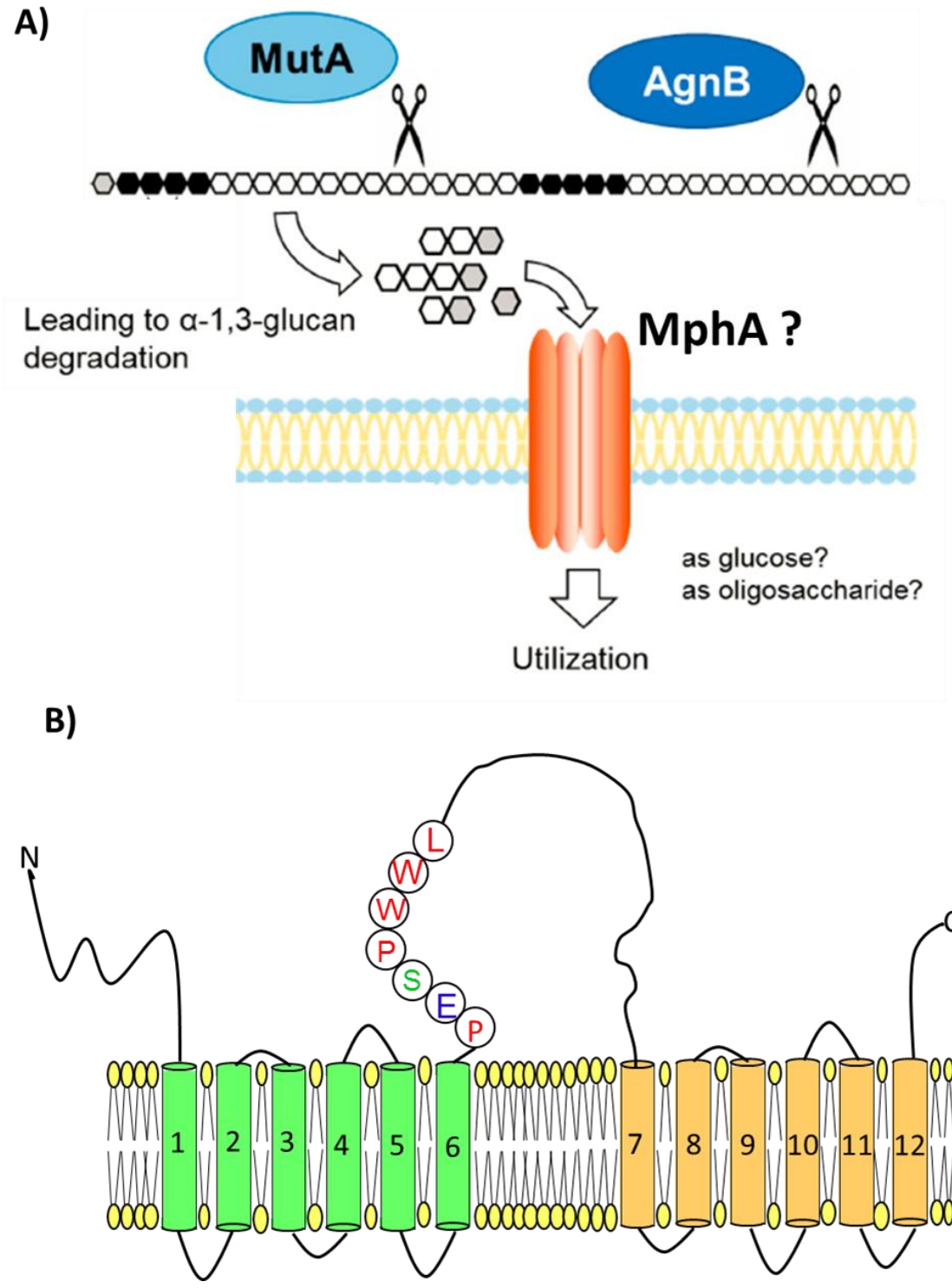



**Figure 51. Model for the maltose permease-like protein MphA in the transportation of α-1,3 glucans in Aspergillus nidulans.**
A) In Hülle cells the α-1,3 glucanases MutA and AgnB are required in the processes of degrading of α-1,3 glucans. MphA might be involved in the transportation of carbohydrates. This could lead to a supporting function during nutrient supply for the growth of the fungus, especially the cleistothecium. Image modified (Yoshimi et al., 2017). B) Model for a possible structure of MphA. The sequence of MphA consists of 540 amino acids and contains twelve transmembrane helices which, in turn consist of two 6-helix bundles connected by a cytoplasmic loop. This loop contains a sequence motif (PESP(W)L) which is specific for all sugar transporters (Horák 1997). MphA might have a central role regarding transportation of carbohydrates.

MphA effects the maturation of cleistothecia and ascospores. Interestingly MphA also influences other developmental processes such as the development of asexual structures like conidiophores. This finding differs to previous studies (Wei et al., 2004, Dos reis et al., 2017, Pantazopoulou et al., 2007). The deletion of the gene *mphA* has an unexpected negative effect on supporting fungal growth, asexual and sexual development. MphA has a central role in supporting fungal structures especially during surface growth. Hülle cells are associated with cleistothecia which need the growth on surfaces. In submerged liquid cultures where cleistothecia are not normally formed, the activity of MphA seems to be reduced.

During vegetative growth $\alpha$-1,3 glucans are stored in the cell wall and $\alpha$-1,3-glucanase like MutA found mainly in Hülle cells during surface growth are required for the degradation of these glucans (Zonneveld 1973, Wei et al., 2001, He et al., 2017, Yoshimi et al., 2017). This finding aligns to the observation of this thesis since $\alpha$-1,3-glucanase MutA is unrepresentative during initial vegetative growth and was found in Hülle cells as well as in sexual mycelium.

Hülle cells are known to nurse the cleistothecia during maturation since abolished Hülle cells correlate with a reduced size of the cleistothecia (Sarikaya-Bayram et al., 2010). The deletion of *mphA* appears to correlate with a stalled development of cleistothecia. The addition of higher concentrations of carbohydrates to the *mphA*$\Delta$ strain effects the development of the fungus negatively. This suggests that MphA could be crucial for the transportation of carbohydrates since the deletion of gene *mphA* causes a significant reduction of the development regarding cleistothecia and other fungal structures. This finding could support the existence of effective transport systems during development (Pöggeler et al., 2006). The development of thick spherical cells called chlamydospores are produced at the very end of the hyphae in *Candida albicans* and are reduced in higher concentration of carbohydrates (Böttcher et al., 2016). This supports the suggestion and aligns to the observation of this thesis that spore-like structures like chlamydospores and Hülle cells are influenced by carbohydrate metabolism.

Taken together, this study shows that sexual differentiation increases monodictyphenone (*mdp*) / xanthone (*xpt*) biosynthetic protein levels localized in differentiated sexual cell types. Hülle cells formed in surface and liquid cultures contain shared proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene

clusters. Proteins encoded by these gene clusters are found in a sexual mycelium with high amounts of Hülle cells grown on surface agar plate cultures. Monodictyphenone and xanthones are antimicrobial substances against fungivory and other environmental threats and thereby protecting the fungus (Regulin and Kempken 2018, Chen et al., 2017, Bok et al., 2009). This suggests that Hülle cells and sexual mycelium are involved in the production of an antimicrobial agent.

Studies of the deletion strain *mphA*Δ demonstrate that MphA protein promotes fungal growth, asexual and sexual development. MphA was identified in enriched Hülle cells from surface growth and was underrepresentend in submerged liquid cultures. This points out that Hülle cells are supporting fungal growth (Figure 52).

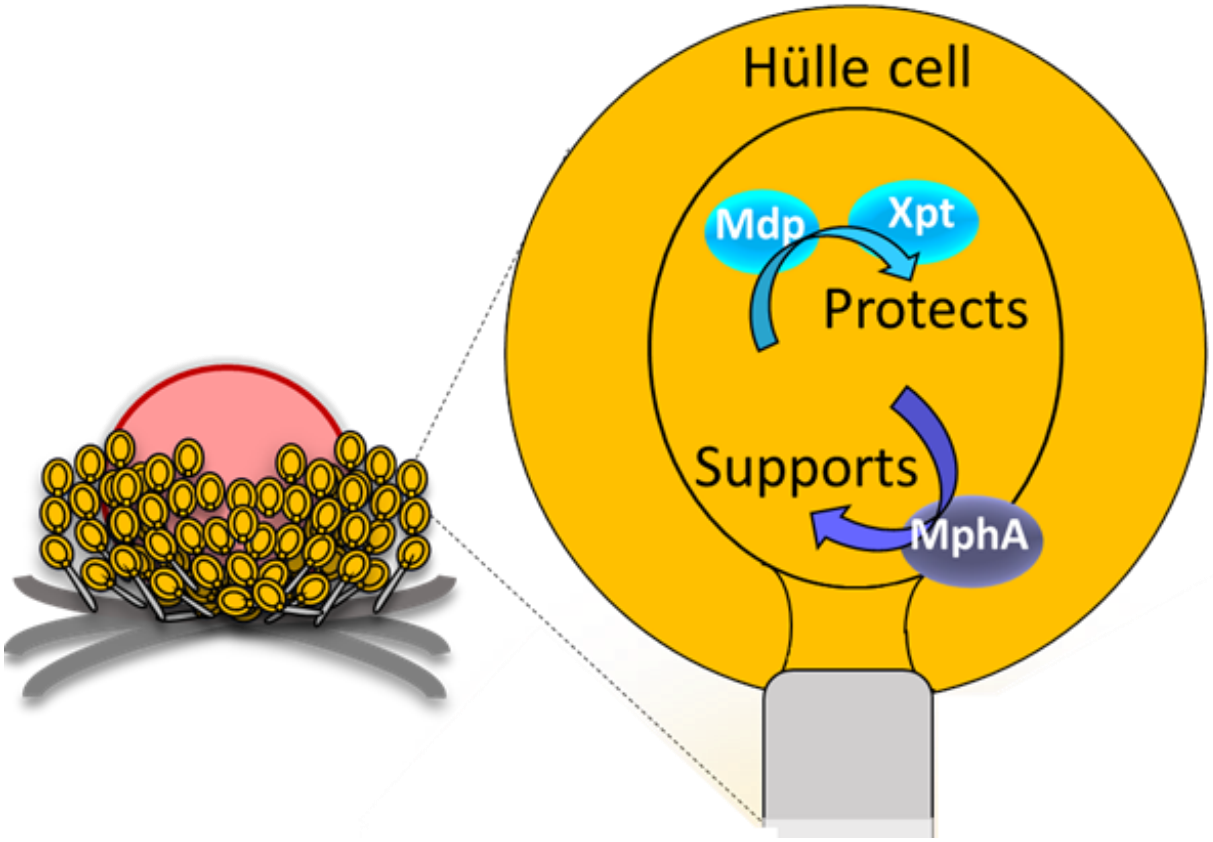



**Figure 52. Hülle cells protect and support the cleistothecia.**
The picture shows left a cleistothecium surrounded by Hülle cells. On the right a single enlarged Hülle cell is shown. Sexual differentiation of *A. nidulans* increases monodictyphenone (*mdp*) / xanthone (*xpt*) biosynthetic protein levels. Enzymes encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters are found in Hülle cells. Monodictyphenone and xanthones are antimicrobial substances. This suggests that the synthesis of an antimicrobial substance is crucial during maturation of the cleistothecia. The MphA maltose permease-like protein was found in Hülle cells. MphA promotes fungal growth and development.

## 4.6. Conclusion

The central findings of this thesis lead to the conclusion that sexual differentiation of *Aspergillus nidulans* increases biosynthetic proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) secondary metabolite gene clusters. These proteins are present in a sexual mycelium with high amounts of Hülle cells from solid agar plate cultures. Proteins encoded by the monodictyphenone (*mdp*) / xanthone (*xpt*) secondary metabolite gene clusters are present in Hülle cells and in other fungal cell types. Bayram and co-workers showed that the transcripts of the monodictyphenone (*mdp*) / xanthone (*xpt*) clusters are specifically expressed during sexual development (Bayram et al., 2016). The presence of the prenyltransferase XptB and the oxidoreductase XptC encoded by the xanthone (*xpt*) secondary metabolite gene cluster was studied in this thesis and their localization in Hülle cells and other fungal tissues could be confirmed. Monodictyphenone and xanthones are antimicrobial agents that protect most probably the fungus especially sexual structures against fungivory and other environmental threats (Regulin and Kempken 2018, Chen et. al., 2017, Bok et al., 2009) The cleistothecia of *A. nidulans* covered with Hülle cells are more resistant against fungivory when comparing to vegetative or asexual tissue (Döll et. al, 2013). In this study it was shown that proteins required for the synthesis of antimicrobial substances such as monodictyphenone and xanthones, are present in Hülle cells. This confirms the assumption that Hülle cells have a capacity to protect the cleistothecia.

Secondly, this thesis shows that protein quantities of prenyltransferase NptA and the serine/threonine kinase RfeA are reduced in a *laeA*Δ strain. These proteins are localized in Hülle cells and in other fungal tissues. For this analysis a parental *nkuA*Δ strain with an additional unknown mutation that leads to increased numbers of Hülle cells was compared to a *laeA*Δ strain that forms reduced numbers of Hülle cells both under submerged liquid conditions. Overall, this thesis provides insights that demonstrate that prenyltransferases NptA and serine/threonine kinase RfeA are localized in different fungal cell types including Hülle cells and the protein quantity is reduced in strains lacking LaeA. This aligns to the findings that other prenyltransferases such as *tidB* are not expressed in a *laeA*Δ strain (Bok et al., 2006). RefA is mainly uncharacterized in *A. nidulans* (De Souza et. al., 2013).

Furthermore, the AnkG (AN8434) ankyrin repeat domain protein and the tyrosine domain protein (AN8435) are found in enriched Hülle cells from solid agar plate as well as in submerged liquid cultures. Ankyrin repeat domain proteins are adaptor proteins associated with membranes (Islam et al., 2017). Additionally, observations in this study aligned to the findings that AnkG (AN8434) ankyrin repeat domain protein is associated to the membrane and furthermore, is localized to the membrane of the subtending hyphae of Hülle cells. Beyond that, in this thesis the localization of tyrosinase domain protein (AN8435) was studied. Probably AN8435 is localized to Hülle cells and other fungal tissues. Tyrosinases are known to be involved in the process of pigmentation (Chai et al., 2017, Hsu et al., 2016). The absence of Hülle cells in *Aspergillus tonophilus* and *Aspergillus fisheri* correlates with non-pigmented cleistothecia (Hermann et al., 1983). This indicates that Hülle cells are potentially important for pigmentation and, therefore, tyrosinases are most probably required during the maturation process of the cleistothecia.

Lastly the findings of this thesis reveal that the MphA protein supports fungal growth and development and that MphA protein is specially enriched in Hülle cells. During the thesis research the corresponding gene of MphA was deleted and observation demonstrated that MphA promotes fungal development and growth. MphA protein supports also the maturation of the cleistothecium. Abolished Hülle cell formation correlates with a significantly reduced size of the cleistothecia (Sarikaya-Bayram et al., 2010). This supports the view that Hülle cells have a nursing function to nourish the growing cleistothecia.

Taken together the biological function of Hülle cells is highly likely to have a protective and a nursing function especially during sexual development. Further research is hence needed to determine the biological function of Hülle cells during sexual development in more detail.

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# Abbreviations

°C…….....celsius

Δ…… deletion

µ…… micro

Σ…… sum

aa…….....amino acid

Blast……..Basic local alignment search tool

bp…….....base pairs

cm…….....centimeter

*comp*$^{+}$ ……...complementation

C-terminus……...carboxy terminus

Da…….....dalton

DIC……..... differential interference contrast

DNA…….....deoxyribonucleic acid

et al.,…….....et alii, and others

e.g……..... exempli gratia, for example

ER……. endoplasmic reticulum

FGSC ……...Fungal Genetics Stock Center

PSM…….....peptide spectral match

g……... gram

gDNA…… genomic DNA

GFP……green fluorescent protein

ID……...identification

LC-MS…….. liquid chromatography–mass spectrometry

l……..liter

$log_2$…… logarithm to the base 2

kDa……..... kilodalton

MS…….....mass spectrometry

ms……. millisecond

min…….....minute(s)

mg……..... milligram

ml…….....milliliter

mm……..... millimeter

mM…… millimolar

M……molar

N-terminus ……... amino-terminus

µg…….. microgram

µl……microliter

µm……. micrometer

PAGE ……...polyacrylamide gelelectrophoresis

PCR…….. polymerase chain reaction

pH……..power of hydrogen

sp…….. spore

RNA…….. ribonucleic acid

rpm…… revolutions per minute

tRNA……..transfer ribonucleic acid

Tris…… tris(hydroxymethyl)aminomethane

UTR…….. untranslated region

UV……. ultraviolet

V…… Volt

v…….volume

v/v……..volume per volume

w…… weight

wt……...wildtype

w/v…….weight per volume

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## Acknowledgements

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# Curriculum vitae

## Private data

Name: Benedict Dirnberger
Data of birth: 22. April 1984
Birthplace: Vienna
Civil status: unmarried

## Academic achievements

02/2014 – ongoing **PhD student / Doctor rerum naturalium in Biochemistry and Microbiology**
Place of education: Georg August University of Goettingen,
Institute: Department for Molecular Microbiology and Genetics
Title of the thesis: Proteomics of *Aspergillus nidulans* sexually differentiated cells
Supervisor: Prof. Dr. Gerhard H. Braus

11/2010 – 01/2013 **Diploma (corresponding to M.Sc.) in Biology, Major in Genetics and Microbiology**
Place of education: University of Vienna
Institute: Department for Molecular Systems Biology
Title of the thesis: A subcellular fractionation that allows the measurement of the proteome and the metabolome
Supervisor: Prof. Dr. Wolfram Weckwerth & Ass. Prof. Dr. Stefanie Wienkoop

10/2004 - 11/2010 **Prediploma in Biology**
Place of education: University of Vienna

## International experience

| | |
|---|---|
| 2/2013- ongoing | University of Goettingen (Ger) |
| 07/2009 - 08/2009 | University of Cambridge (UK) |
| 07/2007 | University of Bristol (UK) |

## Publications in peer reviewed international journals

Büttel, Z., Díaz, R., Dirnberger, B., Flak, M., Grijseels, S., Kwon, M. J., Nielsen, J. C. F., Nygård, Y., Phule, P., Pohl, C., Prigent, S., Randelovic, M., Schütze, T., Troppens, D., & Viggiano. A. 2015. Unlocking the potential of fungi: the QuantFung project. *Fungal Biology and Biotechnology* **2**: 6.

## Presentations in scientific meetings (talks and posters)

**[1] Benedict Dirnberger**, Oliver Valerius, Enikő Fekete-Szücs, Cindy Meister, Ines Teichert, Jennifer Gerke, Danielle Troppens, Gerhard H. Braus **(2017).** A quantitative comparative proteomic approach to investigate Hülle cells from the filamentous fungus *Aspergillus nidulans*. **16th Human Proteome Organization World Congress (HUPO2017), Dublin, Ireland (poster A-016).**

**[2] Benedict Dirnberger**, Oliver Valerius, Enikő Fekete-Szücs, Cindy Meister, Ines Teichert, Jennifer Gerke, Danielle Troppens, Gerhard H. Braus **(2016).** A comparative proteomic approach to investigate secondary metabolite gene cluster expression in various *Aspergillus nidulans* cell types. **13th European Conference on Fungal Genetics, Paris, France, p 567 (poster CS7W41).**

**[3]** Danielle Troppens, **Benedict Dirnberger**, Gerhard Braus **(2016).** Investigating the potential of Hülle cells in *Aspergillus nidulans.* **13th European Conference on Fungal Genetics, Paris, France, p 134 (poster CS1M28).**

**[4] Benedict Dirnberger**, Oliver Valerius, Enikő Fekete-Szücs, Cindy Meister, Ines Teichert, Jennifer Gerke, Danielle Troppens, Gerhard H. Braus **(2016).** A comparative proteomic approach to investigate secondary metabolite gene cluster expression in various *Aspergillus nidulans* cell types. **The Thirteenth International *Aspergillus* Meeting, Paris, France, p 17 (poster 23).**

**[5] Benedict Dirnberger et al., (2015).** Investigation of Hülle cells from the filamentous fungus *Aspergillus nidulans*. **Proteomics Forum 2015, Berlin, Germany, p48 (poster 091).**

**[6]** Danielle Troppens, Özgür Bayram, **Benedict Dirnberger**, Gerhard Braus **(2015).** Investigating the potential of Hülle cells in *Aspergillus nidulans.* **Annual Conference of the Association for General and Applied Microbiology, Marburg, Germany p 218 (poster SMaP07).**

**[7] Benedict Dirnberger et al., (2015).** Investigation of Hülle cells from the filamentous fungus *Aspergillus nidulans.* **Annual Conference 2015 of the Association for General and Applied Microbiology, Marburg, Germany p 228 (poster SCP01).**

**[8] Benedict Dirnberger (2015).** Investigation and quantification of secondary metabolite gene cluster expression in Aspergillus nidulans. **Annual Conference 2015 of the Association for General and Applied Microbiology, Marburg, Germany (oral presentation).**

**[9] Benedict Dirnberger,** Oliver Valerius, Ines Teichert, Holm Frauendorf, Danielle Troppens, Bastian Jöhnk, Gerhard H. Braus **(2014).** Investigation of Hülle cells from the filamentous fungus *Aspergillus nidulans*. **ESF-EMBO Symposium Synthetic biology of antibiotic production II, Sant Feliu de Guixols, Spain, p14 (poster013).**

**[10] Benedict Dirnberger** & Gerhard H. Braus **(2014).** Exploration of molecular switches from filamentous growth to single Hülle cells as tool for synthetic biology. **Proceeding of the 12th European Conference of Fungal Genetics (ECFG), Seville, Spain, p78 (poster 018).**

## Fellowships, scholarships and awards

| | |
|---|---|
| 03/2018 | TOP scholarship foreign countries, Lower Austria, awarded for Doctoral thesis |
| 11/2017 | Travel award, 16th Human Proteome Organization World Congress (HUPO2017) 17.09-21.09.2017, awarded by European proteomics association (EUPA) |
| 08/2017 | Travel award, 16th Human Proteome Organization World Congress (HUPO2017), 17.09-21.09.2017, awarded by German society for proteomics |
| 11/2016 | GGNB scholarship (DFG Grant GSC 226/2), awarded for Doctoral thesis |
| 04/2016 | Award for best poster presentation, The Thirteenth International *Aspergillus* Meeting, Paris, France, p 17 (poster 23), awarded by Novozymes |
| 07/2015 | Travel award (offered), 9th European Summer School – Advanced Proteomics, 02.08-08.08. 2015 Kloster Neustift Brixen, awarded by German society for proteomics |
| 02/2015 | Travel award, Proteomics Forum 2015, 22.03-25.03.2015 Berlin Germany, awarded by Austrian society for proteomics |
| 02/2014 | Marie Skłodowska-Curie Fellowship (ITN), awarded for Doctoral thesis (University of Goettingen) |
| 08/2013 | Leonardo da Vinci scholarship, awarded for research in the Mass Spectrometry – Yeast Group (University of Goettingen) |
| 01/2013 | University of Goettingen scholarship, awarded for research in the Department of Plant Biochemistry |

# Supplements

**Supplementary Table 1:**
Overwiew of the proteome data evalution using Proteome Discoverer 1.4.

| Proteome experiment 1 | |
|---|---|
| No. | Description |
| 1 | **Vegetative mycelium: 20 hours after germination in liquid shake flasks**<br>Three biological replicates |
| 2 | Filter: proteins identified in two or more biological replicates and with more than two peptides per proteins |
| 3 | **Asexual mycelium: 3 days after germination on solid agar plate cultures**<br>Three biological replicates |
| 4 | Filter: proteins identified in two or more biological replicates and with more than two peptides per proteins |
| 5 | **Asexual mycelium: 5 days after germination on solid agar plate cultures**<br>Three biological replicates |
| 6 | Filter: proteins identified in two or more biological replicates and with more than two peptides per proteins |
| 7 | **Asexual mycelium 7 days after germination on solid agar plate cultures**<br>Three biological replicates |
| 8 | Filter: proteins identified in two or more biological replicates and with more than two peptides per proteins |
| 9 | **Sexual mycelium: 3 days after germination on solid agar plate cultures**<br>Three biological replicates |
| 10 | Filter: proteins identified in two or more biological replicates and with more than two peptides per proteins |
| 11 | **Sexual mycelium: 5 days after germination on solid agar plate cultures**<br>Three biological replicates |
| 12 | Filter: proteins identified in two or more biological replicates and with more than two peptides per proteins |
| 13 | **Sexual mycelium: 7 days after germination on solid agar plate cultures**<br>Three biological replicates |
| 14 | Filter: proteins identified in two or more biological replicates and with more than two peptides per proteins |
| 15 | **Hülle cells: 3 days after germination on solid agar plate cultures**<br>Three biological replicates |
| 16 | Filter: proteins identified in two or more biological replicates and with more than two peptides per proteins |
| 17 | **Hülle cells: 5 days after germination on solid agar plate cultures**<br>Three biological replicates |
| 18 | Filter: proteins identified in two or more biological replicates and with more than two peptides per proteins |
| 19 | **Hülle cells: 7 days after germination on solid agar plate cultures**<br>Three biological replicates |
| 20 | Filter: proteins identified in two or more biological replicates and with more than two peptides per proteins |
| 21 | **Insert lists into a Venn diagram** |
| 22 | **Additional steps were performed** |

**Supplementary Table 2:**
Overwiew of the proteome data evalution using Perseus 1.5.0.15.

| Proteome experiment 2 | | |
|---|---|---|
| No. | Command | Description |
| 1 | Generic matrix upload | ProteinGroup.txt normailzed ratio |
| 2.1 | Filter rows based on categorical columns | Remove rows with + in reverse column |
| 2.2 | Filter rows based on categorical columns | Remove rows with + in only identified by site |
| 3 | Select rows manually | Remove rows with contaminants |
| 4 | Transform | Inverse ratio (1/x) when *lysA*Δ; *nkuA*Δ is not in the denominator |
| 5 | Transform | $Log_2$ (X) |
| 6 | Categorical annotation rows | Group biological replicates |
| 7 | Normalization | Substract column median of ratio |
| 8 | Average groups | Calculate median of each group |
| 9 | Sample t-test | One - sample t-test, side both, p value 0.05 |
| 10 | Select rows manually | Selected proteins with t-test significants (+) |
| 11 | Select rows manually | Filter protein ratio for values outside -0.5 to +0.5 |
| 12 | Additional steps and analyses were performed beside using Perseus 1.5.0.15. | |

**Supplementary Table 3.**
The list of the 401 proteins found in enriched Hülle cells. Hülle cells enriched from solid agar plate cultures were analyzed within three different time points. Proteins were sorted according to the criteria of the Venn diagram shown in Figure 24. For each time point three biological replicates were considered. Only proteins identified in two or more biological replicates and with two or more peptides per protein were considered for the analysis. Numbers represent the average of spectral counts from three biological replicates. Spectral counts were obtained from enriched Hülle cells and are listed. Proteins that were common between Hülle cells and different type of mycelia are also listed. Putative function or role proteins are listed under Description. Different sources were used, mainly AspGD (Cerqueira et al., 2014).

| **Found only in Hülle cells (6 proteins)** | | | | |
|---|---|---|---|---|
| | | **Spectral counts, found in Hülle cells** | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN2601 (MphA) | Major facilitator superfamily | | 8 | 8 |
| AN9392 | Major facilitator superfamily | 7 | 4 | |
| AN3407 | Protein with a predicted serine-type peptidase domain | | 25 | 29 |
| AN4695 (HexA) | Woronin body protein | | 8 | 7 |
| AN8434 (AnkG) | Ankyrin repeat domain protein | | 4 | 4 |
| AN8435 | Tyrosinase domain protein | | 2 | 3 |
| **Shared in Hülle cells & sexual mycelium (24 proteins)** | | | | |
| | | **Spectral counts, found in Hülle cells** | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN7349 (MutA) | Alpha-1,3-glucanase, specifically expressed in Hülle cells | 3 | 43 | 28 |
| AN0941 (AgdE) | Alpha-glucosidase activity, predicted role in maltose metabolism | 6 | 32 | 24 |
| AN3790 (AgnB) | Putative alpha-1,3-glucanase | | 18 | |
| AN9443 | Predicted cataytic activity and role in carbohydrate metabolic process | | | 11 |
| AN7345 (AgdC) | Glucosidase activity, involved in degradation of glucans | 6 | 32 | 24 |
| AN11143 (GlaA) | Putative Glucoamylase | | 4 | |
| AN11049 | NmrA-like, predicted secondary metabolite member | | 35 | 49 |
| AN10023 (MdpL) | Member of the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters | 25 | | 42 |
| AN7999 | Oxoreductase, member of the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters | | 8 | 47 |
| AN7998 (XptC) | GMC oxoreductase, member of the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters | 7 | 6 | |
| AN12402 (XptB) | Prenytransferase, member of the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters | 5 | | 8 |
| AN10022 | Member of the monodictyphenone (*mdp*) / xanthone (*xpt*) gene clusters | | 5 | 6 |
| AN0150 (MdpG) | Polyketide synthase, member of the monodictphenone (*mdp*) / xanthone (*xpt*) gene clusters | 4 | 3 | |
| AN7812 (StcN) | Putative versicolorin B synthase | 3 | 4 | |
| AN7641 | Putative copper amine oxidase | 22 | 31 | |
| AN6314 | Oxidoreductase | 5 | 27 | 48 |
| AN8203 | Hydrolase | | 5 | |
| AN1142 | Oxidoreductase | | 4 | |
| AN11897 | Ribonuclease | | 6 | |
| AN5669 | Putative succinyl-CoA:3-ketoacid-coenzyme A transferase | 8 | | 2 |
| AN10977 | Ortholog(s) have extracellular region localization | | 6 | |
| AN6930 | Predicted transaminase activity | | 9 | |
| AN5488 | Protein of unknown function | 2 | 20 | |
| AN1863 | Protein of unknown function | 2 | | 4 |

**Supplementary Table 3.**
Continued.

| Shared in Hülle cells & asexual mycelium (10 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN5324 (DlpA) | Dehydrin-like protein | 9 | 9 | 6 |
| AN5004 | Induced in light | 2 | 30 | 7 |
| AN6856 | Upregulated in *atm*Δ | 5 | 6 | |
| AN5015 (ConJ) | Induced in light | 2 | 8 | 2 |
| AN5971 | Induced in light | 7 | 4 | |
| AN1362 | Cue5 ortholog | | 4 | |
| AN0635 | Intracellular localization | 8 | 4 | 8 |
| AN0691 | Protein of unknown function | 6 | 16 | |
| AN4646 | Protein of unknown function | | 13 | |
| AN5764 | Induced by light | 2 | | |
| **Shared in Hülle cells & vegetative mycelium (8 proteins)** | | | | |
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN1182 (BenA) | Beta-tubulin | 5 | 4 | 23 |
| AN4236 | Proteasome regulatory particle | 3 | | 5 |
| AN6688 (AspB) | Putative Septin B | 7 | | 2 |
| AN2918 (Cct4) | Chaperonin complex component | 3 | | 3 |
| AN4159 (GlnA) | Glutamate-ammonia ligase | 4 | | |
| AN2149 (Cct1) | Chaperonin | 2 | | 2 |
| AN10476 | Dehydrogenase | 7 | 9 | 10 |
| AN4281 (SrgB) | GTPase | | 2 | 3 |
| **Shared in Hülle cells, sexual mycelium & asexual mycelium (73 proteins)** | | | | |
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN0045 | Induced by light | 15 | 55 | |
| AN0158 | 2-alkenal reductase [NAD(P)] activity, | 21 | | 4 |
| AN0472 (EngA) | 1,3-beta-glucosidase | 33 | 9 | 5 |
| AN0567 | Alcohol oxidase | 10 | | 6 |
| AN0693 | Induced by light | | 23 | 7 |
| AN0694 | Intracellular localization | | 66 | |
| AN0779 | Glucan 1,3-beta-glucosidase | 6 | 22 | |
| AN0787 | Mannosyl-oligosaccharide 1,2-alpha-mannosidase | 6 | | 8 |
| AN0950 | Protein of unknown proteins | 4 | 36 | 9 |
| AN10040 | Oxidoreductase | 59 | | 10 |
| AN10281 | Serine/threonine phosphatase | 2 | 20 | 12 |
| AN10499 | Intracellular localization | 6 | 9 | |
| AN10563 (Pho8) | Vacuolar alkaline phosphatase | 4 | | 8 |
| AN10631 | Protein of unknown function | 5 | 10 | |
| AN10930 | UDP-N-acetylmuramate dehydrogenase activity | 16 | 66 | |
| AN11080 (NptA) | Prenyltransferase | 11 | | 26 |

**Supplementary Table 3.**
Continued.

| Shared in Hülle cells, sexual mycelium & asexual mycelium (73 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN12027 | Protein of unknown function | 6 | | |
| AN1502 (NagA) | Chitin hydrolysis | 6 | | 7 |
| AN1616 | Oxidoreductase | 7 | 15 | |
| AN1675 | Lysophospholipase | 2 | 17 | |
| AN1733 (PrnC) | Delta-1-pyrroline-5-carboxylate dehydrogenase | 13 | | |
| AN1918 (AcuF) | Phosphoenolpyruvate carboxykinase | 25 | 44 | |
| AN2000 (Ubi4) | Polyubiquitin | 15 | 9 | |
| AN2017 (AgdA) | Alpha-glucosidase | 2 | 2 | |
| AN2092 | Prolyl aminopeptidase | 3 | 4 | |
| AN2314 | 1,4-alpha-glucane-branching enzyme | 6 | | |
| AN2383 | Protein of unknown function | | 8 | 25 |
| AN2395 | Beta-glucuronidase | | 4 | 2 |
| AN2572 | Dipeptidyl-peptidase | 6 | | |
| AN2828 (BglL) | Beta-glucosidase | 28 | | 10 |
| AN2860 | Protein of unknown function | 4 | | 5 |
| AN2866 | Oxidoreductase | 2 | | 8 |
| AN2936 | Alpha-mannosidase | 17 | 2 | |
| AN3121 | Protein of unknown function | | 8 | 4 |
| AN3402 (AmyB) | Alpha-amylase | 6 | 5 | |
| AN3416 (SsoA) | Syntaxin protein | 2 | 15 | |
| AN3512 | Protein of unkown function | 5 | 4 | |
| AN3983 | Protein of unknown function | | 9 | 21 |
| AN4058 | Dihydroxy acid dehydratase | 2 | | 10 |
| AN4102 (BglA) | Beta-glucosidase | 16 | 2 | |
| AN4577 (fmdS) | Kynurenine formamidase | | 2 | |
| AN4825 | Glucan 1,3-beta-glucosidase | 2 | | 8 |
| AN4871 (ChiB) | Chitinase | 78 | 111 | 16 |
| AN4940 | Meiotic recombination | 13 | 64 | 12 |
| AN4988 | Intracellular localization | 16 | 55 | 2 |
| AN5217 (PilA) | Protein similarity to sphingolipid | 8 | 65 | |
| AN5281 | Pyranose oxidase | 8 | 44 | |
| AN5422 | Beta-lactamase family protein | 11 | 8 | 7 |
| AN5442 | Carboxypeptidase Y | 7 | | |
| AN5446 | Protein of unknown function | 14 | 62 | |
| AN5634 (AcuB) | Isocitrate lyase | 39 | | |
| AN6024 (GstB) | Glutathione S-transferase | 6 | 10 | 4 |
| AN6273 | Intracellular localization | | 4 | 8 |
| AN6279 (AcuJ) | Carnitine acetyltransferase | 16 | | 8 |
| AN6398 | Intracellular localization | 4 | 8 | |
| AN6796 | Intracellular localization | 2 | 22 | |

**Supplementary Table 3.**
Continued.

| Shared in Hülle cells, sexual mycelium & asexual mycelium (73 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN6844 | 3-hydroxyisobutyryl-CoA hydrolase activity | 6 | | 10 |
| AN6963 | Oxidoreductase | 4 | 36 | |
| AN7269 | UDP-N-acetylmuramate dehydrogenase | | 7 | |
| AN7307 | Catalytic activity | 5 | 5 | |
| AN7836 | Protein of unknown function | 2 | 13 | 4 |
| AN7912 (OrsC) | Tyrosinase | 2 | 2 | |
| AN7914 | Alcohol dehydrogenase | 19 | 10 | 2 |
| AN7962 (PepJ) | Extracellular deuterolysin-type metallo-proteinase | | 8 | |
| AN8007 (AbnC) | Arabinan endo-1,5-alpha-L-arabinosidase activity | 2 | 12 | 8 |
| AN8330 | Oxidoreductase | 7 | | 14 |
| AN8553 | Catalase | 29 | 69 | 10 |
| AN8829 | Protein of unknown function | | 17 | |
| AN9002 | Monooxigenase, secondary metabolite enzyme | 13 | 54 | |
| AN9094 | UDP-N-acetylglucosamine pyrophosphorylase | 17 | | 10 |
| AN9180 | Transketolase | 20 | 34 | |
| AN9194 (CetL) | Transcript enriched in dormant conidia | 20 | 20 | |
| AN9340 (TreA) | Alpha-trehalase | 4 | 5 | 20 |
| **Shared in Hülle cells, sexual mycelium & vegtative mycelium (21 proteins)** | | | | |
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN10276 | Nucleic acid binding | 2 | | 4 |
| AN10614 | G-quadruplex DNA binding | 4 | 3 | |
| AN10946 | Protein of unknown function | 7 | 9 | |
| AN1222 | S-adenosylmethionine synthetase | 18 | 6 | 4 |
| AN1358 | Serine/threonine phosphatase activity | 2 | 4 | 4 |
| AN1990 | Homocitrate synthase | 5 | | 5 |
| AN2142 (kapA) | Karyopherin (importin) alpha | 4 | | 2 |
| AN2455 | Zinc ion binding activity | 6 | | 2 |
| AN2980 | Ribosome activity | 6 | 15 | 2 |
| AN4575 | Protein of unknown function | 6 | 9 | 8 |
| AN4667 (AspA) | Septin | 11 | | |
| AN4727 | Involved in galactose metabolism | 2 | 30 | |
| AN4775 | Cullin deneddylation | 10 | | 9 |
| AN5134 (GltA) | Glutamate synthase | 12 | 2 | |
| AN5601 | Saccharopine dehydrogenase | 16 | | |
| AN5602 | Chaperone | 6 | 66 | |
| AN5740 | Rho family GTPase | 6 | | 2 |
| AN5895 | Rab GDP-dissociation inhibitor | 16 | 19 | 19 |
| AN7570 (tubB) | Alpha-tubulin | 2 | | 8 |
| AN8182 (AspC) | Septin | 12 | | |
| AN8863 | Nap/SET family protein | 4 | | 9 |

**Supplementary Table 3.**
Continued.

| Shared in Hülle cells, asexual mycelium & vegetative mycelium (6 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN0316 (tubA) | Alpha-tubulin | 19 | | 16 |
| AN1126 | ADP ribosylation factor | 6 | 2 | 2 |
| AN1971 | ATP-dependent 3'-5' DNA helicase | 2 | 3 | 6 |
| AN2012 | Protein of unknown function | 2 | 4 | 3 |
| AN6631 | F1F0-ATPase complex subunit | 8 | 8 | |
| AN7350 | Nucleic acid binding | 8 | 9 | 3 |
| **Shared in Hülle cells, sexual mycelium, asexual mycelium & vegetative mycelium (253 proteins)** | | | | |
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN0046 | Protein of unknown function | 13 | | 19 |
| AN0084 | Ran GTPase binding activity | 8 | 11 | |
| AN0170 (TrxA) | Thioredoxin | | 6 | 6 |
| AN0240 (PppA) | Transaldolase | 64 | 25 | 30 |
| AN0241 (SodA) | Cu/Zn-superoxide dismutase | 5 | 50 | 11 |
| AN0252 | F1F0-ATPase complex gamma | 9 | 12 | 2 |
| AN0262 | Ribosomal large subunit assembly | 4 | 16 | 6 |
| AN0285 | 6-phosphogluconolactonase | 8 | 16 | 3 |
| AN0297 | Protoplast secreted protein 2 | 8 | 6 | 9 |
| AN0359 (SgdA) | Translation initiation factor 3 (eIF3) | 8 | 3 | |
| AN0432 | Cytochrome-b5 reductase activity | 4 | 3 | |
| AN0433 | Ribosome activity | | 4 | 10 |
| AN0443 | Alcohol dehydrogenase | 6 | 7 | 21 |
| AN0465 | Protein of unknown function | 9 | 10 | 4 |
| AN0554 (AldA) | Aldehyde dehydrogenase | 57 | 43 | 89 |
| AN0579 | Isopentenyl-diphosphate delta-isomerase | 6 | | 9 |
| AN0641 | Osmoadaptation | 11 | 8 | 8 |
| AN0687 (SpdA) | Spermidine synthase | | 4 | 4 |
| AN0688 | Transketolase | 52 | 57 | 63 |
| AN0734 | Histone H4 | 5 | | 6 |
| AN0747 | Ubiquinol-cytochrome-c reductase | 10 | 10 | 3 |
| AN0776 | Ribosome activity | 4 | 8 | 10 |
| AN0843 | Ribosome activity | | 8 | 3 |
| AN0870 | Transporter | 6 | 6 | 14 |
| AN0893 | Adenylosuccinate synthase | 16 | 5 | 3 |
| AN0932 (GlrA) | Glutathione oxidoreductase | 3 | 4 | 7 |
| AN1003 | Isocitrate dehydrogenase (NAD+) | 12 | 2 | |
| AN10030 | Serine protease | 4 | 18 | 5 |
| AN1013 | 60S ribosomal protein L5 | 29 | 7 | 25 |
| AN1015 | Phosphorylase | 7 | 10 | 8 |
| AN10170 | ATPase activity and cytoplasm localization | 28 | | |
| AN10202 | ATP binding activity | 20 | 5 | 7 |
| AN10223 | Peroxiredoxin | 6 | 7 | 2 |

**Supplementary Table 3.**
Continued.

| Shared in Hülle cells, sexual mycelium, asexual mycelium & vegetative mycelium (253 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN10266 | Ubiquitin-activating enzyme | 7 | 3 | |
| AN10273 (Gst3) | Glutathione S-transferase | 16 | | |
| AN10279 | Nucleic acid binding | 5 | 2 | 11 |
| AN10311 (MnpA) | Mannoprotein | 4 | 6 | 2 |
| AN10351 | Aminopeptidase activity | 19 | 3 | 23 |
| AN10416 | 60s ribosomal protein similar to subunits L15 and L27 | 10 | | 6 |
| AN1047 | Heat shock protein | 33 | 32 | 5 |
| AN10474 | Protein of unknown function | 6 | | |
| AN10540 | Dipeptidyl-peptidase activity | 14 | 12 | 4 |
| AN10675 | Mitochondrion localization | 5 | 3 | |
| AN10709 (GfaA) | Glutamine-fructose-6-phosphate transaminase | 13 | | 6 |
| AN10745 | Glycine hydroxymethyltransferase activity | 10 | 10 | 9 |
| AN1084 | Elongation factor EF-Tu | 8 | 9 | |
| AN11005 | Metalloexopeptidase activity | 5 | 5 | |
| AN11045 | D-lactate dehydrogenase (cytochrome) activity | 2 | | 5 |
| AN11125 | Protein of unknown function | 8 | 6 | |
| AN1122 | Ribosome activity | 3 | 7 | |
| AN11227 | 70 kilodalton heat shock protein | 26 | 15 | 20 |
| AN1152 | Protein of unknown function | | 7 | |
| AN1162 | Guanyl-nucleotide exchange factor | 26 | 20 | 3 |
| AN1166 | Ribosome activity | 10 | 9 | 9 |
| AN11898 | Protein of unknown function | 2 | | 10 |
| AN1228 | Maturation of rRNA | 2 | 8 | 10 |
| AN1246 (PgkA) | Phosphoglycerate kinase | 53 | 10 | 16 |
| AN12465 | Intracellular localization | 35 | 18 | 33 |
| AN1263 | Adenosylhomocysteinase | 31 | 3 | 7 |
| AN1342 | Alanine-glyoxylate transaminase | 9 | 6 | 4 |
| AN1378 | Protein of unknown function | | 10 | |
| AN1380 | tRNA ligase | 4 | | |
| AN1409 | Acetyl-CoA C-acetyltransferase | 30 | 7 | 14 |
| AN1430 | Oxidoreductase | 2 | | 4 |
| AN1523 | F1F0-ATPase complex | 62 | 35 | 77 |
| AN1543 | Succinate dehydrogenase | 13 | 7 | 7 |
| AN1547 | Carboxylate CoA-transferase | 27 | 13 | 4 |
| AN1638 | Metalloaminopeptidase | 21 | 24 | 3 |
| AN1689 | Oxidoreductase | 4 | 10 | 2 |
| AN1757 (ssfA) | 20s core proteosome | | 8 | |
| AN1810 | Ornithine transaminase | 19 | 2 | |

**Supplementary Table 3.**
Continued.

| Shared in Hülle cells, sexual mycelium, asexual mycelium & vegetative mycelium (253 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN1883 | Argininosuccinate synthase | 19 | 10 | |
| AN1923 | Alanine transaminase | 12 | 35 | 26 |
| AN1964 | Protein of unknown function | 8 | 4 | |
| AN1993 | Aspartate transaminase | 24 | 10 | 12 |
| AN2062 (BipA) | Chaperone | 14 | 15 | 14 |
| AN2150 | Protein of unknown function | 5 | 2 | |
| AN2208 | Galactose 1-dehydrogenase | 9 | 2 | 6 |
| AN2225 | Role in cytoplasmic translation | 7 | 4 | |
| AN2248 (GatA) | 4-aminobutyrate transaminase | 23 | 16 | |
| AN2272 | Protein of unknown function | 10 | | 4 |
| AN2275 | Ribosome activity | 8 | | |
| AN2286 (AlcC) | Alcohol dehydrogenase III | 11 | 2 | 8 |
| AN2295 | Succinate-CoA ligase | 4 | 6 | 6 |
| AN2304 | Protein of unknown function | 8 | 9 | |
| AN2315 | DNA-directed RNA polymerase activity | 120 | 33 | |
| AN2435 (AlcA) | Citrate synthase | 49 | | 40 |
| AN2436 (AclB) | ATP citrate synthase | 64 | 3 | 13 |
| AN2526 | Ketol-acid reductoisomerase | 44 | 5 | 2 |
| AN2734 | rRNA binding activity | 3 | 8 | 10 |
| AN2867 (PgmB) | Phosphoglucomutase | 24 | 29 | 39 |
| AN2875 (FbaA) | Fructose-bisphosphate aldolase | 80 | 36 | 26 |
| AN2903 (PepE) | Aspartic protease | 22 | 9 | 22 |
| AN2914 | Argininosuccinate lyase | 14 | | 3 |
| AN2916 | Succinate dehydrogenase | 19 | 14 | 3 |
| AN2932 | Eukaryotic initiation factor 4A | 17 | | 4 |
| AN2954 | Protein of unknown function | 4 | | 4 |
| AN2968 (IppA) | Inorganic diphosphatase | 20 | 24 | 25 |
| AN2981 (GsdA) | Glucose 6-phosphate 1-dehydrogenase | 18 | | 12 |
| AN2999 (IdpA) | Isocitrate dehydrogenase | 22 | 7 | 7 |
| AN3031 | Threonine synthase | 7 | | 2 |
| AN3058 | Glycine hydroxymethyltransferase | 23 | | 23 |
| AN3059 (PgmA) | Phosphoglycerate mutase | 24 | 2 | 7 |
| AN3112 (UgmA) | UDP-galactopyranose mutase | 19 | | 14 |
| AN3172 | Protein of unknown function | 11 | 11 | 7 |
| AN3413 | Ribosomal protein S2 and S5 | 3 | 2 | |
| AN3459 | Metallodipeptidase activity | 7 | 2 | |
| AN3466 (KgdB) | Dihydrolipoamide S-succinyl transferase | 21 | | 10 |
| AN3592 (ClxA) | Calnexin | | 5 | 15 |
| AN3674 | Protein of unknown function | 40 | 22 | 43 |

**Supplementary Table 3.**
Continued.

| Shared in Hülle cells, sexual mycelium, asexual mycelium & vegetative mycelium (253 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN3814 (CypA) | Peptidyl-prolyl cis-trans isomerase | 6 | 6 | 2 |
| AN3829 | Succinate-semialdehyde dehydrogenase | 7 | | 15 |
| AN3873 | Oxidoreductase | 15 | 8 | |
| AN3918 | Aminopeptidase activity | 3 | 9 | |
| AN3928 (ThiF) | Thiazole synthase | 26 | 10 | 6 |
| AN3954 | Phosphogluconate dehydrogenase | 102 | 20 | 46 |
| AN4000 (FabM) | Poly(A)-binding proteins | 9 | 10 | 5 |
| AN4064 | ADP/ATP carrier protein | 5 | 8 | 17 |
| AN4087 | 40S ribosomal protein subunit | 30 | 29 | 17 |
| AN4163 (CpcB) | Response to amino acid starvation | 18 | 15 | 17 |
| AN4218 | Translation elongation factor activity | 58 | 41 | 33 |
| AN4259 | Protein of unknown function | 10 | | 12 |
| AN4282 | Intracellular localization | 16 | 19 | 4 |
| AN4288 | Protein of unknown function | 27 | 3 | |
| AN4323 | Aminotransferase | 4 | 4 | 4 |
| AN4376 | NADP-linked glutamate dehydrogenase | 42 | 9 | 37 |
| AN4390 (EcmA) | GPI-anchored protein,role in cell wall biosynthesis | 3 | 5 | |
| AN4409 (ArgB) | Ornithine carbamoyltransferase | 11 | | 3 |
| AN4414 | Diphosphomevalonate decarboxylase | 17 | | |
| AN4443 (MetH) | Methionine synthase | 95 | 64 | 115 |
| AN4462 (PycA) | Pyruvate carboxylase | 46 | 16 | 16 |
| AN4464 (PurH) | Bifunctional enzyme | 19 | | 9 |
| AN4470 | Role in positive regulation of translaion | 6 | | 10 |
| AN4475 | Ribosomal large subunit assembly | 2 | 9 | 4 |
| AN4501 (ArtA) | 14-3-3 protein | 24 | 24 | 56 |
| AN4594 | Maturation of rRNA | | 5 | 5 |
| AN4616 | Protein of unknown function | 5 | | 4 |
| AN4769 | ATP sulfurylase | 18 | 5 | 9 |
| AN4793 | Aspartate semialdehyde dehydrogenase | 6 | 4 | 8 |
| AN4794 | Ribosomal protein | | 11 | 9 |
| AN4802 | 60S ribosomal protein L21 | 3 | 7 | 2 |
| AN4862 | Ran GTPase activating | 4 | | |
| AN4888 (PdcA) | Decarboxylase | 16 | | 9 |
| AN4905 (GstA) | Glutathione S-transferase | | 6 | 6 |
| AN4916 | Ribosome biogenesis | 10 | 6 | 9 |
| AN5014 | Ribosome activity | | 4 | 2 |
| AN5129 | Heat shock protein | 104 | | 75 |
| AN5162 (PdhB) | Pyruvate dehydrogenase | 17 | | 2 |
| AN5210 (PkiA) | Pyruvate kinase | 15 | 5 | 6 |
| AN5441 | Endonucleolytic cleavage | | 21 | 3 |

**Supplementary Table 3.**
Continued.

| Shared in Hülle cells, sexual mycelium, asexual mycelium & vegetative mycelium (253 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN5520 | Ribosome biogenesis | 8 | 15 | 5 |
| AN5523 | Alpha-trehalose-phosphate synthase | 6 | | 5 |
| AN5525 (AcoA) | Aconitate hydratase | 52 | 49 | 9 |
| AN5563 | Dehydrogenase | 22 | | 19 |
| AN5564 | Phosphatidyl synthase | 10 | 2 | |
| AN5566 | Protein of unknown function | 10 | | 11 |
| AN5571 (KgdA) | Oxoglutarate dehydrogenase | 18 | | 2 |
| AN5626 | Acetyl-CoA synthase | 22 | 31 | 4 |
| AN5686 (TpmA) | Tropomysin | | 10 | 3 |
| AN5701 (AroF) | 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase | 7 | | 3 |
| AN5715 | 40s ribosomal protein S26 | | 5 | 2 |
| AN5719 | Ribosome activity | 2 | 3 | 14 |
| AN5744 | 14-3-3-like protein | 19 | 17 | 19 |
| AN5746 (AcuN) | Phosphopyruvate hydratase | 91 | 29 | 59 |
| AN5800 | Ribosome activity | 6 | 20 | 7 |
| AN5810 (PepP) | Prolidase | 7 | | 10 |
| AN5872 | Proteasom | | 6 | 8 |
| AN5907 | Ribose-5-phosphate isomerase | | | 7 |
| AN5960 | Ribosomal small subunit assembly | | 9 | 4 |
| AN5975 | Mannitol-1-phosphate 5-dehydrogenase | 45 | 24 | 64 |
| AN5996 | Ribosome activity | | 9 | 6 |
| AN5997 | Role in rRNA export from nucleus | 5 | | 5 |
| AN5999 | Arginine or pyrimidine metabolism | 16 | 5 | |
| AN6004 | Protein of unknown function | 6 | 6 | 2 |
| AN6010 (SgdE) | Hsp70-family protein | 73 | 21 | 22 |
| AN6037 (SwoB) | Glucose-6-phosphate isomerase | 37 | 10 | 71 |
| AN6048 | Aspartate transaminase | 17 | 9 | 4 |
| AN6089 | Heat shock protein | 48 | 10 | 5 |
| AN6202 | Ribosomal protein L3 | 20 | 7 | 8 |
| AN6209 | 5'-phosphoribosyl-4-(N-succinocarboxamide)-5-aminoimidazole lyase | 5 | 7 | 10 |
| AN6232 (VmaB) | F1F0-ATPase complex subunit | 15 | | 9 |
| AN6330 | Elongation factor 2 | 98 | 28 | 9 |
| AN6341 | Similarity to Saccharomyces cerevisiae Crn1p | 15 | | 6 |
| AN6346 | Dihydroxy-acid dehydratase | 10 | 8 | 5 |
| AN6499 (MdhC) | Malate dehydrogenase | 35 | 27 | 86 |
| AN6500 | Ribosome activity | 35 | 8 | 2 |
| AN6525 (AciA) | Formate dehydrogenase | 41 | 23 | 29 |
| AN6542 (ActA) | Actin A | 41 | 25 | 29 |
| AN6547 | Proteasom | | 25 | 5 |
| AN6563 | Translation elongation factor EF-1 gamma | 50 | 6 | 73 |

**Supplementary Table 3.**
Continued.

| Shared in Hülle cells, sexual mycelium, asexual mycelium & vegetative mycelium (253 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN6629 | Ribosomal protein L14 | 12 | 23 | 10 |
| AN6630 | Polypeptide-associated complex | 6 | 12 | 7 |
| AN6639 (McdB) | 2-methylcitrate dehydratase | 28 | 9 | 27 |
| AN6653 (AcuE) | Malate synthase | 28 | 7 | 10 |
| AN6679 | DNA binding activity and nucleus localization | | 10 | |
| AN6699 | Electron carrier activity | | 4 | 19 |
| AN6700 | ATPase activity | 23 | 2 | |
| AN6708 (PdhA) | Dihydrolipoamide S-acetyltransferase | 8 | 2 | 2 |
| AN6717 (MdhA) | Mitochondrial malate dehydrogenase | 76 | 87 | 72 |
| AN6840 | Hydroxyacylglutathione hydrolase | | 11 | 10 |
| AN6900 (TpiA) | Triose-phosphate isomerase | 13 | 24 | 7 |
| AN6921 | Chaperone | 3 | 2 | 5 |
| AN7003 | Ribosome activity | | 5 | 2 |
| AN7105 | Translation initiation factor 3 (eIF3) | 8 | 9 | |
| AN7107 | Ribosome activity | | 9 | 10 |
| AN7141 | NAD-dependent aldehyde dehydrogenase | 4 | 4 | 11 |
| AN7193 | NADPH-dependent glycerol dehydrogenase | 8 | | 4 |
| AN7254 | Cell division protein | 44 | | |
| AN7262 | Protein of unknown function | 2 | 11 | 2 |
| AN7299 | Role in cellular process | | 7 | 4 |
| AN7354 | Ribosome activity | 2 | 6 | 10 |
| AN7388 (CpeA) | Laccase | 24 | 40 | 89 |
| AN7436 (PdiA) | Protein disulfide isomerase | 19 | 16 | |
| AN7451 | NAD-glutamate dehydrogenase | 9 | 23 | |
| AN7459 (hxkA) | Hexokinase | 15 | 10 | 27 |
| AN7590 | Reductase | 4 | 6 | 2 |
| AN7600 | Iron-sulfur cluster binding | 4 | 8 | |
| AN7625 | Myo-inositol-1-phosphate synthase | 32 | 22 | |
| AN7632 | Dehydrogenase | 15 | 12 | |
| AN7657 | 1,3-beta-transglycosidase | 6 | 10 | 2 |
| AN7708 | NADP+ 1-oxidoreductase activity | 2 | 3 | 3 |
| AN7710 | Intracellular localization | 3 | 19 | 13 |
| AN7725 (PyroA) | Protein required for biosynthesis of pyridoxine | 3 | 5 | |
| AN7950 (EglC) | Glucan endo-1,3-beta-D-glucosidase | 3 | 17 | |
| AN8009 (Nmt1) | Protein of unknown function | 21 | 20 | 29 |
| AN8041 (GpdA) | Glyceraldehyde-3-phosphate dehydrogenase | 106 | 58 | 47 |
| AN8054 | 20S core proteasome | | 5 | 10 |
| AN8170 (NapB) | Nap/SET family member | 7 | | 4 |
| AN8176 | Ribosome activity | 63 | 22 | 30 |
| AN8187 | Protein of unknown function | 4 | | 3 |

**Supplementary Table 3.**
Continued.

| Shared in Hülle cells, sexual mycelium, asexual mycelium & vegetative mycelium (253 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts, found in Hülle cells | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN8216 (SwoH) | Nucleoside diphosphate kinase | 10 | 19 | 19 |
| AN8218 (TrxB) | Thioredoxin reductase | 10 | 16 | 3 |
| AN8269 (Hsp90) | Heat shock protein | 47 | 22 | 55 |
| AN8273 | Ubiquinol-cytochrome-c reductase | 19 | 10 | 10 |
| AN8275 | Mitochondrial citrate synthase | 69 | 24 | 17 |
| AN8277 (CysD) | Bifunctional enzyme | | 13 | 4 |
| AN8605 (Cyp1) | Peptidyl-prolyl cis-trans isomerase | 5 | | 11 |
| AN8637 (CatA) | Catalase | 8 | | 6 |
| AN8689 (GlkA) | Glucokinase | 15 | 10 | 16 |
| AN8692 | Thioredoxin-dependent peroxidase | 11 | 8 | 7 |
| AN8704 | Ribosomal protein | 4 | 6 | 6 |
| AN8707 | Fumarate dehydratase | 20 | 8 | 9 |
| AN8770 | Bifunctional enzyme | 3 | | 4 |
| AN8856 | RNA binding activity | 9 | 5 | 6 |
| AN8866 | Phosphoglycerate dehydrogenase | 7 | 6 | 9 |
| AN8867 | Serine-tRNA ligase activity | 17 | 3 | 9 |
| AN8953 (AgdB) | Alpha-glucosidase | 21 | 17 | 2 |
| AN9042 (AgnC) | Alpha-1,3-glucanase | | 21 | 6 |
| AN9103 (AifA) | Oxidoreductase | 14 | 5 | 6 |
| AN9148 | UTP-glucose-1-phosphate uridylyltransferase | 32 | 16 | 31 |
| AN9339 (CatB) | Catalase | 18 | 34 | 10 |
| AN9403 (PdhC) | Pyruvate dehydrogenase (lipoamide) | 7 | 5 | 7 |
| AN9465 | Ribosome activity | 2 | 14 | 4 |

**Supplementary Table 4.**
The list of protein found in sexual, asexual and vegetative mycelium from solid agar plates (Figure 24). Sexual and asexual mycelium from solid agar plate cultures were analyzed within three different time points. Vegetative mycelium was analyzed 20 hours after inoculation (submerged liquid cultures). For each time points three biological replicates were considered for the different mycelia types. Only proteins identified in two or more biological replicates and with two or more peptides per protein were considered for the analysis. Proteins that were common between the different mycelia types are also listed. Numbers represent the average of spectral counts from three biological replicates. Putative function or role proteins are listed under Description. Different sources were used, mainly AspGD (Cerqueira et al., 2014).

| Found in sexual mycelium (203 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts found in sex. mycelium | | |
| Gene ID | Description | 3 day | 5 day | 7 day |
| AN6000 (AptA) | Polyketide synthase | 153 | 60 | 127 |
| AN7806 (StcU) | Versicolorin reductase | 13 | 43 | 22 |
| AN8162 | Oxidoreductase activity | 20 | 63 | 58 |
| AN8466 | Cell wall macromolecule catabolic process | 20 | 21 | 2 |
| AN5348 | Nucleotide binding activity | 17 | 5 | 40 |
| AN5558 | Protease | 63 | 10 | 3 |
| AN10965 | Protein of unknown function | 18 | 25 | 15 |
| AN6002 (AptC) | Monooxygenase | 3 | 22 | 21 |
| AN4105 | Erythromycin esterase | 16 | 22 | 5 |
| AN8514 (TdiB) | Prenyltransferase | 14 | 16 | 7 |
| AN12267 | UDP-N-acetylmuramate dehydrogenase | 15 | 10 | 33 |
| AN6784 (XptA) | Prenyltransferase | 5 | | 3 |
| AN0146 (MdpC) | Monodictyphenone secondary metabolite cluster | 4 | 10 | |
| AN2704 | Aryl-alcohol oxidase-related | 9 | 8 | 4 |
| AN3206 | Aryl-alcohol oxidase-related | 10 | 35 | |
| AN10952 | Monooxygenase activity | 6 | 31 | 6 |
| AN0052 | Oxidoreductase acitivity | 16 | 26 | |
| AN3573 | Oxidoreductase activity | 2 | 12 | 34 |
| AN4016 | 60S ribosomal protein L40 | 30 | 42 | |
| AN6082 | Ribosome activity | 15 | 31 | 22 |
| AN3432 | Aldose 1-epimerase | 21 | 24 | |
| AN3601 | Catalytic activity | 15 | 21 | 5 |
| AN5698 | Fatty acid metabolism | | 37 | 33 |
| AN8004 | Cytochrome P450 | 2 | 4 | 30 |
| AN8086 | Protein of unknown function | 18 | 12 | 2 |
| AN8106 | Protein of unknown function | 9 | 19 | 3 |
| AN9322 | Nucleotide binding activity | 7 | 17 | 10 |
| AN0034 | Glycerone kinase | 5 | 2 | 2 |
| AN0212 | Oxidoreductase | 8 | 8 | |
| AN0221 | Chitinase | 12 | 8 | 8 |
| AN0270 | Kinase | 4 | | 6 |
| AN0380 (Cyp10) | Peptidyl-prolyl cis-trans isomerase | 4 | | 8 |
| AN0385 | Aminotransferase | 4 | 7 | |
| AN0558 (GelB) | 1,3-beta-transglycosidase | 7 | | 8 |
| AN0593 | Dehydrogenase | 2 | 17 | |
| AN0596 (DdbA) | DNA damage binding protein | 11 | | 5 |
| AN0602 | Protein of unknown function | 5 | | 5 |
| AN0628 | D-lactate dehydrogenase | 2 | 3 | |
| AN0788 | Protein of unknown function | 8 | 5 | |
| AN0806 | Protein of unknown function | 14 | 2 | |
| AN0839 | oxidoreductase | 4 | 2 | |
| AN0875 | Protein of unknown function | | 9 | 8 |
| AN0887 (LamA) | Urea amidolyase | 5 | | 5 |
| AN10130 | Ribosome activity | 2 | 4 | |
| AN10214 | Protein of unknown function | 6 | 19 | |
| AN10290 | Pyridoxamine-phosphate oxidase | 7 | | 2 |
| AN10326 | Protein of unknown function | 4 | | 4 |
| AN10358 | Oxidoreductase activity | 2 | 3 | 9 |
| AN10370 | Oxidoreductase | 4 | | 6 |
| AN10379 | Glutatthione transferase | 17 | 8 | 4 |
| AN10429 | Phosphatase | 8 | 6 | |
| AN10444 | Involved in sexual development, gamma glutamyl transpeptidase | 9 | 3 | |

**Supplementary Table 4.**
Continued.

| Found in sexual mycelium (203 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts,found in sex. mycelium | | |
| Gene ID | Description | 3 day | 5 day | 7 day |
| AN10863 | Oxidoreductase | 4 | 9 | |
| AN10973 | Citrate synthase | | 4 | 8 |
| AN11035 | Monooxygenase activity | 10 | 5 | |
| AN11097 | Dioxygenase activity | 10 | 33 | |
| AN1115 | Purine nucleosidase | 3 | | 9 |
| AN11246 | Protein of unknown function | 16 | 15 | 4 |
| AN11419 | Ribosome activity | 2 | 8 | 4 |
| AN11917 | Methyltransferase activity | 4 | 4 | |
| AN1204 | Protein of unknown function | 8 | 2 | |
| AN12050 | Nucleotide binding activity | 3 | 3 | |
| AN12090 | Methyltransferase activity | 5 | 14 | |
| AN12335 (AcdA) | Acyl-CoA dehydrogenase domain | 3 | 6 | |
| AN12369 | Protein of unknown function | | 6 | 6 |
| AN12446 | Protein of unknown function | 14 | 5 | |
| AN1271 | Arylformamidase activity | 6 | 5 | |
| AN1277 (AdfC) | Alpha-arabinofuranosidase activity | 2 | 4 | |
| AN1291 | Carbon-sulfur lyase activity | 2 | 4 | |
| AN1446 (MecB) | Cystathionine gamma-lyase | 9 | 6 | |
| AN1477 | Beta-1,4-xylosidase | 4 | 2 | |
| AN1611 | Flavin adenine dinucleotide binding | 2 | 8 | |
| AN1639 | Oxidoreductase | 14 | 2 | |
| AN1688 | Protein of unknown function | | 5 | 5 |
| AN1699 | Acyl-coA dehydrogenase | 2 | 7 | 9 |
| AN1763 | Oxidoreductase | 6 | 12 | 5 |
| AN1837 | Hydrophobin | 12 | | 2 |
| AN1849 | Protein of unknown function | 12 | 2 | |
| AN1979 | Protein of unknown function | 3 | 4 | |
| AN2161 (Ngn1) | GNAT-type acetyltransferase | 13 | 2 | |
| AN2166 | Protein of unknown function | 4 | 7 | 8 |
| AN2180 (XprF) | Hexokinase-like protein | 13 | 13 | |
| AN2311 | Phosphomevalonate kinase | | 5 | 6 |
| AN2351 | Alcohol dehydrogenase | | 9 | 7 |
| AN2359 (XlnD) | Beta-xylosidase | 6 | 8 | |
| AN2366 | Trypsin-like protease | 14 | 8 | 5 |
| AN2405 | Protein of unknown function | 5 | 7 | |
| AN2416 | Ubiquitin-activating enzyme | 7 | | 4 |
| AN2426 | Protein of unknown function | 13 | 11 | |
| AN2439 (SldB) | Spindle assembly checkpoint protein | 4 | 19 | |
| AN2441 | Nucleotide binding activity | 6 | 5 | |
| AN2463 (LacF) | Beta-galactosidase | 5 | 5 | |
| AN2469 | Oxidoreductase | 2 | 13 | 4 |
| AN2493 | Alkaline phosphatase | 17 | 5 | 3 |
| AN2577 | Oxidoreductase | | 5 | 5 |
| AN2622 (IpnA) | Isopenicillin-N synthase | 6 | 5 | |
| AN2762 | Acyl-coA dehydrogenase | 8 | 2 | |
| AN2801 | Oxidoreductase activity | 2 | 8 | |
| AN2853 | Protein of unknown function | 8 | 3 | |
| AN2859 | Lyase activity | 5 | 21 | 4 |
| AN2878 | Protein of unknown function | 17 | 2 | |
| AN2921 | Protein of unknown function | | 13 | 2 |
| AN3135 | Protein of unknown function | | 2 | 3 |
| AN3184 | Aldose 1-epimerase | 6 | | 2 |
| AN3191 | Hydrolase activity | 3 | 2 | 3 |
| AN3201 (LacD) | Beta-galactosidase | 17 | 14 | |
| AN3312 | L-xylulose reductase | 2 | 2 | |
| AN3375 | Heat shock protein binding | 8 | 8 | |
| AN3419 | Acetyltransferase activity | 6 | 4 | |
| AN3488 | Flavin adenine dinucleotide binding | 4 | | 8 |
| AN3499 | Phosphotransferase activity | 4 | 4 | |
| AN3565 | Hydrolase activity | 2 | 2 | 6 |
| AN10723 | Acetyltransferase activity | 2 | 2 | |
| AN10581 | Protein of unknown function | 2 | 10 | 4 |
| AN1059 (FacC) | Carnitine acetyltransferase | 6 | 3 | |
| AN10673 | Serine-type peptidase activity | 10 | 5 | |

**Supplementary Table 4.**
Continued.

| Found in sexual mycelium (203 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts in sex. mycelium | | |
| Gene ID | Description | 3 day | 5 day | 7 day |
| AN3577 | Citrate lyase | 2 | 10 | |
| AN3590 | Protein of unknown function | 12 | 6 | |
| AN3598 (FprA) | Peptidyl-prolyl cis-trans isomerase | 2 | 6 | |
| AN3706 | Role in rRNA export from nucleus | 4 | 6 | 2 |
| AN3708 | Hydrolase activity | 25 | | 4 |
| AN3741 (AlcB) | Alcohol dehydrogenase | 2 | 18 | 8 |
| AN3779 | Exopolyphosphatase activity | 7 | 2 | |
| AN3789 | Protein of unknown function | 19 | 12 | |
| AN3805 | Oxaloacetase | | 22 | 23 |
| AN3867 | Pheromone precursor processing | 6 | 2 | |
| AN3916 | Glycerol kinase | 3 | 5 | |
| AN3972 | Cytosol localization | 15 | 20 | |
| AN4011 | 5'-phosphoribosyl-4-(N-succinocarboxamide)-5-aminoimidazole lyase | 2 | 2 | |
| AN4041 | Protein of unknown function | 2 | 3 | |
| AN4177 | Catalytic activity | 14 | 6 | |
| AN4231 | Protein required for riboflavin biosynthesis | 4 | 5 | 6 |
| AN4290 | S-methyl-5-thioribose-1-phosphate isomerase | 4 | 31 | |
| AN4299 | Protein of unknown function | 4 | 5 | 7 |
| AN4317 | Nuclear pore complex protein | 8 | 6 | |
| AN4353 | 3-oxoacyl-[acyl-carrier-protein] synthase | 4 | 26 | |
| AN4396 | Ras guanine nucleotide exchange factor | 2 | 2 | |
| AN4458 | Ubiquitin thiolesterase activity | 5 | 4 | |
| AN4494 | Ribosome activity | 2 | 22 | 4 |
| AN4504 (DfgG) | Endo-mannanase | 10 | 23 | |
| AN4522 | Ribosome activity | 6 | 7 | 3 |
| AN4532 | Catechol oxygenase | 12 | 12 | 20 |
| AN4687 (MccB) | 3-methylcrotonyl-CoA carboxylase | 13 | 4 | |
| AN4691 | Oxidoreductase | 24 | | 10 |
| AN4777 | Ribosome activity | 11 | 5 | 4 |
| AN4822 | Tartrate dehydrogenase | 11 | 5 | |
| AN4830 | Coenzyme A biosynthetic process | 4 | 4 | |
| AN4901 | Glutaminase A | 11 | 21 | |
| AN4967 | Protein of unknown function | 2 | 8 | |
| AN5019 | 5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase | 2 | 16 | 5 |
| AN5109 | Oxidoreductase activity | | 10 | 13 |
| AN5144 (PfkZ) | 6-phosphofructo-2-kinase | 2 | 4 | |
| AN5178 | Glycerol dehydrogenase | 2 | 2 | |
| AN5193 | 1-aminocyclopropane-1-carboxylate synthase | 7 | | 9 |
| AN5373 | Oxidoreductase activity | 8 | 21 | |
| AN5421 | Protein of unknown function | | | |
| AN5480 | RNA binding protein | 9 | 15 | 2 |
| AN5556 | Oxidoreductase activity | 3 | 13 | |
| AN5718 | Fumarate hydratase | 22 | | 13 |
| AN5737 | Oxidoreductase | 21 | 3 | |
| AN5777 | Triglyceride lipase activity | 26 | 4 | |
| AN5830 | Hydrolase activity | 2 | 11 | 5 |
| AN5937 | Manganese ion binding | 2 | 2 | 6 |
| AN5977 | Carbonyl reductase (NADPH) activity | 5 | 2 | 2 |
| AN5986 | Reductase | 6 | 6 | 4 |
| AN6031 | Monooxygenase activity | 4 | 6 | 7 |
| AN6035 | Catalytic activity | 4 | 5 | |
| AN6197 | Nuclear migration protein | | 8 | 23 |
| AN6329 | Protein of unknown function | 4 | 9 | |
| AN6362 | Arsenate reductase activity | 4 | 4 | |
| AN6394 | Acyl-coA dehydrogenase | 9 | 8 | |
| AN6423 | Protein of unknown function | 11 | | 13 |
| AN6470 | Carbohydrate catabolism | 12 | | 6 |
| AN6535 | Protein of unknown function | 21 | 4 | 8 |
| AN6632 | 40S ribosomal protein S28 | 2 | | 8 |
| AN6687 | DNA binding activity | 11 | | 6 |
| AN6933 (MaeB) | Malate dehydrogenase | 7 | 9 | |
| AN7044 | Histidinol-phosphatase | 6 | 4 | |
| AN7128 | Oxidoreductase activity | 2 | 4 | |

**Supplementary Table 4.**
Continued.

| Found in sexual mycelium (203 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts found in sex. mycelium | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN7259 (DcnA) | NEDD8 ligase activity, ubiquitin bindin | 4 | 3 | |
| AN7274 | Fumiquinazoline C biosynthetic protein | 5 | 15 | |
| AN7367 | Hydrolase activity | 2 | 18 | 17 |
| AN7564 | Threonine aldolase | 7 | | 8 |
| AN7704 | Catabolic process | 16 | | 6 |
| AN7705 | Protein of unknown function | 3 | 3 | |
| AN7711 | ADP-ribose diphosphatase | | 8 | 5 |
| AN8016 | ATP-dependent RNA helicase | | 4 | 22 |
| AN8045 | Protein of unknown function | 8 | 5 | |
| AN8163 | Short-chain dehydrogenase/reductase | 12 | 18 | |
| AN8415 (ApdG) | Acyl-coA dehydrogenase | 7 | 2 | |
| AN8592 | Monooxygenase activity | 6 | 3 | 4 |
| AN8606 (GudA) | Glucose 1-dehydrogenase | 2 | 2 | 3 |
| AN8654 | Aminomethyltransferase | 4 | 3 | 4 |
| AN8657 | Oxidoreductase | 12 | | 8 |
| AN8797 | Protein of unknown function | 20 | | 14 |
| AN8808 | Phosphoric diester hydrolase | 9 | 18 | |
| AN8899 | 1-aminocyclopropane-1-carboxylate deaminase | 2 | 2 | |
| AN8988 | oxidoreductase | | 9 | 10 |
| AN9116 | Phosphoribosyltransferase | 3 | 6 | 22 |
| AN9227 | Dioxygenase | 12 | 12 | |
| AN9285 | Glucose repressible | | 4 | 22 |
| AN9323 | Protein of unknown function | 4 | 3 | 13 |
| AN10762 | Protein of unknown function | 8 | 4 | |

| Found in asexual mycelium (118 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts found in asex. mycelium | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN7558 | Transcript induced by light in developmentally competent mycelia | | 3 | 37 |
| AN8339 | Transcript induced by light in developmentally competent mycelia | | 29 | 29 |
| AN1553 | Role in fruiting body development | 29 | 29 | |
| AN10378 | bZIP transcription factor | 4 | 8 | |
| AN2946 | Pheromone precursor processing | 41 | 41 | |
| AN7734 | Transcription factor | 4 | | 5 |
| AN9171 | Protein of unknown function | | 10 | 15 |
| AN4852 | Glucan 1,3-beta-glucosidase | 68 | 20 | |
| AN1754 | Protein of unknown function | 3 | 8 | 3 |
| AN1035 (AfoF) | Asperfuranone biosynthesis | | 9 | 10 |
| AN4742 | Translation initiation factor | 4 | 6 | |
| AN3239 | Oxidoreductase | 26 | | 26 |
| AN0357 | Ubiquinol-cytochrome-c reductase subunit | 5 | | 5 |
| AN0483 | Protein of unknown function | | 6 | 8 |
| AN0941 (AgdE) | Alpha-glucosidase activity | 13 | 13 | |
| AN10113 | Protein of unknown function | 8 | 8 | |
| AN10174 | Protein of unknown function | 3 | | 3 |
| AN10219 | Transcript induced in response to calcium dichloride in a CrzA-dependent | 4 | 4 | |
| AN10308 | Protein of unknown function | 5 | 15 | |
| AN10510 | Protein of unknown function | 3 | | 3 |
| AN10522 | Peptidase | 4 | 4 | 4 |
| AN1063 | NADH dehydrogenase | 8 | 2 | 8 |
| AN11149 | Arylsulfatase activity | 2 | 10 | 2 |
| AN11533 | Protein of unknown function | 3 | 5 | 3 |
| AN11698 | Exopeptidase activity | | 33 | 22 |
| AN11718 | Mitochondrial intermembrane spce localization | | 11 | 11 |
| AN11764 | Binding activity | 2 | 4 | 2 |
| AN11946 | Protein of unknown function | 2 | 4 | 2 |
| AN12376 | Nucleotide binding | 4 | 6 | 2 |
| AN1396 | Role in glycerol metabolism | 9 | | 9 |
| AN1403 | Protein of unknown function | 14 | 15 | 10 |
| AN1426 | Serine-type carboxypeptidase activity | 5 | 5 | |

**Supplementary Table 4.**
Continued.

| Found in asexual mycelium (118 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts found in asex. mycelium | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN1433 | Protein of unknown function | | 9 | 8 |
| AN1532 | Protein of unknown function | 3 | 4 | |
| AN1683 | Oligosaccharyltransferase delta | 2 | | 10 |
| AN1778 (PantoB) | Ketopentoate hydroxymethyl transferase | | 2 | 10 |
| AN1857 | Kynureninase | 4 | 4 | |
| AN1986 | Ferroxidase activity | 6 | 2 | 6 |
| AN2005 (YpdA) | Histidine-containing phosphotransfer | 2 | 6 | 2 |
| AN2047 | Calmodulin | 3 | 5 | 3 |
| AN2050 | Cell wall organization and Golgi apparatus | | 10 | 10 |
| AN2069 | Cytochrome b5 | 2 | 2 | 8 |
| AN2070 | Protein of unknown function | 4 | 10 | |
| AN2110 | Oxidoreductase activity | 3 | 3 | 3 |
| AN2119 | Sphingolipid transporter activity | 5 | 2 | 5 |
| AN2140 | Endoplasmic reticulum localization | 2 | 5 | 2 |
| AN2185 | Protein of unknown function | | 3 | 10 |
| AN2237 | Carboxypeptidase | | 4 | 8 |
| AN2325 | Carbohydrate binding | 4 | 23 | |
| AN2431 (Nup49) | Nuclear pore complex | | 2 | |
| AN2472 | Serine/threonine phosphatase activity | 2 | | 2 |
| AN2576 | Monooxygenase activity | 14 | 2 | 14 |
| AN2662 | Carbon-nitrogen ligase activity | | 13 | 15 |
| AN2776 | Protein of unknown function | | 4 | 6 |
| AN3022 | Protein of unknown function | | 6 | 8 |
| AN3163 (StoA) | Stomatin ortholog | 2 | | 2 |
| AN3367 | Sequence-specific DNA binding activity | 2 | 5 | 2 |
| AN3388 (AmyF) | Alpha-amylase | 5 | 16 | |
| AN3687 | Protein of unknown function | | 18 | 10 |
| AN3783 | Protein of unknown function | 2 | 10 | |
| AN3931 (PilB) | Conserved eisosome protein | | 2 | 10 |
| AN4034 (HapC) | Component of AnCP/AnCF CCAAT-binding complex | 4 | 6 | |
| AN4081 | Cysteine dioxygenase activity | 2 | 4 | 2 |
| AN4277 | Glucose transmembrane transporter activity | 12 | | 10 |
| AN4292 | Possible pseudogene | 3 | 3 | 6 |
| AN4305 | Catalytic activity | 3 | | 3 |
| AN4499 | Nuclear transport protein | 5 | | 8 |
| AN4515 (CrhB) | Transglycosidase | 4 | 4 | 5 |
| AN4525 | Transglycosidase | | 10 | 5 |
| AN4553 | Mitochondrial outer membrane | 5 | 6 | 8 |
| AN4615 | Transmembrane domain-containing protein | 5 | 6 | 11 |
| AN4636 | Lipid particle localization | 5 | 10 | 5 |
| AN4911 | Protein of unknown function | 4 | 4 | |
| AN4927 | F1F0-ATPase complex | 5 | | 5 |
| AN4946 | Protein of unknown function | 6 | 5 | |
| AN5485 | Nuclear pore complex | 6 | 4 | 3 |
| AN5624 | DNA binding activity | 9 | | 10 |
| AN5670 | Protein transporter activity | 8 | | 8 |
| AN5671 | Protein of unknown function | 2 | 5 | 2 |
| AN6078 (NadA) | Adenine deaminase | 3 | | 3 |
| AN6203 | Protein of unknown function | 2 | 5 | |
| AN6228 | Cytosol localization | | 13 | 6 |
| AN6256 | Nucleotide binding activity | | 6 | 8 |
| AN6337 | Protein of unknown function | 2 | 2 | 3 |
| AN6382 | Phospholipase C activity | | 4 | 8 |
| AN6445 | Paryl-alcohol oxidase-related protein | 22 | 36 | |
| AN6450 | Oxidoreductase | 5 | | 5 |
| AN6473 | Protein of unknown function | 2 | 6 | 2 |
| AN6518 | Hydrolase activity | 2 | | 2 |
| AN6847 | Sulfuric ester hydrolase | | 9 | 10 |
| AN7102 | Protein of unknown function | 5 | 2 | 11 |
| AN7153 | UDP-N-acetylmuramate dehydrogenase | 12 | 12 | |
| AN7201 | Role in proteolysis | 8 | 7 | |
| AN7214 | Oxidoreductase activity | 9 | 29 | |
| AN7488 | Endoplasmic reticulum, fungal-type vacuole localization | 2 | 2 | 5 |

**Supplementary Table 4.**
Continued.

| Found in asexual mycelium (118 proteins) | | | | |
|---|---|---|---|---|
| | | Spectral counts found in asex. mycelium | | |
| **Gene ID** | **Description** | **3 day** | **5 day** | **7 day** |
| AN7567 | Role in actin cytoskeleton organization | 4 | | 4 |
| AN7579 | K48-linked polyubiquitin binding | | 4 | 5 |
| AN7595 | Protein of unknown function | | 4 | 8 |
| AN7669 | Hydrolase activity | 6 | 4 | |
| AN7680 | Protein of unknown function | | 11 | 8 |
| AN7864 (BxID) | Beta-1,4-xylosidase | 7 | 7 | 9 |
| AN7960 | Protein of unknown function | 8 | 2 | |
| AN7990 | Protein of unknown function | 4 | 4 | 5 |
| AN7992 | Protein of unknown function | 5 | 5 | |
| AN8043 | Protein of unknown function | 8 | 10 | |
| AN8234 | Protein phosphatase inhibitor activity | | 8 | 6 |
| AN8242 | Lipase | | 8 | 5 |
| AN8546 | Phospholipase C | 8 | 10 | |
| AN8607 | Protein of unknown function | 2 | 10 | 10 |
| AN8690 | Protein expressed at increased levels during osmoadaptation | 5 | | 3 |
| AN8805 | Unfolded protein binding activity | | 5 | 8 |
| AN8974 | Protein of unknown function | | 4 | 8 |
| AN9004 | Oxidoreductase activity | 13 | 4 | 13 |
| AN9027 | Hydrolase activity | 3 | | 3 |
| AN9037 | Oxidoreductase | | 2 | 10 |
| AN9163 | Protein of unknown function | 2 | 2 | 2 |
| AN9235 | Catalytic activity | | 2 | 12 |
| AN0328 | Nucleus localisation | 3 | 4 | |

| Found in vegetative mycelium (299 proteins) | | | | | |
|---|---|---|---|---|---|
| **Gene ID** | **Description** | **Spectral counts found in veg. mycelium** | **Gene ID** | **Description** | **Spectral counts found in veg. mycelium** |
| | | **20h** | | | **20h** |
| AN0117 | Cellular response to oxidative stress | 32 | AN10480 | Protein of unknown function | 19 |
| AN0133 | RNA helicase | 30 | AN10489 | Peptidyl-prolyl cis-trans isomerase | 25 |
| AN0183 | Molybdopterin binding domain protein | 13 | AN10519 | Binding activity | 50 |
| AN0242 | Hydrolase activity | 68 | AN10535 | Translation initiation factor | 4 |
| AN0327 | Role in ribosomal small subunit | 41 | AN1055 | Formation of translation preinitiation complex | 12 |
| AN0381 | Unfolded protein binding activity | 71 | AN10557 | ATP-dependent RNA helicase | 91 |
| AN0411 | Role in ER to Golgi transport | 36 | AN10595 (AspE) | Septin | 23 |
| AN0490 | CTP synthase | 10 | AN10734 | Role in regulation of translational elongation | 87 |
| AN0565 | Multifunctional enzyme | 422 | AN10743 | Establishment or maintenance of actin cytoskeleton | 19 |
| AN0692 | Cytoplasm localization | 13 | AN10765 | Nucleic acid binding | 11 |
| AN0707 | 5'-3' exoribonuclease activity | 5 | AN10837 | Amidophosphoribosyltransferase activity | 25 |
| AN0708 | Polypeptide | 86 | AN10848 | Nucleic acid binding | 26 |
| AN0717 | Histidinol-phosphate aminotransferase | 47 | AN11008 | Squalene monooxygenase activity | 18 |
| AN0753 | Intracellular localization | 106 | AN11052 | 5'-3' exonuclease activity | 35 |
| AN0757 | tRNA (cytosine-5-)-methyltransferase | 54 | AN11128 | Binding activity | 19 |
| AN0809 | DNA binding | 25 | AN11168 | Nucleus localization | 13 |
| AN0840 | Alpha-isopropylmalate synthase | 131 | AN11238 | IMP dehydrogenase/GMP reductase | 23 |
| AN0879 | Protein of unknown function | 29 | AN1158 | mRNA 5'-UTR binding | 128 |
| AN0917 | Nucleotide binding activity | 7 | AN1163 | Chaperone | 22 |

**Supplementary Table 4.**
Continued.

| Found in vegetative mycelium (299 proteins) | | | | | |
|---|---|---|---|---|---|
| **Gene ID** | **Description** | **Spectral counts found in veg. mycelium** | **Gene ID** | **Description** | **Spectral counts found in veg. mycelium** |
| | | **20h** | | | **20h** |
| AN1558 (MyoA) | Myosin I, required for secretion and polarized growth | 17 | AN2523 (ChsB) | Class III chitin synthase | 6 |
| AN1606 | Calcium ion binding | 31 | AN2682 | Oxidoreductase activity | 35 |
| AN1636 | Protein of unknown function | 7 | AN2731 | ATPase activator activity | 46 |
| AN1667 | Protein of unknown function | 19 | AN2751 | topoisomerase II-associated protein | 15 |
| AN1700 | 26S proteasome regulatory | 53 | AN2756 (SalB) | Actin binding protein | 126 |
| AN1709 | Mitochondrial tyrosyl-tRNA synthetase | 5 | AN2765 | Localized to chromosomes | 14 |
| AN1752 | Sulfite reductase | 210 | AN2862 | CAP-Gly domain protein | 41 |
| AN1851 | Unfolded protein binding activity | 90 | AN2871 (McnC) | CUE-domain containing phosphoprotein | 22 |
| AN1873 | Protein of unknown function | 9 | AN2904 | 26S proteasome regulatory subunit | 64 |
| AN1901 (PdmA) | Sterol 14 alpha-demethylase | 3 | AN2907 | Protein of unknown function | 135 |
| AN1904 | Chaperonin-containing T-complex | 55 | AN2909 | ATP binding | 16 |
| AN1911 | Mannose-1-phosphate guanyltransferase | 60 | AN2917 | Role in positive regulation of RNA polymerase II | 37 |
| AN1922 | Proteasome regulatory particle | 31 | AN2954 | Protein of unknown function | 8 |
| AN1929 | DNA-dependent ATPase activity | 9 | AN2967 | tRNA dihydrouridine synthase | 4 |
| AN1949 | RNA helicase activity | 12 | AN2976 | Intracellular localization | 6 |
| AN1954 | Protein of unknown function | 13 | AN2992 | Eukaryotic translation initiation factor 2 complex | 15 |
| AN1965 | Ribose-phosphate pyrophosphokinase | 11 | AN3019 | Ubiquitin-dependent protein | 56 |
| AN1966 (HulE) | HECT ubiquitin ligase | 37 | AN3029 | Protein transporter activity | 23 |
| AN2014 | 7S RNA binding activity | 12 | AN3055 | Ribosome binding | 23 |
| AN2042 | Oxidoreductase | 23 | AN3070 | ATP binding, unfolded protein | 45 |
| AN2068 | RNA binding activity | 124 | AN3080 | Role in ER to Golgi vesicle-mediated transport | 38 |
| AN2080 | Intracellular localization | 58 | AN3122 | Transcript levels increase during the unfolded-protein | 42 |
| AN2120 (KapJ) | Karyopherin (importin) beta 3 | 181 | AN3134 | ATP binding, unfolded protein | 65 |
| AN2126 | Actin capping protein | 32 | AN3147 | Intracellular localization | 28 |
| AN2147 | Ribosomal RNA processing protein | 37 | AN3156 | Eukaryotic translation initiation factor 2 complex | 47 |
| AN2164 | Protein transporter activity | 90 | AN3169 | Ribose-phosphate pyrophosphokinase | 22 |
| AN2210 | ABC-transporter | 80 | AN3188 | Phosphatidylserine decarboxylase | 21 |
| AN2213 | ATPase activity | 25 | AN3344 (Ngn27) | PGNAT-type acetyltransferase | 10 |
| AN2226 | Protein of unknown function | 6 | AN3628 | Peptide alpha-N-acetyltransferase | 34 |
| AN2278 | Helicase activity | 30 | AN3709 | Protein of unknown function | 20 |
| AN2284 (HemA) | 5-aminolevulinic acid synthase | 46 | AN3716 | Protein of unknown function | 34 |
| AN2412 | NADH dehydrogenase | 11 | AN3719 | Protein of unknown function | 6 |
| AN2458 | Protein of unknown function | 56 | AN3720 | Putative dehydrogenase | 61 |
| AN2516 (AmpA) | Localized to peripheral patches and to hyphal tips | 11 | AN3739 | Intracellular localization | 45 |
| AN2518 | Involved in exocytosis | 16 | AN3744 | Ribosome activity | 35 |

**Supplementary Table 4.**
Continued.

| Found in vegetative mycelium (299 proteins) | | | | | |
|---|---|---|---|---|---|
| **Gene ID** | **Description** | **Spectral counts found in veg. mycelium** | **Gene ID** | **Description** | **Spectral counts found in veg. mycelium** |
| | | **20h** | | | **20h** |
| AN3804 | IgE binding activity | 25 | AN4865 | Nucleic acid binding | 35 |
| AN3807 | Lipid binding activity | 7 | AN4890 | Role in Golgi to plasma membrane transport | 13 |
| AN3824 | Phenylalanine-tRNA ligase activity | 65 | AN4891 | Histone binding activity | 10 |
| AN3827 | Role in cell proliferation | 17 | AN4892 | Role in mRNA processing | 13 |
| AN3830 (IleA) | L-threonine dehydratase | 38 | AN4908 | Eukaryotic translation initiation factor subunit | 184 |
| AN3832 | Mitochondrial translation | 11 | AN4923 | 3-hydroxy-3-methylglutaryl coenzyme A synthase | 147 |
| AN3839 (SwoF) | Control of polar growth | 25 | AN4947 | Protein of unknown function | 5 |
| AN3933 | DNA-directed RNA polymerase activity | 42 | AN5068 | Aryl formamidase activity | 16 |
| AN4025 | Tubulin-tyrosine ligase | 10 | AN5141 | Protein of unknown function | 28 |
| AN4038 | Translation initiation factor | 133 | AN5186 | Ubiquitin thiolesterase activity | 20 |
| AN4051 | Heme binding | 36 | AN5318 | Non-ribosomal peptide synthetase | 68 |
| AN4094 | Role in sterol metabolism | 5 | AN5521 (AlpA) | Microtubule stabilizing | 15 |
| AN4187 | Helicase activity | 21 | AN5529 (CotA) | Protein involved in cell polarity | 24 |
| AN4192 | Heat shock protein binding | 16 | AN5629 | NADH dehydrogenase | 7 |
| AN4207 | Protein transporter activity | 34 | AN5635 (TreB) | Trehalase | 23 |
| AN4258 | Role in phospholipid metabolism | 24 | AN5652 | Hydantoinase/oxoprolinase | 29 |
| AN4300 | Protein of unknown function | 5 | AN5666 (MpkA) | Mitogen-activated protein kinase (MAP kinase) | 25 |
| AN4346 | Nucleotide binding activity | 10 | AN5688 | Role in lipid biosynthetic process | 11 |
| AN4380 | Ribonucleotide reductase | 77 | AN5702 | Role in ribosomal large subunit biogenesis | 11 |
| AN4412 | Nuclear exosomal DEAD-box family RNA helicase | 10 | AN5705 | Protein of unknown function | 12 |
| AN4430 | Acetolactate synthase | 34 | AN5713 | chaperonin complex component | 65 |
| AN4431 | RNA binding activity | 5 | AN5716 | Inosine-5'-monophosphate dehydrogenase | 17 |
| AN4453 | Protein of unknown function | 22 | AN5717 (KapI) | Required for normal hyphal growth | 117 |
| AN4460 | 18S rRNA processing complex subunit | 4 | AN5743 | Potassium-transporting ATPase | 8 |
| AN4483 | Serine/threonine kinase activity | 6 | AN5747 | Protein domain specific binding | 20 |
| AN4492 | Ubiquitin-specific protease activity | 25 | AN5775 | DNA binding transcription factor | 4 |
| AN4523 | Spliceosome involved in pre-mRNA splicing | 37 | AN5823 | Protein of unknown function | 38 |
| AN4546 | RNA binding activity | 33 | AN5865 | GTP-binding protein | 20 |
| AN4547 | Role in establishment or maintenance of cell polarity | 90 | AN5883 (MetF) | Methylenetetrahydrofolate reductase | 59 |
| AN4563 | Protein serine/threonine kinase activity | 30 | AN5894 | RNA polymerase II core binding | 11 |
| AN4564 (TeaA) | Cell-end marker protein | 11 | AN5918 (CatC) | Catalase with a role in gluconic acid | 6 |
| AN4602 | Tubulin binding activity | 9 | AN5931 | ATP-dependent RNA helicase | 13 |
| AN4717 (PkaB) | Cyclic AMP-dependent protein kinase A (PKA) | 9 | AN5957 | Amino acid aminotransferase | 78 |
| AN4762 | Protein of unknown function | 11 | AN5970 | Disulfide isomerase, ER localization | 13 |
| AN4774 | Uroporphyrin-III C-methyltransferase | 48 | AN5972 | Ubiquitin binding activity | 24 |

**Supplementary Table 4.**
Continued.

| Found in vegetative mycelium (229 proteins) | | | | | | |
|---|---|---|---|---|---|---|
| **Gene ID** | **Description** | **Spectral counts found in veg. mycelium** | **Gene ID** | **Description** | **Spectral counts found in veg. mycelium** | |
| | | **20h** | | | | **20h** |
| AN5973 (PkcB) | Similarity to protein kinase C | 26 | AN6980 | Component of the nuclear pore complex | | 10 |
| AN5992 | ATP binding | 9 | AN6993 | Phosphoribosyltransferase | | 27 |
| AN6006 (KapD) | Karyopherin and nuclear receptor | 75 | AN7005 | Protein of unknown function | | 7 |
| AN6014 (FaaA) | Long-chain-fatty-acid-CoA ligase | 12 | AN7028 | Thymidylate synthase | | 6 |
| AN6033 | Role in ER to Golgi vesicle-mediated transport | 28 | AN7146 | Role in sterol metabolism | | 24 |
| AN6058 | Menadione stress-induced protein | 20 | AN7169 (FhbA) | NirA-dependent flavohemoprotein | | 167 |
| AN6067 | GTPase activator activity | 43 | AN7199 | Cytoplasm localization | | 29 |
| AN6126 (AccA) | Acetyl-CoA carboxylase | 377 | AN7208 | Protein of unknown function | | 19 |
| AN6168 (MaeA) | Malate dehydrogenase | 40 | AN7297 | Role in chromatin remodeling | | 4 |
| AN6193 | ATP-dependent peptidase activity | 37 | AN7422 | ubiquitin carboxyl-terminal hydrolase | | 12 |
| AN6207 | ATP binding | 14 | AN7441 | ATP-dependent 3'-5' DNA helicase activity | | 25 |
| AN6231 (TrpB) | Tryptophan synthase | 149 | AN7474 | RNA binding | | 8 |
| AN6266 | Ribosome biogenesis and nucleus localization | 6 | AN7498 | Deoxyhypusine monooxygenase activity | | 26 |
| AN6267 | Nucleotide binding activity | 11 | AN7540 | Translation initiation factor | | 93 |
| AN6366 | Oxidoreductase activity | 8 | AN7544 | Zinc ion binding | | 7 |
| AN6489 | Ribosome activity, role in translation | 19 | AN7659 | RNA helicase activity | | 60 |
| AN6508 | Protein kinase | 52 | AN7687 | Membrane translocase complex | | 31 |
| AN6510 | Translocase of outer mitochondrial membrane complex | 12 | AN7699 | Protein of unknown function | | 4 |
| AN6515 | Protein targeting to endoplasmic reticulum membrane | 35 | AN7706 | Nucleic acid binding | | 8 |
| AN6521 (LysF) | Homoaconitate hydratase | 65 | AN7752 | Ferrochelatase activity | | 5 |
| AN6643 (BioB) | Biotin synthase | 48 | AN7928 | Possible pseudogene | | 4 |
| AN6651 | Ribosomal small subunit biogenesis | 33 | AN7995 | Role in ribose metabolism | | 9 |
| AN6676 | Nucleic acid binding | 9 | AN8023 (VpsA) | Required for vacuole biogenesis | | 85 |
| AN6705 | DNA-dependent ATPase activity | 35 | AN8038 | Nucleic acid binding | | 10 |
| AN6712 (PldA) | Phospholipase D | 7 | AN8072 | Protein of unknown function | | 9 |
| AN6731 (SdeA) | Delta-9-stearic acid desaturase | 22 | AN8073 | RNA binding, S-adenosylmethionine-dependent | | 7 |
| AN6734 (KapF) | Karyopherin | 73 | AN8102 (PepAc) | Pepsin-like aspartic protease | | 4 |
| AN6753 | NADH-dependent flavin oxidoreductase | 35 | AN8118 | cytochrome c oxidase | | 4 |
| AN6838 (TubC) | Beta-tubulin | 149 | AN8225 | Ribosomal protein L1 | | 6 |
| AN6853 | Phosphatidylinositol transporter activity | 25 | AN8233 | Role in phospholipid transport | | 51 |
| AN6865 | Protein of unknown function | 7 | AN8268 | RasGAP SH3 binding protein | | 47 |
| AN6866 (AroC) | Role in aromatic amino acid biosynthesis | 12 | AN8346 | Oxidoreductase activity | | 49 |
| AN6903 | Nucleic acid binding | 13 | AN8488 | Palmitoyltransferase activity | | 13 |
| AN6922 | Role in cytoplasmic translation | 11 | AN8706 (GnaA) | Glucosamine-phosphate N-acetyltransferase | | 13 |
| AN6978 | Nucleotide exchange factor | 17 | AN8709 | Aspartate transaminase | | 34 |

**Supplementary Table 4.**
Continued.

| Found in vegetative mycelium (229 proteins) | | | | | |
|---|---|---|---|---|---|
| **Gene ID** | **Description** | **Spectral counts found in veg. mycelium** | **Gene ID** | **Description** | **Spectral counts found in veg. mycelium** |
| | | **20h** | | | **20h** |
| AN8722 | ATP-dependent RNA helicase | 39 | AN1177 | Structural molecule activity | 75 |
| AN8748 | Palmitoyl-(protein) hydrolase activity | 12 | AN1194 | Adenylyl phosphosulfate kinase | 18 |
| AN8794 | RNA binding | 6 | AN12101 | NADPH dehydrogenase | 21 |
| AN8815 | Isoflavone reductase family protein | 40 | AN12272 | Protein of unknown function | 5 |
| AN8819 | Dehydrogenase | 7 | AN12419 | DNA-directed RNA polymerase activity | 9 |
| AN8836 | PAK (p21-activated kinase) family protein | 14 | AN1270 | Translation initiation factor 3 | 40 |
| AN8843 | Homoserine kinase | 54 | AN1281 | Chaperone binding, protein binding | 27 |
| AN8853 (SnpA) | Eukaryotic polypeptide releasing | 33 | AN1287 (StoB) | Stomatin-like protein | 4 |
| AN8859 | Aspartate kinase | 32 | AN1288 | Protein of unknown function | 77 |
| AN8862 (MyoA) | Involved in the movement of vesicles to the hyphal tip | 30 | AN0922 | Golgi to ER and COPI vesicle coat localization | 37 |
| AN8869 | Uracil phosphoribosyltransferase | 10 | AN0999 (CapA) | Adenylate cyclase-associated protein | 31 |
| AN8874 | GTPase activity | 38 | AN1006 (NiaD) | Nitrate reductase | 38 |
| AN9067 (TinC) | NIMA-interacting protein | 37 | AN10088 | Protein of unknown function | 9 |
| AN9090 (RrmA) | RNA binding protein | 50 | AN10103 | DNA binding activity | 19 |
| AN9149 | Protein of unknown function | 14 | AN10156 | Phosphotransferase activity | 27 |
| AN9304 (ElfA) | Glutathione S-transferase | 31 | AN10173 | RNA binding activity | 24 |
| AN9357 | Cytoplasm localization | 9 | AN1023 (SagA) | Sensitivity to DNA-damaging agents | 12 |
| AN9467 | Serine/threonine phosphatase activity | 13 | AN10257 | mRNA binding activity | 42 |
| AN9497 | Hydrolase activity | 5 | AN1306 | Role in actin filament severing | 18 |
| AN10301 | Nucleotide binding activity | 10 | AN1328 (NnaA) | GNAT-type acetyltransferase | 6 |
| AN10337 | Enzyme regulator activity | 28 | AN1401 (KapK) | Essential exportin 1 | 62 |
| AN10350 | Phosphatidylinositol binding activity | 55 | AN1405 | Protein of unknown function | 4 |
| AN10352 | rRNA processing | 26 | AN1408 | U5 snRNP-specific protein | 42 |
| AN10396 | Farnesyl-diphosphate farnesyltransferase | 5 | AN1466 | Nucleus localization | 25 |
| AN1045 | RNA binding | 30 | AN1524 | Methylenetetrahydrofolate dehydrogenase | 21 |
| AN10475 | Tryptophan-tRNA ligase | 41 | | | |

**Supplementary Table 4.**
Continued.

| Shared in sexual and asexual mycelium (221 proteins) | | | | |
|---|---|---|---|---|
| Gene ID | Description | Spectral counts found in sex. mycelium | | |
| | | 3 day | 5 day | 7 day |
| AN0050 | Picolinic acid decarboxylase | 29 | 28 | |
| AN0076 | Role in interspecies interaction | 11 | 2 | 4 |
| AN0089 (AvaA) | Small GTPase | 4 | | 10 |
| AN0131 | Cytoplasm localization | 2 | 6 | |
| AN0184 | Role in actin filament | 6 | 2 | |
| AN0193 | Hydrolase activity | 11 | 12 | 10 |
| AN0224 | Dipeptidase activity | 15 | 18 | 18 |
| AN0231 (IvoB) | Conidiophore-specific phenol oxidase | 15 | 9 | 15 |
| AN0248 (PdiB) | Disulfide isomerase | 13 | 2 | |
| AN0391 | Protein of unknown function | 58 | 69 | |
| AN0400 | Protein of unknown function | | 10 | 10 |
| AN0447 | Iron ion binding | 11 | | 12 |
| AN0733 (HhtA) | Histone H3; core histone protein | 2 | | 2 |
| AN0768 | Protein of unknown function | 31 | 60 | 26 |
| AN0774 | Nucleotide binding | 7 | 10 | |
| AN0775 | Oxidoreductase activity | 5 | 20 | |
| AN0783 | Carbon-nitrogen ligase | | 32 | 4 |
| AN0824 (ScdA) | Short-chain fatty acid beta-oxidation | 15 | 54 | 8 |
| AN0880 | Peroxisomal targeting signal 2 | 21 | 21 | |
| AN0886 | Role in nitrogen metabolism | | 16 | 14 |
| AN0895 | Oxidoreductase | 41 | 87 | 19 |
| AN0933 (CrhC) | Transglycosidase | 10 | 10 | 4 |
| AN0942 (LadA) | L-arabinitol 4-dehydrogenase | 15 | 43 | 14 |
| AN10054 | Phosphotransferase activity | 8 | 9 | |
| AN10060 | Alpha-amylase | 41 | 116 | 11 |
| AN10109 | Protein of unknown function | 19 | 32 | |
| AN10131 (PyrD) | Dihydroorotase | 4 | 16 | |
| AN10146 | Protein of unknown function | 8 | 20 | |
| AN10197 | Role in coenzyme M biosynthetic | 12 | 4 | |
| AN10217 | Oxidoreductase activity | 3 | 4 | |
| AN10233 | Phosphogluconate dehydrogenase | 8 | 119 | |
| AN10260 | Menadione stress-induced protein | 14 | 61 | |
| AN10335 | Nucleotide binding | 6 | 9 | |
| AN10421 | Protein of unknown function | 3 | 13 | |
| AN10437 | Mitochondrion localization | 22 | 21 | |
| AN10482 | Beta-glucosidase | | 5 | 4 |
| AN10494 | Nucleotide binding | 12 | 13 | 5 |
| AN1050 | 3-ketoacyl-CoA thiolase | 23 | 91 | |
| AN10507 | Unfolded protein binding activity | | 6 | 10 |
| AN10520 | Alpha/beta hydrolase | 3 | 32 | |
| AN10626 (RmtA) | Arginine methyltransferase | 3 | 146 | 18 |
| AN10695 (AspE) | Septin | 4 | 3 | 4 |
| AN10700 | Protein of unknown function | 7 | 10 | |
| AN10708 | Role in proteasomal ubiquitin | 15 | 42 | 6 |
| AN1074 | Glycine dehydrogenase | 9 | 6 | |
| AN10797 | Catalytic activity | 8 | 10 | 4 |
| AN10839 | Role in retrograde transport | 8 | 16 | |
| AN11039 | Carbon-sulfur lyase activity | 4 | 20 | 4 |
| AN11062 | Protein of unknown function | 11 | 9 | 8 |
| AN11161 | Phosphatidylserine decarboxylase | 14 | 14 | 4 |
| AN11187 | Monooxygenase activity | 17 | 10 | |
| AN11233 | Chitinase activity | 23 | 3 | |
| AN11778 | Exoinulinase | 13 | 29 | |
| AN1184 | Ribosome localization | 13 | | 8 |
| AN11862 | TRC complex localization | 11 | 9 | |
| AN11908 | Hydrolase activity | 4 | 7 | |
| AN1191 (SumO) | Small ubiquitin-like modifier (SUMO) protein | | 2 | 5 |
| AN11945 | Protein of unknown function | 2 | 18 | 4 |
| AN1197 | Catalytic activity | 16 | 5 | 4 |
| AN11985 | Protein of unknown function | | 4 | 13 |
| AN12004 | Protein of unknown function | 7 | 7 | |
| AN12070 | Carbon-nitrogen ligase activity | 3 | 5 | |
| AN12198 | Protein of unknown function | | 4 | 8 |
| AN12221 | Deoxyribose-phosphate aldolase activity | 9 | 19 | 4 |

**Supplementary Table 4.**
Continued.

| Shared in sexual and asexual mycelium (221 proteins) | | | | |
|---|---|---|---|---|
| Gene ID | Description | Spectral counts found in sex. mycelium | | |
| | | 3 day | 5 day | 7 day |
| AN12229 | Zinc ion binding activity | 13 | | 14 |
| AN12466 | Glutathione synthase activity | 7 | 11 | |
| AN1418 | Glucosamine-6-phosphate deaminase activity | 22 | 4 | |
| AN1427 | Role in transmembrane transport | 8 | 10 | 2 |
| AN1428 | N-acetylglucosamine-6-phosphate deacetylase | 42 | 47 | 2 |
| AN1503 | Dihydrodipicolinate synthase | 10 | 19 | |
| AN1659 | Amino acid transporter | | 10 | 5 |
| AN1715 | Mannose-6-phosphate isomerase | 31 | 87 | 30 |
| AN1734 | Protein of unknown function | 5 | 45 | |
| AN1742 | Fumarylacetoacetase activity | 38 | 4 | |
| AN1809 | Fumarylacetoacetase | | 34 | 66 |
| AN1868 | Glycerol dehydrogenase | 15 | 12 | |
| AN1882 | Role in cell redox homeostasis | | 58 | 7 |
| AN1896 (FahA) | Fumarylacetoacetate hydrolase | 41 | 79 | 27 |
| AN1897 (HmgA) | Homogentisate 1,2-dioxygenase | 41 | | 16 |
| AN1899 (HpdA) | Role in aromatic amino acid biosynthes | 24 | 7 | 4 |
| AN1947 | Hydrolase activity | 5 | 12 | |
| AN2085 | Endopeptidase activity | 3 | | 5 |
| AN2091 | Carboxy-lyase activity | 9 | 4 | |
| AN2133 | Uracil phosphoribosyltransferase | 7 | 45 | 8 |
| AN2332 | Succinate dehydrogenase | 5 | 49 | |
| AN2393 | Nucleotide binding | 10 | 25 | |
| AN2404 | Protein of unknown function | 5 | 6 | |
| AN2470 | Nucleotide binding | 32 | 45 | 4 |
| AN2471 | Protein of unknown function | 15 | 8 | |
| AN2479 (Ngn16) | GNAT-type acetyltransferase | 4 | 4 | |
| AN2532 | Copper ion binding | 11 | 12 | |
| AN2548 (EasC) | Required for emericellamide biosynthesis | 4 | 2 | 4 |
| AN2720 | Catalytic activity | 8 | 30 | 4 |
| AN2815 | Mannitol 2-dehydrogenase | 11 | 81 | 3 |
| AN2847 | DNA binding activity | 15 | 13 | |
| AN2879 | Carbon-nitrogen ligase activity | 10 | | 15 |
| AN2896 | Catalytic activity | 10 | 20 | 10 |
| AN2977 | Role in small molecule transport | 2 | 4 | |
| AN3017 | Epoxide hydrolase | 25 | 11 | |
| AN3091 | Metalloendopeptidase | 44 | 12 | 9 |
| AN3104 | Allantoicase | 10 | 12 | |
| AN3200 | Beta-glucuronidase | 4 | 16 | |
| AN3222 | IMP 5'-nucleotidase activity | 22 | 2 | |
| AN3299 | Protein of unknown function | 11 | 7 | |
| AN3305 | Nucleotide binding activity | 18 | 47 | |
| AN3334 | Nucleotide binding activity | 37 | 145 | 19 |
| AN3351 | UDP-N-acetylmuramate dehydrogenase activity | 28 | 18 | 9 |
| AN3361 (NopA) | Rhodopsin family G-protein coupled receptor-like | 8 | 19 | 9 |
| AN3431 | Nicotinate-nucleotide diphosphorylase | 9 | 21 | |
| AN3605 | Oxidoreductase activity | 7 | 7 | 9 |
| AN3627 | Intracellular localization | 47 | 2 | 2 |
| AN3634 | Phosphoribosyltransferase | 11 | 25 | 7 |
| AN3679 | Nucleotide binding | 6 | 44 | |
| AN3756 | Endopeptidase activity | 9 | 2 | 2 |
| AN3869 | Role in sterol metabolism | 44 | 10 | 6 |
| AN3987 | Tetratricopeptide | 7 | 4 | |
| AN4018 | Role in protein targeting to vacuole | 28 | 13 | 19 |
| AN4048 | Endodeoxyribonuclease activity | 4 | | 19 |
| AN4052 (ExgC) | Glucan 1,3-beta-glucosidase | 9 | | 29 |
| AN4245 | Ceramidase | 18 | 10 | |
| AN4268 | Monooxygenase activity | 2 | 28 | |
| AN4397 | Protein of unknown functionp | 9 | 15 | |
| AN4421 | Oxidoreductase activity | 46 | 123 | 11 |
| AN4452 | Ribosome activity | 2 | 3 | 2 |
| AN4467 (CypB) | Cyclophilin B | 4 | 5 | |
| AN4531 | Protein of unknown function | 10 | 7 | |
| AN4688 (IvdA) | Acyl-coA dehydrogenase | 17 | 23 | 4 |
| AN4690 (MccA) | Alpha subunit of 3-methylcrotonyl-CoA carboxylase | 19 | 10 | |

**Supplementary Table 4.**
Continued.

| Shared in sexual and asexual mycelium (221 proteins) | | | | |
|---|---|---|---|---|
| Gene ID | Description | Spectral counts found in sex. mycelium | | |
| | | 3 day | 5 day | 7 day |
| AN4807 | Protein of unknown function | 6 | 3 | 4 |
| AN4809 (GtaA) | Glutaminase A | 61 | 33 | 27 |
| AN4843 | Alpha-glucosidase | | 6 | |
| AN4913 (PhK) | Phosphoketolase | 5 | 25 | 2 |
| AN4914 | Acetate kinase | 22 | 34 | |
| AN4957 (GalE) | Galactokinase | 11 | 22 | 4 |
| AN4979 | Dihydroneopterin aldolase activity | 8 | 10 | |
| AN4987 (PkaR) | Protein kinase A | 30 | 47 | |
| AN5021 | Role in biosynthetic process | 19 | 21 | 2 |
| AN5110 | Protein of unknown function | 4 | 32 | 2 |
| AN5194 | ATPase activity | 7 | 2 | 3 |
| AN5354 | Oxidoreductase activity | 7 | 12 | |
| AN5387 | Protein of unknown function | 7 | 27 | |
| AN5411 | Metal ion binding, phosphoric diester hydrolase | 9 | 28 | 2 |
| AN5452 | Splicing factor 3b | 25 | 28 | |
| AN5524 | Hydrolase activity | 8 | 5 | |
| AN5607 | Role in proteasome assembly | 26 | 2 | |
| AN5613 (HxA) | Xanthine dehydrogenase | 29 | 2 | |
| AN5658 | Gamma-glutamyltransferase activity | 35 | 16 | |
| AN5704 | Type II fatty acid synthase | 10 | 9 | |
| AN5831 | Chorismate synthase | 8 | 16 | |
| AN5879 | Ribosomal small subunit assembly | 5 | 32 | 5 |
| AN5916 | Mitochondrial enoyl-CoA hydratase | | 55 | 6 |
| AN5942 | Protein of unknown function | | 7 | 2 |
| AN5989 | Epimerase/dehydratase | | 36 | 15 |
| AN5994 | ATP-dependent NAD(P)H-hydrate dehydratase | 56 | 36 | 2 |
| AN6066 | Catalytic activity | 19 | 20 | |
| AN6111 | Exosome | | 6 | 11 |
| AN6169 | Rho guanyl-nucleotide exchange factor | 7 | 9 | 10 |
| AN6274 | Oxidoreductase activity | 50 | 41 | 5 |
| AN6438 | Aromatic-amino-acid transaminase | 20 | 18 | 24 |
| AN6512 | ATPase activity | 6 | 6 | |
| AN6536 (HisB) | Imidazole glycerol-phosphate dehydratase | 7 | 2 | |
| AN6606 | Role in post-translational protein modification | 8 | 10 | |
| AN6635 (YA) | Conidial laccase (p-diphenol oxidase) | 30 | 62 | 22 |
| AN6654 | Glutamate-ammonia ligase activity | 10 | | |
| AN6655 (GudC) | Glucose 1-dehydrogenase | 11 | 44 | 8 |
| AN6723 (DhbD) | 2,3-dihydroxybenzoate carboxylyase | 6 | 13 | 5 |
| AN6792 (GfdB) | Dehydrogenase | 5 | 91 | |
| AN6804 | Transporter of the major facilitator superfamily (MFS) | 7 | 9 | 12 |
| AN6862 | Protein of unknown function | 10 | | |
| AN6918 | Oxidoreductase | 2 | | 26 |
| AN6923 (HxtA) | High-affinity hexose transporter | 5 | 9 | 2 |
| AN6985 | Ribulokinase | 48 | 60 | 37 |
| AN7008 (HadA) | Mitochondrial hydroxyacyl-CoA dehydrogenase | 10 | 10 | |
| AN7011 | Protein of unknown function | 13 | 6 | |
| AN7035 | Peptidase activity | | 8 | 4 |
| AN7140 | Protein of unknown function | 34 | | |
| AN7181 | Protein of unknown function | 38 | 46 | 20 |
| AN7231 | Serine-type peptidase | 15 | 27 | 4 |
| AN7278 | Glutamate decarboxylase | 12 | 15 | |
| AN7298 | Starvation-induced autophagy | 17 | 15 | 16 |
| AN7331 | Cyanate hydratase activity | 12 | 31 | 5 |
| AN7471 | Hydrolase activity | 4 | 3 | |
| AN7517 | Protein of unknown function | 22 | 4 | 6 |
| AN7691 (PlcB) | Phospholipase | 14 | 7 | |
| AN7742 | Single-stranded DNA binding activity | 14 | 8 | 2 |
| AN7805 (StcV) | Role in sterigmatocystin/aflatoxin biosynthesis | 13 | 21 | 24 |
| AN7907 | Glyoxylate-bleomycin resistance protein | 15 | 6 | |
| AN7911 (OrsB) | Member of the F9775 SM gene cluster | 35 | 71 | 9 |
| AN7959 | Protein of unknown function | 4 | | 15 |
| AN8010 | Glycogen (starch) synthase | 14 | 16 | |
| AN8046 | Triacylglycerol lipase | 6 | 25 | 2 |
| AN8050 | Protein of unknown function | 7 | 8 | |

**Supplementary Table 4.**
Continued.

| Shared in sexual and asexual mycelium (221 proteins) | | | | |
|---|---|---|---|---|
| **Gene ID** | **Description** | **Spectral counts found in sex. mycelium** | | |
| | | **3 day** | **5 day** | **7 day** |
| AN8219 | Plasma membrane ATPase | 25 | 9 | |
| AN8335 | Protein of unknown function | 12 | 51 | |
| AN8396 (PdcB) | Pyruvate decarboxylase | 36 | 34 | 4 |
| AN8445 | Aminopeptidase Y | 26 | 35 | 31 |
| AN8496 | Metal ion binding activity | 23 | 16 | 16 |
| AN8561 | Nucleotide binding | 2 | | 15 |
| AN8566 | Catechol 1,2-dioxygenase activity | | 33 | 4 |
| AN8628 | Cofactor binding | 5 | 64 | 4 |
| AN8638 (cetJ) | Transcript enriched in dormant conidia induced by light | 37 | 24 | |
| AN8639 | Alpha-trehalose-phosphate synthase | 19 | 82 | 2 |
| AN8737 (MstA) | Sugar transporter | 3 | 2 | 9 |
| AN8744 | Oxidoreductase activity | 2 | 5 | |
| AN8755 (MclA) | Methylisocitrate lyase | 24 | 43 | 6 |
| AN8777 (amdS) | Acetamidase | 13 | 3 | 6 |
| AN8782 | S-formylglutathione hydrolase | 13 | 44 | 10 |
| AN8803 (RodA) | Hydrophobin | 26 | 9 | 11 |
| AN8908 | Phosphoric diester hydrolase | 9 | 53 | 14 |
| AN8932 | TIM-barrel enzyme family protein | 10 | 9 | |
| AN8968 | Isoflavone reductase | 5 | 38 | |
| AN8977 (AlcP) | Gluconolactonase | 18 | 6 | 4 |
| AN9011 | Aryl-alcohol oxidase-related | 15 | 23 | 4 |
| AN9054 | Protein of unknown function | 5 | 53 | 26 |
| AN9064 | Xylitol dehydrogenase | 25 | 30 | 2 |
| AN9130 (AifA) | Apoptosis-inducing factor (AIF)-like | 53 | 17 | 10 |
| AN9183 (BglR) | Beta-glucosidase | 3 | 4 | 4 |
| AN9348 | Aryl-alcohol oxidase-related protein | 13 | 16 | |
| AN9380 | Chitin deacetylase | 13 | 17 | 4 |
| AN9425 | Carbon-carbon lyase | 6 | 6 | 22 |
| AN9434 | Nucleus localization | | 10 | 10 |

| Shared in sexual, asexual and vegetative mycelium (164 proteins) | | | | |
|---|---|---|---|---|
| **Gene ID** | **Description** | **Spectral counts found in sex. mycelium** | | |
| | | **3 day** | **5 day** | **7 day** |
| AN0121 (HemC) | Porphobilinogen deaminase | 7 | | 9 |
| AN0140 | Role in actin assembly | 24 | 15 | 5 |
| AN0163 | Rho GDP-dissociation inhibitor activity | | 12 | |
| AN0182 (RasA) | Small monomeric GTPase | 5 | | |
| AN0261 (Sec23) | COPII coat component | 13 | 5 | |
| AN0271 | dUTP pyrophosphatase | 15 | 3 | 10 |
| AN0306 | Role in regulation of actin filament polymerization | 29 | 23 | 4 |
| AN0317 | Ubiquitin binding activity | 20 | 8 | 12 |
| AN0410 (BimG) | Required for the completion of anaphase | 22 | 8 | |
| AN0445 | Ribosome activity | 16 | 79 | 10 |
| AN0570 | RNA binding | 6 | 11 | 22 |
| AN0648 (TrpC) | Involved in tryptophan biosynthesis | 41 | 41 | 21 |
| AN0667 (ManA) | Mannose-6-phosphate isomerase | 22 | 28 | 8 |
| AN0705 | Isoleucine-tRNA ligase activity | 10 | 26 | 3 |
| AN0797 | Multifunctional enzyme | 65 | 29 | 57 |
| AN0847 | Chaperone | 3 | 8 | 19 |
| AN0858 (Hsp104) | Chaperone | 4 | 2 | |
| AN0906 (KapB) | Essential nuclear transport protein | 12 | | 7 |
| AN0912 | Beta-isopropylmalate dehydrogenase | 11 | 23 | 17 |
| AN0952 | Acid phosphatase activity | 7 | | 13 |
| AN10020 | Regulator activity | 25 | 13 | 6 |
| AN10079 (UreB) | Urease | 7 | 7 | 13 |
| AN10087 | Similar to proliferating cell nuclear antigen (PCNA) | 24 | 46 | 8 |
| AN10188 | Protein of unknown function | 2 | | |
| AN10194 | Helicase activity | 9 | | 2 |
| AN10195 | Valine-tRNA ligase activity | 13 | 15 | 9 |
| AN10220 (Ccp1) | Cytochrome c peroxidase | 14 | | 8 |

**Supplementary Table 4.**
Continued.

| Shared in sexual, asexual and vegetative mycelium (164 proteins) | | | | |
|---|---|---|---|---|
| Gene ID | Description | Spectral counts found in sex. mycelium | | |
| | | 3 day | 5 day | 7 day |
| AN10222 | Glucose-6-phosphate 1-epimerase activity | 31 | 31 | |
| AN10230 | S-methyl-5-thioadenosine phosphorylase activity | 20 | 14 | 9 |
| AN10284 | Glutathione synthase | 12 | 8 | 7 |
| AN10296 | Fumarate reductase (NADH) activity | 32 | 10 | 14 |
| AN10298 | 3-phosphoserine aminotransferase | 37 | 160 | 5 |
| AN10512 (MthA) | Mitochondrial ketoacyl-CoA thiolase | 26 | 92 | 5 |
| AN10533 | Trehalose-6-phosphate synthase | | 2 | 2 |
| AN10681 | Ribosome activity | 5 | 10 | 2 |
| AN10710 | Rhosphomannomutase activity | 13 | 19 | |
| AN10718 (RiboG) | 6,7-mimethyl-8-ribityl-lumazine synthase | 23 | 46 | 5 |
| AN10740 | Ribosome activity, role in translati | 14 | 75 | 25 |
| AN10783 | Coenzyme binding | 15 | 44 | |
| AN10901 | Glycine dehydrogenase | 11 | 51 | 37 |
| AN10942 | Actin depolymerizing | 15 | 35 | 13 |
| AN10981 | Bifunctional GTP cyclohydrolase II | 16 | 10 | 2 |
| AN11054 | Alpha-1,4-glucosidase | 12 | 6 | 10 |
| AN11070 | Metalloaminopeptidase activity | 45 | 163 | 7 |
| AN11094 | Zinc-binding oxidoreductase | 4 | 67 | |
| AN1198 | Aminomethyltransferase | 12 | 38 | 11 |
| AN12237 (AbpA) | Actin-binding protein | 8 | 13 | 5 |
| AN1274 | NADP+ 1-oxidoreductase activity | 33 | 79 | |
| AN1345 | Structural constituent of ribosome activity | 6 | 17 | 10 |
| AN1429 (CodA) | Choline oxidase | 26 | 46 | |
| AN1485 | Serine/threonine kinase activity | 27 | 6 | 3 |
| AN1534 | Succinate dehydrogenase | 46 | 90 | 10 |
| AN1677 | Short-chain dehydrogenase | 10 | 90 | 10 |
| AN1805 (CanB) | Carbonic anhydrase | 19 | | 21 |
| AN1913 | Lysyl-tRNA synthetase | | 78 | 8 |
| AN2306 | Ubiquinol-cytochrome-c reductase | 4 | 2 | |
| AN2317 | Actin depolymerizing protein | 5 | 3 | |
| AN2414 | NADH dehydrogenase (ubiquinone) | 11 | 24 | 2 |
| AN2440 | Ribose-5-phosphate isomerase | 18 | 16 | |
| AN2873 (LysA) | Saccharopine dehydrogenase | 22 | 81 | 3 |
| AN2882 | Homoserine dehydrogenase | 59 | 129 | 9 |
| AN2930 | Mitochondrion localization | 9 | 14 | 2 |
| AN2964 (PdhX) | Dehydrogenase complex component | 19 | 7 | |
| AN2970 | Phosphatase activity | 10 | 9 | |
| AN2998 | Multifunctional enzyme | 18 | 133 | 5 |
| AN3034 (SuAprgA1) | Regulation of penicillin biosynthesis | 7 | 31 | 2 |
| AN3223 (PfkA) | 6-phosphofructokinase | 36 | 171 | 19 |
| AN3469 (H2B) | Histone H2B | 3 | 5 | 2 |
| AN3581 (TrxR) | Thioredoxin reductase | 34 | 58 | 4 |
| AN3591 | Propionyl-CoA-yielding methylmalonate semialdehyde | 74 | 77 | |
| AN3593 | Aldolase/adducin domain protein | 9 | 27 | |
| AN3626 | Phosphoribosylamino-imidazole-carboxylase | 18 | 30 | 10 |
| AN3695 | Anthranilate synthase | 8 | 9 | 11 |
| AN3702 | Leucine-tRNA ligase activity | 2 | 14 | 5 |
| AN3712 | Dienelactone hydrolase | 25 | 79 | |
| AN3748 | Phosphoribosyltransferase | 44 | 39 | 14 |
| AN3853 | Metalloendopeptidase activity | 22 | 13 | 19 |
| AN3932 | Proteasome beta-5 subunit | 19 | 46 | 13 |
| AN3973 | Peroxiredoxin | 15 | 27 | |
| AN4015 | Translation elongation factor activity | | 41 | 15 |
| AN4060 (Rps16) | Ribosomal protein S16 | 16 | 40 | |
| AN4073 | Cytosolic small ribosomal subunit protein S12 | 7 | 18 | 2 |
| AN4085 | Intracellular localization | 14 | | 4 |
| AN4127 | Intracellular localization | 4 | 6 | |
| AN4174 | Lactoylglutathione lyase | 39 | 68 | 18 |
| AN4178 (Myg1) | UPF0160 domain-containing protein | 15 | 26 | 12 |
| AN4202 (Rpl16A) | Ribosomal protein of the large (60S) ribosomal subunit | 8 | 46 | 38 |

**Supplementary Table 4.**
Continued.

| Shared in sexual, asexual and vegetative mycelium (164 proteins) | | | | |
|---|---|---|---|---|
| **Gene ID** | **Description** | **Spectral counts found in sex. mycelium** | | |
| | | **3 day** | **5 day** | **7 day** |
| AN4222 | Ribosome activity | 29 | 54 | 16 |
| AN4234 (PcmA) | Phosphoacetylglucosamine mutase | 22 | 54 | 48 |
| AN4402 | Voltage-gated anion channel activity | 9 | 7 | 9 |
| AN4449 | Endopeptidase activator activity | 21 | 8 | 3 |
| AN4457 | Endopeptidase activator activity | 4 | 13 | 7 |
| AN4550 (Cyp7) | Peptidyl-prolyl cis-trans isomerase D | 29 | | 4 |
| AN4583 | Peptidyl-prolyl cis-trans isomerase D | | 53 | 7 |
| AN4591 | Phosphopentomutase activity | | 50 | 16 |
| AN4803 | rRNA binding | 12 | 101 | 49 |
| AN4859 (PmaA) | Plasma membrane ATPase | 10 | | |
| AN4869 | 20S proteasome component | 19 | 59 | 13 |
| AN5028 (PpoC) | Fatty acid oxygenase | 69 | | 10 |
| AN5121 | Protein similar to proteasome regulatory | 5 | 11 | 13 |
| AN5122 | Kinase | 5 | | 10 |
| AN5222 | Ribosome activity | 12 | 52 | |
| AN5226 (AcpA) | Acetate permease | 5 | 7 | 8 |
| AN5376 | Protein of unknown function | 7 | 10 | 10 |
| AN5482 | Ran GTPase | 27 | 4 | 2 |
| AN5547 | Oxidoreductase | | 8 | 2 |
| AN5577 | Manganese-superoxide dismutase | | 36 | 10 |
| AN5586 | Mannose-1-phosphate guanylyltransferase | | 5 | |
| AN5604 (AcuG) | Fructose-bisphosphatase | 45 | | 28 |
| AN5610 | L-aminoadipate-semialdehyde dehydrogenase | 12 | 62 | 11 |
| AN5616 | Pyridoxal phosphate binding | 9 | 23 | 5 |
| AN5646 | Role in fatty acid degradation | 15 | 42 | 13 |
| AN5662 | Threonine-tRNA ligase activity | 24 | 59 | 23 |
| AN5749 | Metallopeptidase activity | 27 | 32 | 11 |
| AN5784 | Role in proteasomal ubiquitin-dependent protein | 17 | 51 | 14 |
| AN5790 | Isocitrate dehydrogenase (NAD+) | 3 | 18 | |
| AN5793 | 20S proteasome beta-type subunit | 22 | 51 | 22 |
| AN5799 | Gamma-glutamyl phosphate reductase | 18 | 9 | 22 |
| AN5803 (FimA) | Fimbrin protein | 22 | 8 | 10 |
| AN5812 | Epoxide hydrolase activity | 39 | 48 | 24 |
| AN5884 (PyrF) | Orotate phosphoribosyltransferase | 16 | | |
| AN5886 (LuA) | Alpha-isopropylmalate isomerase | 10 | | 10 |
| AN5979 | Role in ribosomal small subunit | 5 | 34 | 2 |
| AN6083 | Role in ribosomal large subunit assembly | 15 | 70 | 11 |
| AN6157 (PyrG) | Orotidine-5'-phosphate decarboxylase | 15 | 36 | 14 |
| AN6182 (GalD) | Hexose-1-phosphate uridylyltransferase | 8 | 9 | 2 |
| AN6214 (Nha1) | Hat1 acetyltransferase | 4 | | 7 |
| AN6227 | Catalytic activity | 29 | 10 | 19 |
| AN6248 | Adenyl-nucleotide exchange factor activity | | 22 | 5 |
| AN6257 | Protein of unknown function | 12 | 15 | 4 |
| AN6368 | Arginyl-tRNA synthetase | 19 | 6 | |
| AN6490 | Phosphorylase | 14 | 27 | 4 |
| AN6650 (McsA) | Methylcitrate synthase | 85 | 141 | 69 |
| AN6726 | Role in proteasomal ubiquitin-dependent protein | 42 | 91 | 13 |
| AN7000 | Succinate-CoA ligase | 11 | 3 | 2 |
| AN7111 (FoxA) | Peroxisomal multifunctional enzyme | 23 | 4 | |
| AN7194 | Nucleotide binding | 25 | 25 | 3 |
| AN7484 | Increased levels during osmoadaptation | 13 | 7 | 4 |
| AN7511 (GelE) | 1,3-beta-transglycosidase | 8 | | 66 |
| AN7554 | Poly(A) RNA binding activity | 2 | 10 | 10 |
| AN7594 | Protein of unknown function | | 6 | |
| AN7721 | Translocon | 4 | | 5 |
| AN8012 (StcN) | Versicolorin B synthase | 32 | 11 | 6 |
| AN8021 (VmaA) | Vacuolar ATPase (V-ATPase) | 32 | 30 | 20 |
| AN8022 | Protein of unknown function | 4 | | 9 |
| AN8032 | C-3 sterol dehydrogenase | 9 | 22 | |
| AN8044 | Metalloendopeptidase activity | 34 | 3 | 35 |

**Supplementary Table 4.**
Continued.

| Shared in sexual, asexual and vegetative mycelium (164 proteins) | | | | |
|---|---|---|---|---|
| **Gene ID** | **Description** | **Spectral counts found in sex. mycelium** | | |
| | | **3 day** | **5 day** | **7 day** |
| AN8057 (CysB) | Cysteine synthase | 16 | 32 | |
| AN8065 | Role in actin filament organization | 5 | | 6 |
| AN8080 | Thioredoxin peroxidase activity | 17 | 80 | 2 |
| AN8204 (NdxC) | Undix hydrolase | 9 | 2 | 6 |
| AN8664 | Hydrolase activity | 19 | 79 | 5 |
| AN8674 | Vacuolar proton-transporting V-type ATPase | 12 | 2 | |
| AN8698 | Role in actin cortical patch assembly | 8 | 41 | |
| AN8870 | Expression increased in salt-adapted strains | 30 | 140 | 27 |
| AN8979 (AlcA) | Alcohol dehydrogenase | 52 | | 24 |
| AN9085 | Protein of unknown function | 10 | 6 | 2 |
| AN9097 | Ribosomal protein | 14 | 20 | 3 |
| AN9124 | Hsp90 protein binding | 18 | 9 | 10 |
| AN9308 | UDP-N-acetylmuramate dehydrogenase activity | 3 | 2 | 2 |
| AN9401 | Mitochondrian localization | 50 | 69 | |
| AN9407 (FasA) | Fatty acid synthase | 16 | 14 | 2 |
| AN9468 | Maturation of SSU-rRNA | 9 | 45 | 3 |
| AN9470 (UaZ) | 5-hydroxy-isourate | 15 | 10 | 2 |

| Shared in asexual and vegetative mycelium (32 proteins) | | | | |
|---|---|---|---|---|
| **Gene ID** | **Description** | **Spectral counts found in asex. mycelium** | | |
| | | **3 day** | **5 day** | **7 day** |
| AN0075 (TigA) | Disulfide isomerase | | 11 | |
| AN0162 | Sequence-specific DNA binding | 2 | 5 | 2 |
| AN0745 | Nucleolar protein | | 2 | 4 |
| AN0943 | Mitochondrial F1F0-ATP synthase | 2 | 5 | 2 |
| AN10229 | NADH dehydrogenase | | 13 | 12 |
| AN11055 | Phosphatase | 4 | | 2 |
| AN1195 | Hydrolase | | 2 | 2 |
| AN1282 | Role in posttranslational protein targeting to membrane | | 14 | |
| AN2086 | Nuclear pore complex protein | 2 | | |
| AN2279 | Endoplasmic reticulum organization | 3 | | 2 |
| AN2316 | Cytochrome c oxidase | | 10 | 6 |
| AN2420 | U2 snRNP localization | 2 | | 10 |
| AN2743 | Regulation of translation in response | | 6 | |
| AN2885 | Role in sequence-specific DNA binding | | 6 | 2 |
| AN3843 | Role in cristae formation | 2 | | 2 |
| AN3906 | Protein of unknown function | 27 | | |
| AN4080 | Role in cytoplasmic translation, polysomal ribosome | | 2 | 2 |
| AN4270 | Role in actin cortical patch assembly | | 2 | |
| AN4919 | Role in actin cortical patch assembly | | 3 | 3 |
| AN4929 | Protein of unknown function | | 43 | |
| AN5181 (NudC) | Protein involved in nuclear migration | | 11 | 2 |
| AN5499 | Nuclear pore complex; Spindle-assembly checkpoint | | 12 | |
| AN5606 | Proton-transporting ATPase activity | 9 | 24 | 7 |
| AN5745 | Eukaryotic translation initiation factor 3 | 4 | 12 | |
| AN6060 | Binding activity and role in RNA metabolic process | 5 | 4 | |
| AN6077 | NADH dehydrogenase | 10 | 4 | 3 |
| AN6287 | F1F0-ATPase complex subunit | 3 | 11 | 6 |
| AN6591 | Essential nuclear export receptor | 3 | 2 | 2 |
| AN7051 (MetG) | Cystathionine beta-lyase | 4 | | |
| AN7159 | Tripeptidyl-peptidase activity | 20 | | |
| AN7177 | Protein of unknown function | | 8 | |
| AN8274 | DNA binding activity | | 4 | 2 |

**Supplementary Table 4.**
Continued.

| Shared in sexual and vegetative mycelium (87 proteins) | | | | |
|---|---|---|---|---|
| Gene ID | Description | Spectral counts found in sex. mycelium | | |
| | | 3 day | 5 day | 7 day |
| AN0057 | Tyrosine-tRNA ligase activity | | 4 | 5 |
| AN0179 | Oxidoreductase | 2 | | 2 |
| AN0351 (GfdA) | Glycerol-3-phosphate dehydrogenase | | 16 | |
| AN0354 (GroG) | 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase | 10 | | |
| AN0495 | Formyltetrahydrofolate deformylase | | 11 | 3 |
| AN0651 (FadA) | Heterotrimeric G protein | | 4 | 5 |
| AN0665 | Involved in vesicle transport | 10 | 7 | 7 |
| AN0673 | Role in actin assembly | 5 | 6 | |
| AN0907 | Ribosome activity | 2 | 11 | 23 |
| AN0997 | Ribosome activity | | 2 | 5 |
| AN10148 (ChpA) | Cysteine- and histidine-rich-domain [CHORD]-containing protein | | 6 | 3 |
| AN10182 | Translation initiation factor 3 | 8 | 7 | |
| AN10278 | Cytosol, mitochondrion, nucleus localization | 5 | 6 | |
| AN10282 | Nucleus localization | | 11 | 5 |
| AN10418 | Protein of unknown function | 2 | 9 | |
| AN10472 | Hydroxyethylthiazole kinase activity | 2 | 5 | |
| AN10526 (RmtA) | Arginine methyltransferase | 2 | 3 | |
| AN11058 | Intracellular localization | 7 | 7 | |
| AN1150 | Role in arginine metabolism | 10 | 7 | |
| AN12246 | Ribosome activity | | 2 | 9 |
| AN1256 | ATPase activity | 10 | 24 | 4 |
| AN1379 | Role in cysteine metabolism | 3 | 18 | |
| AN1394 (AspD) | Septin | 6 | 8 | |
| AN1769 | Role in cysteine metabolism | 3 | 19 | 7 |
| AN2051 | Hsp90p co-chaperone | | 3 | 8 |
| AN2243 | Carbamoyl-phosphate synthase | 10 | 2 | |
| AN2343 | Protein of unknown function | 2 | 10 | 5 |
| AN2733 | Uroporphyrinogen decarboxylase activity | 4 | 2 | |
| AN2775 | Protein of unknown function | | 11 | 5 |
| AN2997 | Protein of unknown function | 2 | 7 | |
| AN3026 (CopA) | Alpha-COP coatamer-related protein | 7 | 6 | |
| AN3456 | Cystathionine gamma-synthase | 2 | 4 | 4 |
| AN3823 | Ribosome activity | 9 | 4 | |
| AN3894 | Aconitate hydratase | | 10 | 7 |
| AN3901 | Lactic acid dehydrogenase | | 3 | 5 |
| AN4086 | Phenylalanine-tRNA ligase | 9 | 6 | |
| AN4401 | Asparagine synthase | | 10 | 2 |
| AN4404 | Aminopeptidase activity | | 2 | 10 |
| AN4463 | Vesicle-mediated transport and clathrin coat of coated pit | 2 | 2 | |
| AN4647 | Protein of unknown function | 2 | 4 | 4 |
| AN4739 | Phosphoribosyl amino imidazolesuccinocarbozamide synthetase | | 5 | 8 |
| AN4820 | Succinate-semialdehyde dehydrogenase | 5 | 16 | |
| AN4872 | Protein of unknown function | | 94 | 5 |
| AN4956 | Acetolactate synthase | | 2 | 4 |
| AN4997 | Phosphatidylinositol transporter | 8 | 12 | |
| AN5123 | Oxidoreductase | | 7 | 12 |
| AN5130 | Coproporphyrinogen oxidase | 2 | 8 | |
| AN5206 (LysB) | Homoisocitrate dehydrogenase | 9 | 10 | |
| AN5534 | Glyoxylate reductase activity | | 2 | 4 |
| AN5589 | Glycerol kinase | 10 | 5 | 5 |
| AN5591 | Aminotransferase | 10 | 8 | 10 |
| AN5731 | Chorismate synthase | 3 | 8 | |
| AN5741 | Ribosome activity | | 5 | 10 |
| AN5778 | Actin binding activity | | 9 | 18 |
| AN5782 | Catalytic activity | | 12 | 19 |
| AN5820 (MecA) | Cystathionine beta-synthase | | 13 | 11 |
| AN5904 | Beta-ketoacyl-[acyl-carrier-protein] synthase | | 12 | 6 |
| AN5954 | Eukaryotic translation initiation factor 3 | | 11 | 8 |
| AN6338 | Aromatic-amino-acid transaminase | | 20 | 5 |
| AN6354 | Ubiquitin C-terminal hydrolase | 3 | | 4 |
| AN6391 (PphA) | Phosphatase | 2 | 3 | 4 |
| AN6505 (RcoA) | WD40 repeat protein | 10 | 19 | 3 |
| AN6541 | Ligase with a role in purine metabolism | 2 | 8 | 4 |
| AN6644 (BiA) | Dethiobiotin synthetase | 7 | 6 | |

**Supplementary Table 4.**
Continued.

| Shared in sexual and vegetative mycelium (87 proteins) | | | | |
|---|---|---|---|---|
| **Gene ID** | **Description** | **Spectral counts found in sex. mycelium** | | |
| | | **3 day** | **5 day** | **7 day** |
| AN7010 | Role in biosynthetic process | | 10 | 10 |
| AN7143 | Cytosol localization | 14 | 10 | |
| AN7289 | Actin monomer binding activity | | 2 | 11 |
| AN7387 (PcrA) | Pyrroline-5-carboxylate reductase | 2 | | |
| AN7430 (HisHF) | Glutamine amidotransferase | 3 | 8 | 4 |
| AN7479 | Asparaginyl-tRNA synthetase | 8 | 3 | |
| AN7587 | Protein of unknown function | | 9 | 12 |
| AN7636 | Oxidoreductase | 5 | 9 | |
| AN7722 | Role in arginine metabolism | 9 | 2 | |
| AN7895 (CipB) | Oxidoreductase | | 37 | |
| AN8121 | 5'-phosphoribosylformyl glycinamidine synthetase | | 9 | |
| AN8224 | Glutamate-tRNA ligase | | 15 | 7 |
| AN8406 | Menadione stress-induced | | 10 | 11 |
| AN8547 | Glucose-methanol-choline (GMC) oxidoreductase | | 5 | 6 |
| AN8790 | D-xylulokinase | 3 | 6 | |
| AN8800 (SgdC) | Cysteinyl tRNA synthase | | 4 | 8 |
| AN8820 (CnaA) | Calmodulin-dependent protein phosphatase | | 13 | 9 |
| AN8824 | Ribosomal large subunit binding activity | 2 | 18 | 16 |
| AN9080 | Nucleus localization | | 8 | 5 |
| AN9157 | Glutamine-tRNA ligase | 6 | 5 | 6 |
| AN9408 (FasB) | Fatty acid synthase | | 32 | 8 |
| AN9419 | Alanine-tRNA ligase | | 23 | 11 |
| AN6906 | Ubiquitin ligase E3 | 10 | 5 | |

**Supplementary Table 5**. **Proteins that were unchanged in *laeA*Δ vegetative mycelium**

The protein quantity of the following proteins is unchanged in a *laeA*Δ vegetative mycelium. The $log_2$ SILAC ratio was determined for the strains *laeA*Δ (AGB1074) in comparison to *laeA* (AGB1092). The following threshholds were set. In the range of a $log_2$ SILAC ratio between -0.5 and + 0.5 the protein quantity is unchanged. The $log_2$ SILAC ratio for the complementation strain (*laeAcomp*$^+$, AGB1076) in comparison to the parental *laeA* strain (AGB1092) is also listed. Ø = median of $log_2$ SILAC ratios, SD = standard deviation, NaN = not a number. Biological replicates are numbered 1-3. Spectral counts (PSM) for three biological replicates are shown (Spectral counts were obtained by Proteome Discoverer 1.4.). The color scales represent $log_2$ SILAC ratios

-5 -3.25 -1.5 -1.3 -1 -0.75 -0.5 -0.25 0 0.25 0.5 0.75 1 1.25 1.5 3.25 5

| Gene ID | Description | Spectral counts | | | *laeA*Δ/*laeA* | | | Ø | SD | *laeAcomp*+/*laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| **Actin** | | | | | | | | | | | | | | |
| AN0306 | Actin filament | 80 | 79 | 105 | -0.5 | 0 | -0.5 | **-0.5** | 0.3 | 0.2 | -0.1 | 0.5 | 0.2 | **0.3** |
| AN6542 (ActA) | Actin A | 335 | 425 | 563 | -0.4 | 0 | 0.0 | **0.0** | 0.2 | 0.2 | -0.1 | 0.5 | 0.2 | **0.3** |
| AN0140 | Actin assembly | 53 | 68 | 91 | -0.3 | 0.1 | -0.1 | **-0.1** | 0.2 | 0.2 | -0.1 | 0.5 | 0.2 | **0.3** |
| AN2756 (SlaB) | Actin binding protein | 117 | 100 | 191 | -0.5 | 0 | -0.5 | **-0.5** | 0.3 | 0 | -0.4 | 0.3 | 0.0 | **0.4** |
| AN2862 | Cytoskeleton protein | 69 | 55 | 84 | -0.5 | -0.1 | -0.5 | **-0.5** | 0.2 | 0.5 | 0 | 0.4 | 0.4 | **0.3** |
| **Cellular processes** | | | | | | | | | | | | | | |
| AN3720 | Coat protein complex II | 21 | 43 | 56 | -0.5 | 0.3 | 0.5 | **0.3** | 0.5 | -0.1 | 0.2 | 0.4 | 0.2 | **0.2** |
| AN0117 | Response to oxidative stress | | 6 | 26 | NaN | -0.5 | -0.5 | **-0.5** | 0.3 | NaN | 0 | -0.5 | -0.3 | **0.3** |
| AN1757 (SsfA) | Proteasome subunit | 13 | 20 | 29 | -0.5 | 0.4 | -0.2 | **-0.2** | 0.5 | 0.4 | 0.1 | 0.5 | 0.4 | **0.2** |
| AN0084 | GTPase-activating protein | 118 | 135 | 164 | -0.2 | 0.2 | -0.5 | **-0.2** | 0.4 | 0.4 | 0.1 | 0.3 | 0.3 | **0.2** |
| AN0999 (capA) | Adenylate cyclase | 13 | 32 | 54 | -0.4 | 0.1 | -0.5 | **-0.4** | 0.3 | 0.5 | 0.1 | 0.3 | 0.3 | **0.2** |
| **Metabolic process** | | | | | | | | | | | | | | |
| AN8041 (GpdA) | Glyceraldehyde-3-phosphate dehydrogenase | 1104 | 971 | 1794 | 0 | 0 | -0.3 | **0.0** | 0.2 | -0.3 | -0.1 | 0.3 | -0.1 | **0.3** |
| AN0932 (GlrA) | Oxidoreductase | 15 | 42 | 52 | -0.4 | -0.1 | -0.4 | **-0.4** | 0.2 | 0.3 | 0.1 | 0.4 | 0.3 | **0.1** |
| AN10526 (RmtA) | Arginine methyltransferase | 4 | 15 | NaN | NaN | 0 | 0.2 | **0.1** | 0.1 | 0 | 0 | 0.2 | 0.0 | **0.1** |
| AN1430 | Oxidoreductase | | 3 | 14 | NaN | 0.2 | -0.5 | **-0.2** | 0.4 | NaN | 0 | 0.4 | 0.2 | **0.2** |
| AN7451 | NAD-glutamate dehydrogenase | 244 | 229 | | -0.4 | 0 | NaN | **-0.2** | 0.2 | 0.2 | -0.4 | NaN | -0.1 | **0.3** |
| AN2999 (IdpA) | Isocitrate dehydrogenase | 99 | 69 | 205 | -0.2 | 0.5 | -0.2 | **-0.2** | 0.4 | -0.1 | -0.4 | 0.2 | -0.1 | **0.3** |
| AN8012 | Farnesyl-pyrophosphate synthase | 28 | 41 | 80 | -0.4 | -0.2 | -0.3 | **-0.3** | 0.1 | 0.4 | -0.2 | 0.4 | 0.4 | **0.3** |
| AN7632 | Dehydrogenase | 93 | 83 | 99 | -0.4 | 0.4 | 0.2 | **0.2** | 0.4 | 0.5 | -0.3 | 0.2 | 0.2 | **0.4** |
| AN1342 | Alanine-glyoxylate transaminase | 42 | 47 | 27 | -0.3 | 0.5 | -0.5 | **-0.3** | 0.5 | 0.1 | -0.3 | 0.3 | 0.1 | **0.3** |
| AN0254 | Catalytic activity | | 29 | 4 | NaN | -0.5 | -0.4 | **-0.5** | 0.3 | NaN | -0.5 | -0.5 | -0.5 | **0.3** |
| **Catabolic process** | | | | | | | | | | | | | | |
| AN2903 (PepE) | Aspartic protease | 167 | 184 | 164 | -0.5 | 0.1 | 0.0 | **0.0** | 0.3 | 0.1 | -0.5 | 0.6 | 0.1 | **0.5** |
| AN5442 (CpyA) | Carboxypeptidase Y | 16 | 39 | 94 | -0.5 | 0 | 0.1 | **0.0** | 0.3 | 0.2 | -0.3 | -0.5 | -0.3 | **0.4** |
| AN5810 (PepP) | Prolidase | 11 | 67 | 67 | -0.3 | 0.4 | 0.5 | **0.4** | 0.4 | 0.3 | 0.4 | 0.3 | 0.3 | **0.1** |
| **Vacuole function** | | | | | | | | | | | | | | |
| AN1195 | V-ATP synthase subunit C | 28 | 22 | 28 | -0.3 | 0.3 | -0.2 | **-0.2** | 0.3 | 0.5 | -0.1 | 0.4 | 0.4 | **0.3** |
| AN10563 (Pho8) | Vacuolar alkaline phosphatase | 38 | 10 | 12 | -0.5 | 0.5 | 0.5 | **0.5** | 0.6 | 0.5 | -0.1 | 0.2 | 0.2 | **0.3** |

**Supplementary Table 5, continued. Protein quantity of the following proteins was unchanged or unquantifiable in *laeA*Δ vegetative mycelium.**

The $log_2$ SILAC ratio was determined for the strains *laeA*Δ (AGB1074) in comparison to *laeA* (AGB1092). Additionally, the $log_2$ ratio for the complementation strain (*laeAcomp*$^+$, AGB1076) in comparison to the parental *laeA* strain (AGB1092) is listed. The range between these two ratios are unchanged or unquantifiable. Proteins without a $log_2$ SILAC ratio are listed (marked with NaN). These proteins are unquantifiable. It cannot be ruled out that protein quantities of some of the following identified proteins are quantifiable.

Ø = median of $log_2$ SILAC ratios, SD = standard deviation, NaN = not a number. Biological replicates are numbered 1-3. Spectral counts (PSM) for three biological replicates are shown(Spectral counts were obtained by Proteome Discoverer 1.4.). The color scales represent $log_2$ SILAC ratios.

| -5 | -3.25 | -1.5 | -1.25 | -1 | -0.75 | -0.5 | -0.25 | 0 | 0.25 | 0.5 | 0.75 | 1 | 1.25 | 1.5 | 3.25 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

| Gene ID | Description | Spectral counts | | | *laeA*Δ/*laeA* | | | Ø | SD | *laeAcomp*+/*laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN6700 | ATPase binding activity | 77 | 237 | 638 | -4.2 | -4.4 | -1.5 | **-2.9** | 1.7 | -2.4 | 0.5 | -1.6 | **-1.6** | 1.5 |
| AN5028 (PpoC) | Fatty acid oxygenase | 124 | 99 | 86 | 1.4 | -3.3 | -1.8 | **-2.6** | 2.4 | 1.2 | -0.2 | 3.9 | **1.2** | 2.1 |
| AN8777 (AmdS) | Acetamidase | 17 | 44 | 51 | -2.3 | -2.7 | -1.7 | **-2.2** | 0.5 | -0.2 | -1.0 | 1.8 | **-0.2** | 1.5 |
| AN3017 | Epoxide hydrolase | 83 | 73 | 88 | -3.1 | -2.2 | -1.5 | **-2.2** | 0.8 | -1.8 | -1.1 | 1.5 | **-1.1** | 1.8 |
| AN0870 | Small molecule transport | 74 | 33 | 129 | -2.1 | -0.3 | -2.8 | **-2.1** | 1.3 | -0.8 | -0.2 | -1.3 | **-0.8** | 0.6 |
| AN0354 (AroG) | DAHP synthase | 78 | 91 | 124 | -2.6 | -1.6 | -1.7 | **-2.1** | 0.5 | -1.7 | -0.8 | -0.4 | **-0.8** | 0.7 |
| AN1913 | Lysyl-tRNA synthetase | 62 | 95 | 139 | -2.6 | -1.4 | -2.0 | **-2.0** | 0.6 | -1.1 | -0.4 | -0.7 | **-0.7** | 0.3 |
| AN10745 | Glycine hydroxymethyltransferase | 36 | 90 | 90 | -2.0 | -0.7 | -2.5 | **-2.0** | 0.9 | -0.8 | -0.9 | -1.7 | **-0.9** | 0.5 |
| AN8770 | Acetylglutamate kinase | 66 | 89 | 198 | -1.6 | -0.4 | -3.6 | **-2.0** | 1.6 | -1.1 | -0.3 | -2.3 | **-1.1** | 1.0 |
| AN1990 (LysD) | Homocitrate synthase | 48 | 123 | 191 | -4.1 | -2.0 | -1.3 | **-2.0** | 1.5 | -2.4 | -0.2 | -1.5 | **-1.5** | 1.1 |
| AN1306 | Gelsolin | 17 | 19 | 19 | -3.4 | -2.0 | -1.1 | **-2.0** | 1.2 | -1.3 | -1.1 | -0.9 | **-1.1** | 0.2 |
| AN6227 | Hercynylcysteine sulfoxide lyase | 77 | 110 | 69 | -3.0 | -1.8 | -2.1 | **-1.9** | 0.7 | -1.7 | -0.8 | 3.2 | **-0.8** | 2.6 |
| AN10298 | 3-phosphoserine aminotransferase | 96 | 108 | 181 | -2.3 | -1.1 | -1.9 | **-1.9** | 0.6 | -1.5 | -0.6 | -1.1 | **-1.1** | 0.5 |
| AN11227 (HscA) | Heat shock protein | 165 | 233 | 510 | -1.9 | -0.7 | -1.9 | **-1.9** | 0.7 | -0.5 | -0.1 | -1.7 | **-0.5** | 0.8 |
| AN2295 | Succinate-CoA ligase | 36 | 50 | 93 | -1.8 | -0.4 | -2.0 | **-1.8** | 0.9 | -0.3 | -0.2 | -1.7 | **-0.3** | 0.9 |
| AN0860 | Protein of unknown function | 30 | 21 | 29 | -2.3 | -1.3 | -1.8 | **-1.8** | 0.5 | 1.3 | 0.0 | 1.7 | **1.3** | 0.9 |
| AN2272 | Kinase | 57 | 110 | 170 | -1.7 | -0.8 | -3.0 | **-1.7** | 1.1 | -0.5 | -0.3 | -1.6 | **-0.5** | 0.7 |
| AN5601 | Saccharopine dehydrogenase | 13 | 57 | 132 | -2.7 | -1.4 | -2.1 | **-1.7** | 0.6 | -1.2 | -0.4 | -1.2 | **-1.2** | 0.5 |
| AN4409 (ArgB) | Ornithine carbamoyltransferase | 52 | 206 | 282 | -2.3 | -1.0 | -2.5 | **-1.7** | 0.8 | -1.9 | -0.7 | -0.9 | **-0.9** | 0.6 |
| AN7625 | Myo-inositol-1-phosphate synthase | 104 | 248 | 668 | -1.8 | -0.4 | -3.1 | **-1.7** | 1.3 | -1.5 | -0.3 | -2.8 | **-1.5** | 1.3 |
| AN0443 | Alcohol dehydrogenase | 59 | 111 | 178 | -2.5 | -1.1 | -1.7 | **-1.7** | 0.7 | -0.6 | -5.6 | -1.9 | **-1.9** | 2.6 |
| AN1523 | F1F0-ATPase complex subunit | 250 | 419 | 577 | -1.6 | -0.4 | -1.6 | **-1.6** | 0.7 | -0.4 | -0.5 | -1.6 | **-0.5** | 0.7 |
| AN3695 | Anthranilate synthase | 16 | 39 | 63 | -2.3 | -1.4 | -1.8 | **-1.6** | 0.4 | -1.0 | -0.5 | -0.8 | **-0.8** | 0.3 |
| AN2436 (AclB) | ATP citrate synthase | 407 | 500 | 1057 | -2.0 | -0.7 | -1.6 | **-1.6** | 0.7 | 0.8 | 0.6 | -0.8 | **0.6** | 0.9 |
| AN3059 (PgmA) | Phosphoglycerate mutase | 153 | 235 | 503 | -1.6 | -0.3 | -1.5 | **-1.5** | 0.7 | -0.5 | -0.1 | -1.6 | **-0.5** | 0.8 |
| AN5793 | 20S proteasome beta-type subunit | 9 | 38 | 36 | -1.9 | -0.9 | -2.1 | **-1.5** | 0.7 | -0.2 | 0.0 | -1.0 | **-0.2** | 0.5 |
| AN0240 (PppA) | Pentose-phosphate | 450 | 621 | 820 | -1.5 | -0.4 | -2.2 | **-1.5** | 0.9 | -0.2 | -2.4 | -1.5 | **-1.5** | 1.1 |
| AN2164 | Chromatid cohesion | 17 | 29 | 33 | -1.9 | -0.5 | -1.5 | **-1.5** | 0.7 | 0.5 | 0.2 | -0.7 | **0.2** | 0.6 |

**Supplementary Table 5, is continued from page 185-198.**

| Gene ID | Description | PSM | | | *laeAΔlaeA* | | | Ø | SD | *laeAcomp+laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN6048 | Aspartate transaminase | 169 | 350 | 399 | -2.2 | -0.9 | -2.0 | **-1.5** | 0.7 | -0.9 | -1.2 | -1.5 | **-1.2** | 0.3 |
| AN1177 | Coatomer subunit beta | 34 | 43 | 102 | -1.5 | -0.5 | -2.1 | **-1.5** | 0.8 | -0.1 | -0.5 | -1.4 | **-0.5** | 0.7 |
| AN10675 | Protein of unknown function | 39 | 40 | 75 | -1.5 | -0.6 | -1.6 | **-1.5** | 0.6 | 0.0 | -0.4 | -0.7 | **-0.4** | 0.4 |
| AN10278 | Electron transfer flavoprotein | 8 | 38 | 59 | -1.5 | -0.2 | -1.5 | **-1.5** | 0.7 | -0.1 | -0.3 | -1.5 | **-0.3** | 0.8 |
| AN2126 (KapJ) | Importin | 35 | 19 | 42 | -1.4 | 0.6 | -2.0 | **-1.4** | 1.4 | 0.2 | 0.1 | -1.1 | **0.1** | 0.8 |
| AN2286 (AlcC) | Alcohol dehydrogenase III | 259 | 384 | 437 | -1.4 | 0.0 | -2.1 | **-1.4** | 1.1 | -0.3 | 0.0 | -2.1 | **-0.3** | 1.1 |
| AN8277 (CysD) | Methionin-Synthase | 157 | 262 | 210 | -1.6 | -0.5 | -2.3 | **-1.4** | 0.9 | 0.1 | -0.5 | -1.3 | **-0.5** | 0.7 |
| AN6708 (PhdA) | Acetyltransferase | 87 | 108 | 470 | -1.8 | -0.4 | -2.5 | **-1.4** | 1.1 | -0.5 | -0.3 | -2.1 | **-0.5** | 1.0 |
| AN1263 | Adenosylhomocysteinase | 195 | 433 | 447 | -1.4 | -0.2 | -1.4 | **-1.4** | 0.7 | -0.4 | -0.3 | -1.7 | **-0.4** | 0.8 |
| AN9408 (FasB) | Fatty acid synthase | 81 | 183 | 578 | -2.1 | -0.3 | -2.5 | **-1.4** | 1.2 | 0.0 | 0.8 | -1.5 | **0.0** | 1.2 |
| AN1964 | 40S ribosomal protein S6 | 24 | 21 | 143 | -1.4 | -0.1 | -1.4 | **-1.4** | 0.8 | -0.3 | -0.2 | -2.1 | **-0.3** | 1.1 |
| AN0688 | Transketolase | 558 | 633 | 859 | -1.6 | -0.6 | -1.3 | **-1.3** | 0.5 | 0.6 | 2.4 | -1.0 | **0.6** | 1.7 |
| AN5141 | Protein of unknown function | 6 | 12 | 80 | -1.1 | -0.3 | -2.3 | **-1.3** | 1.0 | -0.4 | -0.3 | -1.5 | **-0.4** | 0.7 |
| AN6168 (MaeA) | Malate dehydrogenase | 12 | 35 | 40 | -3.3 | -1.6 | -1.1 | **-1.3** | 1.2 | -2.4 | -0.6 | -0.6 | **-0.6** | 1.0 |
| AN6010 (SgdE) | Heat shock protein | 305 | 438 | 861 | -2.4 | -1.0 | -1.7 | **-1.3** | 0.7 | -1.1 | -0.6 | -2.3 | **-1.1** | 0.8 |
| AN2896 | Catalytic activity | 10 | 8 | 16 | -1.4 | 0.2 | -1.3 | **-1.3** | 0.9 | -0.5 | -0.4 | -1.4 | **-0.5** | 0.6 |
| AN10148 (CphA) | CHORD-containing protein | 7 | 5 | 17 | -1.7 | -1.3 | -1.1 | **-1.3** | 0.3 | 0.1 | -0.8 | 1.1 | **0.1** | 0.9 |
| AN1013 | 60S ribosomal protein L5 | 72 | 56 | 313 | -1.3 | 0.2 | -1.8 | **-1.3** | 1.0 | -0.4 | -0.2 | -1.3 | **-0.4** | 0.6 |
| AN8722 (Sub2) | RNA helicase | 33 | 42 | 66 | -1.2 | -0.6 | -2.0 | **-1.3** | 0.7 | -0.4 | -0.1 | -1.4 | **-0.4** | 0.7 |
| AN10030 | Alkaline serine protease | 27 | 114 | 150 | -1.3 | -0.4 | -1.9 | **-1.3** | 0.8 | -0.2 | -0.6 | 0.1 | **-0.2** | 0.3 |
| AN3466 (KgdB) | Succinyl transferase | 47 | 117 | 353 | -1.3 | 0.1 | -1.4 | **-1.3** | 0.8 | -0.3 | -0.4 | -2.1 | **-0.4** | 1.0 |
| AN2213 | Proteasome | 39 | 47 | 80 | -1.3 | -0.4 | -1.7 | **-1.3** | 0.7 | 0.3 | -0.5 | -1.2 | **-0.5** | 0.7 |
| AN5210 (PkiA) | Pyruvate kinase | 207 | 258 | 440 | -1.7 | -0.5 | -2.1 | **-1.3** | 0.8 | -0.4 | -0.4 | -2.3 | **-0.4** | 1.1 |
| AN5975 | Dehydrogenase | 459 | 443 | 359 | -1.3 | -0.5 | -2.1 | **-1.3** | 0.8 | 1.0 | 0.4 | 1.7 | **1.0** | 0.7 |
| AN1256 | Protein of unknown function | 52 | 76 | 130 | -1.3 | -0.6 | -1.3 | **-1.3** | 0.4 | -0.2 | -0.2 | -1.4 | **-0.2** | 0.7 |
| AN3112 (UgmA) | UDP-galactopyranose mutase | 91 | 98 | 182 | -1.8 | -0.4 | -1.3 | **-1.3** | 0.7 | 0.4 | 0.2 | -1.9 | **0.2** | 1.3 |
| AN8866 | Phosphoglycerate dehydrogenase | 110 | 141 | 150 | -1.7 | -0.8 | -1.7 | **-1.3** | 0.5 | -0.8 | -0.1 | -1.4 | **-0.8** | 0.6 |
| AN3829 | Dehydrogenase | 33 | 60 | 219 | -1.0 | 0.4 | -2.9 | **-1.3** | 1.6 | -0.8 | -1.1 | -3.8 | **-1.1** | 1.6 |
| AN5884 | Orotate phosphoribosyltransferase | 28 | 33 | 49 | -1.5 | -0.7 | -1.8 | **-1.2** | 0.6 | -0.3 | -0.3 | -1.9 | **-0.3** | 0.9 |
| AN4163 (CpcB) | Protein with seven WD repeats | 84 | 90 | 287 | -1.0 | -0.2 | -2.3 | **-1.2** | 1.1 | 0.0 | -0.3 | -2.0 | **-0.3** | 1.1 |
| AN3906 | Protein of unknown function | 51 | 89 | 182 | -1.4 | 0.2 | -2.7 | **-1.2** | 1.4 | 0.8 | 0.2 | -1.3 | **0.2** | 1.0 |
| AN4380 | Ribonucleotide reductase | 16 | 35 | 45 | -2.0 | -0.8 | -1.6 | **-1.2** | 0.6 | 0.7 | 0.5 | -1.5 | **0.5** | 1.2 |
| AN6058 | Protein of unknown function | 51 | 69 | 102 | -2.6 | -1.4 | -1.0 | **-1.2** | 0.8 | -1.6 | -1.2 | -2.5 | **-1.6** | 0.7 |
| AN0912 | Dehydrogenase | 32 | 58 | 115 | -1.9 | -0.4 | -1.2 | **-1.2** | 0.7 | -0.5 | 0.3 | -1.4 | **-0.5** | 0.8 |
| AN1638 | Serine-type peptidase | 280 | 378 | 473 | -1.2 | 0.0 | -1.3 | **-1.2** | 0.7 | 0.3 | -0.1 | -0.9 | **-0.1** | 0.6 |
| AN0242 | Hydrolase | 56 | 70 | 222 | -1.3 | -0.3 | -1.2 | **-1.2** | 0.5 | 0.0 | -3.5 | -1.6 | **-1.6** | 1.8 |
| AN0840 | Alpha-isopropylmalate synthase | 58 | 107 | 180 | -2.5 | -1.2 | -0.3 | **-1.2** | 1.1 | -1.2 | -0.4 | -1.5 | **-1.2** | 0.6 |
| AN4462 (PycA) | Pyruvate carboxylase | 297 | 369 | 1671 | -1.6 | -0.5 | -1.8 | **-1.2** | 0.7 | -0.2 | -0.2 | -1.0 | **-0.2** | 0.5 |
| AN2909 | Arsenite-transporting ATPase | 6 | 13 | 37 | -1.2 | -0.3 | -1.6 | **-1.2** | 0.7 | 0.5 | 0.3 | -1.2 | **0.3** | 0.9 |
| AN4401 | Asparagine synthase | 14 | 53 | 138 | -2.3 | -1.1 | -1.3 | **-1.2** | 0.6 | -1.6 | -0.3 | -1.7 | **-1.6** | 0.8 |
| AN7010 | Catalytic activity | 54 | 65 | 48 | -3.3 | -1.9 | -0.5 | **-1.2** | 1.4 | -2.6 | -0.4 | 2.3 | **-0.4** | 2.5 |
| AN1282 | Protein of unknown function | 41 | 45 | 114 | -1.8 | -0.5 | -1.2 | **-1.2** | 0.7 | -0.1 | -0.2 | -1.1 | **-0.2** | 0.6 |
| AN4259 | 43S preinitiation complex | 18 | 29 | 107 | -1.8 | -0.6 | -1.7 | **-1.2** | 0.7 | -0.6 | -0.6 | -1.9 | **-0.6** | 0.7 |
| AN1401 (KapK) | Exportin 1 | 32 | 50 | 117 | -1.2 | -0.2 | -1.4 | **-1.2** | 0.6 | 0.3 | 0.0 | -1.0 | **0.0** | 0.7 |
| AN5744 | 14-3-3-like protein | 340 | 443 | 689 | -1.2 | -0.1 | -2.2 | **-1.2** | 1.0 | 0.2 | -0.3 | -1.3 | **-0.3** | 0.8 |
| AN10710 | Phosphomannomutase | 39 | 62 | 79 | -1.2 | -0.3 | -1.5 | **-1.2** | 0.6 | 0.2 | 0.2 | -1.6 | **0.2** | 1.0 |
| AN5206 (LysB) | Homoisocitrate dehydrogenase | 37 | 74 | 117 | -2.2 | -0.6 | -1.7 | **-1.2** | 0.8 | -1.0 | -0.5 | -2.7 | **-1.0** | 1.2 |
| AN12465 | Dihydrolipoamide dehydrogenase | 113 | 241 | 358 | -1.2 | 0.0 | -1.8 | **-1.2** | 0.9 | -0.8 | -0.8 | -2.1 | **-0.8** | 0.7 |
| AN6391 (PphA) | Protein phosphatase | 23 | 27 | 56 | -1.7 | -0.3 | -2.0 | **-1.1** | 1.0 | 0.3 | 0.0 | -1.2 | **0.0** | 0.8 |
| AN8269 (Hsp90) | Heat shock protein | 625 | 758 | 1431 | -1.6 | -0.4 | -1.9 | **-1.1** | 0.8 | 0.1 | -0.1 | -1.5 | **-0.1** | 0.8 |
| AN2914 | Argininosuccinate lyase | 35 | 103 | 151 | -2.1 | -1.1 | -1.1 | **-1.1** | 0.5 | -1.3 | -0.4 | -1.3 | **-1.3** | 0.5 |
| AN10266 | Ubiquitin-activating enzyme E1 | 111 | 245 | 299 | -1.6 | -0.5 | -1.1 | **-1.1** | 0.6 | 0.3 | -0.2 | -0.8 | **-0.2** | 0.6 |
| AN1923 | Alanine transaminase | 157 | 157 | 223 | -1.4 | -0.4 | -1.1 | **-1.1** | 0.5 | -1.6 | -1.2 | -2.1 | **-1.6** | 0.5 |
| AN0359 (SgdA) | Translation initiation factor 3 (eIF3) | 4 | 13 | 217 | -2.5 | 0.2 | 0.5 | **-1.1** | 1.6 | -0.6 | 2.5 | -1.8 | **-0.6** | 2.2 |
| AN4085 | Serine threonine phosphatase | 42 | 62 | 90 | -1.3 | -0.3 | -2.0 | **-1.1** | 0.8 | 0.5 | 0.0 | -1.1 | **0.0** | 0.8 |
| AN6639 (McdB) | 2-methylcitrate dehydratase | 121 | 163 | 220 | -2.0 | -0.6 | -1.6 | **-1.1** | 0.7 | -0.3 | -0.9 | -1.0 | **-0.9** | 0.4 |
| AN0753 | Protein of unknown function | 16 | 58 | 231 | -1.1 | 0.2 | -1.6 | **-1.1** | 1.0 | 2.4 | 1.3 | -4.0 | **1.3** | 3.4 |
| AN3236 | Methyltransferase | 13 | 20 | 14 | -2.6 | -1.1 | 5.0 | **-1.1** | 4.0 | -2.4 | -0.8 | 1.9 | **-0.8** | 2.2 |
| AN1274 | Oxidoreductase | 11 | 41 | 20 | -1.1 | -0.2 | -1.4 | **-1.1** | 0.6 | 0.4 | 0.1 | -1.1 | **0.1** | 0.8 |
| AN1911 | Guanyltransferase | 44 | 42 | 81 | -1.1 | 0.2 | -1.6 | **-1.1** | 0.9 | 0.6 | 0.2 | -1.3 | **0.2** | 1.0 |
| AN4862 (RanGAP) | Ran GTPase activating protein | 10 | 29 | 104 | -1.2 | 0.2 | -2.3 | **-1.1** | 1.2 | 0.3 | 0.2 | -1.9 | **0.2** | 1.3 |
| AN11045 | D-lactate dehydrogenase | 6 | 8 | 48 | -1.0 | -0.5 | -1.8 | **-1.0** | 0.7 | -0.7 | -0.4 | -2.3 | **-0.7** | 1.0 |
| AN3413 | Ribosomal protein S2 | 76 | 55 | 252 | -1.0 | -0.1 | -1.8 | **-1.0** | 0.9 | -0.3 | -0.3 | -2.0 | **-0.3** | 1.0 |
| AN6346 | Dihydroxy-acid dehydratase | 23 | 61 | 6 | -1.6 | -0.2 | -1.9 | **-1.0** | 0.9 | -0.8 | -0.2 | -2.2 | **-0.8** | 1.0 |

| Gene ID | Description | PSM | | | *laeAΔ/laeA* | | | Ø | SD | *laeAcomp+/laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN2875 (FbaA) | Fructose-bisphosphate aldolase | 323 | 507 | 596 | -1.0 | -0.1 | -1.4 | **-1.0** | 0.7 | 0.2 | 0.0 | -1.5 | **0.0** | 0.9 |
| AN7169 (FhbA) | NirA-dependent flavohemoprotein | 170 | 252 | 315 | -1.1 | -0.1 | -1.9 | **-1.0** | 0.9 | 0.4 | -0.2 | -2.0 | **-0.2** | 1.2 |
| AN5989 | Epimerase/dehydratase | 459 | 45 | 41 | -1.3 | -1.9 | -0.2 | **-1.0** | 0.8 | 1.0 | -0.9 | 0.4 | **0.4** | 1.0 |
| AN2210 | ABC-transporter | 58 | 82 | 20 | -2.2 | -1.0 | -0.4 | **-1.0** | 0.9 | -0.7 | 0.1 | -1.6 | **-0.7** | 0.8 |
| AN6202 (Rpl3) | Ribosomal protein L3 | 18 | 32 | 325 | -1.2 | -0.1 | -1.9 | **-1.0** | 0.9 | -0.6 | -0.2 | -2.1 | **-0.6** | 1.0 |
| AN2133 | Actin capping protein | 45 | 18 | 23 | -1.0 | -0.4 | -1.3 | **-1.0** | 0.5 | 0.6 | -0.3 | 0.7 | **0.6** | 0.5 |
| AN2743 | Translation initiation factor 3 | 9 | 19 | 158 | -1.0 | 0.1 | -1.9 | **-1.0** | 1.0 | -0.5 | -0.3 | -2.0 | **-0.5** | 0.9 |
| AN5747 | 26S protease subunit | 12 | 31 | 63 | -0.9 | -0.7 | -1.3 | **-1.0** | 0.3 | 0.3 | -0.5 | -1.2 | **-0.5** | 0.7 |
| AN2932 | Eukaryotic initiation factor 4A | 101 | 245 | 438 | -1.5 | -0.7 | -1.0 | **-1.0** | 0.4 | -0.3 | -0.2 | -1.5 | **-0.3** | 0.7 |
| AN7430 | Glutamine amidotransferase | 17 | 47 | 78 | -2.3 | -1.9 | -0.1 | **-1.0** | 1.2 | -1.2 | -0.7 | -1.4 | **-1.2** | 0.4 |
| AN4464 | Bifunctional purine biosynthesis | 146 | 148 | 338 | -1.5 | -0.6 | -1.4 | **-1.0** | 0.5 | 0.2 | -0.1 | -1.5 | **-0.1** | 0.9 |
| AN1810 (OtaA) | Ornithine transaminase | 293 | 427 | 781 | -1.8 | -1.0 | 0.7 | **-1.0** | 1.3 | -0.9 | 0.1 | -2.3 | **-0.9** | 1.2 |
| AN6547 | Proteasome subunit | 50 | 47 | 51 | -0.9 | 0.0 | -1.9 | **-1.0** | 1.0 | 0.2 | -0.1 | -1.0 | **-0.1** | 0.6 |
| AN5746 | Phosphopyruvate hydratase | 20 | 832 | 963 | -1.0 | -0.2 | -1.7 | **-1.0** | 0.7 | 0.1 | -0.3 | -1.6 | **-0.3** | 0.9 |
| AN10079 (UreB) | Urease | 81 | 57 | 51 | -1.4 | -0.1 | -1.0 | **-1.0** | 0.7 | 1.5 | 0.4 | -0.8 | **0.4** | 1.2 |
| AN12237 (AbpA) | Actin-binding protein | 62 | 52 | 62 | -0.9 | 0.3 | -1.8 | **-0.9** | 1.1 | 0.8 | 0.0 | -1.1 | **0.0** | 0.9 |
| AN4470 | Regulation of translational fidelity | 24 | 35 | 73 | -1.4 | -0.4 | -1.5 | **-0.9** | 0.6 | -0.3 | -0.3 | -1.7 | **-0.3** | 0.8 |
| AN4769 | Sulfurylase | 108 | 263 | 376 | -1.2 | 0.0 | -1.9 | **-0.9** | 1.0 | 0.4 | -0.2 | -2.4 | **-0.2** | 1.5 |
| AN0075 (TigA) | Disulfide isomerase | 19 | 24 | 33 | -0.9 | 0.1 | -1.6 | **-0.9** | 0.9 | 1.0 | 0.6 | -0.9 | **0.6** | 1.0 |
| AN10279 | eIF4G binding | 28 | 27 | 59 | -0.9 | 0.0 | -1.4 | **-0.9** | 0.7 | -0.2 | -0.3 | -1.8 | **-0.3** | 0.9 |
| AN8824 | Translation initiation factor (eIF-6) | 43 | 40 | 29 | -2.0 | -0.8 | -1.1 | **-0.9** | 0.6 | -0.3 | -0.8 | -0.8 | **-0.8** | 0.3 |
| AN2930 | Protein of unknown function | 4 | 23 | 20 | -1.3 | 0.3 | -0.9 | **-0.9** | 0.8 | 0.2 | 0.1 | -1.2 | **0.1** | 0.8 |
| AN2068 | RNA binding effector protein | 52 | 77 | 184 | -1.1 | -0.6 | -0.9 | **-0.9** | 0.3 | 0.0 | -0.4 | -1.6 | **-0.4** | 0.8 |
| AN0554 (AldA) | Aldehyde dehydrogenase | 877 | 828 | 506 | -2.0 | -0.9 | 0.8 | **-0.9** | 1.4 | -1.5 | -0.9 | 2.1 | **-0.9** | 1.9 |
| AN4869 | 20S proteasome component | 30 | 36 | 48 | -1.2 | 0.1 | -1.9 | **-0.9** | 1.0 | 0.0 | 0.2 | -1.1 | **0.0** | 0.7 |
| AN8182 (AspC) | Septin | 139 | 160 | 252 | -0.8 | 0.1 | -1.8 | **-0.9** | 0.9 | 1.1 | 0.4 | -1.1 | **0.4** | 1.1 |
| AN5162 (PdhB) | Pyruvate dehydrogenase | 36 | 70 | 214 | -2.0 | -0.2 | -1.5 | **-0.9** | 0.9 | -0.5 | -0.4 | -2.1 | **-0.5** | 1.0 |
| AN10527 | Trimethyllysine dioxygenase | 9 | 8 | 27 | -1.6 | -0.2 | -0.8 | **-0.8** | 0.7 | -0.7 | -1.2 | -2.1 | **-1.2** | 0.7 |
| AN6267 | Regulation of G0 to G1 transition | 20 | 5 | 14 | -1.3 | -0.2 | -1.5 | **-0.8** | 0.7 | 0.7 | 0.1 | -0.7 | **0.1** | 0.7 |
| AN10222 | Glucose-6-phosphate 1-epimerase | 57 | 52 | 59 | -1.8 | -0.7 | -0.8 | **-0.8** | 0.6 | -0.3 | 0.0 | -0.6 | **-0.3** | 0.3 |
| AN9403 | Pyruvate dehydrogenase | 75 | 57 | 225 | -2.0 | -0.4 | -1.3 | **-0.8** | 0.8 | -0.5 | -0.1 | -2.0 | **-0.5** | 1.0 |
| AN6699 | Electron-transfer flavoprotein | 29 | 94 | 141 | -1.3 | -0.3 | -1.4 | **-0.8** | 0.6 | 0.1 | -0.2 | -1.8 | **-0.2** | 1.0 |
| AN8748 | Palmitoyl-(protein) hydrolase | 10 | 3 | 11 | -1.6 | -0.6 | -1.1 | **-0.8** | 0.5 | 0.1 | -0.3 | -1.2 | **-0.3** | 0.6 |
| AN1017 (HogA) | Mitogen-activated protein kinase | 17 | 14 | 17 | -1.3 | -0.3 | -0.8 | **-0.8** | 0.5 | 0.3 | 0.2 | -1.3 | **0.2** | 0.9 |
| AN2440 | Ribose-5-phosphate isomerase | 7 | 27 | 29 | -0.8 | 0.2 | -1.1 | **-0.8** | 0.7 | 0.3 | 0.3 | -1.1 | **0.3** | 0.8 |
| AN2968 (IppA) | Inorganic diphosphatase | 239 | 358 | 473 | -0.8 | 0.0 | -1.8 | **-0.8** | 0.9 | 0.6 | 0.1 | -1.1 | **0.1** | 0.9 |
| AN3928 (ThiF) | Thiazole synthase | 35 | 132 | 370 | -3.2 | -0.6 | -1.0 | **-0.8** | 1.4 | -1.8 | 0.5 | -1.9 | **-1.8** | 1.4 |
| AN7000 | Succinate-CoA ligase | 60 | 65 | 138 | -1.9 | -0.5 | -1.1 | **-0.8** | 0.7 | -0.2 | 0.1 | -1.4 | **-0.2** | 0.8 |
| AN4888 (PdcA) | Pyruvate decarboxylase | 140 | 499 | 580 | -2.6 | -0.9 | -0.7 | **-0.8** | 1.0 | -0.9 | -0.5 | -2.0 | **-0.9** | 0.8 |
| AN4174 | Lactoylglutathione lyase | 75 | 79 | 116 | -1.0 | 0.0 | -1.6 | **-0.8** | 0.8 | 0.6 | -0.2 | -1.4 | **-0.2** | 1.0 |
| AN1182 (BenA) | Beta-tubulin | 352 | 327 | 648 | -0.8 | 0.0 | -1.4 | **-0.8** | 0.7 | 0.7 | 0.3 | -1.0 | **0.3** | 0.9 |
| AN7107 | Role in translation | 18 | 30 | 210 | -1.0 | 0.0 | -1.6 | **-0.8** | 0.8 | -0.3 | -0.2 | -2.1 | **-0.3** | 1.0 |
| AN6630 | Nascent polypeptide-associated | 118 | 138 | 264 | -1.1 | -0.1 | -1.5 | **-0.8** | 0.7 | -0.1 | -0.2 | -1.7 | **-0.2** | 0.9 |
| AN8435 | Tryrosinase domain protein | 110 | 58 | 73 | 1.5 | -0.2 | -1.4 | **-0.8** | 1.5 | 4.2 | 2.8 | 5.1 | **4.2** | 1.1 |
| AN6591 (Cse1) | Essential nuclear export receptor | 38 | 48 | 113 | -1.0 | -0.5 | -1.1 | **-0.8** | 0.3 | 0.2 | 0.0 | -1.2 | **0.0** | 0.8 |
| AN2212 | Ubiquitin-conjugating enzyme E2 | 7 | 2 | 7 | -0.8 | 0.6 | -1.6 | **-0.8** | 1.1 | 0.8 | 0.0 | -1.2 | **0.0** | 1.0 |
| AN9401 | Oxidoreductase | 7 | 14 | 4 | -2.1 | -0.5 | -1.0 | **-0.8** | 0.8 | -0.4 | -0.7 | -1.7 | **-0.7** | 0.7 |
| AN4739 | SAICAR synthetase | 6 | 6 | 47 | -3.0 | -0.7 | -0.9 | **-0.8** | 1.3 | -0.8 | 0.1 | -1.7 | **-0.8** | 0.9 |
| AN0906 (KapB) | Nuclear transport protein | 22 | 57 | 110 | -1.5 | -0.1 | -0.8 | **-0.8** | 0.7 | 0.0 | 0.1 | -1.5 | **0.0** | 0.9 |
| AN8273 | Ubiquinol-cytochrome-c reductase | 45 | 63 | 126 | -1.6 | -0.2 | -1.3 | **-0.7** | 0.7 | -0.5 | -0.4 | -2.4 | **-0.5** | 1.1 |
| AN2012 | Protein of unknown function | 117 | 148 | 175 | -0.9 | 0.1 | -0.7 | **-0.7** | 0.5 | 0.6 | -0.1 | -0.2 | **-0.1** | 0.5 |
| AN5999 | Carbamoyl-phosphate synthase | 93 | 218 | 481 | -2.6 | -1.2 | -0.3 | **-0.7** | 1.2 | -1.8 | -0.5 | -1.7 | **-1.7** | 0.7 |
| AN10223 | 1-Cys peroxiredoxin | 363 | 335 | 459 | -1.6 | -0.7 | 0.0 | **-0.7** | 0.8 | 0.2 | -0.3 | -1.4 | **-0.3** | 0.8 |
| AN0687 | Arginine metabolism | 60 | 42 | 63 | -1.3 | -0.3 | -0.7 | **-0.7** | 0.5 | 0.3 | 1.6 | -1.1 | **0.3** | 1.4 |
| AN6563 | Translation elongation factor | 330 | 445 | 722 | -1.7 | -0.7 | -0.8 | **-0.7** | 0.6 | -0.1 | 0.0 | -1.2 | **-0.1** | 0.7 |
| AN6726 | Proteasome subunit | 37 | 55 | 51 | -0.9 | 0.0 | -1.5 | **-0.7** | 0.8 | 0.2 | -0.3 | -1.1 | **-0.3** | 0.6 |
| AN0179 | Oxidoreductase | 18 | 63 | 69 | -1.7 | -0.7 | -0.5 | **-0.7** | 0.7 | 0.1 | -2.5 | -1.3 | **-1.3** | 1.3 |
| AN8204 | NAD+ diphosphatase | 6 | 12 | 14 | -1.9 | -0.9 | -0.6 | **-0.7** | 0.7 | 0.1 | -0.6 | -1.3 | **-0.6** | 0.7 |
| AN5745 | Translation initiation factor | 425 | 2 | 35 | -1.2 | -0.3 | -1.1 | **-0.7** | 0.4 | -0.3 | -0.5 | -1.8 | **-0.5** | 0.8 |
| AN7335 | Protein of unknown function | 8 | 3 | | -0.7 | -0.3 | -1.1 | **-0.7** | 0.4 | 0.8 | 0.0 | -1.8 | **0.0** | 1.3 |
| AN5571 (KgdA) | Oxoglutarate dehydrogenase | 71 | 106 | 224 | -1.7 | -0.1 | -1.3 | **-0.7** | 0.9 | -0.5 | -0.4 | -2.0 | **-0.5** | 0.9 |
| AN5713 (Cct7) | Chaperonin | 23 | 24 | 93 | -1.8 | -0.5 | -0.8 | **-0.7** | 0.7 | -0.1 | -0.3 | -1.8 | **-0.3** | 0.9 |
| AN4591 | Phosphoglucomutase | 81 | 60 | 68 | -1.3 | -0.5 | -0.9 | **-0.7** | 0.4 | 0.3 | -0.5 | -0.8 | **-0.5** | 0.6 |
| AN6921 | Co-chaperone | 62 | 61 | 77 | -0.9 | 0.0 | -1.3 | **-0.7** | 0.7 | 0.2 | -0.1 | -0.8 | **-0.1** | 0.5 |
| AN7436 (PdiA) | Disulfide isomerase | 118 | 152 | 204 | -1.7 | -0.7 | -0.6 | **-0.6** | 0.6 | 0.6 | -0.2 | -0.5 | **-0.2** | 0.5 |
| AN2904 | 26S proteasome regulatory subunit | 25 | 69 | 99 | -1.6 | -0.4 | -0.6 | **-0.6** | 0.6 | -0.1 | -0.2 | -1.1 | **-0.2** | 0.5 |
| AN6341 | Coronin | 137 | 110 | 194 | -0.4 | 0.6 | -1.8 | **-0.6** | 1.2 | 1.1 | 0.5 | -1.2 | **0.5** | 1.2 |

| Gene ID | Description | PSM | | | *laeAΔ/laeA* | | | Ø | SD | *laeAcomp+/laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN6508 (GskA) | Kinase | 69 | 53 | 47 | -1.4 | -0.1 | -1.2 | **-0.6** | 0.7 | 0.7 | 0.2 | -0.5 | **0.2** | 0.6 |
| AN3748 | Phosphoribosyltransferase | 43 | 95 | 109 | -2.1 | -0.9 | -0.3 | **-0.6** | 0.9 | -0.6 | -0.3 | -1.1 | **-0.6** | 0.4 |
| AN4667 (AspA) | Septin | 92 | 185 | 226 | -0.9 | 0.2 | -1.4 | **-0.6** | 0.8 | 0.9 | 0.3 | -1.0 | **0.3** | 1.0 |
| AN4102 (BglA | Beta-glucosidase | 138 | 100 | 40 | -0.3 | 0.4 | -1.7 | **-0.6** | 1.1 | 1.4 | -0.8 | 1.1 | **1.1** | 1.2 |
| AN5547 | Oxidoreductase | 28 | 21 | 29 | -1.5 | -0.4 | -0.8 | **-0.6** | 0.6 | -1.2 | -1.2 | -1.6 | **-1.2** | 0.2 |
| AN4402 | Voltage-gated anion channel | 35 | 26 | 82 | -1.6 | -0.3 | -0.9 | **-0.6** | 0.6 | -0.3 | -0.5 | -2.9 | **-0.5** | 1.5 |
| AN7003 | 60S ribosomal protein L13 | 18 | 28 | 159 | -1.2 | 0.0 | -1.3 | **-0.6** | 0.7 | -0.3 | -0.3 | -2.1 | **-0.3** | 1.0 |
| AN5973 (PkcB) | Protein kinase C | 16 | 15 | 36 | -1.6 | 0.1 | -1.3 | **-0.6** | 0.9 | 0.5 | 0.3 | -1.4 | **0.3** | 1.1 |
| AN5122 | Kinase | 69 | 63 | 104 | -1.8 | -0.5 | -0.7 | **-0.6** | 0.7 | -0.8 | -0.5 | -2.0 | **-0.8** | 0.8 |
| AN5529 (CotA) | Serine/threonine protein kinase | 7 | 18 | 55 | -1.4 | 0.0 | -1.1 | **-0.6** | 0.7 | 0.3 | -0.1 | -1.4 | **-0.1** | 0.9 |
| AN5895 (GdiA) | Rab GDP-dissociation inhibitor | 112 | 142 | 251 | -0.9 | 0.2 | -1.4 | **-0.6** | 0.8 | 0.8 | 0.3 | -1.4 | **0.3** | 1.2 |
| AN6900 (TpiA) | Triose-phosphate isomerase | 113 | 189 | 231 | -0.9 | 0.0 | -1.2 | **-0.6** | 0.6 | -0.2 | -0.3 | -1.5 | **-0.3** | 0.7 |
| AN6257 | Protein of unknown function | 87 | 107 | 162 | -1.7 | -0.3 | -0.8 | **-0.6** | 0.7 | 0.1 | 0.0 | -1.4 | **0.0** | 0.8 |
| AN4087 | 40S ribosomal protein subunit | 89 | 78 | 237 | -1.2 | -0.1 | -1.0 | **-0.5** | 0.6 | -0.3 | 0.0 | -2.1 | **-0.3** | 1.1 |
| AN1015 | Phosphorylase | 108 | 80 | 82 | -1.0 | -0.5 | 1.2 | **-0.5** | 1.2 | 1.5 | -0.3 | -0.6 | **-0.3** | 1.1 |
| AN3954 | Phosphogluconate dehydrogenase | 483 | 863 | 944 | -1.5 | -0.3 | -0.7 | **-0.5** | 0.6 | -0.2 | -0.2 | -1.6 | **-0.2** | 0.8 |
| AN3592 (ClxA) | Calnexin | 38 | 33 | 106 | -1.8 | -0.2 | -0.5 | **-0.5** | 0.8 | 0.6 | 0.2 | -1.3 | **0.2** | 1.0 |
| AN1993 | Aspartate transaminase | 151 | 342 | 498 | -1.5 | -0.3 | -0.5 | **-0.5** | 0.6 | -1.4 | -0.9 | -2.3 | **-1.4** | 0.7 |
| AN10718 (RiboG) | Lumazine synthase | 2 | 22 | 27 | -0.5 | 1.1 | -1.6 | **-0.5** | 1.4 | 1.4 | 0.8 | -0.8 | **0.8** | 1.1 |
| AN6004 | Protein of unknown function | 119 | 110 | 119 | -1.3 | -0.2 | -0.8 | **-0.5** | 0.5 | 0.7 | -0.2 | -1.3 | **-0.2** | 1.0 |
| AN7570 (TubB) | Alpha-tubulin | 192 | 263 | 363 | -0.8 | 0.0 | -1.0 | **-0.5** | 0.5 | 0.9 | 0.1 | 0.4 | **0.4** | 0.4 |
| AN2916 | Succinate dehydrogenase | 17 | 40 | 97 | -1.0 | 0.3 | -0.5 | **-0.5** | 0.7 | 0.1 | -0.5 | -2.3 | **-0.5** | 1.3 |
| AN2458 | Cullin binding protein | 39 | 51 | 81 | -1.3 | -0.4 | -0.5 | **-0.5** | 0.5 | 0.1 | -0.4 | -1.2 | **-0.4** | 0.7 |
| AN1733 (PrnC) | Oxidoreductase | 68 | 39 | 37 | -0.5 | 0.2 | -1.0 | **-0.5** | 0.6 | 1.5 | -0.8 | -0.4 | **-0.4** | 1.2 |
| AN6650 (McsA) | Methylcitrate synthase | 57 | 79 | 46 | -1.6 | -0.9 | 0.0 | **-0.5** | 0.8 | 0.2 | -0.9 | 0.9 | **0.2** | 0.9 |
| AN8870 | 40S ribosomal protein S3Ae | 8 | 25 | 123 | -1.3 | 0.0 | -0.9 | **-0.5** | 0.7 | -0.2 | -0.2 | -2.0 | **-0.2** | 1.1 |
| AN10273 (Gst3) | Glutathione S-transferase | 38 | 32 | 37 | -2.4 | 0.5 | -0.5 | **-0.5** | 1.5 | 0.1 | 0.5 | -0.7 | **0.1** | 0.6 |
| AN5602 | Chaperone | 38 | 34 | 47 | -2.1 | -0.3 | -0.6 | **-0.5** | 1.0 | -0.5 | 0.0 | -1.5 | **-0.5** | 0.8 |
| AN6499 (MdhC) | Malate dehydrogenase | 437 | 548 | 429 | -0.9 | 0.0 | -0.9 | **-0.5** | 0.5 | 0.9 | 0.1 | -0.1 | **0.1** | 0.5 |
| AN6840 | Hydroxyacylglutathione hydrolase | 38 | 29 | 19 | -1.1 | -0.3 | -0.6 | **-0.5** | 0.4 | 0.5 | -0.6 | -0.9 | **-0.6** | 0.8 |
| AN4501 (ArtA) | 14-3-3 protein | 427 | 599 | 892 | -1.0 | -0.1 | -0.8 | **-0.4** | 0.5 | 0.7 | 0.0 | -1.0 | **0.0** | 0.9 |
| AN5129 (Hsp70) | Heat shock protein | 604 | 842 | 1322 | -1.6 | -0.5 | -0.4 | **-0.4** | 0.7 | 0.0 | 0.0 | -1.4 | **0.0** | 0.8 |
| AN8022 | Protein of unknown function | 23 | 30 | 28 | -0.9 | 0.0 | -0.8 | **-0.4** | 0.5 | 0.3 | -0.2 | -1.0 | **-0.2** | 0.7 |
| AN9497 | Hydrolase | 141 | 9 | 19 | -1.7 | -0.3 | -0.5 | **-0.4** | 0.7 | -0.8 | -0.2 | -1.1 | **-0.8** | 0.5 |
| AN9308 | Dehydrogenase | 31 | 32 | 12 | -2.0 | -0.6 | -0.2 | **-0.4** | 0.9 | 0.7 | 0.1 | -0.5 | **0.1** | 0.6 |
| AN4820 | Dehydrogenase | 52 | 51 | 22 | 0.5 | 1.1 | -1.9 | **-0.4** | 1.6 | 2.0 | 0.2 | 1.7 | **1.7** | 0.9 |
| AN11070 | Metalloaminopeptidase | 75 | 32 | 20 | -1.2 | -0.4 | 0.1 | **-0.4** | 0.7 | 0.0 | -1.0 | -0.9 | **-0.9** | 0.6 |
| AN5986 | Reductase | 16 | 35 | 13 | -1.6 | 1.2 | -1.9 | **-0.4** | 1.7 | 0.5 | 0.3 | 0.3 | **0.3** | 0.1 |
| AN5790 | Isocitrate dehydrogenase | 9 | 21 | 126 | -0.9 | 0.1 | -0.9 | **-0.4** | 0.6 | 0.2 | -0.3 | -2.2 | **-0.3** | 1.3 |
| AN1426 | Serine-type carboxypeptidase | 34 | 16 | 14 | -0.4 | 0.6 | -0.9 | **-0.4** | 0.8 | 2.7 | 0.0 | -0.1 | **0.0** | 1.6 |
| AN4463 | Clathrin | 102 | 298 | 436 | -1.2 | 0.0 | -0.6 | **-0.3** | 0.6 | 0.4 | 0.1 | -1.2 | **0.1** | 0.8 |
| AN3932 | Proteasome beta-5 subunit | 12 | 28 | 60 | -0.5 | 1.7 | -2.3 | **-0.3** | 2.0 | 0.2 | 0.0 | -1.0 | **0.0** | 0.6 |
| AN5812 | Aminopeptidase | 16 | 19 | 15 | -1.9 | 0.2 | -0.8 | **-0.3** | 1.1 | 0.2 | -0.3 | -1.0 | **-0.3** | 0.6 |
| AN10709 (GfaA) | Transaminase activity | 224 | 451 | 672 | -1.3 | -0.3 | 2.5 | **-0.3** | 2.0 | 0.7 | 0.2 | -1.0 | **0.2** | 0.8 |
| AN4871 (ChiB) | Chitinase | 44 | 62 | 43 | -0.1 | 0.2 | -0.8 | **-0.3** | 0.5 | 0.0 | -1.7 | -0.9 | **-0.9** | 0.9 |
| AN5525 (AcoA) | Aconitate hydratase | 339 | 380 | 55 | -1.4 | -0.1 | -0.4 | **-0.3** | 0.7 | -0.4 | -0.4 | -1.8 | **-0.4** | 0.8 |
| AN3739 | Protein of unknown function | 18 | 10 | 63 | -1.5 | -0.3 | -0.2 | **-0.3** | 0.7 | 0.0 | -0.6 | -0.4 | **-0.4** | 0.3 |
| AN10020 | Proteasome complex localization | 44 | 105 | 105 | -1.5 | -0.3 | 3.0 | **-0.3** | 2.3 | -0.3 | -0.2 | -1.3 | **-0.3** | 0.6 |
| AN2142 | Uracil phosphoribosyltransferase | 43 | 66 | 111 | -1.5 | -0.2 | 1.0 | **-0.2** | 1.3 | 0.1 | -0.2 | -1.6 | **-0.2** | 0.9 |
| AN4794 | Ribosomal protein | 3 | 19 | 129 | -1.8 | 0.2 | -0.6 | **-0.2** | 1.0 | -0.6 | 0.0 | -2.0 | **-0.6** | 1.1 |
| AN4414 | Decarboxylase | 96 | 121 | 209 | -2.0 | -1.0 | 0.5 | **-0.2** | 1.3 | 0.5 | -0.1 | -0.7 | **-0.1** | 0.6 |
| AN6525 (AciA) | Formate dehydrogenase | 384 | 358 | 231 | 0.7 | 1.5 | -1.9 | **-0.2** | 1.7 | 1.9 | 0.5 | 2.4 | **1.9** | 1.0 |
| AN2998 | Tetrahydrofolate synthase | 110 | 143 | 260 | -0.9 | -0.2 | 0.3 | **-0.2** | 0.6 | 0.7 | 0.0 | -1.4 | **0.0** | 1.1 |
| AN7262 | Protein of unknown function | 116 | 84 | 189 | -1.0 | 0.1 | -0.4 | **-0.1** | 0.6 | 1.3 | 0.6 | -1.3 | **0.6** | 1.3 |
| AN2051 (Cdc37) | Cell division cycle 37 | 6 | 10 | 42 | -1.5 | -0.1 | 0.3 | **-0.1** | 1.0 | 0.4 | -0.1 | -0.7 | **-0.1** | 0.6 |
| AN8689 (GlkA) | Glucokinase | 56 | 72 | 102 | -0.1 | 0.9 | -1.1 | **-0.1** | 1.0 | -0.3 | 0.0 | -2.3 | **-0.3** | 1.2 |
| AN3973 | Peroxiredoxin | 45 | 54 | 85 | -2.5 | -0.9 | 0.7 | **-0.1** | 1.6 | -1.5 | -0.7 | -2.8 | **-1.5** | 1.0 |
| AN8674 | V-type ATPase | 36 | 60 | 40 | -2.6 | 0.1 | -0.2 | **0.0** | 1.5 | 0.6 | -0.1 | -1.0 | **-0.1** | 0.8 |
| AN6232 (VamB) | F1F0-ATPase complex subunit | 112 | 173 | 234 | -1.0 | 0.0 | 0.0 | **0.0** | 0.6 | 0.3 | -0.3 | -1.2 | **-0.3** | 0.7 |
| AN2412 (CmkA) | Kinase | 26 | 28 | 50 | -0.4 | 0.6 | 0.0 | **0.0** | 0.5 | 1.2 | 0.5 | -0.8 | **0.5** | 1.0 |
| AN2936 | Alpha-mannosidase | 32 | 31 | 29 | 0.0 | 1.0 | -1.3 | **0.0** | 1.1 | 1.3 | -0.4 | 0.1 | **0.1** | 0.9 |
| AN5604 (AcuG) | Fructose-bisphosphatase | 33 | 43 | 3 | 0.7 | 1.4 | -1.3 | **0.0** | 1.4 | -0.2 | -1.0 | -1.0 | **-1.0** | 0.4 |
| AN6988 | Proteasome regulatory particle | 20 | 46 | 66 | -1.6 | -0.5 | 0.6 | **0.1** | 1.1 | 0.0 | -0.2 | -1.1 | **-0.2** | 0.6 |
| AN1358 | Serine/threonine phosphatase | 41 | 28 | 71 | 0.8 | 0.1 | -1.8 | **0.1** | 1.3 | 1.5 | 0.2 | -0.8 | **0.2** | 1.1 |
| AN5916 (EchA) | Mitochondrial enoyl-CoA hydratase | 2 | 8 | 8 | -0.7 | 0.7 | -0.5 | **0.1** | 0.7 | 0.9 | -0.8 | -1.2 | **-0.8** | 1.1 |
| AN2526 | Ketol-acid reductoisomerase | 149 | 308 | 499 | -1.2 | 0.2 | 0.1 | **0.1** | 0.8 | -0.7 | 0.0 | -2.3 | **-0.7** | 1.2 |
| AN7708 | Oxidoreductase | 63 | 82 | 129 | 0.8 | 0.7 | -0.4 | **0.1** | 0.6 | 5.2 | 0.5 | -1.2 | **0.5** | 3.3 |

| Gene ID | Description | PSM | | | *laeA*Δ/*laeA* | | | Ø | SD | *laeA*comp+/*laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN3716 | 26S proteasome | 13 | 48 | 55 | -1.0 | -0.1 | 0.4 | **0.2** | 0.7 | 0.0 | -0.1 | -1.1 | **-0.1** | 0.6 |
| AN7111 (FoxA) | Role in fatty acid beta-oxidation | 131 | 172 | 40 | 1.4 | 2.3 | -1.9 | **0.2** | 2.2 | 1.7 | -0.5 | -1.3 | **-0.5** | 1.5 |
| AN5994 | Dehydratase | 27 | 7 | 19 | -2.0 | -0.4 | 1.0 | **0.3** | 1.5 | 0.5 | -0.5 | -0.9 | **-0.5** | 0.7 |
| AN2532 | Amine oxidase | 73 | 102 | 85 | 0.3 | 1.7 | -2.3 | **0.3** | 2.0 | 0.8 | 0.1 | -1.8 | **0.1** | 1.3 |
| AN4127 | Protein of unknown function | 29 | 39 | 138 | -2.5 | -1.4 | 2.0 | **0.3** | 2.4 | -1.7 | -0.8 | -3.2 | **-1.7** | 1.2 |
| AN5977 | Carbonyl reductase | 27 | 18 | 34 | -2.0 | -0.6 | 1.4 | **0.4** | 1.7 | 0.5 | -0.2 | 0.4 | **0.4** | 0.4 |
| AN0824 (ScdA) | Dehydrogenase | 6 | 10 | | -1.0 | 0.6 | 1.1 | **0.6** | 1.0 | 0.1 | -0.7 | -1.6 | **-0.7** | 0.8 |
| AN7950 (EglC) | Glucosidase | 13 | 65 | 71 | -2.0 | -0.4 | 1.5 | **0.6** | 1.8 | -0.7 | -0.9 | -1.0 | **-0.9** | 0.1 |
| AN8707 | Fumarate dehydratase | 88 | 172 | 258 | -0.8 | 0.5 | 0.8 | **0.6** | 0.8 | 0.1 | -0.3 | -2.3 | **-0.3** | 1.3 |
| AN6330 | Elongation factor 2 | 896 | 1127 | 1483 | -1.2 | -0.3 | 1.6 | **0.6** | 1.4 | 0.0 | -0.1 | -1.4 | **-0.1** | 0.8 |
| AN9124 | Heat shock protein | 44 | 113 | 219 | -1.4 | -0.3 | 1.7 | **0.7** | 1.6 | 0.6 | 0.1 | -1.2 | **0.1** | 0.9 |
| AN6653 (AcuE) | Malate synthase | 11 | 15 | 24 | 0.4 | 0.4 | 1.8 | **1.1** | 0.8 | 0.1 | -2.3 | -2.9 | **-2.3** | 1.6 |
| AN5872 | Proteasome subunit | 17 | 79 | 64 | -0.9 | 0.0 | 2.7 | **1.4** | 1.8 | 0.1 | -0.2 | -1.0 | **-0.2** | 0.6 |
| AN8953 (AgdB) | Alpha-glucosidase | 68 | 164 | 78 | 3.4 | 3.8 | -1.1 | **1.4** | 2.7 | 2.4 | 2.2 | 4.7 | **2.4** | 1.4 |
| AN5626 (FacA) | Acetyl-CoA synthase | 375 | 351 | 227 | 1.8 | 2.5 | 0.3 | **1.4** | 1.1 | 2.3 | -0.3 | -0.3 | **-0.3** | 1.5 |
| AN8782 | S-formylglutathione hydrolase | 10 | 28 | 12 | -0.1 | 0.7 | 2.4 | **1.6** | 1.3 | 0.7 | -0.3 | 0.6 | **0.6** | 0.5 |
| AN8664 | Hydrolase | 78 | 66 | 89 | -0.8 | 0.3 | 2.9 | **1.6** | 1.9 | -0.5 | -1.0 | -1.3 | **-1.0** | 0.4 |
| AN6231 (TepB) | Tryptophan synthase | 121 | 157 | 277 | -3.1 | -1.5 | 5.0 | **1.7** | 4.2 | -1.8 | -0.5 | -0.7 | **-0.7** | 0.7 |
| AN8233 | Phosphatidylinositol transporter | 94 | 122 | 176 | -0.3 | 0.8 | 3.3 | **2.0** | 1.8 | 1.0 | 0.7 | -1.4 | **0.7** | 1.3 |
| AN3499 | Phosphotransferase | 12 | 26 | 10 | NaN | -1.7 | -1.6 | **-1.7** | 1.0 | -2.1 | -1.6 | -2.1 | **-2.1** | 0.3 |
| AN2977 | Small molecule transport | 17 | 11 | 10 | NaN | -0.4 | -1.4 | **-0.9** | 0.7 | -0.8 | -0.9 | -1.9 | **-0.9** | 0.6 |
| AN3626 | Carboxylase | 3 | 25 | 59 | -2.1 | NaN | -2.6 | **-2.6** | 1.4 | -0.4 | -2.7 | -1.7 | **-1.7** | 1.1 |
| AN10096 | Hydrolase | 29 | 27 | 26 | -3.5 | -2.0 | NaN | **-2.8** | 1.7 | -2.0 | -0.9 | -2.4 | **-2.0** | 0.7 |
| AN0158 | 2-alkenal reductase | 15 | 35 | 38 | -2.9 | -1.9 | NaN | **-2.4** | 1.5 | -1.0 | -0.8 | -1.9 | **-1.0** | 0.5 |
| AN3222 | 5'-nucleotidase | 25 | 25 | 20 | -2.8 | -1.9 | NaN | **-2.3** | 1.4 | -0.6 | -1.1 | -0.5 | **-0.6** | 0.3 |
| AN7074 | Oxidoreductase | 134 | 63 | 60 | -2.5 | -2.2 | NaN | **-2.2** | 1.4 | -2.0 | -1.7 | 1.2 | **-1.7** | 1.8 |
| AN1883 | Argininosuccinate synthase | 92 | 175 | 212 | -2.6 | -1.5 | NaN | **-2.0** | 1.3 | -1.5 | -0.7 | -1.4 | **-1.4** | 0.4 |
| AN2867 (PgmB) | Phosphoglucomutase | 243 | 240 | 417 | -2.0 | -1.0 | NaN | **-1.5** | 1.0 | -0.3 | -0.4 | -0.5 | **-0.4** | 0.1 |
| AN3031 | Threonine synthase | 24 | 101 | 177 | -2.0 | -0.9 | NaN | **-1.4** | 1.0 | -0.9 | -0.5 | -2.0 | **-0.9** | 0.8 |
| AN7141 | Aldehyde dehydrogenase | 71 | 76 | 72 | -2.4 | -1.4 | NaN | **-1.4** | 1.2 | -0.7 | -0.7 | -0.6 | **-0.7** | 0.0 |
| AN1045 | Protein of unknown function | 12 | 17 | 50 | -2.1 | -0.6 | NaN | **-1.4** | 1.1 | -1.1 | -0.3 | -1.7 | **-1.1** | 0.7 |
| AN5610 | Dehydrogenase | 55 | 128 | 200 | -3.1 | -1.3 | NaN | **-1.3** | 1.6 | -1.4 | -0.4 | -1.4 | **-1.4** | 0.5 |
| AN7479 | Asparaginyl-tRNA synthetase | 40 | 122 | 243 | -1.7 | -1.3 | NaN | **-1.3** | 0.9 | -1.6 | -0.4 | -0.6 | **-0.6** | 0.7 |
| AN2435 (AclA) | ATP citrate synthase | 282 | 352 | 540 | -1.8 | -0.7 | NaN | **-1.3** | 0.9 | 0.7 | 0.5 | -0.8 | **0.5** | 0.9 |
| AN9339 (CatB) | Catalase | 637 | 669 | 575 | -1.8 | -1.2 | NaN | **-1.2** | 0.9 | 1.2 | 0.3 | 1.6 | **1.2** | 0.7 |
| AN2964 (PdhX) | Pyruvate dehydrogenase | 17 | 28 | 48 | -2.0 | -0.3 | NaN | **-1.1** | 1.1 | -0.6 | -0.3 | -2.1 | **-0.6** | 1.0 |
| AN5701 (Arof) | DAHP synthase | 76 | 66 | 94 | -2.3 | -1.1 | NaN | **-1.1** | 1.1 | -1.0 | -0.3 | -1.4 | **-1.0** | 0.6 |
| AN1904 | Chaperonin | 4 | 10 | 52 | -1.7 | -0.5 | NaN | **-1.1** | 0.9 | -0.1 | -0.3 | -1.8 | **-0.3** | 0.9 |
| AN10981 | GTP cyclohydrolase II | 69 | 72 | 42 | -1.3 | -0.9 | NaN | **-1.1** | 0.7 | 1.3 | 0.1 | 2.4 | **1.3** | 1.1 |
| AN1922 | Proteosome | 27 | 22 | 68 | -1.8 | -0.4 | NaN | **-1.1** | 0.9 | 0.0 | -0.3 | -1.1 | **-0.3** | 0.6 |
| AN4376 (GdhA) | Glutamate dehydrogenase | 535 | 710 | 1032 | -2.2 | -1.1 | NaN | **-1.1** | 1.1 | -0.7 | -0.2 | -2.0 | **-0.7** | 0.9 |
| AN10946 | Protein of unknown function | 57 | 65 | 216 | -1.7 | -0.4 | NaN | **-1.1** | 0.9 | -0.6 | -0.5 | -1.4 | **-0.6** | 0.5 |
| AN1003 | Isocitrate dehydrogenase | 34 | 151 | 178 | -1.8 | -0.3 | NaN | **-1.0** | 0.9 | -0.3 | -0.3 | -2.3 | **-0.3** | 1.1 |
| AN3058 | Glycine hydroxymethyltransferase | 94 | 159 | 233 | -1.5 | -0.5 | NaN | **-1.0** | 0.8 | -0.6 | -0.4 | -2.0 | **-0.6** | 0.9 |
| AN10540 | Dipeptidyl-peptidase | 166 | 126 | 187 | -1.5 | -0.5 | NaN | **-1.0** | 0.8 | 0.7 | 0.1 | 0.3 | **0.3** | 0.3 |
| AN3172 | 40S ribosomal protein S0 | 52 | 55 | 106 | -1.7 | -0.3 | NaN | **-1.0** | 0.9 | -0.5 | -0.2 | -2.1 | **-0.5** | 1.0 |
| AN10257 | Initiation factor 4G binding | 32 | 45 | 80 | -1.6 | -0.4 | NaN | **-1.0** | 0.8 | -0.4 | -0.3 | -1.5 | **-0.4** | 0.7 |
| AN1700 | 26S proteasome regulatory subunit | 17 | 42 | 139 | -1.3 | -0.6 | NaN | **-1.0** | 0.6 | -0.1 | -0.6 | -1.1 | **-0.6** | 0.5 |
| AN1635 | Protein of unknown function | 14 | 23 | 59 | -1.6 | -0.3 | NaN | **-0.9** | 0.8 | -0.1 | -0.3 | -1.5 | **-0.3** | 0.7 |
| AN0747 | Ubiquinol-cytochrome-c reductase | 28 | 52 | 72 | -1.6 | -0.3 | NaN | **-0.9** | 0.8 | -0.5 | -1.3 | -2.2 | **-1.3** | 0.8 |
| AN2225 | Oxidoreductase | 33 | 48 | 22 | -1.2 | -0.6 | NaN | **-0.9** | 0.6 | 1.1 | -0.3 | -0.1 | **-0.1** | 0.7 |
| AN0858 (Hsp104) | Chaperone | 38 | 100 | 158 | -1.6 | -0.2 | NaN | **-0.9** | 0.9 | -0.3 | -0.5 | -2.0 | **-0.5** | 0.9 |
| AN5563 (GldB) | Dehydrogenase | 150 | 169 | 219 | -1.7 | -0.9 | NaN | **-0.9** | 0.9 | 0.8 | 0.2 | -0.4 | **0.2** | 0.6 |
| AN4793 | Dehydrogenase | 60 | 67 | 96 | -1.9 | -0.9 | NaN | **-0.9** | 1.0 | -1.1 | -0.6 | -0.9 | **-0.9** | 0.3 |
| AN1246 (PgkA) | Phosphoglycerate kinase | 424 | 815 | 1074 | -1.3 | -0.3 | NaN | **-0.8** | 0.7 | -0.7 | -0.6 | -2.1 | **-0.7** | 0.8 |
| AN10797 | Hydroxymethylglutaryl-CoA lyase | 5 | 17 | 13 | -1.4 | -0.3 | NaN | **-0.8** | 0.7 | 0.0 | -0.6 | -1.6 | **-0.6** | 0.8 |
| AN5731 | Chorismate synthase | 20 | 38 | 48 | -2.5 | -0.8 | NaN | **-0.8** | 1.3 | -1.2 | -0.5 | -1.6 | **-1.2** | 0.6 |
| AN0648 (TrpC) | Tryptophan biosynthesis | 31 | 89 | 113 | 0.3 | -1.9 | NaN | **-0.8** | 1.2 | NaN | -0.7 | -1.2 | **-0.9** | 0.4 |
| AN4727 (UrgeA) | UDP-glucose 4-epimerase | 144 | 218 | 272 | -2.0 | -0.8 | NaN | **-0.8** | 1.0 | -0.8 | -0.8 | -1.4 | **-0.8** | 0.3 |
| AN8800 (SgdC) | Cysteinyl tRNA synthase | 41 | 38 | 49 | -2.2 | -0.8 | NaN | **-0.8** | 1.1 | 0.0 | 0.1 | -1.1 | **0.0** | 0.6 |
| AN3712 | Carboxymethylenebutenolidase | 48 | 81 | 52 | -1.5 | -0.7 | NaN | **-0.7** | 0.8 | 1.1 | 0.1 | 0.6 | **0.6** | 0.5 |
| AN0351 (GfdA) | Dehydrogenase | 8 | 6 | 30 | -1.1 | -0.4 | NaN | **-0.7** | 0.5 | -0.1 | -1.5 | -2.0 | **-1.5** | 1.0 |
| AN10351 | Aminopeptidase | 180 | 141 | 158 | -1.0 | -0.2 | NaN | **-0.6** | 0.6 | 0.0 | -0.4 | -1.1 | **-0.4** | 0.6 |
| AN7254 | Cell division control protein 48 | 213 | 207 | 433 | -1.7 | -0.6 | NaN | **-0.6** | 0.9 | 0.1 | -0.2 | -0.7 | **-0.2** | 0.4 |
| AN8434 (AnkG) | Ankyrin repeat doamin protein | 102 | 36 | 62 | -1.6 | -0.6 | NaN | **-0.6** | 0.8 | 4.0 | 2.9 | 1.1 | **2.9** | 1.5 |
| AN2248 (GatA) | 4-aminobutyrate transaminase | 96 | 200 | 513 | -1.1 | 0.0 | NaN | **-0.5** | 0.6 | 0.3 | -0.4 | -2.9 | **-0.4** | 1.7 |
| AN2237 | Carboxypeptidase C | 23 | 14 | 14 | -0.9 | -0.2 | NaN | **-0.5** | 0.4 | 2.4 | 1.2 | 1.5 | **1.5** | 0.6 |

| Gene ID | Description | PSM | | | *laeAΔ/laeA* | | | Ø | SD | *laeAcomp+/laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN7517 | Protein of unknown function | 30 | 4 | 7 | -2.3 | -0.5 | NaN | **-0.5** | 1.2 | 0.8 | -0.1 | 0.1 | **0.1** | 0.5 |
| AN6037 (SwoB) | Glucose-6-phosphate isomerase | 216 | 411 | 615 | -1.7 | -0.5 | NaN | **-0.5** | 0.9 | -0.2 | -0.3 | -1.1 | **-0.3** | 0.5 |
| AN9148 (GalF) | Uridylyltransferase | 173 | 299 | 716 | -1.5 | -0.4 | NaN | **-0.4** | 0.8 | -0.3 | -0.6 | -2.3 | **-0.6** | 1.1 |
| AN7725 (PyroA) | Pyridoxal 5'-phosphate synthase | 231 | 227 | 399 | -1.8 | -0.4 | NaN | **-0.4** | 0.9 | -1.5 | -0.3 | -1.8 | **-1.5** | 0.8 |
| AN1394 (AspD) | Septin | 195 | 170 | 255 | -0.9 | 0.1 | NaN | **-0.4** | 0.6 | 1.0 | 0.4 | -1.0 | **0.4** | 1.0 |
| AN5784 | Proteasome core complex | 32 | 70 | 41 | -1.8 | -0.4 | NaN | **-0.4** | 1.0 | -0.3 | -0.2 | -1.1 | **-0.3** | 0.5 |
| AN10337 | Proteasome regulatory particle | 30 | 40 | 101 | -2.1 | 1.4 | NaN | **-0.4** | 1.7 | -0.3 | -0.2 | -1.2 | **-0.3** | 0.6 |
| AN4583 (Cyp7) | Eptidyl-prolyl cis-trans isomerase D | 53 | 87 | 92 | -1.0 | -0.3 | NaN | **-0.3** | 0.5 | 0.5 | 0.1 | -0.8 | **0.1** | 0.7 |
| AN7299 | DNA-binding protein | 79 | 98 | 229 | -1.2 | -0.3 | NaN | **-0.3** | 0.6 | -0.1 | -0.2 | -1.4 | **-0.2** | 0.7 |
| AN4492 | Protease activity | 18 | 6 | 55 | -1.5 | -0.2 | NaN | **-0.2** | 0.8 | 0.1 | -0.3 | -1.2 | **-0.3** | 0.7 |
| AN2304 | RAD23 homolog | 44 | 52 | 31 | -0.7 | 0.2 | NaN | **-0.2** | 0.5 | 0.9 | 0.1 | -0.8 | **0.1** | 0.9 |
| AN10399 | Oxidoreductase | 89 | 54 | 54 | -1.0 | 0.6 | NaN | **-0.2** | 0.8 | 1.4 | 0.8 | -0.2 | **0.8** | 0.8 |
| AN4443 (MetH) | Methionine synthase | 863 | 1264 | 16 | -1.0 | -0.2 | NaN | **-0.2** | 0.6 | 0.1 | 0.1 | -1.7 | **0.1** | 1.0 |
| AN6717 (MdhA) | Malate dehydrogenase | 488 | 564 | 766 | -1.6 | -0.2 | NaN | **-0.2** | 0.9 | -0.6 | -0.3 | -1.8 | **-0.6** | 0.8 |
| AN4546 | RNA binding activity | 29 | 52 | 96 | -1.2 | -0.2 | NaN | **-0.2** | 0.7 | 0.1 | -0.1 | -0.9 | **-0.1** | 0.6 |
| AN1534 | F1F0-ATPase | 17 | 143 | 7 | -1.9 | 1.7 | NaN | **-0.1** | 1.8 | -0.7 | -0.4 | -2.7 | **-0.7** | 1.3 |
| AN1769 | 3'-5' bisphosphate nucleotidase | 31 | 46 | 25 | -0.6 | 0.4 | NaN | **-0.1** | 0.5 | 1.0 | -0.1 | -1.3 | **-0.1** | 1.2 |
| AN4997 | Phosphatidylinositol transporter | 44 | 85 | 110 | -1.0 | -0.1 | NaN | **-0.1** | 0.6 | 0.3 | 0.2 | -1.6 | **0.2** | 1.1 |
| AN7944 (Ngn3) | GNAT-type acetyltransferase | 62 | 102 | 70 | -1.0 | 0.0 | NaN | **0.0** | 0.6 | -0.7 | 0.1 | 0.8 | **0.1** | 0.8 |
| AN8176 | 60S ribosomal protein L4 | 135 | 126 | 444 | -1.1 | 0.0 | NaN | **0.0** | 0.6 | -0.4 | -0.2 | -2.1 | **-0.4** | 1.0 |
| AN8275 (CitA) | Citrate synthase | 90 | 199 | 401 | -1.6 | 0.0 | NaN | **0.0** | 0.9 | -1.1 | -0.4 | -2.7 | **-1.1** | 1.2 |
| AN6279 (AcuJ) | Carnitine acetyltransferase | 21 | 53 | 64 | -1.3 | 0.1 | NaN | **0.1** | 0.8 | -0.7 | -1.8 | -1.8 | **-1.8** | 0.7 |
| AN3344 (Ngn27) | GNAT-type acetyltransferase | 39 | 62 | 112 | -0.6 | 0.8 | NaN | **0.1** | 0.7 | 1.0 | 0.7 | -2.2 | **0.7** | 1.7 |
| AN4282 | Metalloaminopeptidase | 85 | 137 | 146 | -0.8 | 0.1 | NaN | **0.1** | 0.5 | 0.7 | 0.0 | -1.3 | **0.0** | 1.0 |
| AN8054 | 20S core proteasome | 61 | 75 | 83 | -0.7 | 0.1 | NaN | **0.1** | 0.4 | 0.2 | -0.3 | -1.0 | **-0.3** | 0.6 |
| AN10512 (MthA) | Ketoacyl-CoA thiolase | 18 | 7 | 21 | -0.7 | 1.0 | NaN | **0.1** | 0.9 | 0.9 | -0.6 | -1.6 | **-0.6** | 1.2 |
| AN6089 | Heat shock protein | 107 | 355 | 650 | -1.1 | 0.2 | NaN | **0.2** | 0.7 | -0.9 | -0.5 | -2.9 | **-0.9** | 1.3 |
| AN3299 | Glutathione S-transferase | 15 | 10 | 7 | -0.2 | 0.9 | NaN | **0.3** | 0.6 | 1.4 | -0.4 | 1.3 | **1.3** | 1.0 |
| AN3459 | Metallodipeptidase | 46 | 61 | 99 | 0.0 | 0.7 | NaN | **0.3** | 0.4 | 1.4 | 0.2 | -1.0 | **0.2** | 1.2 |
| AN1689 | Oxidoreductase | 76 | 74 | 37 | 0.1 | 0.6 | NaN | **0.4** | 0.3 | 0.8 | -0.3 | -0.2 | **-0.2** | 0.6 |
| AN3674 | Phospholipid binding activity | 628 | 357 | 615 | -0.5 | 0.4 | NaN | **0.4** | 0.5 | 2.3 | 1.2 | -0.6 | **1.2** | 1.5 |
| AN5803 (FimA) | Fimbrin protein | 76 | 155 | 165 | -0.5 | 0.7 | NaN | **0.7** | 0.6 | 1.2 | 0.1 | -0.8 | **0.1** | 1.0 |
| AN7193 | Glycerol dehydrogenase | 50 | 127 | 113 | -0.2 | 0.8 | NaN | **0.8** | 0.5 | 1.3 | 0.3 | -0.4 | **0.3** | 0.8 |
| AN1543 | Succinate dehydrogenase | 192 | 143 | 132 | 0.3 | 1.7 | NaN | **1.0** | 0.9 | 1.5 | -0.4 | -1.4 | **-0.4** | 1.5 |
| AN8756 | Monooxygenase | 3 | 34 | 78 | -0.5 | 1.3 | NaN | **1.3** | 0.9 | -0.7 | -0.6 | -3.7 | **-0.7** | 1.7 |
| AN2383 | Protein of unknown function | 118 | 150 | 189 | 1.0 | 2.1 | NaN | **1.5** | 1.1 | 3.9 | 4.3 | 2.2 | **3.9** | 1.1 |
| AN6490 | Phosphorylase | 17 | 31 | 27 | -0.8 | 1.7 | NaN | **1.7** | 1.3 | 0.8 | 0.4 | -0.6 | **0.4** | 0.7 |
| AN6031 | Nitronate monooxygenase | 36 | 90 | 32 | 0.8 | 1.9 | NaN | **1.9** | 0.9 | 1.4 | -0.8 | -0.4 | **-0.4** | 1.1 |
| AN8406 | Alcohol dehydrogenase | 67 | 84 | 28 | 0.7 | 1.9 | NaN | **1.9** | 1.0 | -0.6 | -1.5 | -0.9 | **-0.9** | 0.5 |
| AN5646 | Fatty acid degradation | 26 | 46 | 2 | 1.6 | 2.0 | NaN | **2.0** | 1.1 | 1.5 | -0.8 | -0.9 | **-0.8** | 1.4 |
| AN7902 | Monooxygenase | 19 | 31 | 6 | 1.9 | 2.1 | NaN | **2.1** | 1.2 | 0.6 | 1.0 | 1.2 | **1.0** | 0.3 |
| AN2859 | Dihydrodipicolinate synthase | 69 | 47 | 13 | 1.7 | 3.0 | NaN | **2.4** | 1.5 | 1.8 | -0.9 | -1.0 | **-0.9** | 1.6 |
| AN7388 (CpeA) | Laccase | 450 | 319 | 63 | 1.9 | 3.1 | NaN | **3.1** | 1.6 | 3.2 | -0.6 | 4.7 | **3.2** | 2.7 |
| AN7895 (ChiB) | Oxidoreductase | 155 | 340 | 241 | 2.7 | 3.9 | NaN | **3.9** | 2.0 | 1.5 | 1.6 | -0.1 | **1.5** | 1.0 |
| AN10087 | Proliferating cell nuclear antigen | 5 | | 17 | -1.1 | -0.2 | -1.2 | -1.1 | 0.6 | 0.6 | 0.4 | NaN | 0.5 | 0.3 |
| AN7387 (PcrA) | Pyrroline-5-carboxylate reductase | 23 | 23 | 40 | -2.2 | -0.8 | -1.3 | -1.1 | 0.7 | -1.0 | -1.0 | NaN | -1.0 | 0.6 |
| AN1799 | Triglyceride lipase | 32 | 22 | | -1.1 | -0.2 | -1.7 | -1.1 | 0.8 | 0.1 | 0.0 | NaN | 0.1 | 0.1 |
| AN2493 | Alkaline phosphatase | 21 | 42 | 14 | -2.1 | -0.8 | -0.7 | -0.8 | 0.8 | 1.5 | 1.3 | NaN | 1.4 | 0.8 |
| AN4231 | Riboflavin biosynthesis | 21 | 9 | 5 | -1.4 | -0.1 | -1.2 | -0.6 | 0.7 | 0.6 | 0.3 | NaN | 0.4 | 0.3 |
| AN6014 (FaaA) | Long-chain-fatty-acid-CoA ligase | 29 | 9 | | -0.5 | 0.5 | -1.8 | -0.6 | 1.2 | 1.9 | 0.7 | NaN | 1.3 | 0.9 |
| AN4905 (GstA) | Glutathione S-transferase | 36 | 38 | | 0.2 | 1.0 | -2.1 | -0.5 | 1.6 | 2.3 | 0.6 | NaN | 1.4 | 1.2 |
| AN5558 (PrtA) | Alkaline protease | 17 | 102 | 73 | 0.1 | 0.4 | -0.7 | -0.2 | 0.6 | 2.9 | 3.3 | NaN | 3.1 | 1.8 |
| AN1763 | Oxidoreductase | 11 | 10 | | -0.6 | 0.4 | 0.1 | 0.1 | 0.5 | 0.5 | 0.1 | NaN | 0.3 | 0.2 |
| AN2572 | Dipeptidyl-peptidase | 65 | 136 | 29 | 0.2 | 1.4 | -2.7 | 0.2 | 2.1 | 1.1 | -0.1 | NaN | 0.5 | 0.7 |
| AN1378 | Protein of unknown function | 169 | 6 | | 0.2 | 2.5 | -1.0 | 0.2 | 1.8 | 1.9 | 0.9 | NaN | 1.4 | 0.9 |
| AN8415 (ApdG) | Acyl-coA dehydrogenase | 15 | 9 | | 0.4 | 1.4 | -0.1 | 0.6 | 0.8 | 0.9 | -2.6 | NaN | -0.8 | 1.8 |
| AN5421 | Monooxygenase | 146 | 40 | 9 | 0.7 | 1.8 | -0.4 | 0.7 | 1.1 | 3.5 | 0.6 | NaN | 2.0 | 1.9 |
| AN8942 | Protein of unknown function | 21 | 17 | 10 | -1.4 | -0.9 | 2.4 | 0.8 | 2.1 | 0.2 | -0.6 | NaN | -0.2 | 0.4 |
| AN9340 (TreA) | Alpha-trehalase | 27 | 17 | 3 | 0.3 | 1.2 | 1.2 | 1.2 | 0.5 | 1.7 | -0.6 | NaN | 0.6 | 1.2 |
| AN8445 | Aminopeptidase Y | 55 | 58 | 52 | -1.0 | -0.3 | 2.9 | 1.3 | 2.1 | 2.9 | 1.5 | NaN | 2.2 | 1.4 |
| AN6035 | D-fuconate dehydratase | 28 | 11 | 7 | 2.9 | 5.0 | -1.8 | 1.6 | 3.5 | 4.1 | 1.0 | NaN | 2.6 | 2.2 |
| AN2166 | Phytanoyl-CoA dioxygenase | 39 | 30 | 3 | 2.0 | 2.8 | -1.3 | 2.0 | 2.2 | 2.1 | 1.1 | NaN | 1.6 | 1.1 |
| AN1050 | 3-ketoacyl-CoA thiolase | 46 | 33 | | 2.3 | 2.6 | -2.1 | 2.3 | 2.6 | 2.4 | -1.4 | NaN | 0.5 | 1.9 |
| AN6853 | Phosphatidylinositol transporter | | 10 | 33 | NaN | -1.3 | -1.2 | -1.3 | 0.7 | NaN | -0.1 | -1.4 | -0.8 | 0.8 |
| AN3593 | Dehydratase | 6 | 17 | 27 | NaN | -0.5 | -1.8 | -1.2 | 0.9 | NaN | 0.6 | -0.9 | -0.2 | 0.7 |
| AN3719 (MpkB) | Mitogen-activated protein kinase | | 7 | 43 | NaN | -0.9 | -0.9 | -0.9 | 0.5 | NaN | 0.2 | -0.2 | 0.0 | 0.2 |
| AN5954 | Translation initiation factor | 8 | 21 | 116 | NaN | -1.6 | -0.3 | -0.9 | 0.9 | NaN | -0.4 | -1.8 | -1.1 | 1.0 |

| Gene ID | Description | PSM | | | *laeAΔ/laeA* | | | Ø | SD | *laeAcomp+/laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN1673 | DAHP synthase | 4 | 9 | 18 | NaN | -1.2 | -1.1 | -1.1 | 0.7 | -1.6 | NaN | -1.0 | NaN | NaN |
| AN7914 | Alcohol dehydrogenase | 203 | 159 | 75 | -6.2 | -4.9 | NaN | -4.9 | 3.3 | -1.8 | -3.9 | NaN | -2.8 | 1.9 |
| AN2343 | Nitroreductase | 97 | 8 | | -4.9 | -2.8 | NaN | -3.9 | 2.5 | -3.1 | -1.8 | NaN | -2.4 | 1.5 |
| AN7836 | Protein of unknown function | 19 | 36 | 23 | -3.5 | -3.0 | NaN | -3.0 | 1.9 | 1.2 | -1.5 | NaN | -0.2 | 1.4 |
| AN2622 (IpnA) | Isopenicillin-N synthase | 8 | 2 | | -2.1 | -4.0 | NaN | -3.0 | 2.0 | 2.0 | 0.4 | NaN | 1.2 | 1.1 |
| AN7268 | Oxidoreductase | 35 | 17 | 2 | -1.1 | -2.9 | NaN | -2.9 | 1.5 | 2.9 | 0.8 | NaN | 1.8 | 1.5 |
| AN1539 (CsnD) | COP9 signalosome | 3 | | 4 | -1.5 | 1.0 | -2.1 | -1.5 | 1.6 | 0.9 | NaN | NaN | NaN | NaN |
| AN3628 | Peptide alpha-N-acetyltransferase | 5 | 7 | 41 | -1.9 | NaN | -1.2 | -1.2 | 1.0 | -0.6 | NaN | -1.6 | -1.1 | 0.8 |
| AN2315 | F1F0-ATPase complex subunit | 417 | 515 | 842 | -1.7 | -0.3 | NaN | -1.0 | 0.9 | -0.4 | -0.5 | NaN | -0.4 | 0.3 |
| AN1194 | Adenylyl phosphosulfate kinas | 14 | 28 | 50 | -1.5 | -0.4 | NaN | -0.9 | 0.8 | 0.6 | 0.2 | NaN | 0.4 | 0.3 |
| AN2668 | Oxidoreductase | 2 | 3 | | -1.2 | -0.6 | NaN | -0.9 | 0.6 | -0.3 | -1.0 | NaN | -0.6 | 0.5 |
| AN5981 | Myosin I binding activity | 12 | 6 | 28 | -1.2 | NaN | 0.9 | -0.1 | 1.1 | 0.1 | NaN | -1.1 | -0.5 | 0.7 |
| AN3524 | Galactose 1-dehydrogenase | | 29 | 43 | NaN | 2.1 | -1.3 | 0.4 | 1.7 | NaN | NaN | -4.6 | -4.6 | 2.7 |
| AN5878 | Thiolases | 2 | 5 | 4 | 1.0 | 1.8 | -0.7 | 0.6 | 1.3 | -0.9 | NaN | NaN | NaN | NaN |
| AN0034 | Kinase | 84 | 51 | | 0.6 | 1.5 | NaN | 1.0 | 0.8 | 2.6 | -0.4 | NaN | 1.1 | 1.6 |
| AN2828 (BglL) | Beta-glucosidase | 58 | 6 | | 1.1 | 1.3 | -0.3 | 1.1 | 0.9 | 3.4 | NaN | NaN | NaN | NaN |
| AN10335 | Oxidoreductase | 5 | 8 | | 1.0 | 2.0 | NaN | 1.5 | 1.0 | 3.0 | -0.2 | NaN | 1.4 | 1.8 |
| AN0783 | Carbon-nitrogen ligase | 3 | 17 | 10 | 1.7 | 1.8 | -1.8 | 1.7 | 2.1 | 2.6 | NaN | NaN | NaN | NaN |
| AN2577 | Oxidoreductase | 36 | 25 | | 1.3 | 2.3 | NaN | 1.8 | 1.2 | 0.7 | NaN | NaN | NaN | NaN |
| AN1677 | Short-chain dehydrogenase | 45 | 48 | 18 | 2.0 | 3.0 | NaN | 2.5 | 1.5 | 1.1 | NaN | NaN | NaN | NaN |
| AN5695 | Carbon-sulfur lyase | 7 | 2 | | 1.2 | 2.7 | NaN | 2.7 | 1.3 | 1.0 | -0.1 | NaN | 0.5 | 0.6 |
| AN5373 | Oxidoreductase | 49 | 25 | 5 | 1.9 | 2.9 | NaN | 2.9 | 1.5 | 3.5 | 1.5 | NaN | 2.5 | 1.7 |
| AN5883 (MetF) | Reductase | 14 | 5 | 42 | NaN | -1.3 | NaN | NaN | NaN | -1.4 | 0.3 | -1.8 | -1.4 | 1.1 |
| AN3591 | Dehydrogenase | 56 | 78 | 62 | NaN | 0.7 | NaN | NaN | NaN | 1.0 | -0.8 | -1.2 | -0.8 | 1.2 |
| AN0651 (FadA) | Heterotrimeric G protein | 5 | 6 | 3 | NaN | NaN | -0.5 | NaN | 0.3 | NaN | NaN | -1.2 | NaN | NaN |
| AN3594 (SogA) | Vacuolar protein-sorting protein | 7 | 24 | 22 | NaN | NaN | -1.5 | NaN | NaN | NaN | 0.0 | -1.8 | NaN | NaN |
| AN7579 | 26S proteasome regulatory subunit | 5 | | 23 | -1.0 | NaN | -1.7 | NaN | 0.9 | NaN | NaN | -0.8 | NaN | NaN |
| AN1805 (CanB) | Carbonic anhydrase | | 35 | 36 | NaN | -2.0 | NaN | NaN | NaN | NaN | 0.1 | NaN | NaN | NaN |
| AN3349 | Cytochrome P450 | 16 | 8 | 7 | NaN | NaN | -3.8 | NaN | NaN | 0.3 | NaN | NaN | NaN | NaN |
| AN6078 (NadA) | Adenine deaminase | 4 | | 10 | NaN | NaN | -2.3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN6712 (PldA) | Phospholipase D | 14 | 5 | | NaN | NaN | -2.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3573 | Oxidoreductase | 54 | 6 | 5 | 1.8 | NaN | NaN | NaN | NaN | 5.4 | NaN | NaN | NaN | NaN |
| AN9103 (AifA) | Apoptosis-inducing factor (AIF)-like | 48 | 59 | 17 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | 1.8 | NaN | NaN |
| AN7143 | Heat shock protein | 3 | | 103 | NaN | NaN | -5.6 | NaN | NaN | NaN | NaN | -1.8 | NaN | NaN |
| AN8797 (MvlA) | Orsellinic acid gene cluster | | 22 | 14 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN6838 (TubC) | Beta-tubulin | 157 | | 365 | NaN | NaN | -3.7 | NaN | NaN | NaN | NaN | -0.9 | NaN | NaN |
| AN8692 | Thioredoxin-dependent peroxidase | 123 | | 6 | NaN | NaN | -2.4 | NaN | NaN | NaN | NaN | 0.6 | NaN | NaN |
| AN6126 (AccA) | Acetyl-CoA carboxylase | 36 | 156 | 351 | NaN | NaN | -2.4 | NaN | NaN | NaN | NaN | -0.8 | NaN | NaN |
| AN6157 (PyrG) | Decarboxylase | | 6 | 16 | NaN | NaN | -2.2 | NaN | NaN | NaN | NaN | -1.4 | NaN | NaN |
| AN8932 | TIM-barrel enzyme family protein | | 6 | 2 | NaN | NaN | -2.1 | NaN | NaN | NaN | NaN | 1.5 | NaN | NaN |
| AN8072 | Protein of unknown function | 7 | 6 | 21 | NaN | NaN | -2.1 | NaN | NaN | NaN | NaN | -1.1 | NaN | NaN |
| AN6060 | Role in RNA metabolic process | 7 | 22 | 107 | NaN | NaN | -2.0 | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN7008 | Hydroxyacyl-CoA dehydrogenase | | 8 | 6 | NaN | NaN | -2.0 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN6753 | Oxidoreductase | | 15 | 34 | NaN | NaN | -1.9 | NaN | NaN | NaN | NaN | -1.8 | NaN | NaN |
| AN8268 | Ras GTPase-activating protein | 12 | | 45 | NaN | NaN | -1.8 | NaN | NaN | NaN | NaN | -1.8 | NaN | NaN |
| AN6077 | NADH dehydrogenase (ubiquinone) | | 5 | 77 | NaN | NaN | -1.8 | NaN | NaN | NaN | NaN | -2.6 | NaN | NaN |
| AN8874 | Dynamin-related protein | 14 | 8 | 41 | NaN | NaN | -1.8 | NaN | NaN | NaN | NaN | -1.9 | NaN | NaN |
| AN5908 (TpiB) | Triose-phosphate isomerase | 12 | 10 | 11 | NaN | NaN | -1.8 | NaN | NaN | NaN | NaN | -0.7 | NaN | NaN |
| AN8036 | Oxidoreductase | | 15 | 14 | NaN | NaN | -1.7 | NaN | NaN | NaN | NaN | 0.7 | NaN | NaN |
| AN9407 (FasA) | Fatty acid synthase | 41 | 144 | 524 | NaN | NaN | -1.7 | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN7159 | Tripeptidyl-peptidase | | 24 | 22 | NaN | NaN | -1.6 | NaN | NaN | NaN | NaN | -1.4 | NaN | NaN |
| AN3629 | DNA glycosylase | | 4 | 13 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -0.6 | NaN | NaN |
| AN6688 (AspB) | Putative Septin B | 8 | 180 | 314 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -0.8 | NaN | NaN |
| AN7035 | Peptidase | | 10 | 34 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -0.1 | NaN | NaN |
| AN9090 (RrmA) | RNA binding protein | 22 | 21 | 86 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN6006 (KapD) | Karyopherin | 9 | 24 | 79 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN9083 | Adenine phosphoribosyltransferase | 11 | 12 | 11 | NaN | NaN | 0.1 | NaN | NaN | NaN | NaN | -0.8 | NaN | NaN |
| AN6705 | Protein of unknown function | | 12 | 44 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN8203 | Hydrolase | 16 | 10 | 35 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | -0.9 | NaN | NaN |
| AN7590 | Mannitol 2-dehydrogenase | 18 | 23 | 17 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN6741 | Aspartic-type endopeptidase | 2 | | 3 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN6338 | Aromatic-amino-acid transaminase | | 14 | 29 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | -2.3 | NaN | NaN |
| AN7909 (OrsA) | Polyketide synthase | 13 | 15 | 45 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | 0.4 | NaN | NaN |
| AN8755 (MclA) | Methylisocitrate lyase | | 3 | 15 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -1.0 | NaN | NaN |
| AN6193 | Peptidase | | 15 | 59 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -2.3 | NaN | NaN |
| AN6734 (KapF) | Karyopherin | 23 | 31 | 47 | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN7199 | Protein of unknown function | | 2 | 48 | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | -2.8 | NaN | NaN |

| Gene ID | Description | PSM | | | *laeAΔ/laeA* | | | Ø | SD | *laeAcomp+/laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN7194 | Oxidoreductase | 6 | 4 | 14 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN8869 | Uracil phosphoribosyltransferase | 12 | 11 | 15 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -1.0 | NaN | NaN |
| AN9094 (UngA) | Pyrophosphorylase | 147 | 233 | 287 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -0.8 | NaN | NaN |
| AN6507 | Cap binding protein | | 4 | 32 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN8023 (VpsA) | Required for vacuole biogenesis | 17 | 51 | 111 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN8057 (CysB) | Cysteine synthase | | 15 | 63 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | -2.4 | NaN | NaN |
| AN6978 (Rcc1) | Nucleotide exchange factor | | 6 | 49 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | -1.4 | NaN | NaN |
| AN7441 | Histone acetyltransferase | 3 | 4 | 20 | NaN | NaN | -0.7 | NaN | NaN | NaN | NaN | -1.0 | NaN | NaN |
| AN6512 | ATPase | | 8 | 14 | NaN | NaN | -0.7 | NaN | NaN | NaN | NaN | -0.9 | NaN | NaN |
| AN7498 | Deoxyhypusine monooxygenase | 12 | 14 | 14 | NaN | NaN | -0.7 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN6045 | Chaperone | 3 | | 7 | NaN | NaN | -0.6 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN6844 | 3-hydroxyisobutyryl-CoA hydrolase | 8 | 12 | 10 | NaN | NaN | -0.6 | NaN | NaN | NaN | NaN | -2.3 | NaN | NaN |
| AN6054 | Genome maintenance protein | | 2 | 3 | NaN | NaN | -0.5 | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN6505 (RcoA) | WD40 repeat protein | 25 | 39 | 57 | NaN | NaN | -0.3 | NaN | NaN | NaN | NaN | -0.4 | NaN | NaN |
| AN8795 | AP-1 adaptor complex subunit | 2 | 7 | 16 | NaN | NaN | -0.3 | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN7142 (PacA) | Phosphatase | | 7 | 216 | NaN | NaN | 0.0 | NaN | NaN | NaN | NaN | -4.2 | NaN | NaN |
| AN6033 | Coat protein complex I | 17 | | 14 | NaN | NaN | 0.0 | NaN | NaN | NaN | NaN | -1.8 | NaN | NaN |
| AN6182 (GalD) | Uridylyltransferase | 5 | | 4 | NaN | NaN | 0.1 | NaN | NaN | NaN | NaN | -0.4 | NaN | NaN |
| AN7491 | Deubiquitinating enzymes | | 6 | 14 | NaN | NaN | 0.2 | NaN | NaN | NaN | NaN | -0.6 | NaN | NaN |
| AN8945 | Methyltransferase | | 7 | 20 | NaN | NaN | 0.6 | NaN | NaN | NaN | NaN | -0.7 | NaN | NaN |
| AN7540 | Role in translational initiation | | 263 | 141 | NaN | NaN | 0.6 | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN8790 | D-xylulokinase | 10 | 4 | 9 | NaN | NaN | 0.8 | NaN | NaN | NaN | NaN | -1.0 | NaN | NaN |
| AN8045 | Role in mRNA polyadenylation | 3 | | 31 | NaN | NaN | 0.8 | NaN | NaN | NaN | NaN | -1.0 | NaN | NaN |
| AN6325 | 5'-nucleotidase | | 6 | 9 | NaN | NaN | 2.4 | NaN | NaN | NaN | NaN | 0.5 | NaN | NaN |
| AN7105 | Translation initiation factor 3 (eIF3) | 7 | 22 | 237 | NaN | NaN | 2.5 | NaN | NaN | NaN | NaN | -1.9 | NaN | NaN |
| AN9011 | Aryl-alcohol oxidase | 35 | 12 | 12 | NaN | NaN | 2.8 | NaN | NaN | NaN | NaN | 3.9 | NaN | NaN |
| AN7017 | Protein of unknown function | | 6 | 14 | NaN | NaN | 3.5 | NaN | NaN | NaN | NaN | -2.7 | NaN | NaN |
| AN7422 | Deubiquitinating enzymes | 20 | 14 | 38 | NaN | NaN | 4.3 | NaN | NaN | NaN | NaN | -0.5 | NaN | NaN |
| AN3070 | Chaperone | | 21 | 59 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN5737 | Oxidoreductase | | 2 | 10 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | 0.9 | NaN | NaN |
| AN0252 | F1F0-ATPase complex gamma | 43 | | 27 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -2.1 | NaN | NaN |
| AN3080 | Coat protein complex II protein | 20 | 11 | 31 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN1163 | Chaperone | | 13 | 20 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -2.8 | NaN | NaN |
| AN5144 (PfkZ) | 6-phosphofructo-2-kinase | 5 | 12 | 71 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -1.4 | NaN | NaN |
| AN1158 | Cell wall biogenesis protein | 27 | 69 | 130 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN2441 | E1 NeddH/Nedd8-activating | 20 | 12 | 21 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -0.6 | NaN | NaN |
| AN4550 (Dps1) | Asparaginyl-tRNA synthetase | 90 | 108 | 207 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN2970 | Phosphatase | 2 | 10 | 11 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN5123 | Oxidoreductase | | 10 | 7 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -0.5 | NaN | NaN |
| AN5520 | Role in ribosome biogenesis | 30 | 32 | 287 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -2.2 | NaN | NaN |
| AN10229 | Dehydrogenase | 7 | | 9 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -2.6 | NaN | NaN |
| AN4745 | Rho GTPase activating | 4 | 23 | 40 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN3804 | Protein of unknown function | | 3 | 41 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -3.5 | NaN | NaN |
| AN10164 | Polyadenylated RNA-binding | 12 | 15 | 46 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -0.4 | NaN | NaN |
| AN11902 | Formyltetrahydrofolate cyclo-ligase | | 2 | 4 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -0.7 | NaN | NaN |
| AN5957 | Aminotransferase | 18 | 40 | 85 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN5717 (KapI) | Importin subunit | 21 | 35 | 88 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN4178 (Myg1) | Melanocyte proliferating protein | | 8 | 19 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | -2.1 | NaN | NaN |
| AN4288 | Oxidoreductase | 9 | | 101 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | -2.8 | NaN | NaN |
| AN10182 | Translation initiation factor 3 | 5 | 24 | 95 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | -1.4 | NaN | NaN |
| AN3034 | Regulation of penicillin biosynthesis | | 12 | 50 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | -2.6 | NaN | NaN |
| AN2983 (Msb1) | Rho GTPase-activating protein | 3 | | 8 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | -1.9 | NaN | NaN |
| AN5130 | Coproporphyrinogen oxidase | 50 | 125 | 202 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | -4.0 | NaN | NaN |
| AN9509 | Role in transcription termination | 15 | 4 | 28 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | -0.8 | NaN | NaN |
| AN10783 | Phosphogluconate dehydrogenase | 32 | 24 | 28 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | 1.1 | NaN | NaN |
| AN11058 | Protein of unknown function | 14 | 7 | 10 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | 0.6 | NaN | NaN |
| AN4207 | Clathrin adaptor protein | 4 | 6 | 31 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN0317 | Role in endocytosis | 24 | 36 | 49 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | -1.4 | NaN | NaN |
| AN2000 (Ubi4) | Polyubiquitin | 3 | 59 | 90 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | -1.0 | NaN | NaN |
| AN1084 | Elongation factor EF-Tu | 96 | 151 | 8 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN10765 | Translation initiation factor | | 14 | 40 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN0193 | Hydrolase | 14 | | 18 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN0295 | Polyubiquitin binding activity | | 8 | 4 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN4323 | Aminotransferase | | 48 | 108 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | -2.6 | NaN | NaN |
| AN1466 | PWWP domain protein | | 4 | 27 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN2066 | Prospore assembly | 23 | 27 | 35 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -0.9 | NaN | NaN |
| AN10519 | Protein of unknown function | 36 | 43 | 61 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |

| Gene ID | Description | PSM | | | *laeAΔ/laeA* | | | Ø | SD | *laeAcomp+/laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN2981 (GsdA) | Dehydrogenase | 136 | 219 | 266 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN4563 | Serine/threonine kinase | 14 | 15 | 27 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN1409 | Acetyl-CoA C-acetyltransferase | | 206 | 293 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -1.1 | NaN | NaN |
| AN2275 | Large ribosomal subunit | | 22 | 119 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN10281 | Serine/threonine phosphatase | 16 | 11 | 28 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN4397 (FatD) | Coenzyme A synthetase | 11 | 5 | 6 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN10080 | Siderophore biosynthetic | 10 | | 18 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -1.0 | NaN | NaN |
| AN1404 | Diacylglycerol kinase | 3 | 4 | 5 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -0.4 | NaN | NaN |
| AN5566 | GMP synthase | 16 | 38 | 106 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN4258 | Kinase | 28 | 50 | 67 | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN4000 (FabM) | Poly(A)-binding protein | 15 | 67 | 256 | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN3026 (CopA) | Coatomer protein complex | 25 | 44 | 53 | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | -1.4 | NaN | NaN |
| AN9470 | Urate oxidase | | 260 | 159 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN10743 | Role in endocytosis | 9 | 10 | 28 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -1.1 | NaN | NaN |
| AN0717 | Aminotransferase | 28 | 29 | 53 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -0.6 | NaN | NaN |
| AN1162 | Guanyl-nucleotide exchange factor | 212 | 237 | 513 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -1.9 | NaN | NaN |
| AN0106 (PkcA) | Protein kinase C | 11 | 11 | 27 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -0.7 | NaN | NaN |
| AN2907 | Translation initiation factor 3 | | 16 | 98 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN2734 | 60S acidic ribosomal protein P0 | 27 | 29 | 299 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -2.2 | NaN | NaN |
| AN5749 | Metallopeptidase | 8 | 39 | 23 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -0.7 | NaN | NaN |
| AN0673 | Actin assembly | | 32 | 39 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN5972 | Coat protein complex I | 12 | 6 | 48 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | -1.4 | NaN | NaN |
| AN5820 (MecA) | Cystathionine beta-synthase | 63 | 66 | 97 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN5499 (Mlp1) | Nuclear pore complex protein | 2 | | 31 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN7704 | Protein of unknown function | 47 | 15 | 25 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | -0.6 | NaN | NaN |
| AN10469 | Oxidoreductase | | 8 | 5 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN5799 | Reductase | 30 | | 49 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN7721 | Translocon subunit | 12 | | 27 | NaN | NaN | -0.7 | NaN | NaN | NaN | NaN | -1.8 | NaN | NaN |
| AN5677 | Protein of unknown function | 14 | 12 | 29 | NaN | NaN | -0.7 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN5666 (MpkA) | Mitogen-activated protein kinase | 44 | 50 | 98 | NaN | NaN | -0.7 | NaN | NaN | NaN | NaN | -0.4 | NaN | NaN |
| AN1639 | Disulfide oxidoreductase | 11 | 7 | 32 | NaN | NaN | -0.7 | NaN | NaN | NaN | NaN | -0.1 | NaN | NaN |
| AN11702 | Protein of unknown function | | 2 | 102 | NaN | NaN | -0.6 | NaN | NaN | NaN | NaN | 0.2 | NaN | NaN |
| AN4547 | Coat protein complex II | 21 | 34 | 119 | NaN | NaN | -0.6 | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN4430 | Acetolactate synthase | 8 | | 46 | NaN | NaN | -0.5 | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN5613 (HxA) | Xanthine dehydrogenase | 4 | 37 | 27 | NaN | NaN | -0.5 | NaN | NaN | NaN | NaN | 0.5 | NaN | NaN |
| AN5311 | Oxidoreductase | | 15 | 56 | NaN | NaN | -0.5 | NaN | NaN | NaN | NaN | -4.8 | NaN | NaN |
| AN12229 | Protein of unknown function | 9 | 8 | 26 | NaN | NaN | -0.5 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN1150 | Transaminase | 17 | 15 | 62 | NaN | NaN | -0.5 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN0316 (TubA) | Alpha-tubulin | 99 | 199 | 338 | NaN | NaN | -0.4 | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN2243 | Carbamoyl-phosphate synthase | | 9 | 44 | NaN | NaN | -0.4 | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN0076 | Regulation in translation | 8 | 7 | 37 | NaN | NaN | -0.4 | NaN | NaN | NaN | NaN | -0.7 | NaN | NaN |
| AN11102 | Thiolesterase | 3 | 7 | 34 | NaN | NaN | -0.3 | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN10695 | Protein of unknown function | 13 | | 4 | NaN | NaN | -0.3 | NaN | NaN | NaN | NaN | 2.0 | NaN | NaN |
| AN1328 (NnaA) | GNAT-type acetyltransferase | 5 | 5 | 19 | NaN | NaN | -0.2 | NaN | NaN | NaN | NaN | -1.9 | NaN | NaN |
| AN10301 | Protein of unknown function | 5 | 5 | 11 | NaN | NaN | -0.1 | NaN | NaN | NaN | NaN | 0.9 | NaN | NaN |
| AN8831 (AmpB) | Role in actin filament organization | 5 | 9 | 17 | NaN | NaN | 0.0 | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN11055 | Phosphatase | | 4 | 24 | NaN | NaN | 0.0 | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN2871 (McnC) | Phosphoprotein | 4 | 3 | 33 | NaN | NaN | 0.0 | NaN | NaN | NaN | NaN | -1.8 | NaN | NaN |
| AN5743 | Potassium-transporting ATPase | 2 | | 11 | NaN | NaN | 0.0 | NaN | NaN | NaN | NaN | -3.1 | NaN | NaN |
| AN1809 | Fumarylacetoacetase | 17 | 19 | 13 | NaN | NaN | 0.1 | NaN | NaN | NaN | NaN | 1.1 | NaN | NaN |
| AN10614 | G-quadruplex DNA binding activity | | 30 | 175 | NaN | NaN | 0.3 | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN3226 | Oxidoreductase | 21 | 68 | 80 | NaN | NaN | 0.5 | NaN | NaN | NaN | NaN | 2.4 | NaN | NaN |
| AN5435 | Oxidoreductase | | 15 | 25 | NaN | NaN | 0.5 | NaN | NaN | NaN | NaN | -0.7 | NaN | NaN |
| AN9467 | Serine/threonine phosphatase | | 6 | 21 | NaN | NaN | 0.7 | NaN | NaN | NaN | NaN | -0.8 | NaN | NaN |
| AN7659 | RNA helicase | 58 | 63 | 66 | NaN | NaN | 0.7 | NaN | NaN | NaN | NaN | -1.0 | NaN | NaN |
| AN5616 | Aminotransferase | 14 | 28 | 34 | NaN | NaN | 0.9 | NaN | NaN | NaN | NaN | -0.9 | NaN | NaN |
| AN3312 | L-xylulose reductase | | 7 | 7 | NaN | NaN | 1.2 | NaN | NaN | NaN | NaN | 3.2 | NaN | NaN |
| AN10060 | Protein of unknown function | 33 | 3 | | NaN | NaN | 1.2 | NaN | NaN | NaN | NaN | -2.4 | NaN | NaN |
| AN0052 | Oxidoreductase | 6 | 12 | 6 | NaN | NaN | 1.4 | NaN | NaN | NaN | NaN | 2.5 | NaN | NaN |
| AN7600 | Oxidoreductase | 6 | 29 | 283 | NaN | NaN | 1.5 | NaN | NaN | NaN | NaN | -2.3 | NaN | NaN |
| AN10306 | N-terminal subunit of Cand1 | | 7 | 3 | NaN | NaN | 1.7 | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN0788 | Protein of unknown function | | 3 | 4 | NaN | NaN | 1.8 | NaN | NaN | NaN | NaN | -0.2 | NaN | NaN |
| AN6541 | Ligase | 24 | 37 | 60 | NaN | NaN | 1.9 | NaN | NaN | NaN | NaN | -1.8 | NaN | NaN |
| AN11005 | Metalloexopeptidase | | 18 | 27 | NaN | NaN | 2.3 | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN3019 | Proteasome regulatory particle | 28 | 48 | 64 | NaN | NaN | 3.4 | NaN | NaN | NaN | NaN | -0.8 | NaN | NaN |
| AN3987 | Protein of unknown function | 21 | 16 | 60 | NaN | NaN | 4.5 | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN8009 (Nmt1) | Thiamine biosynthesis protein | 64 | 491 | 953 | NaN | NaN | 4.8 | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |

| Gene ID | Description | PSM | | | *laeAΔ/laeA* | | | Ø | SD | *laeAcomp+/laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN0224 | Dipeptidase | 27 | 40 | 33 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0271 | dUTP pyrophosphatase | 19 | 6 | 2 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0182 (RasA) | Small monomeric GTPase | | 10 | 5 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7222 | NACHT domain-containing protein | 40 | 16 | 45 | NaN | NaN | -2.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7264 | Methyltransferase | 5 | 9 | | NaN | NaN | -2.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7177 | Protein of unknown function | 52 | 30 | 63 | NaN | NaN | -2.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN6207 (PkpC) | Kinase | 8 | 16 | 22 | NaN | NaN | -1.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2879 | Carbon-nitrogen ligase | 95 | 4 | 7 | NaN | NaN | -1.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7350 | Translation initiation factor 4B | 45 | 29 | 95 | NaN | NaN | -1.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8074 | Thiolesterase | 6 | 17 | 25 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8168 (NmrA) | Nitrogen metabolite repression | | 4 | 5 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN9138 | Amidase/acetamidase | 13 | 6 | 7 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7469 | Riboflavin kinase | | 8 | 5 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN6515 | Protein of unknown function | 11 | 17 | 37 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7298 | Protein of unknown function | 6 | | 2 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7307 | Protein of unknown function | 13 | 3 | | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN6980 (Nic96) | Nuclear pore complex | 6 | | 9 | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8218 (TrxB) | Thioredoxin reductase | 17 | 15 | 22 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7128 | Oxidoreductase | 21 | 13 | | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7511 (GelE) | 1,3-beta-transglycosidase | 14 | 17 | 30 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN6438 | Exopeptidase | 20 | | 6 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN6272 | Oxidoreductase | | 4 | 10 | NaN | NaN | -0.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN6286 | Protein of unknown function | 4 | 3 | | NaN | NaN | -0.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7998 (XptC) | GMC Oxidoreductase *mdp/xpt* | 7 | 3 | | NaN | NaN | 0.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7051 (MetG) | Cystathionine beta-lyase | | 11 | 23 | NaN | NaN | 0.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8433 | Protein of unknown function | 27 | 24 | 15 | NaN | NaN | 0.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7911 (OrsB) | Amidohydrolase | 6 | | 3 | NaN | NaN | 2.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN9012 | Lyase | 11 | | 6 | NaN | NaN | 3.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7897 | Monooxygenase | | 6 | 12 | NaN | NaN | -4.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10437 | Protein of unknown function | 13 | 14 | 21 | NaN | NaN | -4.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5353 | Protein of unknown function | | 11 | 8 | NaN | NaN | -3.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3565 | Hydrolase activity | 7 | 2 | | NaN | NaN | -3.3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4058 | Dihydroxy acid dehydratase | | 18 | 6 | NaN | NaN | -2.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1849 | Protein of unknown function | | 2 | 5 | NaN | NaN | -2.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10942 | Actin depolymerizing protein | 38 | 39 | 45 | NaN | NaN | -2.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2244 | Cytoplasmic translation | | 6 | 8 | NaN | NaN | -2.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1007 (NiiA) | Nitrite reductase | 7 | 69 | 286 | NaN | NaN | -2.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5688 | Lipid biosynthetic process | | 8 | 10 | NaN | NaN | -2.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN11191 | Polyketide synthase (PKS) | | 188 | 197 | NaN | NaN | -2.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3197 | Oxidoreductase | 9 | 3 | | NaN | NaN | -2.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3057 | Oxidoreductase | 5 | 4 | 9 | NaN | NaN | -2.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN11168 | Protein of unknown function | 3 | 17 | 21 | NaN | NaN | -2.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7722 | Arginine metabolism | 32 | 25 | 63 | NaN | NaN | -2.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8274 | Alpha-ketoglutarate transport | 10 | | 17 | NaN | NaN | -2.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2335 | Dehydrogenase | | 30 | | NaN | NaN | -1.9 | NaN | NaN | NaN | NaN | NAN | NaN | NaN |
| AN12022 | Monooxygenase | | 8 | 2 | NaN | NaN | -1.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3416 | Syntaxin | 39 | 27 | 50 | NaN | NaN | -1.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1211 | Vacuolar ATP synthase subunit H | 22 | 7 | 8 | NaN | NaN | -1.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0628 | D-lactate dehydrogenase | 3 | 4 | 4 | NaN | NaN | -1.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN9193 (LlmJ) | LaeA-like methyltransferase | | 4 | 4 | NaN | NaN | -1.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3456 | Cystathionine gamma-synthase | | 13 | 32 | NaN | NaN | -1.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN9149 | Protein of unknown function | 22 | | 23 | NaN | NaN | -1.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3916 | Glycerol kinase | 50 | 9 | | NaN | NaN | -1.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0948 | ATPase activity | 10 | 6 | 6 | NaN | NaN | -1.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10230 | Phosphorylase | 34 | 28 | 37 | NaN | NaN | -1.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0327 | DNA helicase | | 5 | 22 | NaN | NaN | -1.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5831 | Protein of unknown function | | 44 | 75 | NaN | NaN | -1.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10856 | Protein of unknown function | 6 | 3 | 18 | NaN | NaN | -1.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2091 | Carboxy-lyase | 12 | 30 | 22 | NaN | NaN | -1.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2853 | Transcription regulator protein | | 8 | 2 | NaN | NaN | -1.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4008 | Methyltransferase | 20 | 41 | 33 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3029 | AP-1 adaptor complex | 17 | 23 | | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10533 | Trehalose-6-phosphate synthase | | 28 | 23 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8010 | Glycogen (starch) synthase | 321 | 9 | | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2298 | SUMO activating enzyme | 3 | 14 | 6 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3161 | Oxidoreductase | | 2 | 5 | NaN | NaN | -1.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0447 | Iron-sulfur cluster binding activity | | 6 | 11 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |

| Gene ID | Description | PSM | | | *laeA*Δ/*laeA* | | | Ø | SD | *laeAcomp+*/*laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN4483 | Serine/threonine kinase | | 3 | 11 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1873 | Protein of unknown function | | 10 | 10 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0665 (AneA) | Coatomer subunit epsilon | | 10 | 12 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0697 | Septin cytoskeleton | 7 | | 16 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2414 | NADH dehydrogenase (ubiquinone) | 8 | 3 | 32 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4353 | Protein of unknown function | 33 | 22 | | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4575 | Protein of unknown function | 6 | 25 | 14 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4688 (IvdA) | Acyl-coA dehydrogenase | 10 | 3 | | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN12209 | Protein of unknown function | 2 | | 3 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4916 | Role in ribosome biogenesis | 13 | | 4 | NaN | NaN | -1.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4268 | Nitronate monooxygenase | 5 | 6 | 3 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4965 | Protein of unknown function | | 6 | 24 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3961 | Protein of unknown function | | 38 | 10 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4439 | Adenylyltransferase | 11 | | 8 | NaN | NaN | -1.3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1502 (NagA) | Chitin hydrolysis | 8 | 8 | | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4024 | Protein of unknown function | 8 | 6 | 11 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10417 | RNA helicase | | 11 | 21 | NaN | NaN | -1.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2860 | Alcohol dehydrogenase | 6 | 2 | | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10282 | Protein of unknown function | 3 | 9 | 95 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8396 (PdcB) | Decarboxylase | 22 | 17 | | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8859 | Aspartate kinase | 4 | | 16 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4532 (Prp8) | Catalytic core of the spliceosome | 16 | 8 | 6 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2311 | Phosphomevalonate kinase | 44 | 31 | 44 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10379 | Protein of unknown function | 16 | 17 | 20 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1545 | Phosphatase | 18 | | 19 | NaN | NaN | -1.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5718 | Fumarate hydratase | 8 | 7 | 16 | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN12335 (AcdA) | Acyl-CoA dehydrogenase | 20 | 22 | | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10311 (MnpA) | Cell wall mannoprotein | 20 | 4 | | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7805 (StcV) | Norsolorinic acid reductase | 154 | 4 | | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4779 | Protein of unknown function | 4 | 4 | 4 | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8836 | Serine/threonine-protein kinase | | 4 | 13 | NaN | NaN | -1.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3867 | Pheromone precursor | 14 | 11 | 19 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0423 | D-xylose reductase | 4 | 6 | 35 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10350 | Phosphatidylinositol binding activity | 8 | 9 | 13 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4315 | Translational elongation | 7 | 4 | 15 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10960 | Protein of unknown function | 10 | 9 | 2 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2224 (Sog) | Vacuolar protein-sorting protein | 9 | 18 | 26 | NaN | NaN | -0.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5800 | Role in translation | 160 | | 4 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8801 | Nitronate monooxygenase | 3 | 4 | | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10023 (MdpL) | Member of the *mdp*/*xpt* gene cl. | 19 | 14 | 18 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0847 | Chaperone | | 4 | 14 | NaN | NaN | -0.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5634 (AcuD) | Isocitrate lyase | 6 | 29 | 16 | NaN | NaN | -0.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1524 | Dehydrogenase | 3 | 8 | 28 | NaN | NaN | -0.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN9320 | Protein of unknown function | | 6 | 46 | NaN | NaN | -0.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1611 | U1 snRNP localization | | 17 | 15 | NaN | NaN | -0.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3778 | Gephyrin-related protein | 3 | 9 | 12 | NaN | NaN | -0.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4602 | Role in tubulin complex assembly | 7 | 6 | | NaN | NaN | -0.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3642 | Vacuolar protein sorting protein | 2 | | 8 | NaN | NaN | -0.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0656 | Menaquinone biosynthesis | | 16 | 11 | NaN | NaN | -0.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2017 (AgdA) | Alpha-glucosidase | 6 | | 8 | NaN | NaN | -0.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN11915 | Formyltetrahydrofolate cyclo ligase | | 4 | 12 | NaN | NaN | -0.3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5778 | Cytoskeleton localization | 3 | 11 | 33 | NaN | NaN | -0.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1694 | Protein of unknown function | | 12 | 5 | NaN | NaN | -0.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5606 | ATPase subunit | 14 | | 13 | NaN | NaN | -0.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3709 | Protein of unknown function | | 4 | 6 | NaN | NaN | 0.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2229 (MetE) | Acetyltransferase | | 3 | 3 | NaN | NaN | 0.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1917 (DicB) | Dicarboxylate-tricarboxylate | 17 | 4 | | NaN | NaN | 0.1 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2947 | Phosphodiesterase | 15 | 11 | 8 | NaN | NaN | 0.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4843 (AgdG) | Alpha-glucosidase | 12 | | 2 | NaN | NaN | 0.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5658 | Gamma-glutamyltransferase | 5 | 5 | | NaN | NaN | 0.4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0740 | Oxidoreductase | 9 | 10 | | NaN | NaN | 0.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1675 | Lysophospholipase | 26 | 15 | 15 | NaN | NaN | 0.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2389 | 2-dehydropantoate 2-reductase | | 15 | 4 | NaN | NaN | 1.0 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5635 (TreB) | Alpha-trehalase | 4 | | 27 | NaN | NaN | 1.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0866 | Heat shock protein-like | 9 | 13 | 28 | NaN | NaN | 1.3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0562 | Catalytic activity | | 6 | 3 | NaN | NaN | 2.7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3255 | Glutathione peroxidase | 24 | 24 | 18 | NaN | NaN | 2.8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |

| Gene ID | Description | PSM | | | *laeAΔ/laeA* | | | Ø | SD | *laeAcomp+/laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN1897 (HmgA) | Homogentisate 1,2-dioxygenase | 24 | 15 | | NaN | NaN | 2.9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10788 | Protease | | 9 | 11 | NaN | NaN | 3.2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5068 | Arylformamidase | 18 | 6 | 13 | NaN | NaN | 3.5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3330 | Protein of unknown function | 53 | 61 | 54 | NaN | NaN | 3.6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0234 | Hydrolase | | 21 | 79 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -4.6 | NaN | NaN |
| AN2555 | Serine-type carboxypeptidase | 5 | 11 | 67 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -3.7 | NaN | NaN |
| AN10901 | Dehydrogenase | 17 | 76 | 221 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -3.4 | NaN | NaN |
| AN4908 | Translation initiation factor subunit | 5 | 29 | 185 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -3.3 | NaN | NaN |
| AN0893 (AdB) | Adenylosuccinate synthase | 56 | 65 | 97 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -3.1 | NaN | NaN |
| AN6209 | Adenylosuccinate lyase | 4 | 26 | 38 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.4 | NaN | NaN |
| AN6521 (LysF) | Homoaconitate hydratase | | 13 | 31 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.3 | NaN | NaN |
| AN1558 (MyoA) | Myosin I | 12 | | 11 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.3 | NaN | NaN |
| AN11192 | Cytochrome P450 | | 28 | 18 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.2 | NaN | NaN |
| AN4064 | ADP/ATP carrier protein | 137 | 136 | 263 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.1 | NaN | NaN |
| AN1503 | Dihydrodipicolinate synthase | 3 | 2 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.1 | NaN | NaN |
| AN0952 | Phosphatase | 9 | 2 | 32 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.1 | NaN | NaN |
| AN12070 | Carbon-nitrogen ligase | 7 | 7 | 24 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.1 | NaN | NaN |
| AN10426 | GTPase | 5 | 19 | 56 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.1 | NaN | NaN |
| AN0595 | Reductase | 5 | 3 | 21 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.1 | NaN | NaN |
| AN2332 | Succinate dehydrogenase | | 2 | 8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.1 | NaN | NaN |
| AN10296 | Fumarate reductase | 256 | 297 | 390 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN4038 | Translation initiation factor eIF5B | | 8 | 84 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN4956 | Acetolactate synthase | | 16 | 48 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN8121 | FGAM-Synthase | 7 | 22 | 36 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN3894 | Aconitate hydratase | 4 | 16 | 57 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -2.0 | NaN | NaN |
| AN7386 | Protein of unknown function | | 7 | 5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.9 | NaN | NaN |
| AN4859 (PmaA) | Plasma membrane ATPase | 11 | 4 | 61 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.9 | NaN | NaN |
| AN2516 (AmpA) | Actin-associated protein | | 2 | 32 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.9 | NaN | NaN |
| AN0432 | Cytochrome-b5 reductase | 22 | 30 | 35 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.9 | NaN | NaN |
| AN2120 | Oxidoreductase | 5 | 27 | 201 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.8 | NaN | NaN |
| AN3375 | Heat shock protein | 3 | 6 | 21 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.8 | NaN | NaN |
| AN2731 | Unfolded protein binding activity | 7 | 8 | 53 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.8 | NaN | NaN |
| AN2185 (NucA) | Endonuclease | 16 | 12 | 16 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.8 | NaN | NaN |
| AN12221 | Deoxyribose-phosphate aldolase | 27 | 28 | 20 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN0708 (AromA) | Aromatic amino acid biosynthesis | 29 | 34 | 93 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN1216 (GppA) | Glycerol 3-phosphate phosphatase | | 7 | 11 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN8805 | Ankyrin repeat protein | | 2 | 8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN5447 | Glutamate decarboxylase | 56 | 75 | 183 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.7 | NaN | NaN |
| AN9159 | Protein of unknown function | 6 | 11 | 23 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN2882 | Homoserine dehydrogenase | | 80 | 219 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN0290 | Actin filament | | 18 | 43 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN2847 | Protein of unknown function | | 5 | 2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN8016 (Fal1) | RNA helicase | 7 | 20 | 44 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.6 | NaN | NaN |
| AN0183 | Molybdopterin binding protein | | 23 | 23 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN3163 (StoA) | Stomatin protein | 19 | | 13 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN4234 (PcmA) | Phosphoacetylglucosamine mutase | 21 | 37 | 106 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.5 | NaN | NaN |
| AN1198 | Hydrolase | 9 | 13 | 41 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.4 | NaN | NaN |
| AN0661 | RNA-directed DNA polymerase | 5 | 6 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.4 | NaN | NaN |
| AN4015 | Translation elongation factor | 63 | | 19 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN4317 (Sec13) | Nuclear pore complex protein | 24 | | 7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN8862 (MyoV) | Myosin V | 8 | 18 | 36 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN8021 (VmaA) | Vacuolar ATPase (V-ATPase) | 49 | 57 | 90 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.3 | NaN | NaN |
| AN5662 | Threonine-tRNA ligase | 51 | 107 | 193 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN3445 | Chitin synthase | 12 | 6 | 36 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.2 | NaN | NaN |
| AN7289 | Actin monomer binding protein | 42 | 50 | 59 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.1 | NaN | NaN |
| AN8853 (SnpA) | Polypeptide releasing factor | 17 | 6 | 46 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.1 | NaN | NaN |
| AN4616 | Heat shock protein | 33 | 49 | 138 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.0 | NaN | NaN |
| AN5121 | Proteasome regulatory subunit 8 | 48 | 71 | 78 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -1.0 | NaN | NaN |
| AN3918 | Aminopeptidase | 8 | 24 | 28 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -0.9 | NaN | NaN |
| AN9357 | Short-chain dehydrogenase | 8 | 14 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -0.9 | NaN | NaN |
| AN7334 | Phospholipase | 19 | 25 | 74 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -0.8 | NaN | NaN |
| AN3789 | Protein of unknown function | 26 | 12 | 13 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -0.8 | NaN | NaN |
| AN0667 (ManA) | Mannose-6-phosphate isomerase | | 26 | 27 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -0.8 | NaN | NaN |
| AN2026 | Transcription factor | 4 | 5 | 7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -0.8 | NaN | NaN |
| AN9067 | Protein of unknown function | | 18 | 30 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -0.5 | NaN | NaN |
| AN9297 | Protein of unknown function | | 53 | 67 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -0.5 | NaN | NaN |
| AN5404 | Protein of unknown function | | 4 | 15 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -0.5 | NaN | NaN |

| Gene ID | Description | PSM | | | *laeAΔlaeA* | | | Ø | SD | *laeAcomp+laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN10170 | ATPase activity | 89 | 133 | 180 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -0.4 | NaN | NaN |
| AN0495 | Formyltetrahydrofolate deformylase | | 22 | 34 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | -0.2 | NaN | NaN |
| AN2405 | Methyltransferases | 16 | 6 | 2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | 0.1 | NaN | NaN |
| AN8430 | Protein of unknown function | 11 | 14 | 25 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | 0.1 | NaN | NaN |
| AN6985 | Ribulokinase | 20 | | 2 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | 0.6 | NaN | NaN |
| AN7999 | Oxidoreductase *mdp*/*xpt* | 63 | 63 | 84 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | 0.9 | NaN | NaN |
| AN8604 (Ngn23) | GNAT-type acetyltransferase | 7 | 8 | 9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | 1.6 | NaN | NaN |
| AN0787 (Mns1B) | Mannosidase 1A | 15 | 3 | 10 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | 1.7 | NaN | NaN |
| AN1117 | COPII adaptor activity | 21 | 20 | 22 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | 1.9 | NaN | NaN |
| AN5423 | Protein of unknown function | 16 | 6 | 19 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | 2.1 | NaN | NaN |
| AN0050 | Picolinic acid decarboxylase | 8 | 22 | 4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0051 | Dioxygenase | 14 | 17 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0121 (HemC) | Porphobilinogen deaminase | 6 | 17 | 22 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0133 | RNA helicase | 5 | 2 | 11 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0146 (MdpC) | Versicolorin ketoreductase | 33 | 13 | 23 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0150 (MdpG) | Polketide synthase *mdp*/*xpt* | 17 | 4 | 5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0228 | DNA helicase | 6 | 9 | 9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0261 (Sec23) | Coat protein complex II | 36 | 8 | 133 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0381 | Chaperonin | 15 | 26 | 77 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0410 | Phosphatase | 7 | 9 | 34 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0465 | Protein of unknown function | 42 | | 6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0574 | Nucleotide binding | 17 | 26 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0579 | IPP isomerase | 40 | 43 | 38 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0593 | Dehydrogenase | 21 | 11 | 3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0757 | tRNA methylation | 4 | 6 | 46 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0922 | Golgi vesicle-mediated transport | 11 | 21 | 62 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN0987 | Protein of unknown function | 25 | | 48 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10108 | Oxidoreductase | | 4 | 24 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1019 | SCF complex protein | | 4 | 13 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1023 (SagA) | Cytoskeletal adaptor protein | 7 | | 7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10276 | Initiation factor 4G binding | | 11 | 32 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10284 | Glutathione synthase | 12 | 9 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10476 | Dehydrogenase/GMP reductase | 57 | 39 | 98 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10734 | Regulation translational elongation | 3 | 6 | 35 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN10864 | Phosphatase | 3 | | 3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN11052 | 5'-3' exonuclease | | 12 | 83 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1122 | 60S ribosomal protein L1 | 12 | 22 | 234 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN11246 | Protein of unknown function | | 11 | 31 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1167 | Protein of unknown function | 21 | 14 | 40 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN11981 | Aminomethyltransferase | 2 | 11 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN12027 | Protein of unknown function | 26 | 35 | 16 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN12054 (SsnF) | Transcriptional co-repressor | 10 | 10 | 24 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1222 | S-adenosylmethionine synthetase | 144 | 157 | 357 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN12402 (XptB) | Prenyltransferase (*mpd*/*xpt*) | 10 | 9 | 17 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1546 | Histone deacetylase | | 2 | 4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1547 (CoaT) | Carboxylate CoA-transferase | | 17 | 13 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1606 | Annexin | | 16 | 40 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1851 | Chaperonin | 21 | 34 | 85 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1867 (PhoB) | Cyclin-dependent protein kinase | 2 | | 5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1868 | Glycerol dehydrogenase | 11 | 19 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1882 | Oxidoreductase | | 8 | 4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1896 (FahA) | Fumarylacetoacetate hydrolase | 42 | 49 | 5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1918 (AcuF) | Carboxykinase | 16 | 29 | 39 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1967 | Fatty acid dioxygenase | 22 | 41 | 87 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN1971 | 3'-5' DNA helicase | 16 | 10 | 35 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2080 | Translation release factor eRF3 | | 21 | 39 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2092 (PapA) | Prolyl aminopeptidase | | 8 | 8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2149 (Cct1) | Chaperonin complex component | 12 | 27 | 86 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2208 | Galactose 1-dehydrogenase | 55 | 41 | 91 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2293 | Isomerase activity | | 2 | 7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2314 | Glucan branching enzyme | 43 | 25 | 22 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2336 | Phospholipase C | | 80 | 211 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2439 (SldB) | Spindle assembly checkpoint | | 14 | 14 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2514 | Protein of unknown function | | 9 | 19 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2523 (ChsB) | Chitin synthase | 3 | | 25 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2525 | L-serine dehydratase | | 3 | 14 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2549 (EasD) | Acyl-CoA ligase | 3 | 10 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |

| Gene ID | Description | PSM | | | *laeAΔlaeA* | | | Ø | SD | *laeAcomp+laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN2588 | Protein of unknown function | 46 | 21 | 18 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2682 | Oxidoreductase | 2 | 4 | 93 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2733 | Uroporphyrinogen decarboxylase | | 4 | 5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2775 | eIF2A subunit | 2 | 9 | 43 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2815 | Mannitol 2-dehydrogenase | | 12 | 36 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2917 | Proteasome regulatory particle | 29 | 20 | 49 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2954 | Protein of unknown function | 71 | 131 | 96 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN2993 | Protein of unknown function | 34 | 14 | 11 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3122 | Clathrin-coated vesiclec protein | 19 | 13 | 35 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3134 | T-complex protein 1 | 18 | 24 | 71 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3156 | Translation initiation factor 2 | | 9 | 31 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3331 | Esterase | 22 | 21 | 24 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3339 | Oxidoreductase | | 2 | 7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3351 | Dehydrogenase | 6 | 16 | 19 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3431 | Pyrophosphorylase | 6 | 7 | 13 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3432 | Aldose 1-epimerase | | 7 | 14 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3590 | Protein of unknown function | 25 | 16 | 18 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3830 (IleA) | L-threonine dehydratase | 2 | | 45 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3839 (SwoF) | Peptide tetradecanoyltransferase | 5 | 13 | 22 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3853 | Metalloendopeptidase | 6 | 16 | 77 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3901 | Lactic acid dehydrogenase | 27 | 31 | 96 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN3973 | Peroxiredoxin | 45 | 54 | 85 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4051 | Protein of unknown function | | 16 | 173 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4080 | Role in cytoplasmic translation | | 9 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4081 | Cysteine dioxygenase | 14 | 6 | 24 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4236 | Proteasome regulatory particle | 25 | 32 | 55 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4270 | Actin cortical patch assembly | 22 | 6 | 28 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4290 | Isomerase | 11 | 5 | 16 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4303 | Cytidylyltransferase | 11 | 6 | 40 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4390 | Protein of unknown function | | 67 | 76 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4647 | Phytanoyl-CoA dioxygenase | | 16 | 6 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4774 | Methyltransferase | | 4 | 27 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4775 | Cullin deneddylation | 45 | 47 | 101 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4915 (SrgG) | RAB family GTPase | 16 | 6 | 25 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4929 | Protein of unknown function | 18 | 11 | 20 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4934 | Dehydrogenase | 4 | 24 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4951 | Protein transporter | 4 | 8 | 17 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN4987 (PkaR) | Protein kinase A | 2 | 7 | 8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5021 | Role in conidium formation | | 6 | 35 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5132 | Uncoupling protein (UCP) | 6 | | 24 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5134 (GltA) | Glutamate synthase | 75 | 294 | 789 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5226 (AcpA) | Acetate permease | 67 | | 24 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5257 | Oxidoreductase | | 13 | 14 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5376 | Protein of unknown function | 9 | 5 | 17 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5422 | Beta-lactamase | 43 | 43 | 4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5452 | Splicing factor 3b | 7 | | 29 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5523 (TpsA) | Trehalose-phosphate synthase | 28 | 31 | 31 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5534 | Glyoxylate reductase | 8 | 6 | 29 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5586 | Guanylyltransferase | 36 | 79 | 82 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5607 | Role in proteasome assembly | | 4 | 7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5690 | Amine oxidase | | 3 | 4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN5782 | Fumarylacetoacetate hydrolase | 24 | 31 | 17 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN6314 | Oxidoreductase | | 15 | 10 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN6488 | Tryptophanyl-tRNA synthetase | 9 | 11 | 13 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7181 | Protein of unknown function | 49 | 29 | 32 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7231 | Serine-type peptidase | 21 | 17 | 5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7267 | Oxidoreductase | 179 | 73 | 27 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7269 | Dehydrogenase | 26 | 28 | 25 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7657 (GelA) | 1,3-beta-transglycosidase | 81 | 105 | 101 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7900 | Oxidoreductase | 11 | 22 | 4 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN7912 (OrsC) | Tyrosinase | 14 | 24 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8102 | Pepsin-like aspartic protease | | 5 | 5 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8478 | Protein of unknown function | 10 | 4 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8496 | Metal ion binding activity | 7 | 11 | | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8528 | Protein of unknown function | 22 | 20 | 8 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8815 | Isoflavone reductase | 11 | 3 | 45 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8820 (CnaA) | Calcineurin A | 39 | 28 | 72 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |

| Gene ID | Description | PSM | | | *laeAΔ/laeA* | | | Ø | SD | *laeAcomp+/laeA* | | | Ø | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | | 1 | 2 | 3 | | |
| AN8843 | Homoserine kinase | 8 | 17 | 15 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN8863 | Nap/SET family protein | 12 | 15 | 137 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN9063 | Oxysterol-binding protein | | 4 | 3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN9065 | Translation regulator activity | | 3 | 7 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN9220 | Protein of unknown function | | 9 | 24 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN9322 | Triphenylmethane Reductase | 5 | | 3 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |
| AN9425 | Aldolase | | 10 | 9 | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN | NaN |