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Expression of *otd* orthologs in the amphipod crustacean, *Parhyale hawaiiensis*

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Abstract The arthropod head is a complex metameric structure. In insects, *orthodenticle* (*otd*) functions as a ‘head gap gene’ and plays a significant role in patterning and development of the anterior head ectoderm, the protocerebrum, and the ventral midline. In this study, we characterize the structure and developmental deployment of two *otd* paralogs in the amphipod crustacean, *Parhyale hawaiiensis*. *Photd1* is initially expressed at gastrulation through germband stages in a bilaterally symmetric, restricted region of the anterior head ectoderm and also in a single column of cells along the ventral midline. Late in embryogenesis, *Photd1* is expressed within the developing anterior brain and the expression along the embryonic midline has become restricted to a stereotypic group of segmentally reiterated cells. The second ortholog *Photd2*, however, has a unique temporal–spatial expression pattern and is not detected until after the head lobes have been organized in the developing ectoderm of the germband during late germband stages. Anteriorly, *Photd2* is coincident with the *Photd1* head expression domain; however, *Photd2* is not detected along the ventral midline during formation of the germband and only appears in the ventral midline late in embryonic development in a restricted group of cells distinct from those expressing

Photd1. The early expression of *Photd1* in the anterior head ectoderm is consistent with a role as a head gap gene. The more posterior expression of *Photd1* is suggestive of a role in patterning the embryonic ventral midline. *Photd2* expression appears too late to play a role in early head patterning but may contribute to latter patterning in restricted regions of both the head and the ventral midline. The comparative analysis of *otd* reveals the divergence of gene expression and gene function associated with duplication of this important developmental gene.

Keywords Crustacean · Amphipod · *Parhyale* · *Orthodenticle* · Head development · Brain development · CNS · Midline · Neurogenesis

Introduction

Genes that control the anterior head and nervous system of the fly, *Drosophila melanogaster*, have guided ideas regarding the development and evolution of the arthropod head. In *Drosophila*, a small number of transcription factor genes play a major role in the specification of the anterior head ectoderm and the anterior-most neuromeres that comprise the supraesophageal ganglion (Younossi-Hartenstein et al. 1997). These ‘head gap genes’ are deployed very early in fly development in overlapping functional domains (Cohen and Jürgens 1990; Schmidt-Ott et al. 1994) and their initially broad anterior domains of expression are progressively restricted and sharpened during the processes of both cellularization and gastrulation (Dalton et al. 1989; Finkelstein and Perrimon, 1990; Walldorf and Gehring 1992; Wimmer et al. 1993; Grossniklaus et al. 1994; Wimmer et al. 1997). The resulting anterior expression domains demarcate specific regions of the developing *Drosophila* brain (Hirth et al. 1995). Among these genes, orthologs of *orthodenticle* (*otd*) have been demonstrated to play a central role in anterior head and brain development in disparate bilaterians (e.g., Acampora et al. 1998; reviewed in Arendt and

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Nubler-Jung 1996; Sharman and Brand 1998; Lichtneckert and Reichert 2005).

In *Drosophila* and other insects, the supraesophageal ganglia can be subdivided along the neuroaxis, from anterior to posterior, into three neuromere subunits the protocerebrum (PC), deutocerebrum (DC), and tritocerebrum (TC) (Reichert and Boyan 1997). In *Drosophila*, *otd* is initially expressed in a wide anterior circumferential stripe in the blastoderm embryo (Finkelstein and Perrimon 1990; Cohen and Jürgens 1991). As development progresses, the *otd* expression boundary becomes restricted to the embryonic brain and is detected throughout the developing PC neuromere and in the anterior DC neuromere. Mutant *otd* alleles delete or severely reduce the size of the PC neuromere and the PC-associated preoral medial commissure (Hirth et al. 1995). *otd* appears to, in part, functionally act anteriorly in the specification of cell identity via regulation of proneural gene expression (Younossi-Hartenstein et al. 1997), the intrasegmental regulation of the segment polarity genes *engrailed* (*en*) and *wingless* (*wg*) in embryos (Gallitano-Mendel and Finkelstein 1998), and later in larval and pupal development of medial structures including the ocelli of the adult fly (Wieschaus et al. 1992; Royet and Finkelstein 1995; Hirth et al. 1995). In addition, reciprocal negative regulation between the gene *unplugged* (*unpg*) and *otd* suggests that *otd* plays an important role in specification of the anterior boundary between the DC and TC neuromeres in *Drosophila* (Hirth et al. 2003; Lichtneckert and Reichert 2005). Expression of *otd* is also prominent in the embryonic ventral midline of *Drosophila* and plays an important role in the patterning of the segmentally reiterated ventral nerve cord (VNC) commissures, particularly the posterior commissure (Finkelstein et al. 1990; Klämbt et al. 1991).

The embryonic development of both the head and ventral midline in *Drosophila* is distinctive from most arthropods. Unique to the dipteran flies, the anterior head segments involute in late stages of development and the ventral midline is generated from a distinct group of mesectodermal cells. Given the highly divergent mode of embryonic head development in flies, the expression patterns of *orthodenticle* orthologs have been examined in an insect with less-derived embryonic development, the beetle *Tribolium castaneum*. In contrast to *Drosophila*, *Tribolium* possesses two orthologs of *otd*, *Tcodd-1*, and *Tcodd-2* (Li et al. 1996). The expression of the two *Tribolium otd* paralogs are divergent with respect to each other as well as to their putative ortholog in *Drosophila* (Li et al. 1996). Knockdown of *Tcodd-1* function via parental RNAi produces a headless embryonic phenotype that is more severe than the *Drosophila* gap gene phenotype, which indicates an important anterior determinant role of *Tcodd-1* in *Tribolium* (Schröder 2003). It is notable that the effects of knockdown restricted to zygotic expression of *Tcodd-1* were generally more moderate and did not eliminate the entire head but resemble the *Drosophila* gap gene phenotype (Schröder 2003). Thus, while *Tcodd-1* retains an early anterior gap gene patterning function analogous to its ortholog *otd* in *Drosophila*, *Tcodd-1* has an

additional functional role in specifying the entire head region in *Tribolium*. This observation in *Tribolium* suggests the possibility of *otd* serving as an anterior determinant in other arthropods.

In the absence of equivalent information from additional arthropod taxa, these data can only apply to the most recent common ancestor of holometabolous beetles and flies. Thus, it is problematic to generalize details regarding the mode of ancestral arthropod head development exclusively from data produced in flies and other insects. Among arthropod taxa more distantly related to insects, only limited information is currently available. In the chelicerate *Archezogetes longisetosus*, expression of a single *orthodenticle* ortholog has been reported and cursory examination of expression during late embryogenesis reveals *Alotd* in an anterior head domain as well as along the ventral midline similar to *Tcodd-1* in *Tribolium* (Telford and Thomas 1998). Recent work suggests that the crustaceans are sister taxa to the insects (Friedrich and Tautz 1995; Dohle 2001; Giribet et al. 2001, 2005; Hwang et al. 2001; Richter 2002; Cook et al. 2005; Regier et al. 2005). Comparative neuroanatomy of crustaceans (e.g., Hanström 1928; Sandeman et al. 1992; Gerberding 1997; Strausfeld 1998; Harzsch 2003) suggests that a similar and presumably homologous supraesophageal neuromere ground plan is shared with the insects (Sandeman et al. 1992). Additional comparative studies between crustaceans and insects have suggested both strong similarities and notable differences in neuronal morphology (Whittington et al. 1993; Whittington 1996). These observations have been extended to suggest homologies among neuronal identities by correlating neuronal morphology with the expression of molecular markers (Duman-Scheel and Patel 1999; Browne et al. 2005). Using these studies, our understanding of insect neurogenesis, in combination with the embryonic staging and cell lineage data available for *Parhyale hawaiiensis* (Gerberding et al. 2002; Browne et al. 2005), provides a framework from which to make a detailed analysis of the expression of head patterning orthologs in a model crustacean. At the level of segment morphology and segment specification, the heads of crustaceans, such as *Parhyale*, are quite different from the heads of insects, such as *Drosophila*. For example, insects do not possess paired appendages on the intercalary segment whereas crustaceans possess antennae on the homologous segment (An2). In addition, many aspects of the olfactory systems of insects and crustaceans appear to be considerably divergent (Strausfeld 1998; Strausfeld and Hildebrand 1999). Thus, the a priori expectation would be for gene orthologs controlling the morphology of insects to have divergent temporal and spatial expression in crustaceans.

In this paper, we report detailed expression patterns associated with orthologs of *otd* in the model amphipod crustacean, *P. hawaiiensis*. To further characterize anterior head and ventral midline development in *Parhyale*, we cloned *otd* orthologs and carried out a detailed study of their expression during embryogenesis via in situ mRNA transcript analysis. This analysis, when paired with

available sequence and expression data in other taxa, allowed us to assess the relationship between *otd* orthologs and paralogs among arthropods. Our analysis suggests an evolutionary scenario accounting for the gene duplication and expression characteristics associated with *otd* and is informative with regard to reconstructing the ancestral state of the most recent common ancestor between crustaceans and insects, a marine arthropod stem species existing during the Cambrian ~550 mya.

Materials and methods

Amphipod culture

P. hawaiiensis is maintained as reported in Browne et al. (2005) with the following modifications. The breeding colony is kept in interconnected shallow plastic trays at ~30°C at the Kewalo Marine Lab. Recirculating seawater is provided via magnetic drive pump (Iwaki). Fresh, filtered seawater is added at regular intervals. Animals are fed a liquefied mixture of algae, plankton, fatty acids (Selco), and vitamins (Kent Marine).

Cloning and sequence analysis

Total RNA was isolated with TRIzol Reagent (Gibco BRL) from a pool of mixed-stage *P. hawaiiensis* embryos. First strand cDNA was generated with the SuperScript Pre-amplification System (Gibco BRL). Initial degenerate PCR was completed using conserved nested primers to aligned *otd* proteins (forward primer 5'-GAR MGN CAN CAN TTY AC-3', reverse outer primer 5'-NCK NCK RTT YTT RAA CCA-3', and reverse inner primer 5'-CAR YTN GAY GTN YTN GA-3').

For isolation of additional 5' sequences, polyA RNA was isolated using the Micro Poly(A)Pure Small Scale mRNA Purification Kit (Ambion) from a pool of *P. hawaiiensis* embryos. First strand cDNAs were generated with the SMART PCR cDNA Synthesis Kit (BD Biosciences). Initial degenerate PCR was completed using the forward primer 5'-CAG MGG MGG GAR MGI ACI ACI TTY AC -3' and reverse primer 5'-GC CCK CCK RTT YTT RAA CCA IAC YTG-3'. Sequences 5' of the homeodomain were obtained via 5'RACE (SMART RACE cDNA Amplification Kit, BD Biosciences) with nested sequence-specific primers obtained from the initial degenerate PCR. Sequences 3' of the homeodomain were obtained by performing 3'RACE (Gibco BRL) with sequence-specific nested primers obtained from the initial degenerate PCR (primers available upon request).

Phylogenetic analysis

Maximum parsimony (MP) and maximum likelihood (ML) analyses were executed with PAUP* 4.0Beta (Swofford 2003). Bayesian phylogenetic inference analysis was

executed with MrBayes 3.1.1 (Ronquist and Huelsenbeck 2003). Relevant *orthodenticle* sequences were selected from those available via genbank; all available arthropod sequences were used. Searches were rooted with the *D. melanogaster paired* gene and resulting trees were visualized with TreeView X (Page 1996).

A mutational saturation plot (Philippe et al. 1994; Philippe and Forterre 1999) was generated using a MP analysis with ten random sequence addition MP heuristic searches. The best tree was used to generate a patristic distance matrix. In additional parsimony analyses, MP heuristic searches were subjected to 2,000 bootstrap replicates, each with a random addition MP heuristic search.

For ML analyses, 1,000 random sequence addition ML heuristic searches were run. Searches were then subjected to 2,000 bootstrap replicates, each with a random addition ML heuristic search. The molecular evolution model, HKY + G, was selected using Model-Test 3.7 (Posada and Crandall 1998) for ML analysis.

For Bayesian analyses, all parameters were unlinked except topology. Two different codon partition schemes were generated: (1) codon position partition 1, 2, 3 or (2) codon position partition 1+2, 3. Multiple searches were run from 2 million generations to 5 million generations and trees were sampled every 100 generations. Posterior estimates from runs were analyzed by eye with Tracer v1.3 (Rambaut and Drummond 2003, Tracer v1.3, <http://www.evolve.zoo.ox.ac.uk>) to determine the number of generations to burn in and to assess convergence of data sets. Consensus trees from independent runs within each partition scheme were compared to assess convergence and topology congruence of data sets.

In situ hybridization

P. hawaiiensis embryos were removed from the ventral brood pouch and allowed to develop to the desired stage at 26°C (Browne et al. 2005), then dissected with tungsten wire needles and fixed for 60 min in filtered seawater mixed 9:1 with 37% formaldehyde at room temperature. Fixed embryos were then washed with PT (1× PBS, pH 7.4, and 0.1% Triton X-100) and stored in 100% MeOH at -20°C. Whole-mount in situ hybridization using digoxigenin-labeled RNA was performed as reported in Finnerty et al. (2003), with the following changes. Before hybridization, fixed embryos were incubated for 60 min in a sodium dodecyl sulfate (SDS)-based detergent solution [1% SDS, 0.5% Tween-20, 50 mM Tris-HCl (pH 7.5), 1.0 mM EDTA (pH 8.0), and 150 mM NaCl] at 65°C. After hybridization, embryos were intensively washed with 2× SSC solution at 65°C to remove hybridization buffer and free probe. After the 65°C washes, embryos were then washed at room temperature in PBT (1× PBS containing 0.1% bovine serum albumin and 0.1% Triton X-100). Embryos were then incubated in sheep anti-digoxigenin-AP (1:3,000) overnight at 4°C and subsequently washed with PBT before performing BCIP/NBT color reaction.

After color reaction, embryos were washed with PT and incubated overnight at 4°C in 50% glycerol + 1 mg/ml Hoechst dye. Embryos were stored in 70% glycerol at -20°C. Images were generated from dissected and mounted embryos using an Axioskop2 (Zeiss) and Axio Imager Z1 (Zeiss) and photographed with either an AxioCam (Zeiss) or an ORCA ER (Hamamatsu) using MAC-compatible Openlab and Velocity (Improvision) imaging software.

Antibody staining

Staged *P. hawaiiensis* embryos (Browne et al. 2005) were fixed in the same way as for in situ but for a shorter period of time (15 min). Antibody detection was performed as reported in Patel (1994). All antibody incubations were done overnight at 4°C at the following concentrations: mouse anti-acetylated tubulin primary antibody (Hybridoma Bank, clone E7) 1:1 with PBT, alexa-594 conjugated goat anti-mouse secondary antibody (Molecular Probes) 1:250 with PBT, and HRP conjugated goat anti-mouse secondary antibody (Jackson ImmunoResearch). Embryos were then washed and incubated overnight at 4°C in 50% glycerol + 1 mg/ml Hoechst solution and stored in 70% glycerol at -20°C. Images were generated from dissected and mounted embryos as described above.

Results

Isolation and characterization of *P. hawaiiensis otd* orthologs

Two *P. hawaiiensis otd* sequences, *Photd1* and *Photd2*, were recovered from repeated degenerate PCR screens on independent pools of *Parhyale* cDNA. The isolated fragments (103 bp) were sufficient for unambiguous orthology identification. Nested non-overlapping primers were designed to the two initial degenerate PCR fragments and used to amplify the remaining mRNA transcript sequences both 5' and 3' of the initial homeodomain fragments. All of the *Photd1* sequences are identical with minor variations in the length of the recovered transcripts. The longest recovered complete *Photd1* cDNA was 2 kb. In contrast, the organization of the *Photd2* paralog is significantly more complex. The *Photd2* gene has multiple splice variants both 5' and 3' of an invariant homeodomain core sequence (supplemental data). RACE yielded five unique open reading frame sequences upstream of the homeodomain and three unique open reading frame sequences downstream of the homeodomain. Therefore, the *Photd2* gene appears to be capable of generating potentially 15 unique transcripts; however, the true number of *Photd2* transcripts produced from these recovered alternative splice variants is unknown.

Phylogenetic analysis

The *orthodenticle* homeodomains show extreme amino acid conservation whereas regions both upstream and downstream of the homeodomain are too variable among orthologs to reliably align in distantly related taxa. In addition, due to *Photd2* possessing multiple splice variants both upstream and downstream of the homeodomain, we restricted our phylogenetic analyses of *orthodenticle* genes in disparate taxa to the homeodomain region. Our survey of metazoan taxa revealed different complements of *otd* paralogs in different groups (Fig. 1). In cases in which two paralogs exist, the paralogs appear more similar to each other than to orthologs in other species. We propose two readily distinguishable modes of gene expansion are likely to have occurred to generate the range of *otd* paralogs across metazoans. In the lineage leading to the vertebrates, whole-genome duplication has generated additional paralogous genes (Germot et al. 2001) which, in most cases, are found on different chromosomes. In contrast, in the cnidarian (Finnerty et al., submitted for publication) and arthropod lineages for which genomic information is available, tandem gene duplication appears to have generated paralogous genes in close proximity to one another. However, it is unclear whether duplications occurred independently in each taxon or at particular nodes in the metazoan tree. Specifically in the arthropod lineage leading to the Tetraconata (crustaceans + insects) (Dohle 2001), it is likely that a single duplication of an ancestral *otd* gene occurred to give rise to two paralogs in descendant taxa (Fig. 1). Within any given lineage arising after this duplication, *otd* paralogs are likely to have been subjected to the effects of two homogenizing phenomena: (1) increased probability of gene conversion (mechanism by which mismatched heteroduplexed DNA basepairs between gene copies are repaired) due to the proximity of the paralogs and (2) the effects of mutational saturation. Both of these phenomena would contribute to *otd* paralogs becoming more similar to one another than to *otd* orthologs in related taxa. As a test for mutation saturation among *otd* paralogs, we generated a mutational saturation plot (supplemental data). The presence of a mutational plateau indicates that saturation for mutational changes has occurred, therefore indicating a loss of phylogenetic signal necessary to accurately resolve branch lengths (Philippe et al. 1994; Philippe and Forterre 1999). Thus, node relationships could not be recovered to generate a comprehensive, reliable, gene tree indicating orthology among *otd* paralogs using traditional phylogenetic tools (data not shown). In summary, the orthology of the two *Parhyale* genes to *orthodenticle* is clear; however, whether multiple independent gene duplications have occurred in different lineages or whether an early metazoan ancestor already had separate *otd* paralogs cannot be ruled out based on available evidence (Fig. 1).

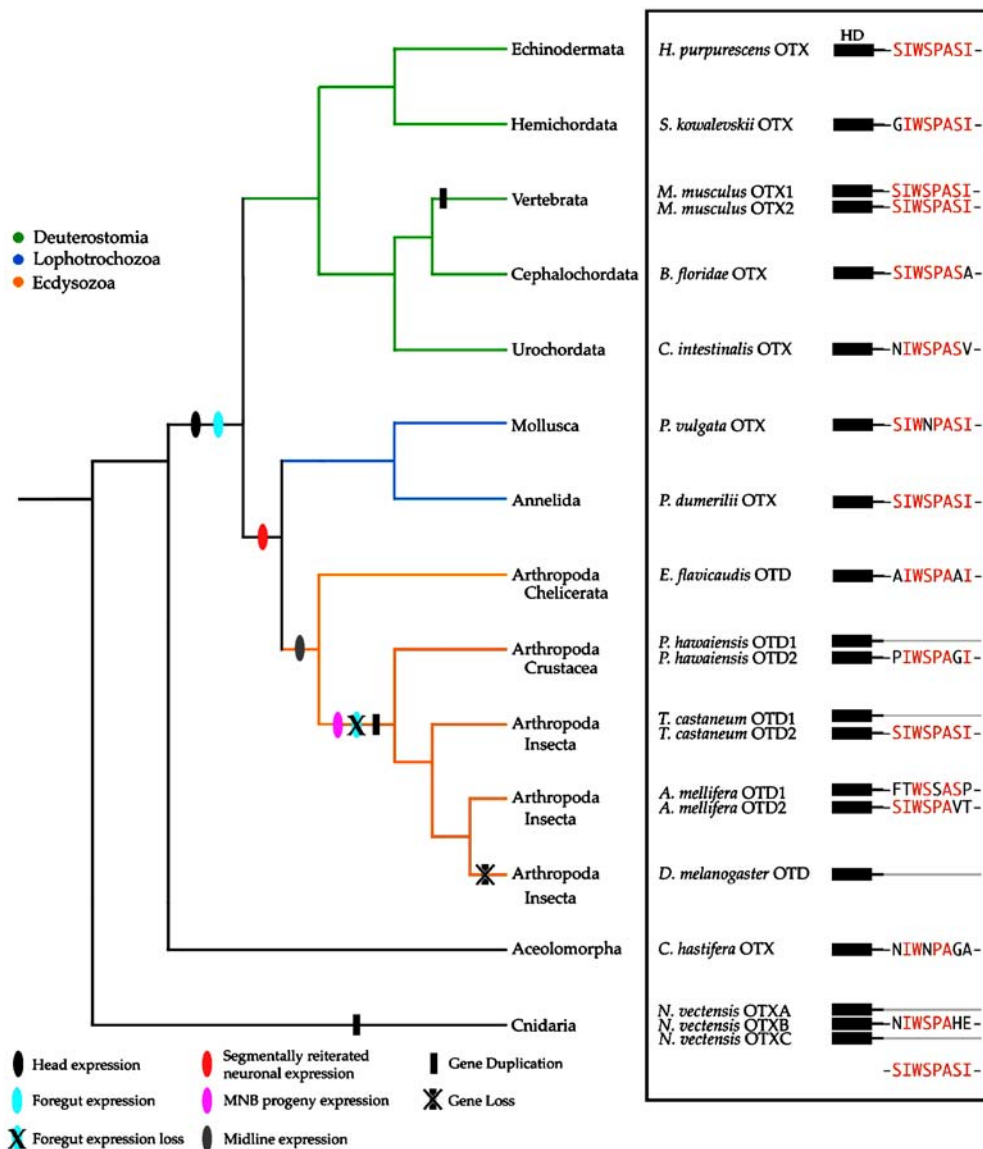


Fig. 1 A model proposing evolutionary duplication and loss of *orthodenticle* paralogs in metazoans. Character legend at the left bottom of figure. Green associated with deuterostome lineages, blue associated with lophotrochozoan lineages, orange associated with ecdysozoan lineages. Cladogram (modified from Adoutte et al. 2000) on the left-hand side indicates evolutionary relationship between species grouped in box on the right-hand side. No implication of polarity is associated with the order of multiple characters occurring on the same branch. For each taxon, orthologs of OTD are included with a schematic of the C-terminal end of each protein that includes the sequence of the short WSP amino acid motif; absence of this motif is indicated with a grey line. Beneath the WSP sequences is the amino acid consensus for the motif. For all taxa, retention of consensus amino acids is indicated with red letters. The WSP motif is detected in at least one *orthodenticle* paralog of all examined taxa with the notable exception of *D. melanogaster*. In this model, an ancestral *orthodenticle* gene was present in the lineage leading to cnidarians + bilaterians and has experienced at least three independent duplication events via two dissimilar duplication processes (black bars). In the lineage leading to the vertebrates, *orthodenticle* gene duplication occurred via entire genome duplication. In the cnidarian lineage leading to *Nematostella vectensis*, *orthodenticle* expansion occurred via local gene duplication generating three closely linked paralogs (*NvOtx* sequences, K. Pang, personal communication). Available data in the arthropod lineage indicates an *orthodenticle* expansion also occurred via local gene duplication in the lineage

leading to the crustaceans + insects (Tetraconata). In taxa for which data is available, at least one *otd* paralog retains an identifiable WSP motif. In the insects, *T. castaneum* and *A. mellifera*, the duplicate paralogs are known to be in close proximity to one another. The dipteran *D. melanogaster* has lost the *otd* paralog containing the WSP motif (crossed black bar). The expression of *orthodenticle* orthologs in the head occurred before the lineage including the Eubilateria (black oval). This expression character is currently placed after divergence of the Aceolomorpha lineage (ChOtx sequences, A. Hejnal, personal communication). Expression of *orthodenticle* in the foregut also appears to be an ancient feature, also occurring before the divergence of the deuterostome and protostome lineages (blue oval). Expression of *orthodenticle* appears in segmentally reiterated neurons in the lineage leading to Lophotrochozoa + Ecdysozoa (red oval). Available data in the arthropod lineage indicates expression of *orthodenticle* in the ventral midline arose before the divergence of the chelicerate + Tetraconata lineage (grey oval). Foregut expression is lost in the lineage leading to the Tetraconata (crossed blue oval). Additional data indicate that *orthodenticle* expression in median neuroblast progeny (MNB) predates the divergence Tetraconata lineage (purple oval). Full species names are *Apis mellifera*, *Branchiostoma floridae*, *Ciona intestinalis*, *Convolutriloba hastifera*, *Drosophila melanogaster*, *Euscorpius flavicaudis*, *Holopneustes purpurescens*, *Mus musculus*, *Nematostella vectensis*, *Parhyale hawaiiensis*, *Patella vulgata*, *Platynereis dumerilii*, *Saccoglossus kowalevskii*, and *Tribolium castaneum*

Expression of the *P. hawaiiensis* *Photd1* paralog

The *Photd1* paralog is first detectable in descendants from each of the three ectoderm clones El, Er, and Ep (Gerberding et al. 2002; Browne et al. 2005) at gastrulation in the early stage 8 embryo (Fig. 2a). There is no maternal *Photd1* transcript contribution to oocytes nor is there detectable *Photd1* zygotic transcription before gastrulation. As the cells that form the germ disc aggregate in the late stage 8 embryo, *Photd1* expression spreads, increases in intensity, and is detected anteriorly in an arc as the head lobes begin to condense laterally. The head lobes are composed of cells from the El (ectoderm left) and Er (ectoderm right) ectoderm clones (Gerberding et al. 2002). *Photd1* expression also remains present in the future

ventral midline cells of the Ep (ectoderm posterior) clone as they converge into a single, tightly associated cluster, at the posterior end of the germ disc (Fig. 2b). In the following stage, stage 9, the anterior lateral-most *Photd1*-expressing cells are beginning to organize into the characteristic bilaterally symmetric head lobe arcs. A persistent arc of less intense staining connects the head lobes across the midline. This staining is associated with cells at the anterior-most region of the developing germ disc. Posteriorly, the medial cluster of *Photd1*-expressing cells have organized as a half circle with a sharply demarcated anterior boundary (Fig. 2c). The formation of this boundary occurs as the posterior ectoderm of the germband begins to organize into a characteristic gridded array (Browne et al. 2005). As parasegment precursor rows (PPRs) begin to

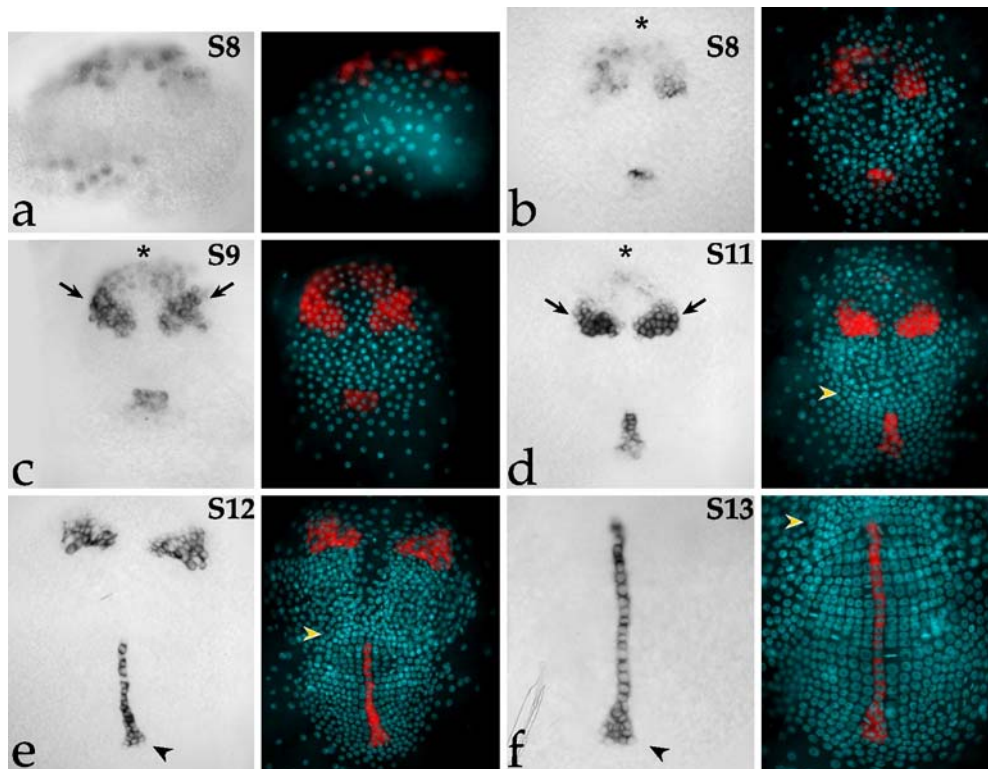


Fig. 2 Expression of *P. hawaiiensis* *otd1*, gastrulation through germband formation. All embryos are mounted ventral side up with the anterior end oriented towards the top. For each panel, a brightfield image is on the left (*Photd1* label is black), and a Hoechst label of the same embryo is on the right (nuclei are blue, *Photd1* is false-colored red). In panels d–f, the yellow arrowhead marks the position of the Mn segment. **a** Early stage 8 embryo (S8), *Photd1*, is present in descendants of El, Er, and Ep blastomeres at gastrulation. **b** Late Stage 8 embryo (S8); anteriorly and medially, the field of El and Er cells expressing *Photd1* has expanded, forming an arch (asterisk). Posteriorly, the few Ep cells expressing *Photd1* have converged medially into a tightly associated cluster. **c** Stage 9 embryo (S9); anteriorly, the lateral-most *Photd1* expressing cells are beginning to organize into the characteristic bilaterally symmetric head lobe arcs (black arrows). Posteriorly, the medial cluster of *Photd1*-expressing cells organizes with a sharply demarcated anterior boundary. Formation of this boundary occurs as the posterior ectoderm of the germband begins to organize into a stereotypic gridded array. **d** Stage 11 embryo (S11); *Photd1*-expressing cells have condensed anteriorly into two bilaterally

symmetric groups largely coincident with the head lobe arcs (black arrows); the more anterior medial expression is fading (asterisk). Posteriorly, *Photd1*-expressing cells begin to form the ventral midline by converging into a single column of cells medially. The anterior boundary of the *Photd1*-expressing midline cells is at the level of PPR1 which is immediately posterior of the Mn segment (yellow arrowhead) and corresponds with the first maxillary segment, Mx1. **e** Stage 12 embryo (S12); anterior medial expression of *Photd1* is undetectable. Posteriorly, as the germband extends, expression in the single cell ventral midline column is maintained via both cell division within forming parasegments as well as convergence and intercalation of additional cells at the posterior extreme of the midline as new PPR rows organize in the region of the wedge-shaped cluster of *Photd1*-expressing cells (black arrowhead). **f** Stage 13 embryo (S13), view of the posterior germband. The posterior-most wedge of *Photd1* cells are indicated with an arrowhead. The ventral midline cells of the single cell column are all derived from the Ep blastomere and express *Photd1* and bisect and maintain the boundary between the posterior El and Er clones in the developing germband

organize in the developing germband in the stage 11 embryo, anterior lateral *Photd1*-expressing cells have condensed into two bilaterally symmetric groups coincident with the developing head lobes; the weaker anterior medial arc of expression has begun to diminish (Fig. 2d). Posteriorly, *Photd1*-expressing cells begin to form the ventral midline by converging medially and extending along the developing ventral midline as a single column of cells (Fig. 2d). The anterior-most *Photd1*-expressing midline cell is positioned at PPR1, this corresponds to the anterior limit of the Ep clone midline and is coincident with the position of the future first maxillary segment, Mx1. The first ectodermal row to form in the germband is PPR0 which corresponds to the future Mn segment (yellow arrowhead, Fig. 2d). As germband extension continues in the stage 12 embryo, the anterior medial arc of *Photd1* expression is no longer detectable. Posteriorly, *Photd1* expression within the extending single cell ventral midline column is retained via both maintenance of expression in PPR progeny and by convergence and intercalation of cells from more posterior PPRs as they organize at the posterior-most extreme of the ventral midline column (black arrowhead, Fig. 2e,f). Throughout germband development, *Photd1* expression in the ventral midline is confined to cells derived from the Ep blastomere. The cells of the Ep midline lineage are distinct from all other ectodermal cells and are observed to both bisect and maintain a precise physical boundary between the posterior El and Er clones in the developing germband from PPR1-posterior (Fig. 2f; Gerberding et al. 2002; Browne et al. 2005). El and Er blastomere progeny intermix considerably across the midline in regions anterior of the Ep midline lineage (Gerberding et al. 2002).

By stage 21, segmentation is complete and the stomodeum is visible medially in the anterior region of the An2 segment (Fig. 3f,i). Expression of *Photd1* appears strongly in the anterior head ectoderm and developing brain, medially in the labrum, and *Photd1* expression in the ventral midline has expanded into ectoderm of the mandibular (Mn) segment anterior of Ep clone descendant cells (Fig. 3a). The region of medial ectoderm between the labrum and the Mn segment has invaginated to form the stomodeum and the lining of the foregut (Fig. 3a; Browne et al. 2005). In the developing anterior head, *Photd1* expression is detected across the midline in the region associated with the formation of the preoral protocerebral commissure (PCC) and the future protocerebral bridge (black arrowhead Fig. 3d,e; white arrowhead Fig. 3g,i).

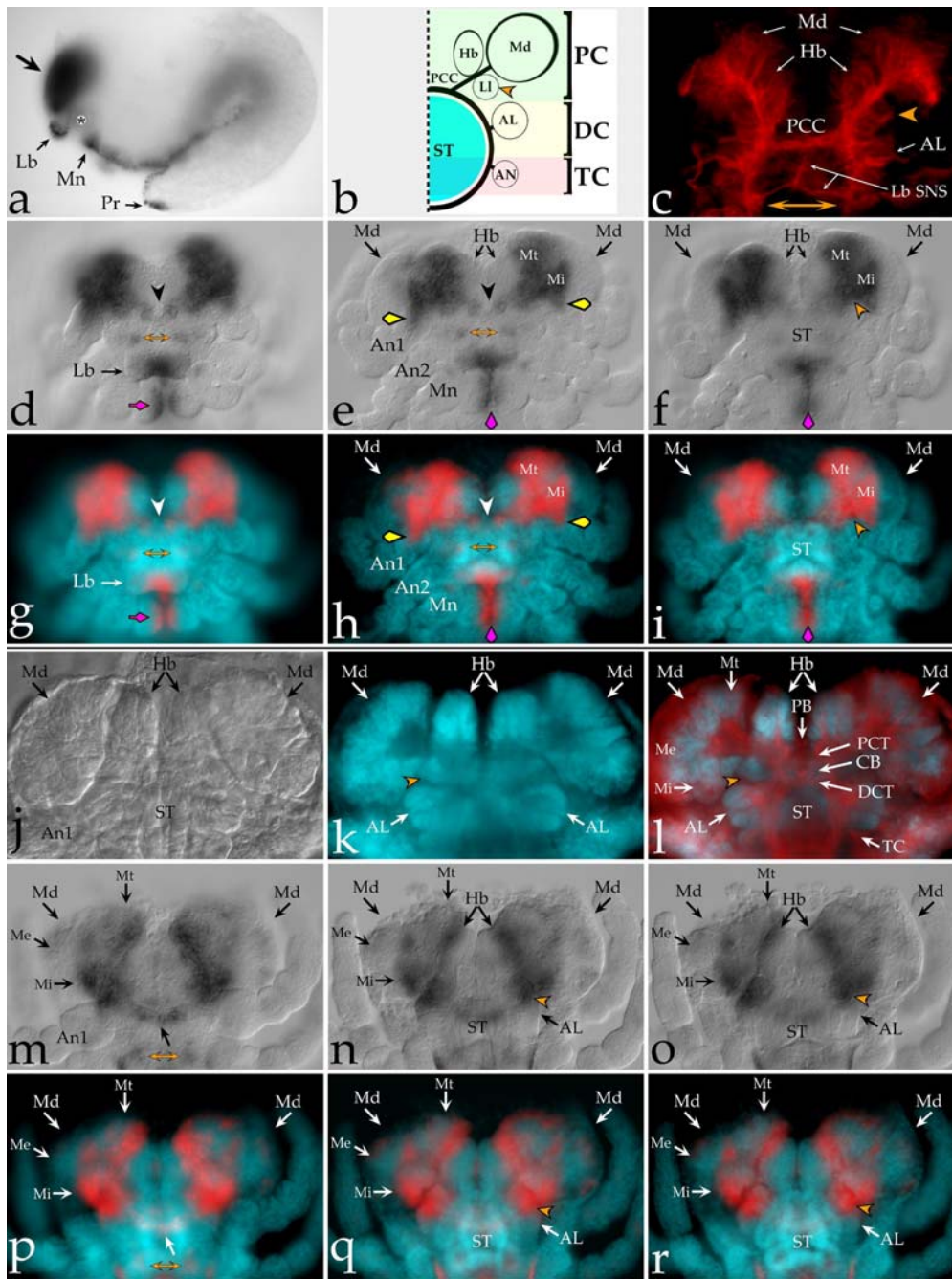
The *Parhyale* supraesophageal ganglia are composed of the protocerebral neuromere (PC), the deutocerebral neuromere (DC), and the tritocerebral neuromere (TC) (Fig. 3b). These three neuromeres together constitute the brain of *Parhyale*. Expression of *Photd1* is clearly regionalized within the developing PC (Fig. 3d–f). In the medulla complex of the PC, *Photd1* expression is detected throughout the medulla terminalis (Mt), in the medulla interna (Mi), and strongly in a few large cell bodies forming the anlagen of the lateral lobes at the proximal base of the Medulla complex (Fig. 3e,f,h,i). The anterior domain

of *Photd1* extends into the proximal base of the An1 antennal appendages that innervate the DC (yellow arrowhead, Fig. 3e,h). *Photd1* is detected neither in the developing anterior medial hemiellipsoid bodies (Hb) nor in the medulla externa (Me). More posteriorly, a de novo domain of *Photd1* is detected in a small bilateral cluster of four to six cells at the base of the labrum and flanking the stomodeum that appear to be positionally associated with receiving/sending axons to the labral nerve of the stomatogastric nervous system (SNS) via the TC neuromere (Fig. 3d,e,g,h). At the posterior extreme of the ventral midline, *Photd1* is also expressed strongly in the developing proctodeum and hindgut ectoderm (Fig. 3a).

By stage 23, the embryonic CNS is well developed, the VNC possesses a full complement of segmentally reiterated anterior and posterior commissures, and many structural landmarks are clearly identifiable in the developing brain and VNC (Figs. 3j–l and 4a–c; Browne et al. 2005). In the developing medulla complex of the PC, *Photd1* expression has retracted (compare Fig. 3e,n). *Photd1* is diminished in the Mt and only remains prominent in cells along the proximal medial boundary adjacent to the Hb (Fig. 3m–r). In contrast, expression of *Photd1* remains strong in the Mi and in the Ll of the PC as these clusters of cells continue to expand (compare Fig. 3f,wk–l,n–r). *Photd1* expression is absent in the Hb and anterior DC (Fig. 3m–r). A narrow band of weak ectodermal expression is detected associated with the anterior border of the labrum (Fig. 3m,p). *Photd1* expression remains detectable in the small bilateral cluster of Lb SNS cells flanking the stomodeum (Fig. 3m,p). These clusters most likely innervate the TC.

Expression of the *P. hawaiiensis Photd1* paralog in the VNC

Expression of *Photd1* at stage 23 in the VNC includes a defined group of segmentally reiterated cells (Fig. 4d,e). These *Photd1*-expressing cells are associated with the anterior commissure (ACom) and the region between the ACom and posterior commissure (PCom) (Fig. 4c–e). The presence of several *Photd1*-expressing cells arrayed along the anterior ACom appears to be a unique attribute not yet reported in any other arthropod (Fig. 4d). The midline glial cells that ensheath the ACom (Gerberding et al. 2001) appear to express *Photd1* (Fig. 4c–e). In *Drosophila*, the positionally analogous glia would be the MGM and MGA glia (Klämbt et al. 1991). The MGM and MGA glia in *Drosophila* do not express *otd*. However, the array of *Photd1*-positive VNC cells shares some features with that of *Drosophila otd* VNC expression; for example, the central cluster of *Photd1* cells between the ACom and the PCom are median neuroblast (MNB) progeny (Fig. 4d, e; Browne et al. 2005). *Photd1* expression in both the MNB progeny and the ACom glia are predictable based on PPR midline lineage studies in a related amphipod (Gerberding et al. 1999, 2001). The expression of *Photd1* in cells associated with the anterior border of the ACom most



likely represents de novo expression flanking the ventral midline lineage. It is also noteworthy that not all ventral midline cell lineages continue to express *Photd1*; for example, expression is absent in the midline glia that ensheath the PCom (Gerberding et al. 2001). Thus, *Photd1* expression is not maintained in all progeny generated by the *Parhyale* Ep blastomere ventral midline lineage.

In summary, the expression of the *Photd1* gene is zygotic, begins during gastrulation, and is associated with the initial organization of the anterior head and ventral midline (Fig. 2). Later in development, *Photd1* becomes restricted anteriorly to defined regions of the PC, anterior DC, and a SNS cell cluster that correlates positionally with innervation of the labral nerves via the TC at the lateral

junction of the labrum and stomodeum (Fig. 3). In the developing VNC, *Photd1* is associated with a subset of ventral midline progeny giving rise to glia ensheathing the ACom, the MNB progeny neurons, and de novo expression in a group of cells arrayed along the anterior border of the ACom (Fig. 4d).

Expression of the *P. hawaiiensis otd2* paralog

The temporal and spatial expression dynamics of the second *otd* paralog, *Photd2*, are very different from that of *Photd1*, and many aspects have no direct parallel in other arthropod taxa. Three in situ probes corresponding to

◀ **Fig. 3** *P. hawaiiensis otd1* brain expression. All panels (except panel **a** in which embryo anterior is left) are oriented anterior towards the top. **a–i** Stage 21 (S21) embryos; **j–r** stage 23 (S23) embryos. **d–f** and **m–o** Nomarski images though progressively more dorsal focal planes of a S21 embryo and a S23 embryo, respectively, with *Photd1* label in black. **g–i** and **p–r** Matching focal plane overlays for Hoechst DNA counterstain (nuclei are blue) and *Photd1* (false-colored red). **a** Segmentation is complete by S21; anteriorly, *Photd1* appears strongly in the anterior head ectoderm (black arrow) and medially in the labrum (*Lb*). Ventral midline expression has expanded anteriorly to include the mandibular segment (*Mn*) (expansion into the mandibular segment is first detected at stage 18, data not shown). The region of medial ectoderm between the labrum and the *Mn* segment invaginates to form the opening of the stomodeum and lumen of the foregut (asterisk). Continuity of ventral midline expression is maintained to the posterior extreme of the embryo where *Photd1* is expressed strongly in the ectoderm of the developing proctodeum (*Pr*) and hindgut. **b** A schematic of the *Parhyale* supraesophageal ganglia showing the neuromere position of the major neuropil substructures in a S21 embryo; the midline is indicated by a vertical dashed line and the stomodeum (*ST*) is indicated in blue and surrounded by a circum-oral neuropil ring of axon tracts (in black). The protocerebral neuromere (*PC*) is indicated in green and includes the preoral protocerebral commissure (*PCC*) medially, followed by the hemiellipsoid body (*Hb*) and lateral lobe (*Ll*, orange arrow) and by the medulla complex (*Md*) laterally. The deutocerebral neuromere (*DC*) is indicated in yellow and includes the antennal lobe (*AL*) neuropil. The tritocerebral neuromere (*TC*) is indicated in red and includes the antenna 2 neuropil (*AN*). **c** S21 embryo stained for acetylated tubulin to detect axon tract morphology in the developing brain. Medially, the *PCC* is detected. Laterally of the *PCC* and projecting anteriorly along the neuroaxis, axon tracts are detected innervating the developing bilateral *Hb*. Posterior of the *Hb* are axon tracts innervating the developing lateral lobes of the *PC* (orange arrowhead, also in **b**, **f**, **i**). Just posterior and lateral of the *PCC*, the *AL* of the anterior *DC* can be seen projecting axons into the circumoesophageal ring. The two major axon tracts associated with the labral stomatogastric nervous system (*Lb SNS*) are visible as a prominent medial descending tract, the nervus connectivus, from the *PCC* and a more posterior lateral tract, the labral nerve, innervating the *TC*. For panels **d–i**, in the developing anterior head, *Photd1* expression (black staining) crosses the midline in the region associated with the formation of the future protocerebral bridge (black arrowhead in **d**, **e** and white arrowhead in **g**, **h**). Bilaterally near the base of the labrum, at the level of the *An2* segment and the tritocerebrum (*TC*), *Photd1* is expressed in a field of four to six cells (orange double-arrow in **d**, **g** and **e**, **h**) associated with the

development of components of the labral stomatogastric nervous system (*Lb SNS*). Ventral midline expression of *Photd1* in the ectoderm is now present in the *Mn* segment (purple arrowhead in **e**, **h** and **f**, **i**). *Photd1* is also present in the distal medial-most region of the developing *Lb* (**d**, **g**) and distal tips of the developing mandibles (*Mn*) (purple arrow in **d**, **g**). *Photd1* expression in the *Md* of the *PC* is detected throughout the proximal most region, the medulla terminalis (*Mt*) (**e**, **h** and **f**, **i**), and also in the medulla interna (*Mi*) (**e**, **h** and **f**, **i**). *Photd1* expression is particularly strong in cell bodies of the forming lateral lobes of the *PC* (orange arrowheads in **f**, **i**). Lateral expression of the anterior *Photd1* expression domain extends posteriorly to the proximal base of the antennae (*An1*) that innervate the anterior *DC* (yellow arrowheads in **e**, **h**). **j–l** Nomarski (**j**), Hoechst DNA counterstain (**k**, nuclei are blue), and matching focal planes for Hoechst and acetylated tubulin (**l**, nuclei in blue, acetylated tubulin in red) to show morphology and position of axon tracts in the developing S23 *Parhyale* brain. Focal plane bisects the neuroaxis of the supraesophageal ganglia. The anterior-most supraesophageal neuromere, the protocerebrum (*PC*) is composed of a number of distinct substructures. At the anterior midline is the protocerebral bridge (*PB*); this is followed posteriorly by a thick bundle of axons forming the protocerebral commissure tract (*PCT*). Immediately posterior of the *PCT* is the central body (*CB*) (**l**). Proceeding laterally, the first anterior bilateral neuropils are the hemiellipsoid bodies (*Hb*) (**j–l**). Posterior of the *PCT* are the lateral lobe neuropils (orange arrowhead (**k**, **l**)). The lateral-most structure of the *PC* is the medulla complex (*Md*) (**j–l**). The *Md* complex can be further subdivided into the proximal-most medulla terminalis (*Mt*), lateral medulla interna (*Mi*), and distal-most medulla externa (*Me*) neuropils (**l**). The second supraesophageal neuromere, the deutocerebrum (*DC*), is positioned anteriorly and laterally of the circumesophageal ring surrounding the stomodeum (*ST*) and includes a thick bundle of axons forming the deutocerebral commissure tract (*DCT*) (**l**). Lateral and posterior of the *DCT* are the antennal lobes (*AL*) that receive nerves from the first antennae (*An1*) (**j–l**). The third supraesophageal neuromere, the tritocerebrum (*TC*), is positioned along the lateral posterior portion of the circumesophageal ring and receives nerves from the second antennae (*An2*) (**l**). **m–r** *Photd1* expression in the *Md* of the *PC* has retracted to the proximal-most region of the *Mt* in cells lying adjacent to the lateral border of the *Hb* and *Photd1* expression remains pervasive throughout the developing *Mi*. Expression also remains pervasive in developing lateral lobes of the *PC* (orange arrowhead, **n**, **q** and **o**, **r**). A transverse ectodermal band of *Photd1* is also detected at the anterior base of the labrum (black arrow **m**, white arrow **p**). *Photd1* expression near the base of the labrum, at the level of the *TC*, associated with the development of the stomatogastric nervous system (*SNS*) is retained (orange double-arrow; **m**, **p**)

unique *Photd2* splice variants were made, one to a 5' splice variant and two to unique 3' splice variants. No variation in expression profile was detected between probes to different splice variants. Expression is shown for the 3' splice variant possessing the WSP motif (Fig. 5). *Photd2* first appears at stage 12 during germband formation in two bilaterally symmetric anterior domains coincident with *Photd1* (compare Fig. 2e with Fig. 5a,b). The initiation of *Photd2* expression, after the anterior ectoderm has been patterned into distinct head lobes, implies that *Photd2*, in contrast to *Photd1*, does not play a major role in patterning the anterior head ectoderm. No *Photd2* expression is detected in the formation ventral midline cell lineage of the *Ep* clone during germband development (Fig. 5a,b). Later in embryonic development (beginning at stage 22), anterior expression of *Photd2* is highly restricted in the anterior head and only appears in the developing *PC* in a small number of cells associated with the base of the *Md* complex

(Fig. 5c,d). *Photd2* also begins to appear in a restricted set of ventral midline cells (Fig. 5c–g). At the level of the *Mn* segment, *Photd2* expression is largely coincident with *Photd1* midline expression (Fig. 5c,d). However, in the *Mx1* and all posterior segments, ventral midline expression of *Photd2* is mutually exclusive of *Photd1* expression (compare Fig. 4 with Fig. 5e–g). *Photd2* cells in the ventral midline appear to have two distinct fates (Fig. 5e,f). At stage 22, the two ventral-most *Photd2*-expressing cells occur in the middle of developing segments and appear to be ectodermal (Fig. 5e), whereas more dorsally, three *Photd2*-expressing cells at the midline junctions of each segment may be glia (Fig. 5f; Gerberding et al. 2001).

In summary, *Photd2* is expressed zygotically and first appears after the formation of the head lobes in an anterior domain in germband embryos (Fig. 5a,b). Late in embryogenesis, the anterior *Photd2* domain has retracted to a few cells at the base of the medulla (Fig. 5c,d). No expression

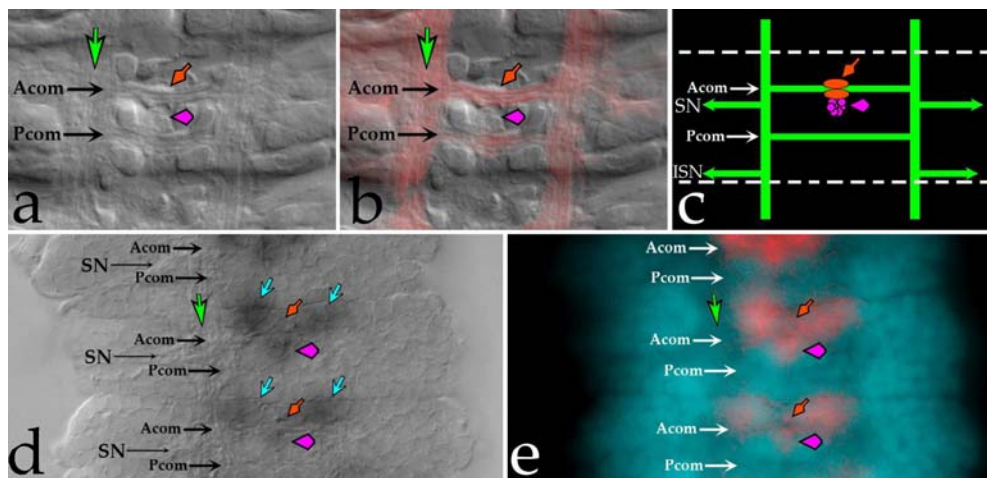


Fig. 4 Ventral nerve cord expression of *P. hawaiiensis otd1*. All panels are dorsal views bisecting a stage 23 embryonic VNC and are oriented anterior towards the top. **a–c** Nomarski (**a**), matching planes for nomarski and acetylated tubulin (**b**, acetylated tubulin in red), and a VNC schematic (**c**). **d** and **e** Nomarski with *Phodt1* in black (**d**) and matching planes for Hoechst DNA counterstain and *Phodt1* (**e**, nuclei are blue; *Phodt1* is false-colored red). The position of the longitudinal axon tracts is indicated with a green arrow. The position of the anterior commissure (*ACom*) and of the posterior

commissure (*PCom*) are indicated (**a–e**). The position of the segmental nerves (*SN*) is indicated in **c** and **d**. The position of the intersegmental nerves (*ISN*) is indicated in **c**. A group of cells located immediately anterior of the *ACom* that express *Phodt1* can be identified (blue arrows, **d**). *Phodt1* is detected in glia ensheathing the *ACom* (red arrow) and in the cluster of median neuroblast (*MNB*) progeny between the anterior and posterior commissures (purple arrowhead)

of *Phodt2* is detected in the ventral midline until late in embryogenesis. Ventral midline expression of *Phodt2* is mutually exclusive of its paralog *Phodt1*. The fates of these *Phodt2*-expressing midline cells are likely to be both ectodermal in the middle of each segment and glial in more dorsal cells at segment boundaries (Fig. 5e–g).

Discussion

The problems imposed on animals by both their environment and respective genetic history have resulted in a diversity of differences in nervous system organization. Considerable variation is observed in the development and overall organization of the brain and nervous systems among insects alone (e.g., Condrón and Zinn 1994; Reichert and Boyan 1997; Urbach and Technau 2003b). In insects and other arthropods, understanding the evolution, development, and segmental homologies of the head and brain has posed long-standing problems in the fields of morphology, paleontology, evolution, and molecular genetics, resulting in what has been described as ‘the endless debate’ (e.g., Rempel 1975; Scholtz and Edgecombe 2005; Browne et al. 2005; Maxmen et al. 2005). In this study, we provide new data from a crustacean that helps clarify the organization and development of the arthropod head and brain.

Expression of paralogous *Parhyale otd* genes in the anterior head

Available evidence suggests that a single *orthodenticle* gene was likely expressed in the head and foregut of the eubilaterian ancestor (Fig. 1; Bruce and Shankland 1998;

Harada et al. 2000; Arendt et al. 2001; Lowe et al. 2003). In the lineage leading to the Lophotrochozoa and Ecdysozoa, *orthodenticle* expression was acquired in segmentally reiterated neurons along the anterior–posterior axis of the body (Fig. 1; Bruce and Shankland 1998). At some point before the divergence of the Tetraconata, the *orthodenticle* gene experienced a number of changes. The number of *orthodenticle* genes expanded via a tandem duplication event (Fig. 1; Li et al. 1996), expression was gained in MNB progeny neurons, and expression was lost in the foregut (Fig. 1). We expect that more comprehensive analysis of *orthodenticle* ortholog expression in taxa diverging before the Tetraconata, including the lophotrochozoans, may reveal that some of these general expression attributes may have first appeared at deeper evolutionary nodes.

Gene duplication is often accompanied by divergence in both gene expression and function. The expression of the *Parhyale otd* paralogs are clearly divergent from *otd* orthologs in both *Tribolium* and *Drosophila*. In contrast to the earliest expression in *Tribolium*, the earliest expression in *Parhyale* (and *Drosophila*) is zygotic and has no maternal component (Fig. 2; Finkelstein and Perrimon 1990). Neither paralog in *Tribolium* is expressed in the DC or TC (Li et al. 1996). The *Parhyale Phodt1* paralog is clearly expressed in the anterior DC (as is *Drosophila otd*) as well as in a restricted region of the TC (Fig. 3). This is a feature, thus far, unique to *Parhyale*. The restriction of the *de novo* TC domain to cell bodies associated with innervation of the labral SNS, coupled with the presence of a prominent nervus connectivus linking the labral SNS to the PCC (Fig. 3c), makes it tempting to speculate that ancestral labral innervation was restricted to the PC in combination with an anterior stomodeum (Rempel 1975; Eriksson and Budd 2000; Browne et al. 2005; Maxmen et al. 2005). This is important

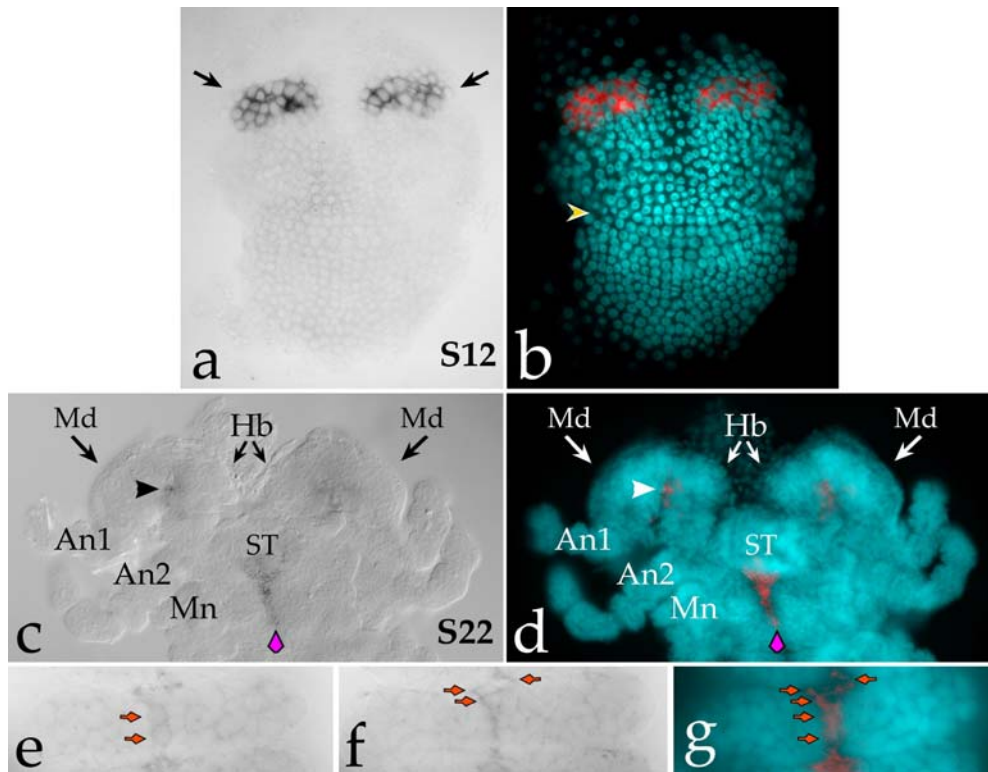


Fig. 5 Expression of *P. hawaiensis otd2*. All embryos are mounted with the anterior end oriented towards the top. **a, b** A stage 12 embryo is mounted ventral side up with a nomarski image on the left and *Phodt2* signal in black. The image on the right is overlay of matching focal planes for Hoechst DNA counterstain with *Phodt2* (nuclei are blue; *Phodt2* is false-colored red). *Phodt2* is first detected at this stage anteriorly in the condensation of cells in the head lobes (black arrows) and is coincident with the anterior *Phodt1* domain at this stage of development (compare with Fig. 2e). The yellow arrowhead marks the position of the Mn segment. *Phodt2* is not expressed in the ventral midline lineage until much later in stage 22 embryos. **c–g** *Phodt2* expression in a stage 22 embryo. **c, d** Embryo mounted dorsal side up, with nomarski image on the left and *Phodt2* signal in black. The image on the right is overlay of matching focal

planes for Hoechst DNA counterstain with *Phodt2* on the right (nuclei are blue; *Phodt2* is false-colored red). In the developing anterior head, *Phodt2* expression is restricted to a small number of cells at the base of the Md. Ventral midline expression of *Phodt2* can now be detected in the midline ectoderm of the Mn segment (purple arrowhead). **e–g** A stage 22 embryonic thoracic segment mounted ventral side up. **e** Ventral nomarski focal plane with *Phodt2* signal in black. **f** Dorsal nomarski focal plane with *Phodt2* signal in black. **g** Collapsed and merged ventral and dorsal focal planes for Hoechst and *Phodt2* (nuclei are blue; *Phodt2* is false-colored red). *Phodt2* is expressed in VNC midline cells exclusive of *Phodt1* (compare with Fig. 4). The two midline cells in **e** appear to be ectodermal. The three slightly more dorsal cells in **f** are at the segmental border and may be glial cells

because the positional origin of the labrum, as the most prominent anterior ectodermal structure in most arthropods, has played a central role in attempts to understand underlying segmental patterning of the arthropod head. Expression analysis of *otd* orthologs in onychophorans would prove particularly useful in helping to resolve questions regarding ancestral features of head patterning and brain development in the Ecdysozoa and may settle the debate regarding the positional origin and innervation patterns associated with the appendicular labrum.

A universal mechanism for patterning both the invertebrate and vertebrate brain has been proposed to account for their tripartite brain organization (Hirth et al. 2003). Based on expression patterns in *Drosophila*, this model proposes that the interface between the posterior boundary of *orthodenticle* expression and anterior expression domain of the gene *unpg* maintains the boundary between the DC and TC via mutual repression. In *Tribolium*, neither *otd* paralog is expressed in the DC during brain development. In *Parhyale*, *Phodt1* is expressed only transiently in the

anterior DC (Fig. 3e,h). In light of this variation in *otd* expression, it would be informative to validate this mechanism by observing the relationship between *Phodt1* and *Parhyale unpg* orthologs in the maintenance of gene expression boundaries correlating with the DC–TC neuromere interface.

The expression of *Phodt2* in the head is radically divergent both from its paralog *Phodt1* as well as from *otd* orthologs in divergent arthropod taxa. The initial anterior domain of *Phodt2* is coincident with *Phodt1* but does not appear until after the head lobes are organized (Fig. 5). As development progresses, the anterior *Phodt2* expression domain decays and, late in development, is only detectable in a few cells of the PC (Fig. 5). *Phodt2* is consistently expressed at a much lower level than *Phodt1*, a phenomenon also observed in *Tribolium*.

Expression of paralogous *Parhyale otd* genes in the ventral midline

Current data suggest *orthodenticle* expression was acquired in the ventral midline in arthropods, (Figs. 1, 2, 4, and 5; Finkelstein et al. 1990; Li et al. 1996; Telford and Thomas 1998). In *Parhyale*, as in *Drosophila* and *Tribolium*, expression of *otd* in the midline cells is apparent at an early stage of development (Fig. 2). The ontological origin of *Tribolium* midline cells are undescribed; however, midline cell lineages are known in *Drosophila* (Klämmt et al. 1991; Bossing and Technau 1994; Schmid et al. 1999), the grasshopper *Schistocerca* (Condrón and Zinn 1994), as well as in a related amphipod crustacean *Orchestia* (Gerberding and Scholtz 1999, 2001). Our data suggests some similarities with *otd* expression in insect midline lineages as well as a number of significant differences.

The ectodermal ventral midline cell lineage in the closely related amphipod, *Orchestia*, generates a specific set of glial and neuronal progeny. For each segment, two glial cells are produced that ensheath the anterior commissure, two glial cells are produced to ensheath the posterior commissure, two to three glia are produced that are positioned at the midline of the intersegmental border, and ~10 median neuroblast progeny are produced that cluster between the ACom and PCom (Gerberding and Scholtz 2001). The midline of *Parhyale* develops in an analogous fashion to that of *Orchestia*. In *Drosophila*, the midline develops from a unique arrangement of mesectodermal cells. The midline lineage produces both the MNB progeny neural cluster and the glia that ensheath the anterior commissure (Klämmt et al. 1991). In insects, the PCom is pioneered ahead of the ACom (Klämmt et al. 1991; Myers and Bastiani 1993), whereas in *Parhyale* the ACom is pioneered ahead of the PCom. *Photd1*-positive midline cells appear in both the MNB progeny neural cluster and in the glia that ensheath the ACom, expression features shared with both *Drosophila* and grasshopper (Fig. 4c–e). The position of the *Parhyale* MNB neural cluster is displaced anteriorly relative to *Drosophila* and grasshopper, but this is likely a pleiotropic effect due largely to the observed heterochrony in commissure formation (reversed order of commissure formation).

In addition to the conserved features, *Photd1* is also uniquely expressed in a group of cells arrayed along the anterior border of the ACom (Fig. 4d). The presence of *Photd1* in these cells represents de novo expression flanking the ventral midline lineage, as well as expression in a group of cells not yet observed in other arthropods. In addition, the late embryonic expression of *Photd2* in a group of midline cells mutually exclusive of *Photd1* has no parallel in other taxa. At least three of these reiterated cells that are found dorsally at the midline junction of segments may represent glial descendants of the ventral midline lineage (Fig. 5e–g; Gerberding and Scholtz 2001).

Implications regarding the evolution and duplication of the *orthodenticle* gene

Understanding the history of *orthodenticle* gene duplication during the course of metazoan evolution presents an interesting dilemma. Expansion of *otd* genes within the vertebrates via genome amplification is straightforward (Germot et al. 2001). However, in all other instances of duplication, *otd* paralogs are in close proximity, suggesting tandem duplication, and the paralogs appear more similar to one another than to *otd* orthologs in other species. This suggests two possible scenarios: (1) *otd* paralogs in each case represent independent duplications or (2) *otd* duplications are very ancient, occurred at deeper nodes, and paralogs in each lineage appear to be different from their orthologs. The history of *otd* duplication could be sufficiently complex that we will not be able to determine the relationships between paralogs from different lineages.

We suggest that two well-known genetic mechanisms, gene conversion and mutational saturation, can be used as support for scenario 2 based on currently available genomic information in the taxa analyzed. Gene conversion is a process by which paralogous genes acquire identical nucleotide sequences via mismatch repair of heteroduplexed DNA (Radding 1982). The likelihood of gene conversion increases with the proximity of the paralogs. Gene conversion would become a problem when attempting to reconstruct the orthology relationships between paralogs due to regions between paralogs becoming more similar to each other than to their orthologs in other lineages and, thus, losing useful phylogenetic signal. The second mechanism, mutational saturation, also results in misleading phylogenetic signal among paralogous sequences (Philippe and Forterre 1999). A manifestation of mutational saturation, for example, is the process of codon usage bias governing the pool of available tRNAs, again acting to make paralogs more similar to one another than to their respective orthologs in other lineages, in this instance via purifying selection (Nei and Kumar 2000).

Among the taxa we sampled, the beetle *T. castaneum* genome contains two *otd* paralogs within ~60 kb of one another (BeetleBase, <http://www.bioinformatics.ksu.edu/beetlebase>). The hymenopteran *Apis mellifera* genome contains two *otd* paralogs within ~40 kb of one another (BeeBase, http://racex00.tamu.edu/bee_resources.html). Preliminary information from the crustacean *Daphnia pulex* genome has revealed the presence of two *otd* paralogs within ~24 kb of one another (F. Poulin and N. Patel, personal communication). Sampling among the chelicerates has only revealed a single *otd* ortholog. Thus, the current evidence suggests a single tandem duplication of an ancestral *otd* gene in the arthropod lineage leading to the Tetraconata (Fig. 1). Within the Tetraconata, two things appear to have happened to *otd* paralogs: (1) loss of one *otd* duplicate in the lineage leading to the dipterans as represented by *Drosophila* (Fig. 1) and (2) physical proximity of the gene duplicates may have led to sequence homogenization within a given lineage via the two distinct genetic mechanisms outlined above, gene

conversion, and/or mutational saturation. Large-scale genomic resources are not yet available for *Parhyale*; however, we predict that the two *P. hawaiiensis orthodenticle* paralogs are physically linked.

The test for mutational saturation suggests that base pair saturation has played a role in *orthodenticle* gene divergence in different taxa, making recovery of paralog orthology among the tandem duplicates present in Tetraconata (crustaceans + insects) particularly problematic with currently available phylogenetic tools. Direct evidence of gene conversion could possibly be demonstrated by investigating *orthodenticle* paralog sequences at the level of populations, revealing instances of allelic variation resulting from heteroduplex DNA mismatch repair. It is tantalizing to hypothesize that the unique retention of the WSP domain in both *A. mellifera* paralogs may represent evidence for this mechanism downstream of the homeo-domain within this lineage (Fig. 1).

In summary, the data presented here represent additional evidence for a single ancestral *orthodenticle* like gene in the common ancestor of the lineage leading to the cnidarians + bilaterians (Fig. 1; Li et al. 1996). In most cases, we also recover an eight-amino-acid diagnostic sequence, the WSP motif, downstream of the homeo-domain in at least one *otd* paralog (Fig. 1). It is highly probable that this motif existed in the single ancestral *orthodenticle* gene before the divergence of the cnidarians and the bilaterians. This ancestral *orthodenticle* gene was independently duplicated in lineages leading to the vertebrates (genome duplication), the cnidarians (three linked copies), and the Tetraconata (tandem duplication). One paralog was subsequently lost in the lineage leading to the dipterans (Fig. 1).

Prospects

Our analysis of *otd* paralog gene expression in *Parhyale* has indicated some of the general boundaries/structures associated with the developing supraesophageal neuromeres that comprise the brain. We are hopeful that, when used in combination with other markers, they will contribute to understanding the distribution and number of neural stem cells that initially specify the *Parhyale* brain (e.g., Urbach and Technau 2003a,b). This type of neuroblast gene expression data will prove to be important in crustaceans as, in sharp contrast to insects, crustacean neuroblasts do not delaminate from the ectoderm and, thus, are often not clearly distinguishable from surrounding non-neural ectodermal cells. In addition, crustacean neuroblasts may switch fates as they divide, generating both neural-fated and non-neural fated ectoderm progeny. Thus, the best means for discriminating between ectodermal cells, neuroblasts, neurons, and glia in crustaceans will be with panels of molecular markers used in concert with neuronal morphology.

Comprehensive characterization of gene expression and, subsequently, genetic function (e.g. Pavlopoulos and Averof 2005) in the *Parhyale* brain and nervous system

will begin to provide us with a framework and new set of tools for exploring diverse aspects related to developmental patterning at three levels: the basic underpinnings of neurogenesis in metazoans, aspects of developmental mechanisms inherent to neurogenesis in arthropods, and specific details related to the unique development of the nervous system of diverse crustaceans.

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Supplement to *Parhyale hawaiiensis otd2*

Supplementary Figure A

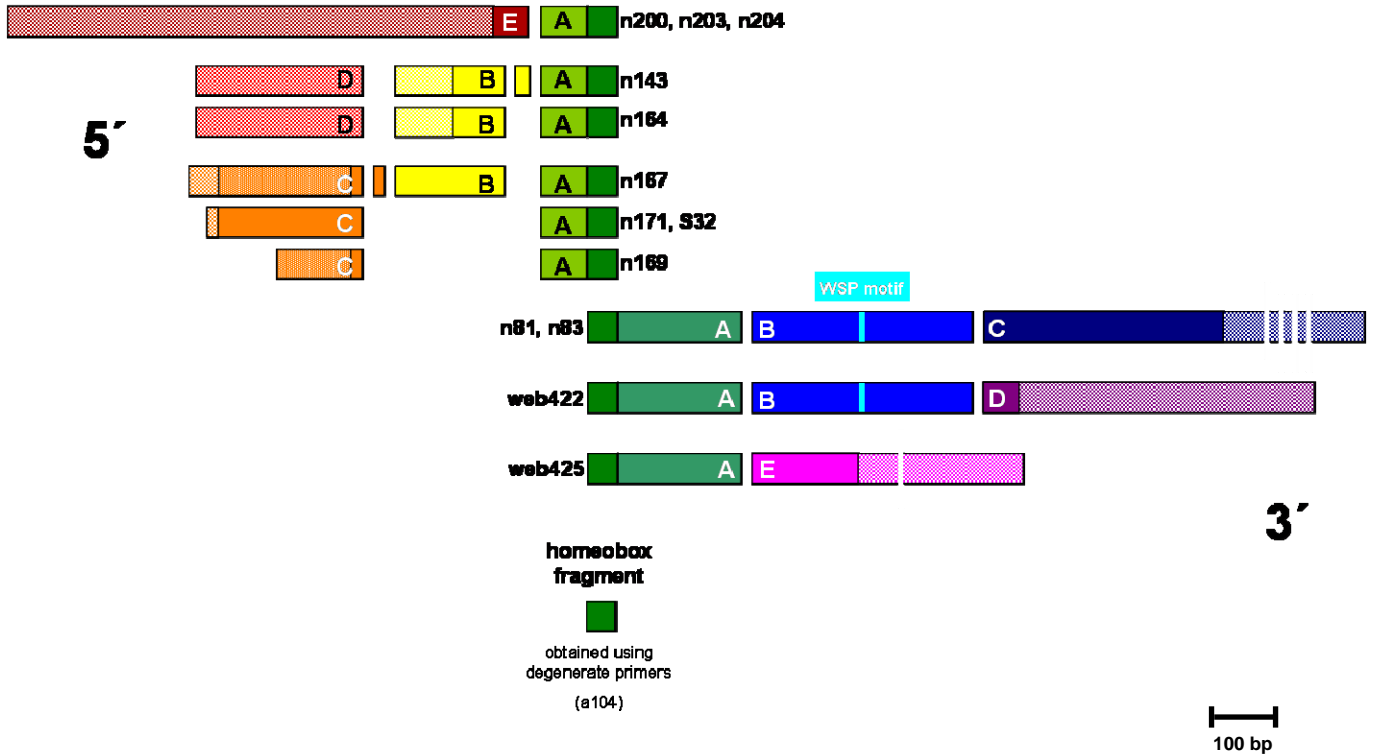


Figure A. Schematic overview over 5' and 3' RACE clones of *Parhyale hawaiiensis otd2* and their putative splicing structure. A schematic alignment of the nucleic acid sequences of 5' RACE clones (top of panel), 3' RACE clones (middle of panel) and the short *otd2* homeobox fragment obtained by PCR using degenerate primers ("fishing" PCR, bottom of panel). Individual putative splicing units are characterized by respective colors and letters: 5'E splicing unit in dark red, 5'D in bright red, 5'C orange, 5'B yellow, 5'A light green; homeobox core fragment green, 3'A splicing unit pale green, 3'B blue, 3'C indigo, 3'D purple and 3'E pink. Each different clone (or group of clones) is comprised of a different combination of respective splicing units.

Open reading frames within the obtained RACE clones were determined by the 5'-most ATG (5' RACE clones) or first 3' stop codon (3' RACE clones, respectively). Coding sequences are in solid colours, non-coding sequences are in hatched colours. A WSP-motif which is present in translated 3'B splicing units is indicated in cyan. For more detailed information about individual RACE clones, see supplementary figure C.

All putative splicing units are displayed in their correct relative scale. Each white perpendicular stroke in clones n81/n83 3'C and clone web425 3'E corresponds to 500 bp of noncoding sequence.

5'C of n169 appears to be truncated as compared to 5'C of n171/S32. In n167, the lack of one adenosine at the 5' end of the sequence changes the open reading frame and thus brings the putative start codon out of frame as compared to n171/S32.

Supplementary Figure B

Figure B. Protein alignments of translated 5' and 3' *otd2* RACE clones. Translated protein sequences that correspond to putative splice units are marked in their respective colours (see also Figure A). The first alignment shows translated 5'RACE sequences (incl. homeodomain of clone a104); the second alignment shows translated 3' RACE sequences (incl. homeodomain of clone a104).

Alignment of translated 5' RACE sequences

```
n203
n143
n164
n167
n171          MPWESHLSFSLKLQSRKKHTHH
n169
a104
```

```
n203
n143
n164
n167
n171  HLSASLLVLTTHIVRRLKCPKQVQSYSSLRRTCSDLPHLSSSRGRNPEACTA
n169
a104
```

```
n203
n143
n164
n167  MWASAQGGGGGGGVAFPFGGSCGAGGSAAAAAGVYLKTPAPYTMNG
n171  DPVMWASAQ
n169  MWASAQ
a104
```

```
n203
n143          MQGTHLLTTRSTQARALTI AATPGRKQRRERTTYT
n164  LGLGVGMGVDSLHPSMGYPPPTDYLYSEYKYSYMAATPGRKQRRERTTYT
n164  LGLGVGMGVDSLHPSMGYPPPTDYLY          TATPGRKQRRERTTYT
n167  LGLGVGMGVDSLHPSMGYPPPTDYLY          TATPGRKQRRERTTYT
n171
n169          AATPGRKQRRERTTYT
a104          AATPGRKQRRERTTYT
```

```
n203  RAQLDILETL
n143  RAQLDILETL
n164  RAQLDILETL
n167  RAQLDILETL
n171  RAQLDILETL
n169  RAQLDILETL
a104  RAQLDILETLFGKTRYPDIFMREEVAIKINLPESRI
```

Alignment of translated 3' RACE sequences

a104 RAQLDILETLFGKTRYPDIFMREEVAIKINLPESRI
n81 EVAIKINLPESRIQVWFKNRRAKCRQQ
web422 IKINLPESRIQVWFKNRRAKCRQQ
web425 IKINLPESRIQVWFKNRRAKCRQQ

a104
n81 QKQQAANGEKAPRTKKVNKSPPPSTNNTITNSSLIGSGAGNNAATTNHHH
web422 QKQQAANGEKAPRTKKVNKSPPPSTNNTITNSSLIGSGAGNNAATTNHHH
web425 QKQQAANGEKAPRTKKVNKSPPPSTNNTITNSSLIGSGAGNNAATTITTTT

a104
n81 HHHTTPNMMNNNSNSNSSTTLTSPLTTSHGARDASPPSPPPSVSSGSS
web422 HHHTTPNMMNNNSNSNSSTTLTSPLTTSHGARDASPPSPPPSVSSGSS
web425 IITITIKVNRKRANITIKRKTESTVQTRKTRVRSRWRREFTTAPKKTERP

a104
n81 LSLPPSTPYNPIWSPAGIPHQSSTLPAMGDLMGGGGLDRGGYGSACYQGY
web422 LSLPPSTPYNPIWSPAGIPHQSSTLPAMGDLMGGGGLDRGGYGSACYQGY
web425 AIWA

a104
n81 GTPSYYSNMDYLAPMTHSQMSSMTSLNPMTSAHHQHLGAHHHSAAAGLSP
web422 GTPSYYSNMDYLAPMTHSQVNTFAHNIDLSILIMLTCLCK
web425

a104
n81 SPLGSSLGSSPLSSSHHLVSSLSSTSLNSGSHAALTHPPLTHSSSLNQDS
web422
web425

a104
n81 PRSPSGAPGSLTPLPQDCLDGYPEDKSAI IAPNSAAAAASAAAAWKNYQGF
web422
web425

a104
n81 QSL
web422
web425

Supplementary Figure C

Figure C. Complete List of DNA-sequences of aforementioned 5' and 3' RACE clones of *Parhyale hawaiiensis otd2* along with their translated protein sequence. Respective putative splice units are coloured as in Figure A.

```
1      ggtgtgttattgtgatggtgagagcagcacagtttgtcgactgcaagtgaacacaag 57
58    tgaatgaggtagaaggtgtgaaagtgaatatgaacacaagtgaacaatgttgtgattata 117
118   aatggactgatggtagtgaatgtggatacaagtgaaataggtagggttgttcatgcaat 177
178   gcgactggaagtggtgaatgtggacacaactgcgctaggtagccgcctcgaacgtggctg 237
238   gctaccgataatgatagtacatgaaagtggggacgataatcagggtgcaacttgggtgga 297
298   agtgacaaaaaacgaaaattgcgggacatagccagatttgttgcttaagaaactgaaagag 357
358   agaagaacaggtgaagaaaaacagatatgggtttattgggaacaatattgaagtggtgcaa 417
418   gtgcgctgacactggtagttggttgaaattgtgaagatactcgtaaaaagaagtgcggaa 477
478   gattatTTAACAGCGACTGAAAATTTCCAGTGTGAAAATACATATAAATATGAGCAGTGT 537
538   agggcagtgactTTTTAACATTAGGTCTTTGATTGTACTGTGCAGATAGGTATGCGGTTG 597
598   agcttctgttaggcgagaaaactgaatcatttcggctagaacatgtacaagttaaca 657
658   acataagaaattcggccggtgtttcagaaatatagtttggttgtttacaaagctgaactt 717
718   gagggcccactcctgcaaagaagattctgctcaagcctcgaataaatctttgttttttc 777
778   taagcaacaaacagacctctaacagaaatttaactgctaataagcgATGCAAGGAACGCAT 837
      M Q G T H
838   L L T T R S T Q A R A L T I A A T P G R
      CTATTAACAACAAGAAGCACGCAGGCAAGAGCACTGACCATTGCGGCCACTCCAGGCCGG 897
      K Q R R E R T T Y T R A Q L D I L E T L
898   AAGCAGCGGCCGGAGCGCACGACGTACACCAGAGCGCAACTGGACATCCTGGAGACCCTG 957
```

DNA-sequence of **Ph_otd2n203** (*Parhyale hawaiiensis otd2* clone n203, exemplary for clones n200, n203 and n204), obtained from *otd2* 5' RACE. Ph_otd2n203 consists of consensus 5' splicing units 5'C and 5'A. Start of open reading frame is marked as **ATG**.

1 ggagcggggcccttctagtcgagtgt 26
 27 ggtggtggtgatgatggttagtggtggcagattgttaccagcgcgctgcctatgagcg 86
 87 acgagttcgtaatgtgttgtaaggggtgatggaggcagagacagggatgaccccgagc 146
 147 taacggacggaggggggtgacgggtggggacgcgtctctagctccatgggacgtcgtcaaag 206
 207 gcgacgtcaggggtgttgagctgttactgctgttactgttattgttggtattattgttgg 266
 267 ggtggtgtgatggtggggtcgcattccccgggttcgggggcagttgcggggctgggggg 326
 327 tcagcagcagcagccgctgggggtctatctcaaaacccccgcccctacaccATGAACGGA 386
 L G L G V G M G V D S L H P S M G Y P P
 387 CTTGGACTCGGTGTCGGCATGGGGGTAGACAGCCTGCACCCAAGCATGGGCTATCCTCCC 446
 P T D Y L Y S E Y K Y S Y M A A T P G R
 447 CCTACCGATTATTTATACAGTGAGTATAAATACTCGTACATGGCGGCCACCCAGGCCGG 506
 K Q R R E R T T Y T R A Q L D I L E T L
 507 AAGCAGCGGCGGGAGCGCACTACGTACACCAGAGCGCAACTGGACATCCTGGAGACCCTG 566

DNA-sequence of **Ph_otd2n143** (*Parhyale hawaiiensis otd2* clone n143), obtained from *otd2* 5' RACE. Ph_otd2n143 consists of consensus 5' splicing units 5'D, 5'B and 5'A. Start of open reading frame is marked as ATG.

```

1                                     gg      2
3  agcggggcccttctagtcgagtggtggtggtgatgatggttagtggtggcagtattgt  62
63  taccagegccgctgcctatgagcgacgagttcgttaattgtgttgtaaggggttgatggag  122
123  gcagagacagggatgacccccgagctaacggacggaggggggtgacggtggggacgcgtctc  182
183  tagctccatgggacgtcgtcaaaggcgacgtcagggttgttgagctgttactgctgttac  242
243  tgttattggttattattggtgggggtggtgtgatggtggggtcgcattccccgggttc  302
303  gggggcagttgccccggctgggggggtcagcagcagcagccgctgggggtctatctcaaaacc  362
      M N G L G L G V G M G V D S L
363  cccgccccctacaccATGAACGGACTTGGACTCGGTGTCGGCATGGGGGTAGACAGCCTG  422
      H P S M G Y P P P T D Y L Y T A T P G R
423  CACCCAAGCATGGGCTATCCTCCCCCTACCGATTATTTATACA  CGGCCACTCCAGGCCGG  482
      K Q R R E R T T Y T R A Q L D I L E T L
483  AAGCAGCGCGGGAGCGCACTACGTACACCAGAGCGCAACTGGACATCCTGGAGACCCTG  542

```

DNA-sequence of **Ph_otd2n164** (*Parhyale hawaiiensis otd2* clone n164), obtained from *otd2* 5' RACE. Ph_otd2n164 consists of consensus 5' splicing units 5'D, 5'B and 5'A. Start of open reading frame is marked as ATG.

```

1                               gattgaaaacttttgccgtggtgtctctctt 30
31 tcaattccagcaagttttaatgccgtgggagtcacatctaagcttctccctaaagttgca 90
91 atcaagaaaaaacacacacaccaccaccttagtgcaagcttgcttggttaactcatata 150
151 gtgcgctcgcttgaagtgcccaaagcaggtacaaagttattcatctctacgtacgtgctct 210
211 gatctgcctcatctatccagcagccgcggccgaaaccagaggcctgcacagcggacca 270
    M W A S A Q G G G G G G G V A F P G F
271 gtaATGTGGGCCTCTGCTCAGGGTGGCGGTGGGGGAGGC GGGGTCGCATTCCCCGGGTTTC 330
    G G S C G A G G S A A A A A G V Y L K T
331 GGGGGCAGTTGCGGGGCTGGGGGGTCAGCAGCAGCAGCCGCTGGGGTCTATCTCAAACC 390
    P A P Y T M N G L G L G V G M G V D S L
391 CCCGCCCTACACCATGAACGGACTTGGACTCGGTGTCGGCATGGGGGTAGACAGCCTG 450
    H P S M G Y P P P T D Y L Y T A T P G R
451 CACCCAAGCATGGGCTATCCTCCCCCTACCGATTATTTATACA CGGCCACTCCAGGCCGG 510
    K Q R R E R T T Y T R A Q L D I L E T L
511 AAGCAGCGGCGGGAGCGCACTACGTACACCAGAGCGCAACTGGACATCCTGGAGACCCTG 570

```

DNA-sequence of **Ph_otd2n167** (*Parhyale hawaiiensis otd2* clone n167), obtained from *otd2* 5' RACE. Ph_otd2n167 consists of consensus 5' splicing units 5'C, 5'B and 5'A. Start of open reading frame is marked as ATG.


```

1          gaattccagcaagttttaM P W E S H L 39
          ATGCCGTGGGAGTCACATCTA
40  S F S L K L Q S R K K H T H H H L S A S 99
  AGCTTCTCCCTAAAGTTGCAATCAAGAAAAAACACACACACCACCACCTTAGTGCAAGC
100 L L V L T H I V R R L K C P K Q V Q S Y 159
  TTGCTTGTGTTAACTCATATAGTGCGTCGCTTGAAGTGTCCAAAGCAGGTACAAAGTTAT
160 S S L R T C S D L P H L S S S R G R N P 219
  TCATCTCTACGTACGTGCTCTGATCTGCCTCATCTATCCAGCAGCCGCGGCCGAAACCCA
220 E A C T A D P V M W A S A Q A A T P G R 279
  GAGGCCTGCACAGCGGACCCAGTAATGTGGGCCTCTGCTCAGGCGGCCACTCCAGGCCGG
280 K Q R R E R T T Y T R A Q L D I L E T L 339
  AAGCAGCGCGGGAGCGCACTACGTACACCAGAGCGCAACTGGACATCCTGGAGACCCTG

```

DNA-sequence of **Ph_otd2n171** (*Parhyale hawaiiensis otd2* clone n171, exemplarily for clones n171 and S32), obtained from *otd2* 5' RACE. Ph_otd2n171 consists of consensus 5' splicing units 5'C and 5'A. Start of open reading frame is marked as ATG.

```

1          gagtgcgtcgcttgaagtgtccaaagcaggtacaaagtat      41
42  tcattctctacgtacgtgctctgatctgcctcatctatccagcagccgcgccgaaacca  101
          M W A S A Q A A T P G R
102  gaggcctgcacagcggaccagtaATGTGGGCCTCTGCTCAGGCGGCCACTCCAGGCCGG  161
          K Q R R E R T T Y T R A Q L D I L E T L
162  AAGCAGCGGCGGGAGCGCACTACGTACACCAGAGCGCAACTGGACATCCTGGAGACCCTG  221

```

DNA-sequence of **Ph_otd2n169** (*Parhyale hawaiiensis otd2* clone n169), obtained from *otd2* 5' RACE. Ph_otd2n169 consists of consensus 5' splicing units 5'C and 5'A. Start of open reading frame is marked as ATG.

1	R A Q L D I L E T L	31
	CAGAGCGCAACTGGACATCCTGGAGACCCTG	
32	F G K T R Y P D I F M R E E V A I K I N	91
	TTCGGCAAGACGCGGTATCCCGACATCTTTATGAGAGAGGAAGTTGCGATCAAGATCAAT	
92	L P E S R I	109
	CTCCCTGAGAGTAGAATA	

DNA-sequence of **Ph_otd2a104** (*Parhyale hawaiiensis otd2* clone a104, exemplary for clones a104 and a105), obtained from *otd2* "fishing" PCR using degenerate primers. Ph_otd2a104 consists of a part of the *otd2* homeobox. Note that the *otd2* homeobox sequence obtained from "fishing" PCR overlaps with 5'A and 3'A sequences.

1 E V A I K I N L P E S R I Q V W F K N R 60
 GGAAGTTGCGATCAAGATCAATCTCCCTGAGAGTAGAATACAGGTTTGGTTTAAGAATCG
 61 R A K C R Q Q Q K Q Q A A N G E K A P R 120
 TAGAGCAAAGTGCCGGCAGCAACAGAAGCAGCAGGCCGCCAACGGCGAGAAGGCTCCACG
 121 T K K V N K S P P P S T N N T I T N S S 180
 CACCAAGAAAGTGAACAAATCTCCTCCTCCATCCACCAACAACAATTACGAACCTCGTC
 181 L I G S G A G N N A A T T N H H H H H H 240
 GCTCATAGGCAGCGGGCTGGTAACAATGCTGCCACCACTAACCACCATCACCACCATCA
 241 T T P N N N N N N N S N S S N S S T T L 300
 CACCACCCCAACAATAATAACAACAACAGTAACAGCAGTAACAGCTCAACAACCTCT
 301 T S P L T T S H G A R D A S P P S P P P 360
 GACGTCGCCTCTGACGACGTCCCATGGAGCTAGAGACGCGTCCCCACCGTCACCCCTCC
 361 S V S S G S S L S L P P S T P Y N P I W 420
 GTCCGTTAGCTCGGGGTCATCCCTGTCTCTGCCTCCATCAACCCCTTACAACCCCATTTG
 421 S P A G I P H Q S S T L P A M G D L M G 480
 GTCTCCAGCTGGGATACCCACCAGTCTCCACGTTACCCGCAATGGGGACTTGATGGG
 481 G G G L D R G G Y G S A C Y Q G Y G T P 540
 TGGTGGTGGGTGGACAGAGGGGTACGGGAGCGCTTGCTACCAGGGCTACGGTACTCC
 541 S Y Y S N M D Y L A P M T H S Q M S S M 600
 ATCCTATTACTCGAACATGGACTACCTCGCACCTATGACCCATTCTCAGATGAGTTCAAT
 601 T S L N P M T S A H H Q H L G A H H H S 660
 GACCTCATTGAACCCCTATGACCTCGGCTCACCACCAGCATCTTGGTGCTCATCACCCTC
 661 A A A G L S P S P L G S S L G S S P L S 720
 AGCGGCTGCGGGTCTGAGTCCCTCACCCTCGGTTCACTACTGGGCTCCTCCCCTCTGAG
 721 S S H H L V S S L S S T S L N S G S H A 780
 CTCATCACATCACCTCGTCTCATCACTAAGCTCAACATCCCTCAACAGCGGTAGTCACGC
 781 A L T H P P L T H S S S L N Q D S P R S 840
 TGCCCTCACTCACCTCCCCTCACCCATTCTTCGTCTTGAATCAAGACAGCCCTCGTAG
 841 P S G A P G S L T P L P Q D C L D G Y P 900
 TCCGTCTGGAGCCCCAGGGTTCGCTTACCCCTCTGCCCCAGGACTGCCTGGACGGTTATCC
 901 E D K S A I I A P N S A A A A S A A A A 960
 AGAAGATAAGTCAGCCATTATTGCACCGAACTCCGCGCCGCAGCATCAGCGGCAGCGGC
 961 W K N Y Q G F Q S L Stop 1020
 GTGGAAGAACTACCAAGGATTTTCAGAGTTTGTGAagtaacgaaggtcacgtaatagtgac
 1021 gaattcttagccatgaaaacaatgtatagtttgaattattttatgatgtttcatgaac 1080
 1081 gcccgcttactcttgaagagtttctgtaaaaactattgcgattttttccagtaatattage 1140
 1141 ttatgactgttcaaatattatogaacagtaattaatgtggctgatattcaatacgaataa 1200
 1201 tttttgtagatgaacttgggaagcaacacagtgtaacctacacgatcacggaggacagtt 1260
 1261 ttccatatggtaggctaaatttgaaatctaacgcggatctgggatacgtcacgtggaac 1320
 1321 acgagggaaattcatttagccttagccatatataacgtccagggggtctaatagggaacgt 1380
 1381 ttcatattaacaatgctcttactccgggaaacggaagtttcaattggctactacatagtt 1440

1441 aaaacaaatccaacttgaaagtaaatctcatgagtgccctccgtgctacttcaatgtgaag 1500
1501 ttagcacttttgggtattgaaaaataactggtggatgccactacacagtgcttagagcgcc 1560
1561 gacactcttaacttttgggtggtgtaactgtacatgcaataacaataagtacatca 1620
1621 gaaatacgcataatgggaggggaaaacttgtcactgattctactacagccctcaccacagta 1680
1681 tgtgcttagcaatggtattatttttgtttttacatacaattccaatagttccctggtggt 1740
1741 atagagttggcctggaacatacttttggctgcaagaaaattcttcattttcaaaaatagcgtg 1800
1801 aacaagattacttataccaacgatgaaagcttagcacttgaaaatacatcgttccacatca 1860
1861 aggatctattggcgagacccttctttgggtcacaacgtgcgctgacaaaacataacgtgaa 1920
1921 gatgagatgagagacagaatcgtgagtggttgggtgaccgtaagagttataagagtcc 1980
1981 ccatgaaaacagcagcagccttgcgatggactcaggccctagtgtgcttaggtgcgat 2040
2041 acgaaactaggcatgcaagctatttgcgacactaggagcactaaatccgaggagataggt 2100
2101 atgcactgctgcctggaggcacttaccttcttcagaggtggggagagtaggggtgctgt 2160
2161 tcggtgaaccttccgtcgatgacgctatagttcttgatgacttatgaccgactgcagccg 2220
2221 tacttgctcctctggggcaagttgggttatgtctgcagcacgaaccagtgctgtggatt 2280
2281 tcccagttaggggtgggggtcaagttgactttacctttgcccgagtggggcatgggagag 2340
2341 gccatgcataggctatagggttaatttagtcaaattcgctcatactatggtatggggagc 2400
2401 agcgccttcaggggaactgtggaggttaccttaactatcagtagtctttcgggacgtct 2460
2461 gtccgagcccactgggcttcttgctatttcatacttctacctgatgttactttcgtctg 2520
2521 tttttccttgccgatatgctctctctgagcgttgatcctgtgtgggaacacgggtcgtgca 2580
2581 tggatcgattccacgtttatcttagcttacaatatgtcccttcttggccgctcttagaa 2640
2641 cagcttctgtaccgatttagggcggcttaaactatcttatatacctatacctagttaagca 2700
2701 agtcccagccttgttaccagatggaaagtgaaagctactctgctacctaactcttcagc 2760
2761 ttactttccagctcctcggtcacgggtccaggaggctagtgccctaccaggaataaaaagggtc 2820
2821 gtaacgttgctacgtccagtagatgtgggtggcgggttgctacgtccagtaggtgtggc 2880
2881 ggcaggttctgaccatgcacttcaccccacctgtgtatcagtaggctgtactactcat 2940
2941 tcggatgtgggaggaggttgcgagaaaattgttgtgaaatactggagacactaaagtga 3000
3001 tgtattaacggcagaaatgtcaaacgctgaactctttagatactgacgggtgtcttctgct 3060
3061 aaaatcagttttctttttcttcatcagcagcgaactgggttcccttctttgatgtttctgat 3120
3121 ggcttctctaattggccaaccggaaaccggtgacaaaattttgctatttattgcagctggta 3180
3181 tcggcacaatacagagttattgtaataaaaatagatgcattggctctgtaataccaaaaaaa 3240
3241 aaaaaaaaaaaaaaaaaa 3255

DNA-sequence of **Ph_otd2n81** (*Parhyale hawaiiensis otd2* clone n81, exemplary for clones n81 and n83), obtained from *otd2* 3' RACE. Ph_otd2n81 consists of consensus 3' splicing units **3'A**, **3'B** and **3'C**. Stop of open reading frame is marked as **TGA**.

1 I K I N L P E S R I Q V W F K 60
 ATCAAGATCAATCTCCCTGAGAGTAGAATACAGGTTTGGTTTAA
 61 N R R A K C R Q Q Q K Q Q A A N G E K A 120
 GAATCGTAGAGCAAAGTGCCGGCAGCAACAGAAGCAGCAGGCCCAACGGCGAGAAGGC
 121 P R T K K V N K S P P P S T N N T I T N 180
 TCCACGCACCAAGAAAGTGAACAAATCTCCTCCTCCATCCACCAACAACAATTACAAA
 181 S S L I G S G A G N N A A T T N H H H H 240
 CTCGTCGCTCATAGGCAGCGGGCTGGTAACAATGCTGCCACCACTAACCATCATCACCA
 241 H H T T P N N N N N N N S N S S N S S T 300
 CCATCACACCACCCCAACAATAATAACAACAATAACAGTAACAGCAGTAACAGCTCAAC
 301 T L T S P L T T S H G A R D A S P P S P 360
 AACCTGACGTCGCCTCTGACGACGTCCCATGGAGCTAGAGACGCGTCCCCACCGTCACC
 361 P P S V S S G S S L S L P P S T P Y N P 420
 CCCTCCGTCCGTTAGCTCGGGGTCATCCCTGTCTCTGCCTCCATCAACCCCTTACAACCC
 421 I W S P A G I P H Q S S T L P A M G D L 480
 CATTGGTCTCCAGCGGGGATACCCACCAGTCTCCACGTTACCTGCTATGGGGACCT
 481 M G G G G L D R G G Y G S A C Y Q G Y G 540
 GATGGGTGGTGGTGGTTGGACAGAGGAGGTACGGGAGCGCTTGCTACCAGGGCTACGG
 541 T P S Y Y S N M D Y L A P M T H S Q V N 600
 TACTCCATCCTATTACTCGAACATGGACTACCTCGCACCTATGACCCATTCTCAGGTAAA
 601 T F A H N I D L S I L I M L T C I C K Stop 660
 TACCTTTGCTCATAATATAGATCTTTCCATATTGATAATGCTAACGTGTATTTGCAAATA
 661 Ataacaatttaagtttcaatatttagcacacgcacggttcataaccctcagtgcttttct 720
 721 taaaaatgtgatcagttgtaatgcggtgcaatatgaaaaagataggttaataaattgtccc 780
 781 tattttgttttgatatctttcacgtatcagtataaacttaaaatgctccctctgctgcy 840
 841 aaatcgttgttccactccaaaaatttcattctgatttagcagtttcatctcagccaaatt 900
 901 attttactgtttacattaacaggttagtgcgatcattacgtacttcagccgtggccatt 960
 961 actaacaacactgttacgtacacagcaataatttggtttatatcgaactgctataatac 1020
 1021 agaatgaaatttttggctgtagaacaactattttggaaacagaggggtataaagggaatgg 1080
 1081 cacgtggacctcattgcattacatttattgcaaagttgactatggagataaactctgaaa 1140
 1141 cgtttttcgtctaagaaaaaaaaaaaaaaaaa 1172

DNA-sequence of *Parhyale hawaiiensis otd2* clone **web422**, obtained from *otd2* 3' RACE. **web422** consists of consensus 3' splicing units **3'A**, **3'B** and **3'D**. Stop of open reading frame is marked as **TAA**.

1	I K I N L P E S R I Q V W F K N R ATCAAGATCAATCTCCCTGAGAGTAGAATACAGGTTTGGTTTAAGAATCG	60
61	R A K C R Q Q Q K Q Q A A N G E K A P R TAGAGCAAAGTGCCGGCAGCAACAGAAGCAGCAGGCCGCCAACGGCGAGAAGGCTCCACG	120
121	T K K V N K S P P P S T N N T I T N S S CACCAAGAAAGTGAACAAATCTCCTCCTCCATCCACCAACAACACAATTACGAACTCGTC	180
181	L I G S G A G N N A A T I T T T T I I T GCTCATAGGCAGCGGGCTGGTAACAATGCTGCCACCATCACCACCA	240
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361	P K K I E R P A I W A Stop TCCGAAGAAGATCGAACGTCGCCGATATGGGCGTGA	420
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661	aaaagtaaagcaaagatgggatgctggtggaaggcaaagcaggcgaagagaagagcaag	720
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961	cacagcgcggtgctgcagctgacgccagatgcccttttgatagagcaggagtaccggcag	1020
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1141	gagaccggcgggtgcttcagtggaaggagaagctaag	1192

DNA-sequence of *Parhyale hawaiiensis* *otd2* clone **web425**, obtained from *otd2* 3' RACE. **web425** consists of consensus 3' splicing units **3'A** and **3'E**. Stop of open reading frame is marked as **TGA**.

[PhD_A2_Parhyale_goi_sequences]
[PhD_A2.1_Ph_otd1]
[PhD_A2.1.1_Ph_otd1_sequences]

"Ph_otd1_general_read_me.txt"

General aspects:

- (i) All sequences are in FASTA format.
- (ii) [bp] indicates nucleic acid sequences; [aa] indicates amino acid sequences
- (iii) Comments are preceded by an apostrophe (')
- (iv) All sequences without oligonucleotide sequence are indicated (e.g. "w/o 1013, 1016"). In these cases, only novel Parhyale sequence is presented.

Parhyale hawaiiensis otd1 terminology:

- (i) In general, sequence denotation begins with: ">Ph_otd1_"
- (ii) The above (i) is followed by: "vsdP", in case sequence originates from vsdPCR (5.2.3.1)
"5R" or "3R", in case sequence derives from 5' or 3' RACE, respectively
"ORF" for coherent ORF sequences
"cDNA" for complete cDNA sequences

"ref" if reference sequence is presented
"HD" if homeobox/homeodomain fragment is presented
- (iii) The above (ii) is followed by clone id.

Parhyale hawaiiensis otd1 synopsis:

cDNA: 2036 bp
5' UTR: 255 bp (1-255)
ORF: 1191 bp (256-1446), 396 aa
3'UTR: 590 bp (1447-2036)
HD: 180 bp (517-696), 60 aa

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For a detailed list of *Parhyale hawaiiensis* otd2 (Ph otd2) sequences and the corresponding alignments, see 3.2.1.4 as well as Browne et al., 2006 (supplementary data).

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>Ph_hbn_3RHH361 [aa] 'seq gap 35 bp 'N' added, w/o 1504
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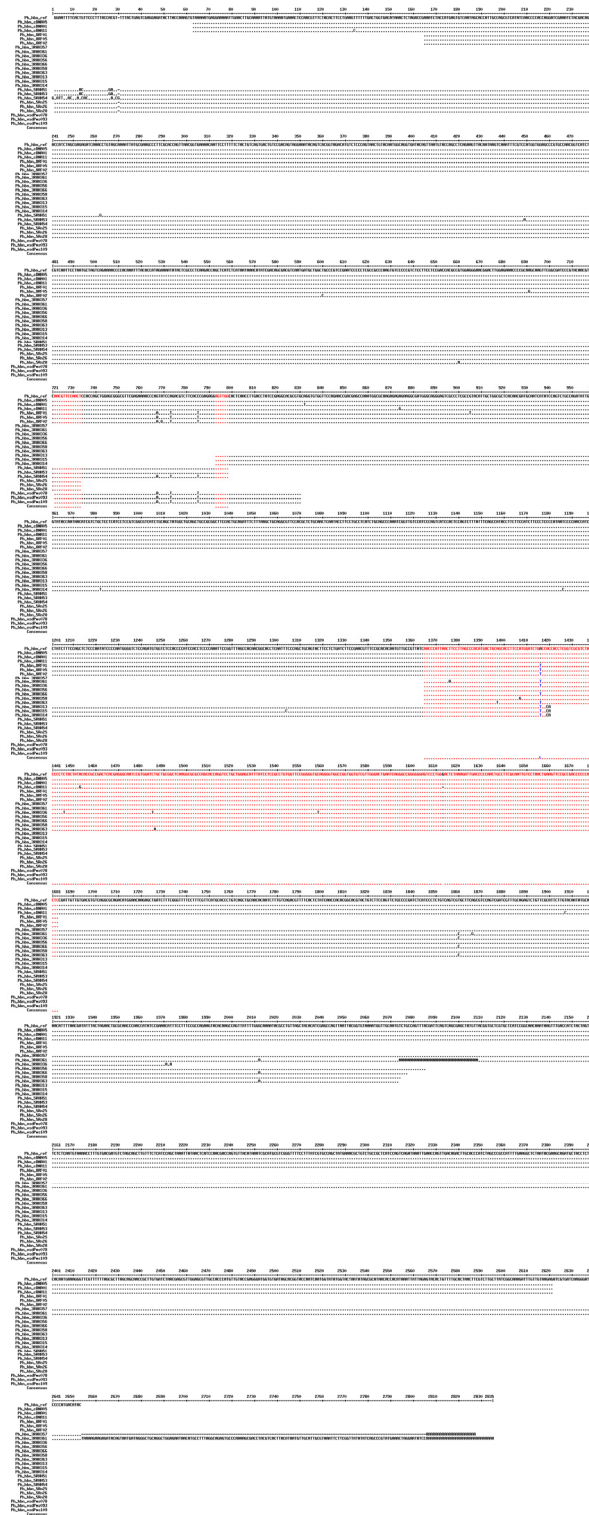
>Ph_hbn_3RHH366 [aa] 'one direction usable seq only, w/o 1504
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[PhD_A2.4_Ph_al1]
[PhD_A2.4.1_Ph_al1_sequences]

"Ph_al1_general_read_me.txt"

General aspects:

- (i) All sequences are in FASTA format.
- (ii) [bp] indicates nucleic acid sequences; [aa] indicates amino acid sequences
- (iii) Comments are preceded by an apostrophe (')
- (iv) All sequences without oligonucleotide sequence are indicated (e.g. "w/o 1213, 1216"). In these cases, only novel Parhyale sequence is presented.

Parhyale hawaiensis all terminology:

- (i) In general, sequence denotation begins with: ">Ph_al1_"
- (ii) The above (i) is followed by: "vsdP", in case sequence originates from vsdPCR (5.2.3.1)
 - "5R" or "3R", in case sequence derives from 5' or 3' RACE, respectively
 - "ORF" for coherent ORF sequences
 - "cDNA" for complete cDNA sequences
 - "ref" if reference sequence is presented
 - "HD" if homeobox/homeodomain fragment is presented
 - "OAR" if OAR (encoding) fragment is presented
- (iii) The above (ii) is followed by clone id.

Parhyale hawaiensis all synopsis:

cDNA: 1849 bp
5'UTR: 169 bp (1-169)
ORF: 1533 bp (170-1702), 510 aa
3'UTR: 147 bp (1703-1849)
HD: 180 bp (791-970), 60 aa
OAR 63 bp (1529-1591), 21 aa

>Ph_al1_HD [bp]
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>Ph_al1_OAR [bp]
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>Ph_al1_ref [bp] 'Ph_al1_cDNA01, extended 5' by 35 bp of Ph_al1_5Rn2, 3' by 62 bp of Ph_al1_3Rn8, 1 nuc exchange (1048 T>C)
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>Ph_al1_OAR [aa]
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>Ph_al1_ref [aa]
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[PhD_A2.4_Ph_al1]

[PhD_A2.4.1_Ph_al1_sequences]

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>Ph_al1_cDNA01 [bp] 'w/o 1213, 1216
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>Ph_all_vsdP_Pha092 [bp] 'w/o fw_QRR, re_QVW

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>Ph_all_3Rn5 [bp] 'w/o 1203

CGTATCCAGGTATGGTTCAAATAATCGGCGAGCAAAATGGCGCAAGCAAGAGAAGTTCGGACCGAGCCATCACTTGGCGGGACCTTCCCATCGTCTTCTTCCGTTCCCACTTCACTCTCTCCCTCCACTTCTCT
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TACCTCACCGGTCTTGTGCCGAGCTCCCTGCCCAGCTTGGGAGTCTCTCCAGTCTAGCGGGTGGGCGCTGTGTCATAAGAACGGACTTCAATAGCCACATTCCGCGAGATTGAGTAGTCACCTC
AGAAACGATCTGGGGAGCCCACTGAGAGGAGATTAGTCCACGACTTCCCTACTTCCCAAACTCTGCCTCAGTCTTACCCCCGGTGTCCAGCAGATTCTGGTGGTCTTCTGCTCCCCGCTCAAT
CCCGAACTCAACGACTATAGTCTCACTTGGCGAGCTCGGCAGCTCTCTCGACGGCATCGGATTTGGACCCAATAATCTGACAGCAAGAAACAAGAACGACGGGCTTCTTATAGCGGAGCTGCGA
CAAGAGCCGAGAGCATGAGAAAAGACTGCGCTCCCTCAGCCGAGAAACAAGATTTGGAGGGACAGACGGAGACGACAGGTCTTCTACAGCGAGATCCCGCGGATTTACCCAAAAGAGAGATC
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>Ph_all_3Rn7 [bp] 'w/o 1203

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CAAGAGCCGAGAGCATGAGAAAAGACTGCGCTCCCTCAGCCGAGAAACAAGATTTGGAGGGACAGACGGAGACGACAGGTCTTCTACAGCGAGATCCCGCGGATTTACCCAAAAGAGAGATC
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>Ph_all_3Rn8 [bp] 'w/o 1203

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>Ph_all_ref [aa]

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>Ph_all_cDNA01 [aa] 'w/o 1213, 1216

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>Ph_all_cDNA04 [aa] 'w/o 1213, 1216

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RGDLAPRLPLLPQTLQSYPPVFFQIILAGLRPPSIPELNDYSSILASSAALSTASDVGPNNSDSKEQDRASSIAELRQRAREHEKRLASLRKNKDSGGTDGDDRSPTRRSPRCSPKREIKT&

>Ph_all_cDNA05 [aa] 'w/o 1213, 1216

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>Ph_all_ORF13 [aa] 'w/o 1214, 1215

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>Ph_all_ORF14 [aa] 'w/o 1214, 1215

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>Ph_all_ORF15 [aa] 'w/o 1214, 1215

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>Ph_all_ORF16 [aa] 'w/o 1214, 1215

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>Ph_all_ORF17 [aa] 'w/o 1214, 1215

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>Ph_all_ORF18 [aa] 'w/o 1214, 1215

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>Ph_all_5Rn2 [aa] 'w/o 1208

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>Ph_all_5Rn3 [aa] 'w/o 1208

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>Ph_all_5Rn4 [aa] 'w/o 1208

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>Ph_all_5Rn118 [aa] 'w/o 1208

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>Ph_all_5Rn119 [aa] 'w/o 1208, id to Ph_all_5Rn149

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>Ph_all_vsdP_Phad16 [aa] 'w/o fw_QRR, re_QVW

SFQLEELEKAFSRTHYPDVFTREELAMKIGLTEARI

>Ph_all_vsdP_Pha092 [aa] 'w/o fw_QRR, re_QVW

SFQLEELEKAFSRTHYPDVFTREELAMKIGLTEARI

>Ph_all_vsdP_Pha097 [aa] 'w/o fw_QRR, re_QVW

SFQLEELEKAFSRTHYPDVFTREELAMKIGLTEARI

>Ph_all_vsdP_Pha102 [aa] 'w/o fw_QRR, re_QVW

SFQLEELEKAFSRTHYPDVFTREELAMKIGLTEARI

>Ph_all_3Rn5 [aa] 'w/o 1203

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>Ph_all_3Rn7 [aa] 'w/o 1203

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>Ph_all_3Rn8 [aa] 'w/o 1203

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[PhD_A2_Parhyale_goi_sequences]
[PhD_A2.5_Ph_al2]
[PhD_A2.5.1_Ph_al2_sequences]

"Ph_al2_general_read_me.txt"

General aspects:

- (i) All sequences are in FASTA format.
- (ii) [bp] indicates nucleic acid sequences; [aa] indicates amino acid sequences
- (iii) Comments are preceded by an apostrophe (')
- (iv) All sequences without oligonucleotide sequence are indicated (e.g. "w/o 1314, 1315"). In these cases, only novel Parhyale sequence is presented.

Parhyale hawaiensis al2 terminology:

- (i) In general, sequence denotation begins with: ">Ph_al2_"
- (ii) The above (i) is followed by: "vsdP", in case sequence originates from vsdPCR (5.2.3.1)
 - "5R" or "3R", in case sequence derives from 5' or 3' RACE, respectively
 - "ORF" for coherent ORF sequences
 - "cDNA" for complete cDNA sequences
 - "ref" if reference sequence is presented
 - "HD" if homeobox/homeodomain fragment is presented
 - "OAR" if OAR (encoding) fragment is presented
- (iii) The above (ii) is followed by clone id.

Parhyale hawaiensis al2 synopsis:

cDNA: 2594 bp
5'UTR: 566 bp (1-566)
ORF: 1761 bp (567-2327), 586 aa
3'UTR: 567 bp (2328-2594)
HD: 180 bp (1464-1643), 60 aa
OAR: 63 bp (2226-2288), 21 aa

>Ph_al2_HD [bp]

CAAAGGAGATATCGAAGTACCTTTCATACCACTCGAAGAATTAGAGAAAGTGTTCCTCAGGACTCATTATCCGGATGTGTTTACCAGGGAGGAGTTGGCAATGAAGATAGGCCTGACGGAGGCA
CGCATACAGGCTGTGGTCCAGAAACCGACGTGCAAAAGTGGCCAGCAAGAA

>Ph_al2_OAR [bp]

GCAGAGCGGCGCAGGCGTCTCTCCACGACCTCAGACTTAAGGCACGAAATCAGGAAGTGCGA

>Ph_al2_ref 'Ph_al2_ORF05, extended 5' by 593bp of Ph_al2_5Rn11 and 3' by 294 bp of Ph_arx3_3Rfwx05; exch nuc (1103 T>C, 1193 A>G, 1208 T>C, 1232 T>C, 1344 A>T, 1478 G>A, 1723 C>T, 1862 C>G, 1892 A>G, 1898 T>C, 2045 C>T, 2087 T>G, 2243 T>G, 2264 C>T, 2267 A>G, 2270 G>A)

GAGTTTACCACCTGGCTTCGTTCTGGAAGCACTGCGCGTATTGCTCCATTGAACTGCAAGGCAATATACCTGGGCGAGTGTGCTTGTTCGCGAGTTATTTGGTACCTTACGGGGTGAATAAAAAC
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CGTGAGGTAGAAGTAGTTTACTGGGTTCTTCAACGTAACCTTAGGGAACAACCAATAAAGAAATCAATACCATAGTTGCACCAATAATACAGTTATAAATTTTTCTGTAAATGGTATATGCTATACTTTGTT
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AAAAA

>Ph_al2_HD [aa]

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>Ph_al2_OAR [aa]
AERRQASLHDLRLKARNQEV

>Ph_al2_ref [aa]
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ALTPPSPSSTFPSPIPSTSSPPINGLGLATPPGLSVEAERRQASLHDLRLKARNQEVRLMLRKAGEITA&

[PhD_A2_Parhyale_goi_sequences]

[PhD_A2.5_Ph_al2]

[PhD_A2.5.1_Ph_al2_sequences]

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>Ph_al2_ref 'Ph_al2_ORF05, extended 5' by 593bp of Ph_al2_5Rn11 and 3' by 294 bp of Ph_arx3_3Rfwx05; exch nuc (1103 T>C, 1193 A>G, 1208 T>C, 1232 T>C, 1344 A>T, 1478 G>A, 1723 C>T, 1862 C>G, 1892 A>G, 1898 T>C, 2045 C>T, 2087 T>G, 2243 T>G, 2264 C>T, 2267 A>G, 2270 G>A)

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>Ph_al2_vsdP_Pha089 [bp] 'w/o fw_QRR, re_QVW; id to Ph_al2_vsdP_Pha095
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>Ph_al2_vsdP_Pha111 [bp] 'w/o fw_QRR, re_QVW
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>Ph_al2_3Rn227 [bp] 'w/o 1301
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>Ph_al2_3Rfwx01 [bp] 'w/o 1304
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>Ph_al2_3Rfwx05 [bp] 'w/o 1304
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>Ph_al2_ORF03 [aa] 'w/o 1314, 1315
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>Ph_al2_ORF04 [aa] 'w/o 1314, 1315
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>Ph_al2_ORF05 [aa] 'w/o 1314, 1315
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>Ph_al2_5Rg1 [aa] 'w/o 1305, id to 2-4
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>Ph_al2_5Rp11 [aa] 'w/o 1305
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>Ph_al2_5Rp13 [aa] 'w/o 1305
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>Ph_al2_5Rn9 [aa] 'w/o 1306
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>Ph_al2_5Rn11 [aa] 'w/o 1306
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>Ph_al2_5Rn12 [aa] 'w/o 1306

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>Ph_al2_vsdP_Pha089 [aa] 'w/o fw_QRR, re_QVW; id to Ph_al2_vsdP_Pha095
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>Ph_al2_3Rn227 [aa] 'w/o 1301
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>Ph_al2_3Rfw01 [aa] 'w/o 1304
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>Ph_al2_3Rfw05 [aa] 'w/o 1304
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[PhD_A2.6_Ph_pby1_pby2]
[PhD_A2.6.1_Ph_pby1_pby2_sequences]

"Ph_pby1_general_read_me.txt"

General aspects:

- (i) All sequences are in FASTA format.
- (ii) [bp] indicates nucleic acid sequences; [aa] indicates amino acid sequences
- (iii) Comments are preceded by an apostrophe (')
- (iv) All sequences without oligonucleotide sequence are indicated (e.g. "w/o 1614, 1615"). In these cases, only novel Parhyale sequence is presented.

Parhyale hawaiensis pby1 terminology:

- (i) In general, sequence denotation begins with: ">Ph_pby1_"
- (ii) The above (i) is followed by: "A" or "B" in case presented sequences represent isoform A or B
 - "vsdP", in case sequence originates from vsdPCR (5.2.3.1)
 - "5R" or "3R", in case sequence derives from 5' or 3' RACE, respectively
 - "ORF" for coherent ORF sequences
 - "cDNA" for complete cDNA sequences

 - "ref" if reference sequence is presented
 - "HD" if homeobox/homeodomain fragment is presented
 - "PAX" if paired/Pax domain (encoding fragment) is presented
- (iii) The above (ii) is followed by clone id.

Parhyale hawaiensis pby1 synopsis:

pby1 isoform A:

cdNA: 2149 bp
5'UTR: 99 bp (1-99)
ORF: 1653 bp (100-1752), 550 aa
3'UTR: 397 bp (1753-2149)
PAX: 396 bp (250-645), 132 aa
HD: 180 bp (727-906), 60 aa

pby1 isoform B:

cdNA: 1791 bp
5'UTR: 99 bp (1-99)
ORF: 1191 bp (100-1290), 396 aa
3'UTR: 501 bp (1291-1791)
PAX: 396 bp (250-645), 132 aa
HD: 180 bp (727-906), 60 aa

shared cDNA fraction of both isoforms:

cdNA: 1174 bp
5'UTR: 99 bp (1-99)
ORF: 1075 bp (100-1174), 358 aa
3'UTR: none
PAX: 396 bp (250-645), 132 aa
HD: 180 bp (727-906), 60 aa

>Ph_pby1_PAX [bp] 'shared by both isoforms

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>Ph_pby1_HD [bp] 'shared by both isoforms

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>Ph_pby1A_ref [bp]

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>Ph_pby1B_3Rf21 [aa] 'w/o 1602
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FSDGDDEPENYSGNPPEASY@

>Ph_pby1B_3Rf316 [aa] 'w/o 1602
LTEARVQVWFSNRRARQRKASNSPEEAAQRPANNRNGGSPVAAISPFGPAVPGPSHGLDGGYGMFNRYGLQAPASDAVVPVKIEHSPSSCEDSSSSPNQPSYHPPAYQDGGPGLFPMQQAAVLNMS
FSDGDDEPENYSGNPSEASY@

[PhD_A2_Parhyale_goi_sequences]
[PhD_A2.6_Ph_pby1_pby2]
[PhD_A2.6.1_Ph_pby1_pby2_sequences]

“Ph_pby2_vsdPCR.txt”

```
>Ph_pby2_vsdP_Phad081 [bp] 'w/o fw_QRR, re_QVW  
TGCAGAGCAGCTGGAAATCCTTGAGAGATCCTTCGAGAAGACGCAATATCCTGACGTCTACACTAGGGAGGAGCTCGCACAGAAGGGCGCCTGACGGAGGCTAGGGTG  
  
>Ph_pby2_vsdP_Phad081 [aa] 'w/o fw_QRR, re_QVW  
AEQLEILERSFEKTQYPDVYTRELAQKARLTEARV
```

[PhD_A2_Parhyale_goi_sequences]
 [PhD_A2.6_Ph_pby1_pby2]
 [PhD_A2.6.2_Ph_pby1_pby2_alignments]

“alignment_Ph_pby1_pby2_vsdPCR_aa_sequences.gif”

	1 10 20 30 36
	-----+-----+-----+-----+-----
Ph_pby1_vsdP_PhQ411	AIQLEILEQEFCSNQYPDITTREHLAERTGLTEARY
Ph_pby1_vsdP_Phad02	AIQLEILEQEFCSNQYPDITTREHLAERTGLTEARY
Ph_pby1_vsdP_Phad08	AIQLEILEQEFCSNQYPDITTREHLAERTGLTEARY
Ph_pby1_vsdP_Phad06	AIQLEILEQEFCSNQYPDITTREHLAERTGLTKARY
Ph_pby1_vsdP_Phad14	AIQLEILEQEFCSNQYPDITTREHLAERTGLTEVRY
Ph_pby2_vsdP_Phad081	AEQLEILERSFEKTQYPDYVYTRELAQKARLTEARY
Consensus	AIQLEILEQEFCSNQYPDITTREHLAERTGLTEARY

[PhD_A2_Parhyale_goi_sequences]
 [PhD_A2.6_Ph_pby1_pby2]
 [PhD_A2.6.2_Ph_pby1_pby2_alignments]

“alignment_Ph_pby1_pby2_vsdPCR_sequences.gif”

	1 10 20 30 40 50 60 70 80 90 100 109
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----
Ph_pby1_vsdP_PhQ411	TGCATTCAGCTGGAATTCCTGGAGCAGGAGTTCTGCAGCAACCAATACCCGGACATTACGACTCGCGAGCACCTGGCAGAGAGGACTGGGCTCACTGAGGCTAGAGTG
Ph_pby1_vsdP_Phad02	TGCATTCAGCTGGAATTCCTGGAGCAGGAGTTCTGCAGCAACCAATACCCGGACATTACGACTCGCGAGCACCTGGCAGAGAGGACTGGGCTCACTGAGGCTAGAGTG
Ph_pby1_vsdP_Phad08	TGCATTCAGCTGGAATTCCTGGAGCAGGAGTTCTGCAGCAACCAATACCCGGACATTACGACTCGCGAGCACCTGGCAGAGAGGACTGGGCTCACTGAGGCTAGAGTG
Ph_pby1_vsdP_Phad06	TGCATTCAGCTGGAATTCCTGGAGCAGGAGTTCTGCAGCAACCAATACCCGGACATTACGACTCGTGAGCACCTGGCAGAGAGGACTGGGCTCACTGAGGCTAGAGTG
Ph_pby1_vsdP_Phad14	TGCATTCAGCTGGAATTCCTGGAGCAGGAGTTCTGCAGCAACCAATACCCGGACATTACGACTCGTGAGCACCTGGCAGAGAGGACTGGGCTCACTGAGGCTAGAGTG
Ph_pby2_vsdP_Phad081	TGCAGAGCAGCTGGAATTCCTGGAGCAGGAGTTCTGCAGCAACCAATACCCGGACATTACGACTCGCGAGCACCTGGCAGAGAGGACTGGGCTCACTGAGGCTAGAGTG
Consensus	TGCATTCAGCTGGAATTCCTGGAGCAGGAGTTCTGCAGCAACCAATACCCGGACATTACGACTCGCGAGCACCTGGCAGAGAGGACTGGGCTCACTGAGGCTAGAGTG

[PhD_A2_Parhyale_goi_sequences]

[PhD_A2.6_Ph_pby1_pby2]

[PhD_A2.6.2_Ph_pby1_pby2_alignments]

"alignment_Ph_pby1A_all_aa_sequences.gif"

```
1 10 20 30 40 50 60 70
|-----|
Ph_pby1A_rnf MECHYSFRPQPFGRMGVSVPFSSRSRFPFAPLPSITANLDEHNDQDNHANSRVNQLGGKFNAGRPLPS
Ph_pby1A_ORF13 PFGMGVSVPFSSRSRFPFAPLPSITANLDEHNDQDNHANSRVNQLGGKFNAGRPLPS
Ph_pby1A_ORF16 PFGMGVSVPFSSRSRFPFAPLPSITANLDEHNDQDNHANSRVNQLGGKFNAGRPLPS
Ph_pby1A_ORF09 PFGMGVSVPFSSRSRFPFAPLPSITANLDEHNDQDNHANSRVNQLGGKFNAGRPLPS
Ph_pby1A_3R2219
Ph_pby1A_3Rf27
Ph_pby1A_3Rf29
Ph_pby1_S0101 MECHYSFRPQPFGRMGVSVPFSSRSRFPFAPLPSITANLDEHNDQDNHANSRVNQLGGKFNAGRPLPS
Ph_pby1_5s54 MECHYSFRPQPFGRMGVSVPFSSRSRFPFAPLPSITANLDEHNDQDNHANSRVNQLGGKFNAGRPLPS
Ph_pby1_5s55 MECHYSFRPQPFGRMGVSVPFSSRSRFPFAPLPSITANLDEHNDQDNHANSRVNQLGGKFNAGRPLPS
Ph_pby1_vsdP_Ph0M11
Ph_pby1_vsdP_Ph008
Ph_pby1_vsdP_Ph002
Ph_pby1_vsdP_Ph014
Ph_pby1_vsdP_Ph006
Consensus
71 80 90 100 110 120 130 140
|-----|
Ph_pby1A_rnf NYRREIILAHQGIKPCNSKRLKYSNGYSKILNRYVEIGSIPKGLREVNRSLPPRLDERIVQRIDHSF
Ph_pby1A_ORF13 NYRREIILAHQGIKPCNSKRLKYSNGYSKILNRYVEIGSIPKGLREVNRSLPPRLDERIVQRIDHSF
Ph_pby1A_ORF16 NYRREIILAHQGIKPCNSKRLKYSNGYSKILNRYVEIGSIPKGLREVNRSLPPRLDERIVQRIDHSF
Ph_pby1A_ORF09 NYRREIILAHQGIKPCNSKRLKYSNGYSKILNRYVEIGSIPKGLREVNRSLPPRLDERIVQRIDHSF
Ph_pby1A_3R2219
Ph_pby1A_3Rf27
Ph_pby1A_3Rf29
Ph_pby1_S0101 NYRREIILAHQGIKPCNSKRLKYSNGYSKILNRYVEIGSIPKGLREVNRSLPPRLDERIVQRIDHSF
Ph_pby1_5s54 NYRREIILAHQGIKPCNSKRLKYSNGYSKILNRYVEIGSIPKGLREVNRSLPPRLDERIVQRIDHSF
Ph_pby1_5s55 NYRREIILAHQGIKPCNSKRLKYSNGYSKILNRYVEIGSIPKGLREVNRSLPPRLDERIVQRIDHSF
Ph_pby1_vsdP_Ph0M11
Ph_pby1_vsdP_Ph008
Ph_pby1_vsdP_Ph002
Ph_pby1_vsdP_Ph014
Ph_pby1_vsdP_Ph006
Consensus
141 150 160 170 180 190 200 210
|-----|
Ph_pby1A_rnf NNCHGVHTLEQLREKLVAEFTGTPWVWVPLVGLGKLRPYRQDLPLECTINDSSEDETEPGLTIRKKQ
Ph_pby1A_ORF13 NNCHGVHTLEQLREKLVAEFTGTPWVWVPLVGLGKLRPYRQDLPLECTINDSSEDETEPGLTIRKKQ
Ph_pby1A_ORF16 NNCHGVHTLEQLREKLVAEFTGTPWVWVPLVGLGKLRPYRQDLPLECTINDSSEDETEPGLTIRKKQ
Ph_pby1A_ORF09 NNCHGVHTLEQLREKLVAEFTGTPWVWVPLVGLGKLRPYRQDLPLECTINDSSEDETEPGLTIRKKQ
Ph_pby1A_3R2219
Ph_pby1A_3Rf27
Ph_pby1A_3Rf29
Ph_pby1_S0101 NNCHGVHTLEQLREKLVAEFTGTPWVWVPLVGLGKLRPYRQDLPLECTINDSSEDETEPGLTIRKKQ
Ph_pby1_5s54 NNCHGVHTLEQLREKLVAEFTGTPWVWVPLVGLGKLRPYRQDLPLECTINDSSEDETEPGLTIRKKQ
Ph_pby1_5s55 NNCHGVHTLEQLREKLVAEFTGTPWVWVPLVGLGKLRPYRQDLPLECTINDSSEDETEPGLTIRKKQ
Ph_pby1_vsdP_Ph0M11
Ph_pby1_vsdP_Ph008
Ph_pby1_vsdP_Ph002
Ph_pby1_vsdP_Ph014
Ph_pby1_vsdP_Ph006
Consensus
211 220 230 240 250 260 270 280
|-----|
Ph_pby1A_rnf RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1A_ORF13 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1A_ORF16 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1A_ORF09 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1A_3R2219 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1A_3Rf27 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1A_3Rf29 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1_S0101 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1_5s54 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1_5s55 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1_vsdP_Ph0M11 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1_vsdP_Ph008 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1_vsdP_Ph002 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1_vsdP_Ph014 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Ph_pby1_vsdP_Ph006 RRSRTSFTIQLELEDEFCSNQYPIITREHLAERTGLTEARVQVFNRRARQRKRSNPFEEARRRPPA
Consensus
291 290 300 310 320 330 340 350
|-----|
Ph_pby1A_rnf NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1A_ORF13 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1A_ORF16 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1A_ORF09 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1A_3R2219 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1A_3Rf27 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1A_3Rf29 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1_S0101 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1_5s54 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1_5s55 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1_vsdP_Ph0M11 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1_vsdP_Ph008 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1_vsdP_Ph002 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1_vsdP_Ph014 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Ph_pby1_vsdP_Ph006 NNRRNGSPVARIISPTDPAVPPGSHGLDGGVGHNRVGLQAPASDVAVPPVVKLEHSPSSSEEDSSSPNQP
Consensus
351 360 370 380 390 400 410 420
|-----|
Ph_pby1A_rnf SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1A_ORF13 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1A_ORF16 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1A_ORF09 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1A_3R2219 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1A_3Rf27 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1A_3Rf29 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1_S0101 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1_5s54 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1_5s55 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1_vsdP_Ph0M11 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1_vsdP_Ph008 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1_vsdP_Ph002 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1_vsdP_Ph014 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Ph_pby1_vsdP_Ph006 SVHPYAYGTSLLYPRQVVDGFGWNTYTPNPAVHPGYPFGDPTGQVGLGAVRNRQRFQAPRSGSSE
Consensus
421 430 440 450 460 470 480 490
|-----|
Ph_pby1A_rnf VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1A_ORF13 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1A_ORF16 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1A_ORF09 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1A_3R2219 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1A_3Rf27 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1A_3Rf29 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1_S0101 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1_5s54 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1_5s55 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1_vsdP_Ph0M11 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1_vsdP_Ph008 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1_vsdP_Ph002 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1_vsdP_Ph014 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Ph_pby1_vsdP_Ph006 VVTPHSGDRSPVAGDELNDNRHSRSLDFPQPSTYGYVQPLSMNTSDVTRALSPPCTSYRGRSEQQDQG
Consensus
491 500 510 520 530 540 550
|-----|
Ph_pby1A_rnf PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1A_ORF13 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1A_ORF16 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1A_ORF09 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1A_3R2219 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1A_3Rf27 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1A_3Rf29 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1_S0101 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1_5s54 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1_5s55 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1_vsdP_Ph0M11 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1_vsdP_Ph008 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1_vsdP_Ph002 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1_vsdP_Ph014 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Ph_pby1_vsdP_Ph006 PVVTSRRNFHQPNSLKPFGTSSLGSSSFATRANGGSFCENQGTGTSRRDQQRPHSVGLR
Consensus
```


[PhD_A2_Parhyale_goi_sequences]

[PhD_A2.6_Ph_pby1_pby2]

[PhD_A2.6.2_Ph_pby1_pby2_alignments]

"alignment_Ph_pby1A_pby1B_pbyshared_aa.gif"

```
1      10      20      30      40      50      60      70      80      90      100
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
Ph_pby1A_ref  MECHYSFRPQPF GPMGYSVPF PSASAF PPTAPLPSTANLDHENNDFDNAHANSRYNQLGGKFVNGRPLPSNVRRREIALAAQGIRPCNISKALKYSHGCV
Ph_pby1B_ref  MECHYSFRPQPF GPMGYSVPF PSASAF PPTAPLPSTANLDHENNDFDNAHANSRYNQLGGKFVNGRPLPSNVRRREIALAAQGIRPCNISKALKYSHGCV
Ph_pby1_shared_ref MECHYSFRPQPF GPMGYSVPF PSASAF PPTAPLPSTANLDHENNDFDNAHANSRYNQLGGKFVNGRPLPSNVRRREIALAAQGIRPCNISKALKYSHGCV
Consensus     MECHYSFRPQPF GPMGYSVPF PSASAF PPTAPLPSTANLDHENNDFDNAHANSRYNQLGGKFVNGRPLPSNVRRREIALAAQGIRPCNISKALKYSHGCV

101     110     120     130     140     150     160     170     180     190     200
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
Ph_pby1A_ref  SKILNRYQETGSIKPGVLAGVARS LPPRLDERIVQRIMSFNNCHGVNTLEQLRERLVAEFTGTPVNVVPLPYLGKGLRPYRDQLPFLECTNTDSDSEEDT
Ph_pby1B_ref  SKILNRYQETGSIKPGVLAGVARS LPPRLDERIVQRIMSFNNCHGVNTLEQLRERLVAEFTGTPVNVVPLPYLGKGLRPYRDQLPFLECTNTDSDSEEDT
Ph_pby1_shared_ref SKILNRYQETGSIKPGVLAGVARS LPPRLDERIVQRIMSFNNCHGVNTLEQLRERLVAEFTGTPVNVVPLPYLGKGLRPYRDQLPFLECTNTDSDSEEDT
Consensus     SKILNRYQETGSIKPGVLAGVARS LPPRLDERIVQRIMSFNNCHGVNTLEQLRERLVAEFTGTPVNVVPLPYLGKGLRPYRDQLPFLECTNTDSDSEEDT

201     210     220     230     240     250     260     270     280     290     300
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
Ph_pby1A_ref  EPGLTIRRKQRSRTSFTAIQLEILEQEFC SNQYPDIT TREHLAERTGL TEARVQYWF SNRRARQRKASNSPEEAAQR PANNRNGGSPVAIISPFDPAYP
Ph_pby1B_ref  EPGLTIRRKQRSRTSFTAIQLEILEQEFC SNQYPDIT TREHLAERTGL TEARVQYWF SNRRARQRKASNSPEEAAQR PANNRNGGSPVAIISPFDPAYP
Ph_pby1_shared_ref EPGLTIRRKQRSRTSFTAIQLEILEQEFC SNQYPDIT TREHLAERTGL TEARVQYWF SNRRARQRKASNSPEEAAQR PANNRNGGSPVAIISPFDPAYP
Consensus     EPGLTIRRKQRSRTSFTAIQLEILEQEFC SNQYPDIT TREHLAERTGL TEARVQYWF SNRRARQRKASNSPEEAAQR PANNRNGGSPVAIISPFDPAYP

301     310     320     330     340     350     360     370     380     390     400
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
Ph_pby1A_ref  GPSHGLDGGYG MFNRYGLQAPASDAV VPPVKIEHSPSSCEDSSSSPNQPSYHPPAYQDGTSLLYPRQQVDGFGFN YNTPYNPAVHPGYFPGDPGYPGQHY
Ph_pby1B_ref  GPSHGLDGGYG MFNRYGLQAPASDAV VPPVKIEHSPSSCEDSSSSPNQPSYHPPAYQDGGPGLFPMHQQAARV L NMSFSDGDEPENYSGNPPEASY
Ph_pby1_shared_ref GPSHGLDGGYG MFNRYGLQAPASDAV VPPVKIEHSPSSCEDSSSSPNQPSYHPPAYQ
Consensus     GPSHGLDGGYG MFNRYGLQAPASDAV VPPVKIEHSPSSCEDSSSSPNQPSYHPPAYQ.....Q.....N.....

401     410     420     430     440     450     460     470     480     490     500
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
Ph_pby1A_ref  LGHVAAQQAFQAPGRSGQSEVQTPHSGDRPSAGQELNQRHRSRDSLQFPGPQSTYGYQP LSNNTSDVTATRLSP PCTSYAGASEQQQGPVITSAANFM
Ph_pby1B_ref  LGHVAAQQAFQAPGRSGQSEVQTPHSGDRPSAGQELNQRHRSRDSLQFPGPQSTYGYQP LSNNTSDVTATRLSP PCTSYAGASEQQQGPVITSAANFM
Ph_pby1_shared_ref LGHVAAQQAFQAPGRSGQSEVQTPHSGDRPSAGQELNQRHRSRDSLQFPGPQSTYGYQP LSNNTSDVTATRLSP PCTSYAGASEQQQGPVITSAANFM
Consensus     LGHVAAQQAFQAPGRSGQSEVQTPHSGDRPSAGQELNQRHRSRDSLQFPGPQSTYGYQP LSNNTSDVTATRLSP PCTSYAGASEQQQGPVITSAANFM

501     510     520     530     540     550
|-----|-----|-----|-----|-----|
Ph_pby1A_ref  QPSNLKPEPGTSSLGSSSFATAANGGSFCENQGTG TSSSADQQRPHSVGLA
Ph_pby1B_ref  QPSNLKPEPGTSSLGSSSFATAANGGSFCENQGTG TSSSADQQRPHSVGLA
Ph_pby1_shared_ref QPSNLKPEPGTSSLGSSSFATAANGGSFCENQGTG TSSSADQQRPHSVGLA
Consensus     QPSNLKPEPGTSSLGSSSFATAANGGSFCENQGTG TSSSADQQRPHSVGLA
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[PhD_A2_Parhyale_goi_sequences]

[PhD_A2.6_Ph_pby1_pby2]

[PhD_A2.6.2_Ph_pby1_pby2_alignments]

"alignment_Ph_pby1B_all_aa_sequences.gif"

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1      10      20      30      40      50      60      70
|-----|-----|-----|-----|-----|-----|-----|
Ph_pby1B_ref MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1B_0RF01 PFGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1B_0RF05 PFGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1B_0RF03 PFGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1_5R0101 MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1_5N54 MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1_5N55 MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1B_3R1413 MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1B_3R1316 MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1B_3R121 MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1_vsdP_Ph0411 MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1_vsdP_Ph02 MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1_vsdP_Ph08 MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1_vsdP_Ph014 MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Ph_pby1_vsdP_Ph06 MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS
Consensus MECHYSFRPQPFPGPHGVSVPFSPASAFPTAPLPSTANLDHENNDFDHAHANSRYNQLGKGFVNGRPLPS

71     80     90     100    110    120    130    140
|-----|-----|-----|-----|-----|-----|-----|
Ph_pby1B_ref NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1B_0RF01 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1B_0RF05 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1B_0RF03 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1_5R0101 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1_5N54 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1_5N55 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1B_3R1413 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1B_3R1316 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1B_3R121 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1_vsdP_Ph0411 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1_vsdP_Ph02 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1_vsdP_Ph08 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1_vsdP_Ph014 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Ph_pby1_vsdP_Ph06 NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF
Consensus NVRREIILAAQGIKPCNISKALKVSHGCVSKILNRYQETGSIKPGVLGAVARSLPPLDERIVQRIINSF

141    150    160    170    180    190    200    210
|-----|-----|-----|-----|-----|-----|-----|
Ph_pby1B_ref NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1B_0RF01 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1B_0RF05 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1B_0RF03 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1_5R0101 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1_5N54 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1_5N55 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1B_3R1413 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1B_3R1316 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1B_3R121 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1_vsdP_Ph0411 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1_vsdP_Ph02 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1_vsdP_Ph08 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1_vsdP_Ph014 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Ph_pby1_vsdP_Ph06 NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ
Consensus NNCHGVNTLEQLRELVAEFTGTPVNVVPLPVLGKGLRPYRDQLPFLFECTINTDSOSEDTEPGLTIRKQ

211    220    230    240    250    260    270    280
|-----|-----|-----|-----|-----|-----|-----|
Ph_pby1B_ref RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1B_0RF01 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1B_0RF05 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1B_0RF03 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1_5R0101 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1_5N54 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1_5N55 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1B_3R1413 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1B_3R1316 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1B_3R121 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1_vsdP_Ph0411 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1_vsdP_Ph02 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1_vsdP_Ph08 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1_vsdP_Ph014 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Ph_pby1_vsdP_Ph06 RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA
Consensus RRSRTSFTAIQLLEIQEFCSNQYDITTREHLAERTGLTEARVQVHFSNRARRQRKASNSPEEAQRPA

281    290    300    310    320    330    340    350
|-----|-----|-----|-----|-----|-----|-----|
Ph_pby1B_ref NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1B_0RF01 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1B_0RF05 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1B_0RF03 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1_5R0101 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1_5N54 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1_5N55 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1B_3R1413 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1B_3R1316 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1B_3R121 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1_vsdP_Ph0411 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1_vsdP_Ph02 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1_vsdP_Ph08 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1_vsdP_Ph014 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Ph_pby1_vsdP_Ph06 NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP
Consensus NNRNGGSPVAATSPFDPAVPGPSHGLDGGYGHNRYGLQAPASDAVPPVVKIEHSPSSCEDSSSSPNQP

351    360    370    380    390    396
|-----|-----|-----|-----|-----|-----|
Ph_pby1B_ref SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1B_0RF01 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1B_0RF05 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1B_0RF03 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1_5R0101 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1_5N54 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1_5N55 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1B_3R1413 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1B_3R1316 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1B_3R121 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1_vsdP_Ph0411 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1_vsdP_Ph02 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1_vsdP_Ph08 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1_vsdP_Ph014 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Ph_pby1_vsdP_Ph06 SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
Consensus SYHPPAYQDGGPGLFPMQGGARVLMNSFSDGDEPENYSGNPSEARY
```


[PhD_A2_Parhyale_goi_sequences]
 [PhD_A2.6_Ph_pby1_pby2]
 [PhD_A2.6.2_Ph_pby1_pby2_alignments]

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1      10      20      30      36
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Ph_pby1_vsdP_PhQW11  AIQLEILEQEFCSNQYPOITTREHLAERTGLTEARV
Ph_pby1_vsdP_Phad02  .....
Ph_pby1_vsdP_Phad08  .....
Ph_pby1_vsdP_Phad14  .....V.
Ph_pby1_vsdP_Phad06  .....K.
Ph_pby2_vsdP_Phad081 .E.....RS.EKT...VY...E..QKAR.....
Consensus           .....
  
```

[PhD_A2_Parhyale_goi_sequences]
 [PhD_A2.6_Ph_pby1_pby2]
 [PhD_A2.6.2_Ph_pby1_pby2_alignments]

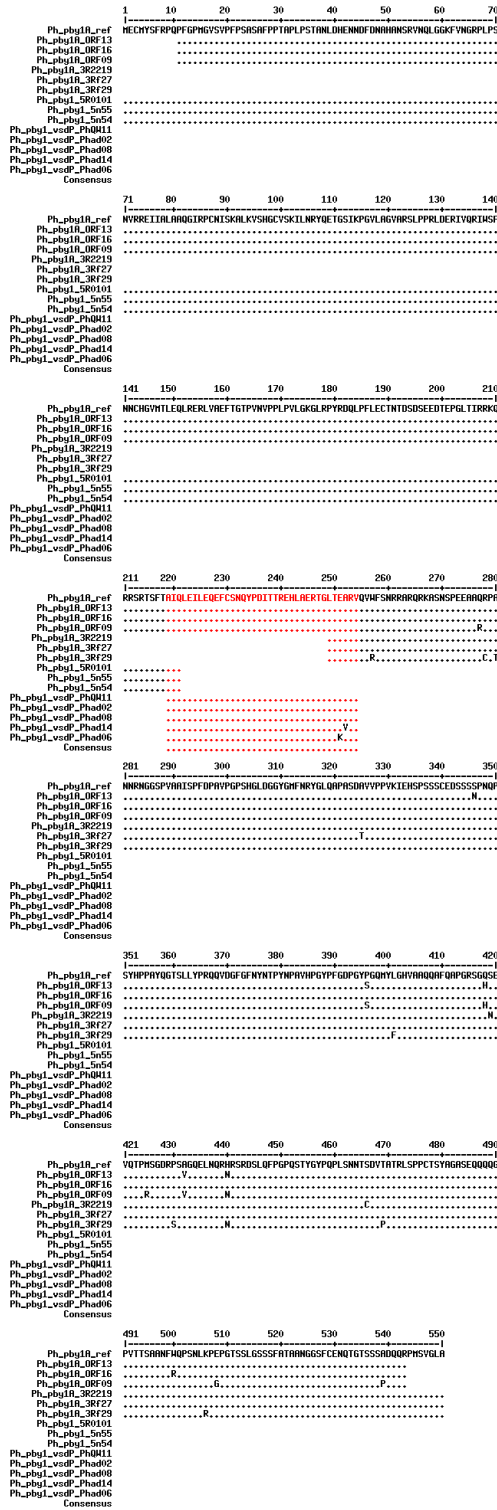
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1      10      20      30      40      50      60      70      80      90      100     109
|-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----|
Ph_pby1_vsdP_PhQW11  TGCRAATTCAGCTGGAAATCTGGAGCAGGAGTTCTGCAGCAACCAATACCCGGACATTACGACTCGCGAGCACCTGGCAGAGAGGACTG66GCTCACTGAGGCTAGAGTG
Ph_pby1_vsdP_Phad02  .....
Ph_pby1_vsdP_Phad08  .....C.....
Ph_pby1_vsdP_Phad06  .....T.....T.....A.....
Ph_pby1_vsdP_Phad14  .....T.....T.....
Ph_pby2_vsdP_Phad081 .GAG.....C.T..AGATCC..T..GAG.AG.CG...T..T..G.CTAC...A.G...G.G..C..C..A.G.GC.C..G.G...T...A...
Consensus           .....
  
```


[PhD_A2_Parhyale_goi_sequences]
[PhD_A2.6_Ph_pby1_pby2]
[PhD_A2.6.2_Ph_pby1_pby2_alignments]

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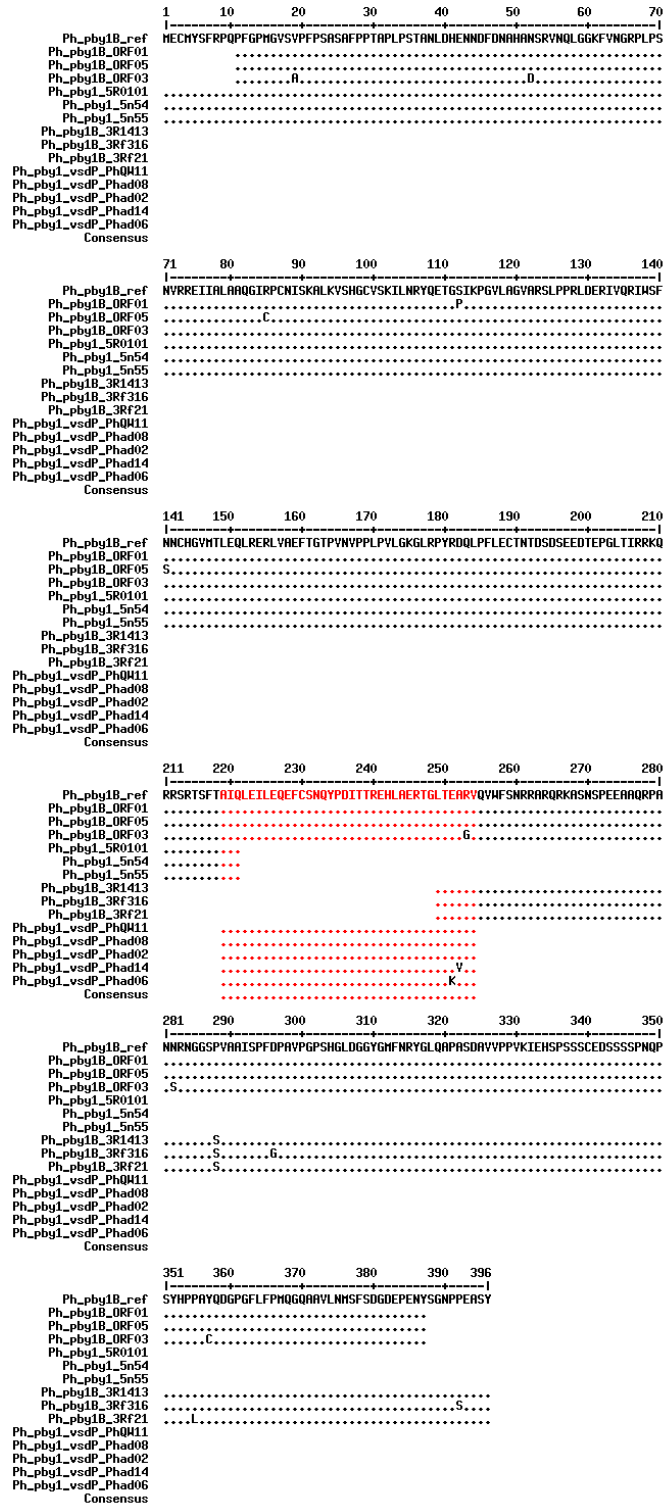


[PhD_A2_Parhyale_goi_sequences]

[PhD_A2.6_Ph_pby1_pby2]

[PhD_A2.6.2_Ph_pby1_pby2_alignments]

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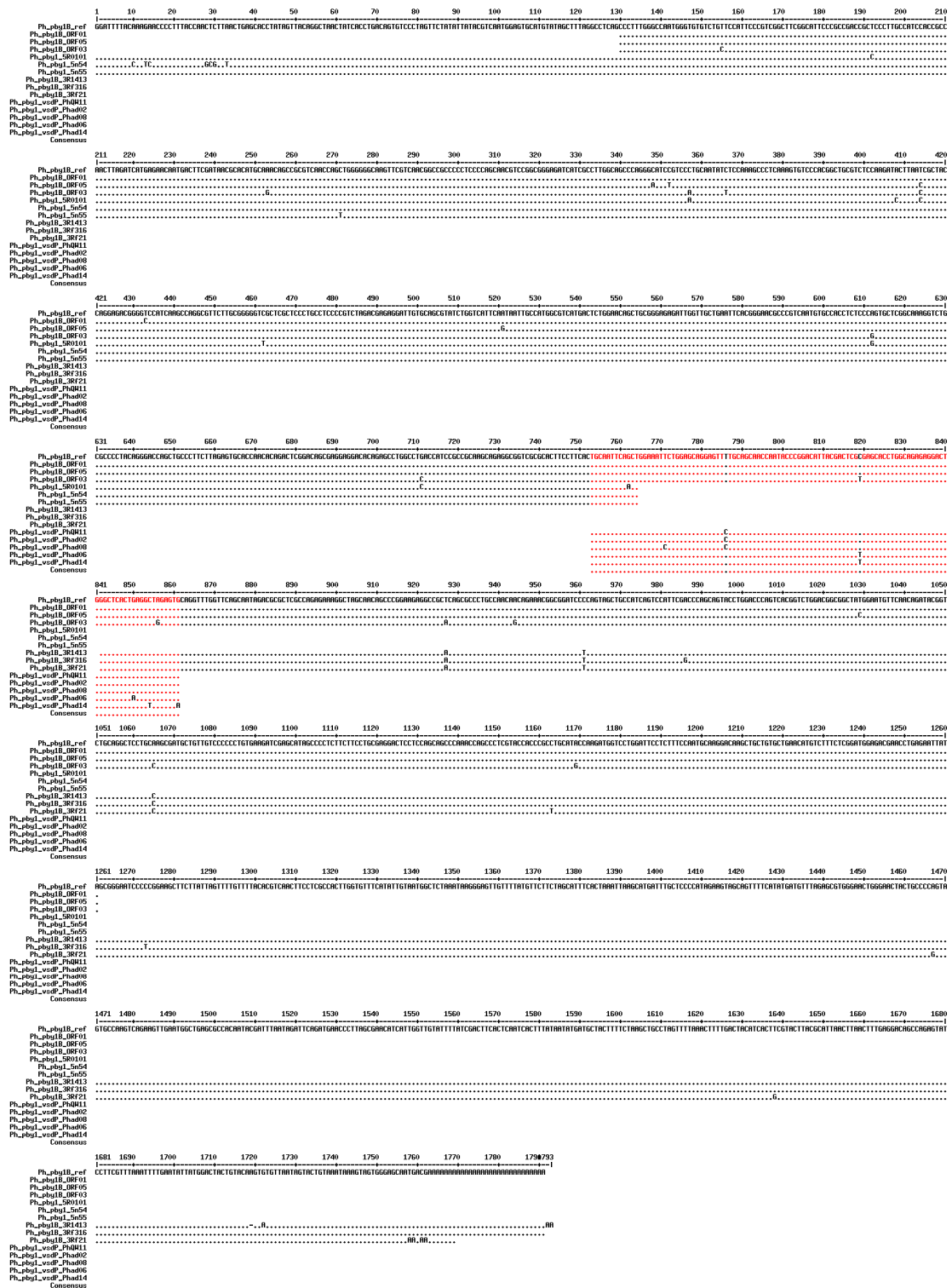


[PhD_A2_Parhyale_goi_sequences]

[PhD_A2.6_Ph_pby1_pby2]

[PhD_A2.6.2_Ph_pby1_pby2_alignments]

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AATGGAAGAATTACACAACAGTACGCGGGCGATGAGTTGCAGCAAAATACGACACCGTGGTCATCTGGAAATGTTCTGCACAGCTTCTACATCAAGAGTACCCTAGGTGGGGCACGGACCGTCAGAGAG
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>Ph_awh_LIM1 [aa]
CAGCARIITDRFLLRVNCLSWHQSLRCCVCQLALDRQPSCFIRDQNIYCKSD

>Ph_awh_LIM2 [aa]
CAKCCRAIGSTDWVRRARDRVYHLACFACDACKRQLSTGEEFALHESRVLCQH

>Ph_awh_HD [aa]
SKRVRTTFTDEQLQVLQANFQIDSNPDSQDLERIAQLTGLSKRVTVVWFQNSRARQKKHM

>Ph_awh_ref [aa]
MMASATVLLPMNRIKAERNSPVSSPEASSGNTGDGDPSPCCMGGSVKLTVSLQHTARERRGLCLPLSVNNNNNSSTNNNNNSNSASAPHGVEGGGVRLSGGGCGAPTTECAGCARIITDRFL
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>Ph_ahw_ref [aa] 'Ph_ahw_ORF21 w/o primers, extended 5' by Ph_ahw_5RCR02 (1210 bp); extended 3' by Ph_ahw_3Rn21 (1298 bp)

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>Ph_ahw_ORF20 [bp] 'w/o 1414/1415

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>Ph_ahw_ORF21 [bp] 'w/o 1414/1415

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>Ph_ahw_ORF22 [bp] 'w/o 1414/1415

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>Ph_ahw5RCR5 [bp] 'w/o 1406

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>Ph_ahw_5Rn121 [bp] 'w/o 1406

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>Ph_ahw_5Rn122 [bp] 'w/o 1406

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>Ph_ahw_5Rn150 [bp] 'w/o 1406

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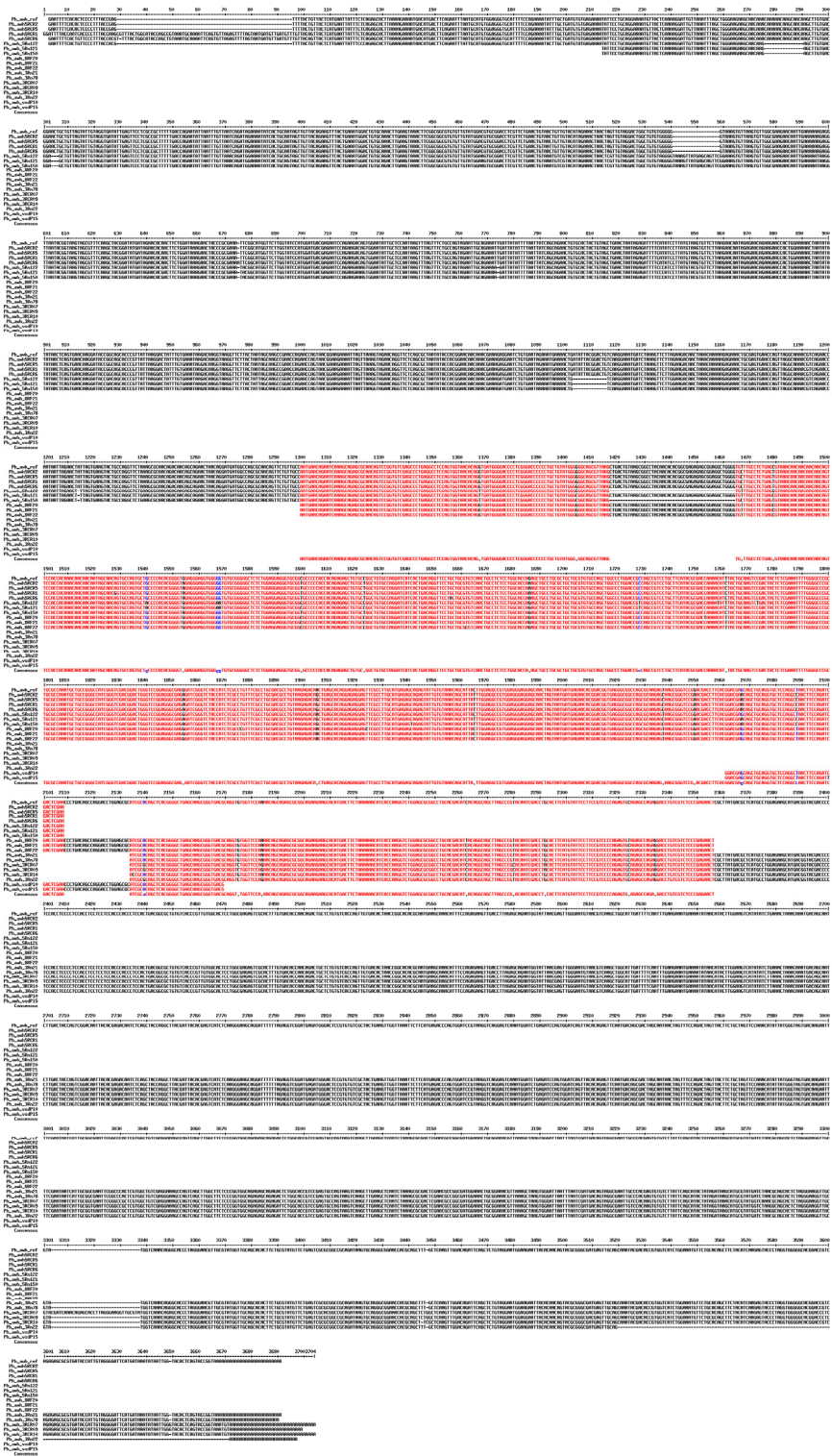
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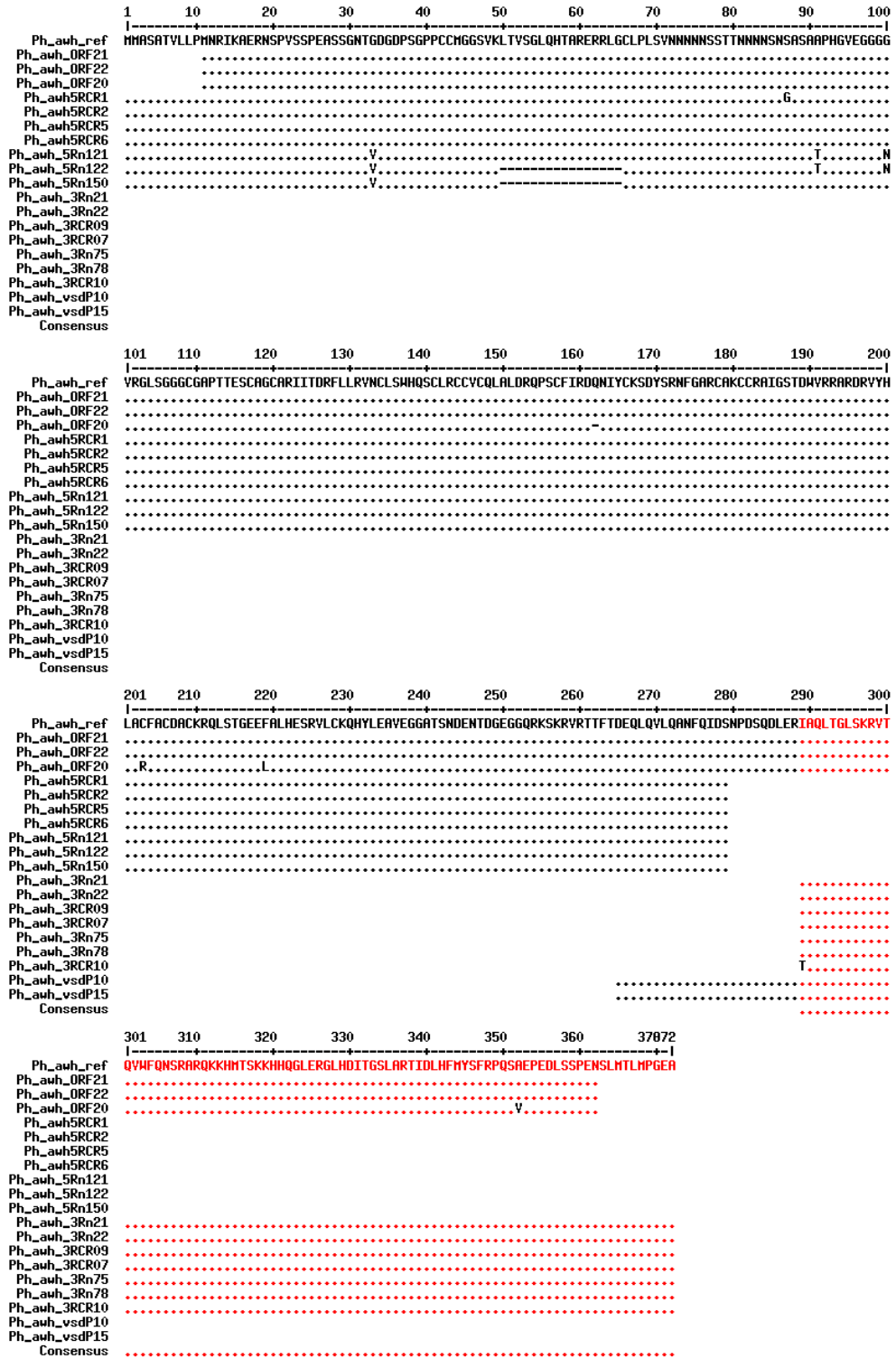
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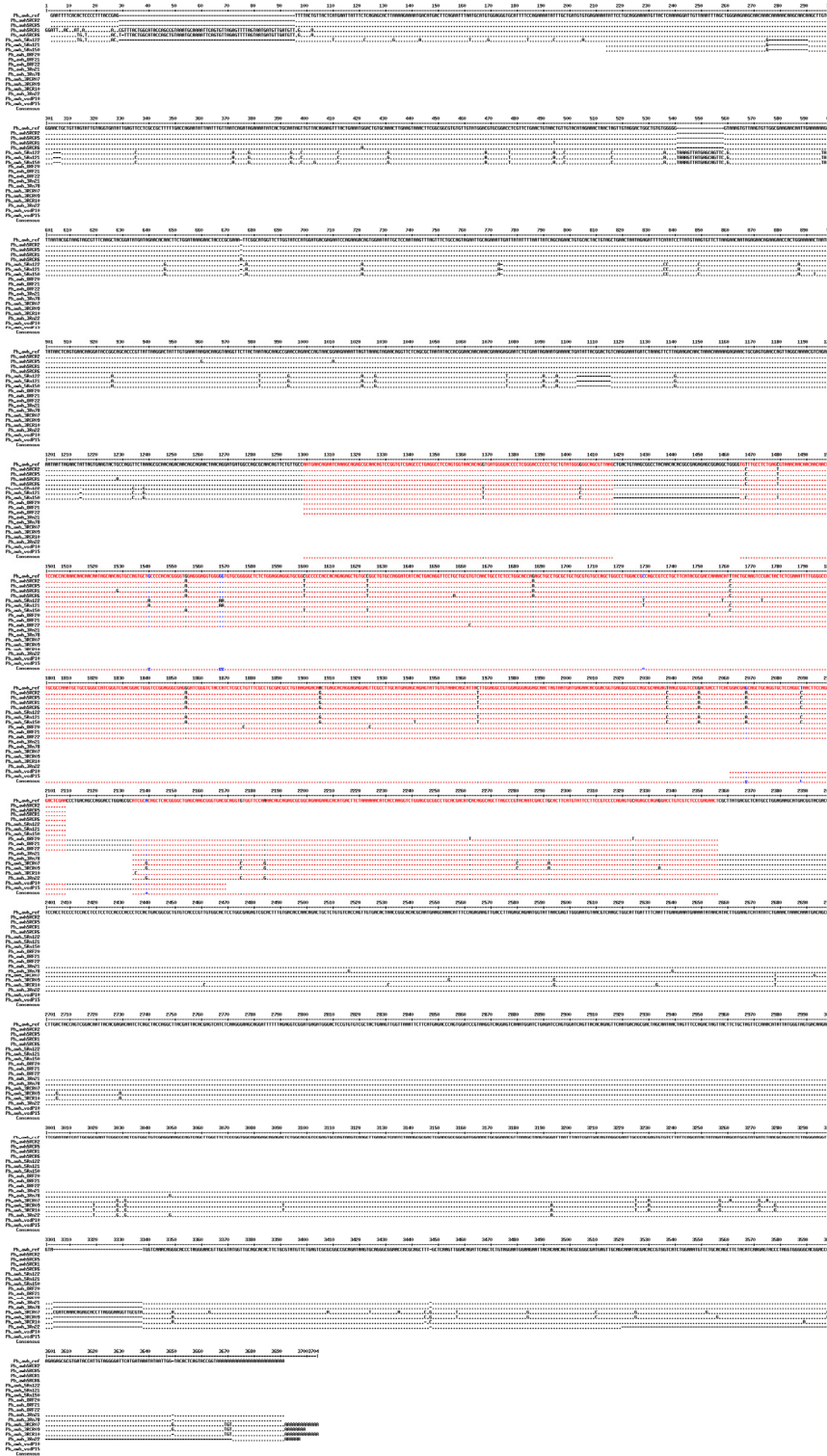
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[PhD_A2.8.1_Ph_six3_sequences]

"Ph_six3_general_read_me.txt"

General aspects:

- (i) All sequences are in FASTA format.
- (ii) [bp] indicates nucleic acid sequences; [aa] indicates amino acid sequences
- (iii) Comments are preceded by an apostrophe (')
- (iv) All sequences without oligonucleotide sequence are indicated (e.g. "w/o 2013, 2016"). In these cases, only novel Parhyale sequence is presented.

Parhyale hawaiensis six3 terminology:

- (i) In general, sequence denotation begins with: ">Ph_six3_"
- (ii) The above (i) is followed by: "vsdP", in case sequence originates from vsdPCR (5.2.3.1)
 - "5R" or "3R", in case sequence derives from 5' or 3' RACE, respectively
 - "ORF" for coherent ORF sequences
 - "cDNA" for complete cDNA sequences

 - "ref" if reference sequence is presented
 - "HD" if homeobox/homeodomain fragment is presented
 - "SD" if Six-domain (encoding) fragment is presented
- (iii) The above (ii) is followed by clone id.

Parhyale hawaiensis six3 synopsis:

cDNA: 3655 bp
5'UTR: 77 bp (1-77)
ORF: 1632 bp (78-1709), 343 aa
3'UTR: 1946 bp (1710-3655)
SD: 357 bp (195-551), 119 aa
HD: 180 bp (552-731), 60 aa

Parhyale six3 has been annotated as: "Parhyale hawaiensis optix; GenBank: EU908055.1"

>Ph_six3_SD [bp]

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>Ph_six3_HD [bp]

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>Ph_six3_ref [bp]

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>Ph_six3_3R10 [bp] 'w/o 2001

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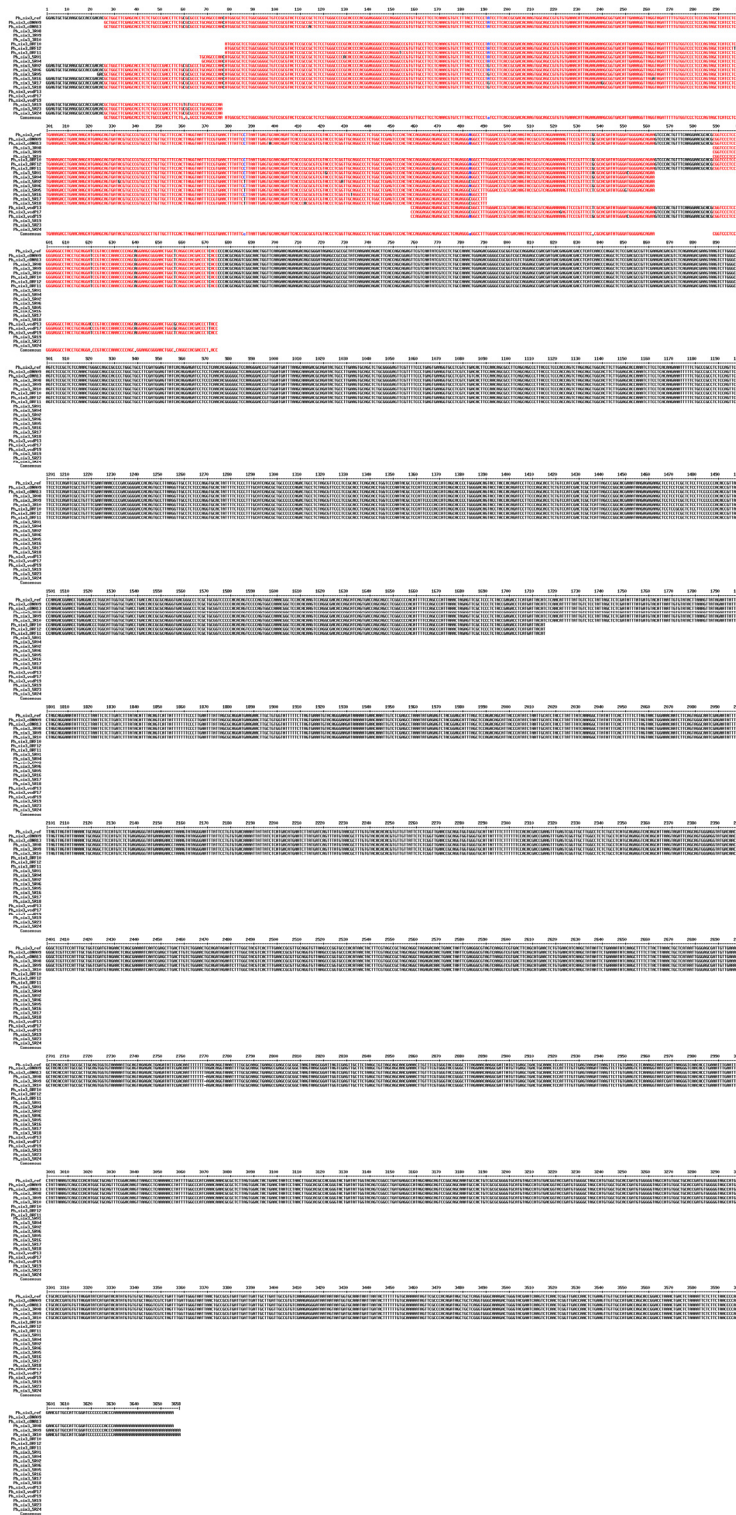
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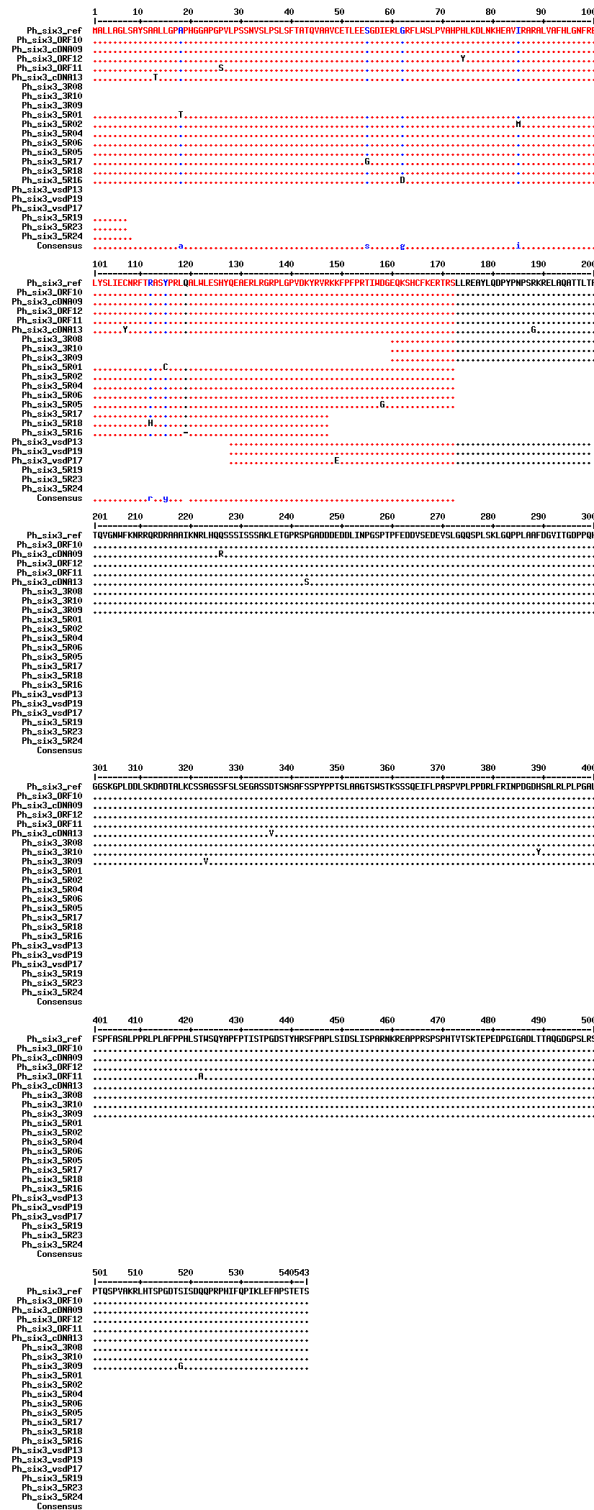


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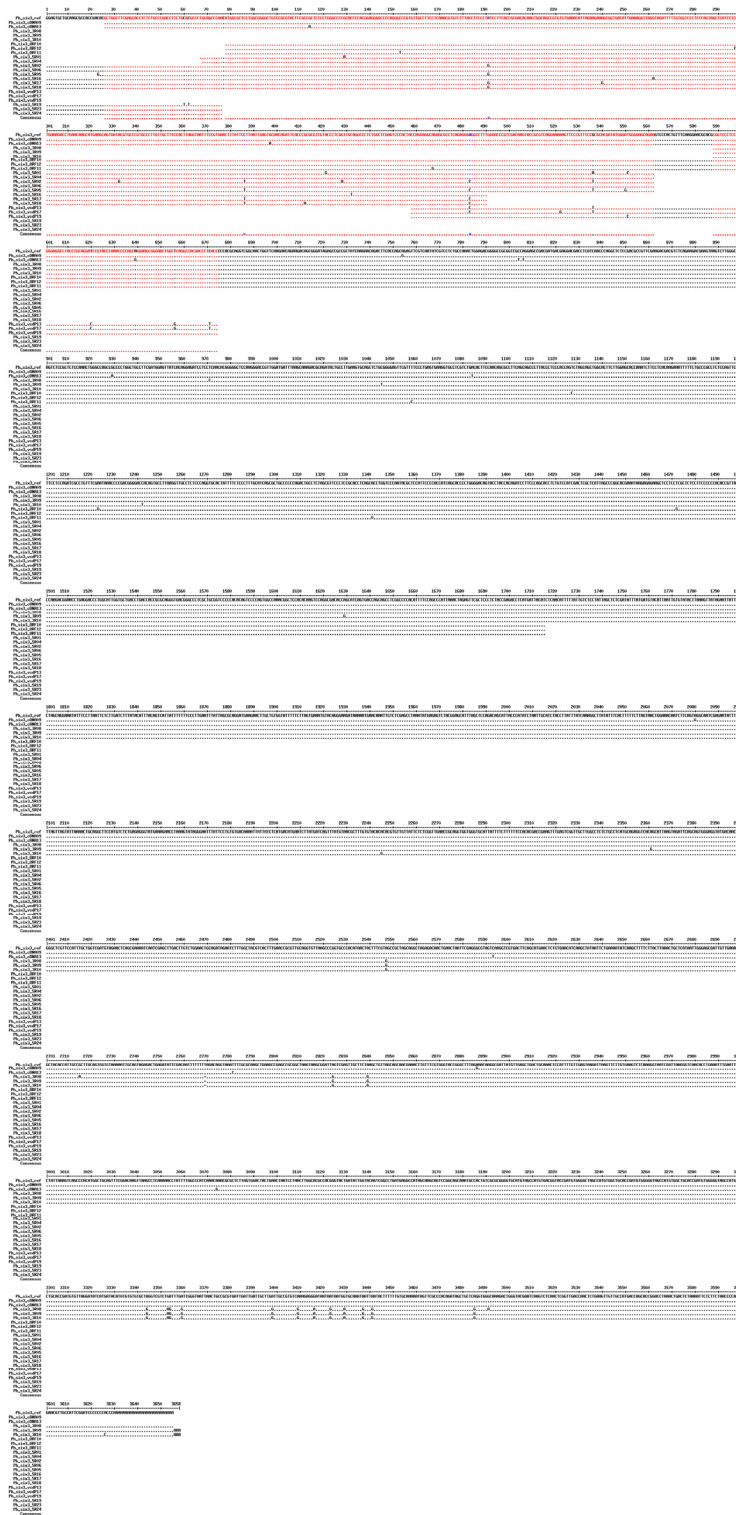


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General aspects:

- (i) All sequences are in FASTA format.
- (ii) [bp] indicates nucleic acid sequences; [aa] indicates amino acid sequences
- (iii) Comments are preceded by an apostrophe (')
- (iv) All sequences without oligonucleotide sequence are indicated (e.g. "w/o 2114, 2115"). In these cases, only novel Parhyale sequence is presented.

Parhyale hawaiiensis six4 terminology:

- (i) In general, sequence denotation begins with: ">Ph_six4_"
- (ii) The above (i) is followed by:
 - "vsdP", in case sequence originates from vsdPCR (5.2.3.1)
 - "5R" or "3R", in case sequence derives from 5' or 3' RACE, respectively
 - "ORF" for coherent ORF sequences
 - "cDNA" for complete cDNA sequences

 - "ref" if reference sequence is presented
 - "HD" if homeobox/homeodomain fragment is presented
 - "SD" if Six-domain (encoding) fragment is presented
- (iii) The above (ii) is followed by clone id.

Parhyale hawaiiensis six4 synopsis:

cDNA: 2711 bp
5'UTR: 590 bp (1-590)
ORF: 1515 bp (591-2105), 504 aa
3'UTR: 606 bp (2106-2711)
SD: 351 bp (1227-1577), 117 aa
HD: 180 bp (1578-1757), 60 aa

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Ph_six4_5R13 NLPASHFATDAVSEVILRAKVEVAFKGNKYEVNILEATSFHANYHTALQGMKYKHYKEAEVRRQRLGAVDKYRIRK
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Ph_six4_3R19 NLPASHFATDAVSEVILRAKVEVAFKGNKYEVNILEATSFHANYHTALQGMKYKHYKEAEVRRQRLGAVDKYRIRK
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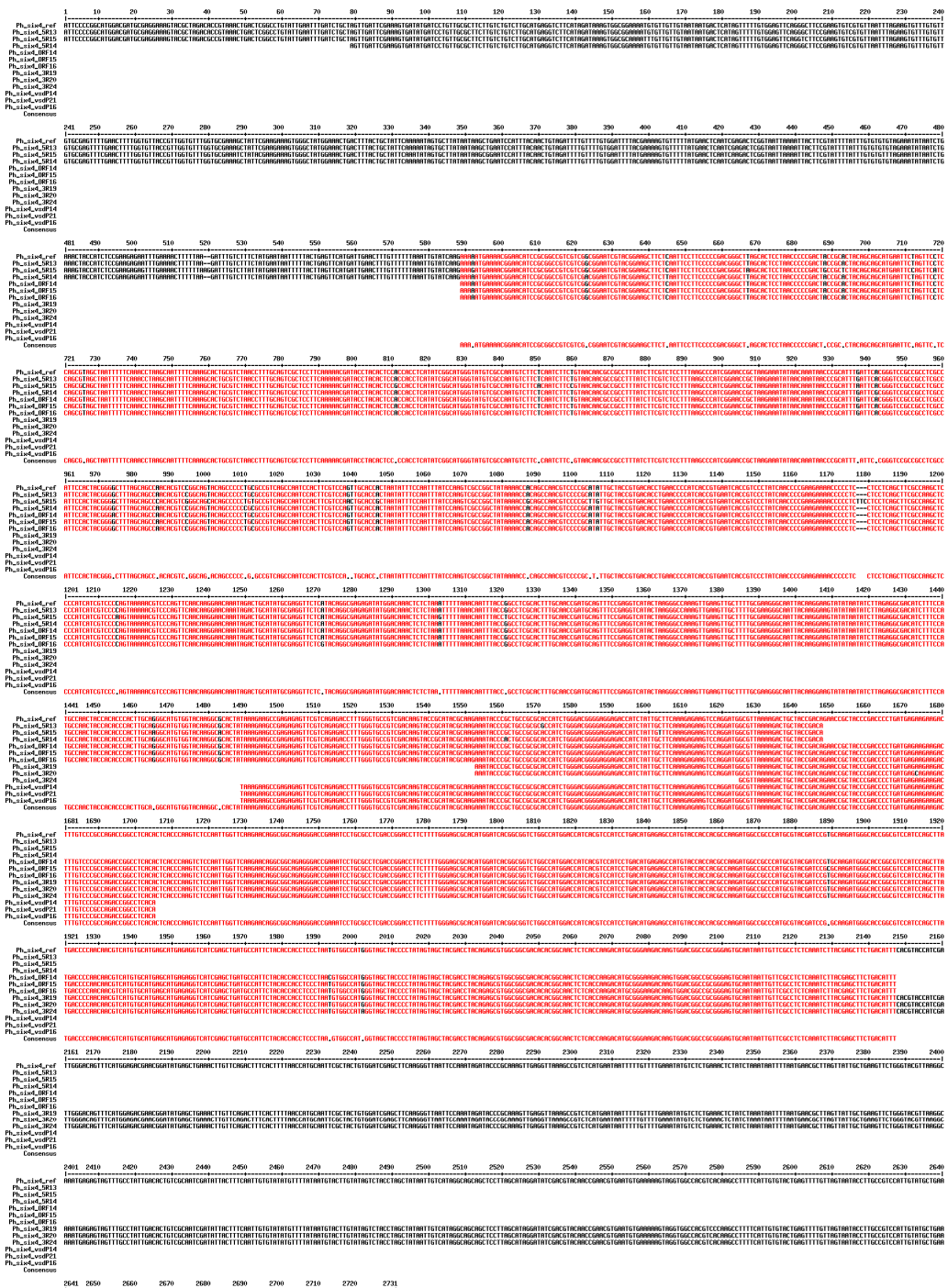
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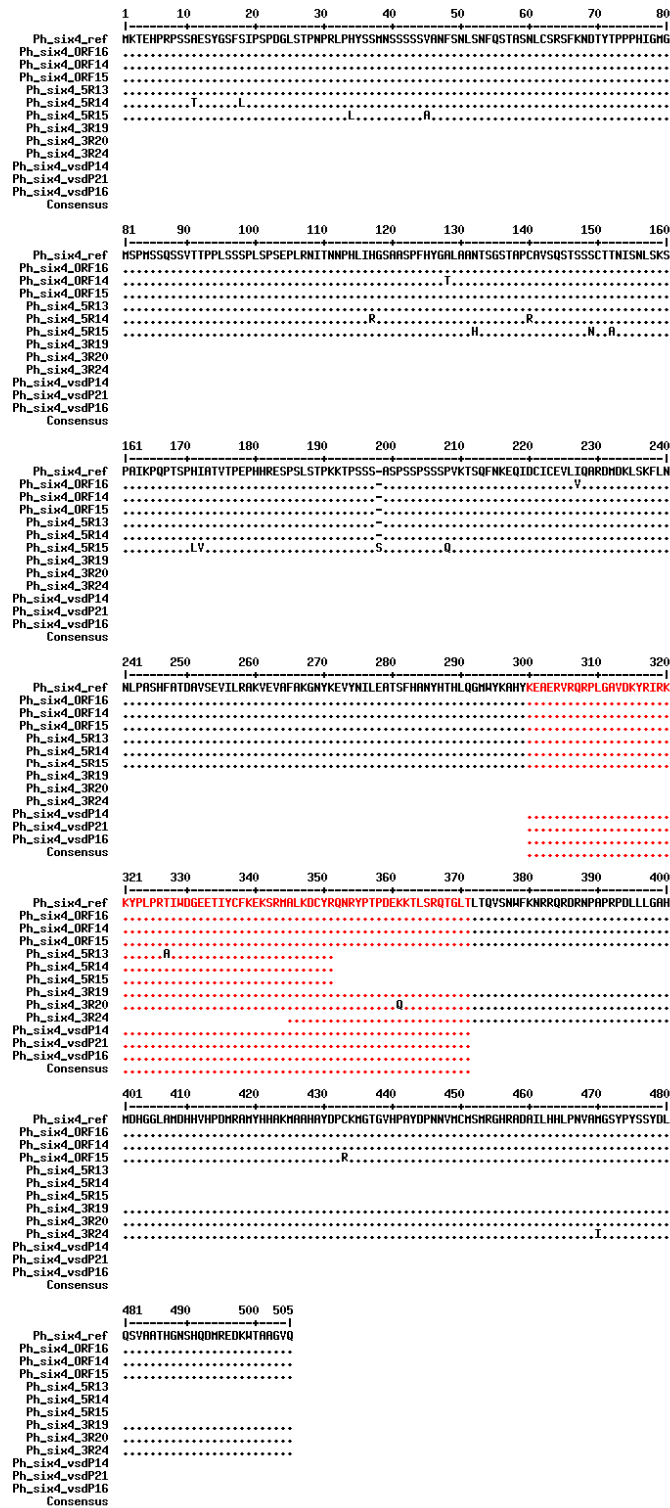


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General aspects:

- (i) All sequences are in FASTA format.
- (ii) [bp] indicates nucleic acid sequences; [aa] indicates amino acid sequences
- (iii) Comments are preceded by an apostrophe (')
- (iv) All sequences without oligonucleotide sequence are indicated (e.g. "w/o 2314, 2315"). In these cases, only novel Parhyale sequence is presented.

Parhyale hawaiensis gbx terminology:

- (i) In general, sequence denotation begins with: ">Ph_gbx_"
- (ii) The above (i) is followed by:
 - "vsdP", in case sequence originates from vsdPCR (5.2.3.1)
 - "5R" or "3R", in case sequence derives from 5' or 3' RACE, respectively
 - "ORF" for coherent ORF sequences
 - "cDNA" for complete cDNA sequences

 - "ref" if reference sequence is presented
 - "HD" if homeobox/homeodomain fragment is presented
 - "VPIPV" if VPIPV (encoding) fragment is presented
- (iii) The above (ii) is followed by clone id.

Parhyale hawaiensis gbx synopsis:

cDNA 3456 bp
5'UTR: 505 bp (1-505)
ORF: 1665 bp (506-2170), 554 aa
3'UTR: 1286 bp (2171-3456)
HD: 180 bp (1346-1525), 60 aa
VPIPV 69 bp (1598-1666), 23 aa

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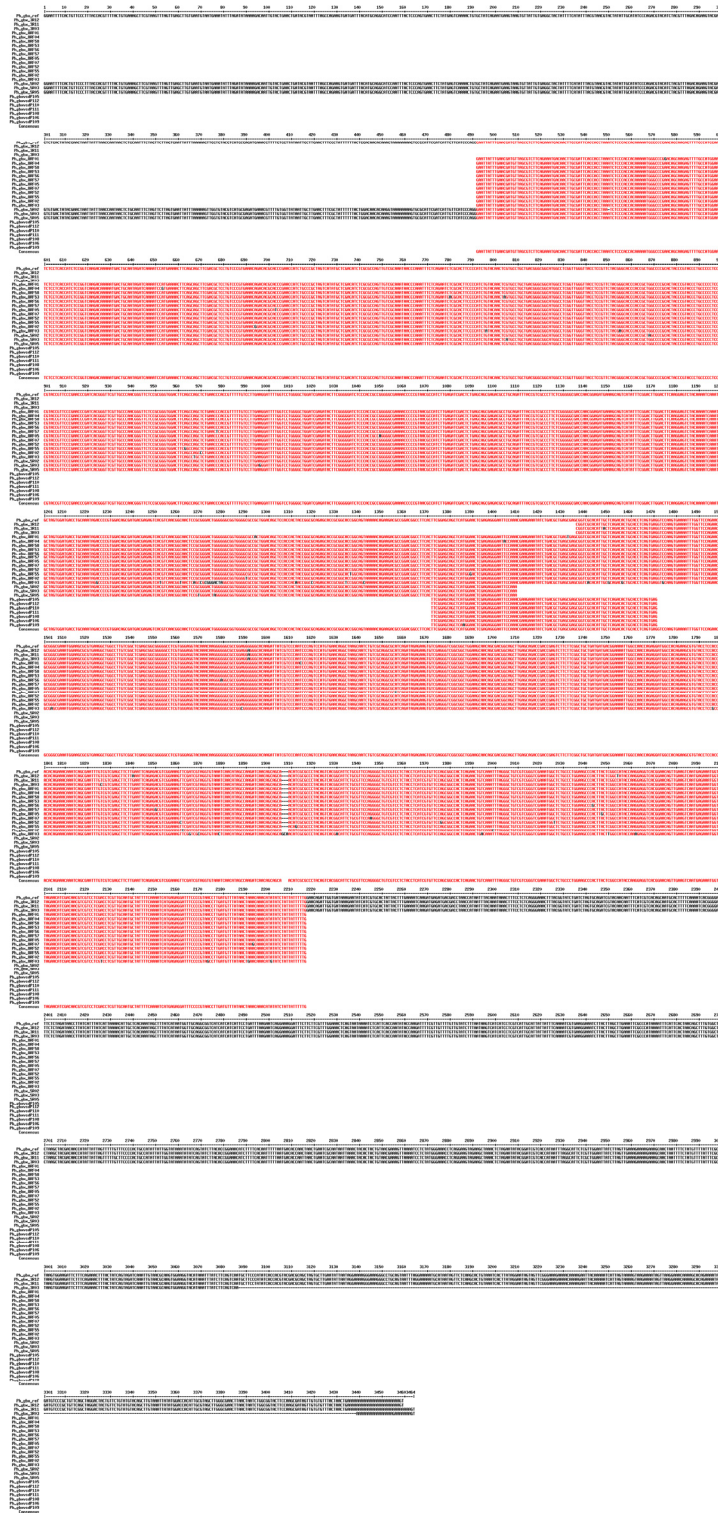
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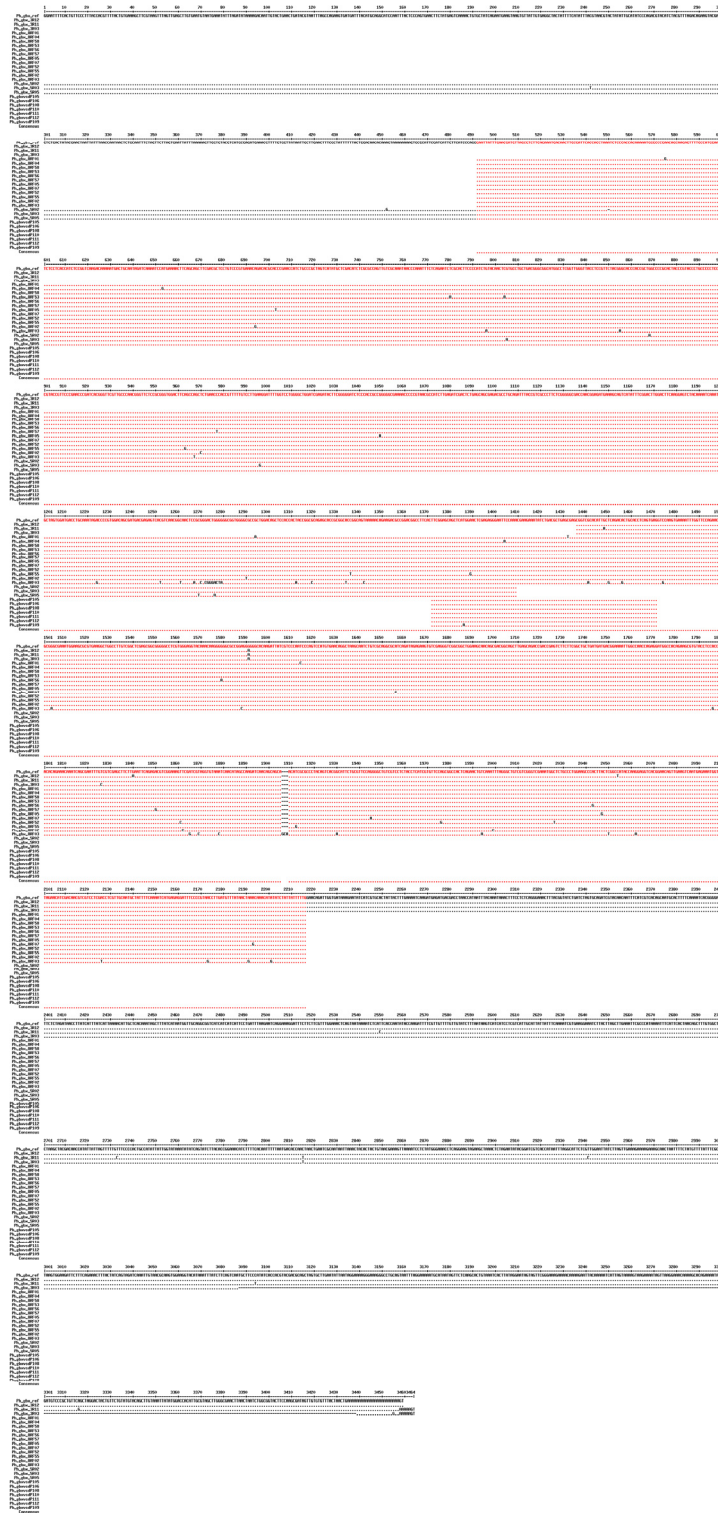
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Ph_gbxvsP112 .....
Ph_gbxvsP108 .....
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Ph_gbxvsP106 .....
Ph_gbxvsP109 .....
Ph_gbx_S802 .....
Consensus .....
101 |-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
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Ph_gbx_08F58 .....
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Ph_gbx_08F04 .....
Ph_gbx_08F05 .....
Ph_gbx_08F07 .....
Ph_gbx_08F56 .....
Ph_gbx_08F02 .....
Ph_gbx_08F01 .....
Ph_gbx_08F57 .....
Ph_gbx_08F55 .....
Ph_gbx_08F53 .....
Ph_gbx_5B05 .....
Ph_gbx_3B03 .....
Ph_gbx_3B03 .....
Ph_gbx_3B03 .....
Ph_gbx_3R11 .....
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Ph_gbxvsP105 .....
Ph_gbxvsP111 .....
Ph_gbxvsP112 .....
Ph_gbxvsP108 .....
Ph_gbxvsP110 .....
Ph_gbxvsP106 .....
Ph_gbxvsP109 .....
Ph_gbx_S802 .....
Consensus .....
201 |-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
Ph_gbx_ref  HMLPFPYSGHTNKGSSHSLSLDFKSTYSNELYDGLDIPVDSIDEHWNHSNGLGGGGPLDSSTTIGREHRTGSKNRRPRTHTFSEGLNELREF
Ph_gbx_08F58 .....
Ph_gbx_08F52 .....
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Ph_gbx_08F05 .....
Ph_gbx_08F07 .....
Ph_gbx_08F56 .....
Ph_gbx_08F02 .....
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Ph_gbx_08F57 .....
Ph_gbx_08F55 .....
Ph_gbx_08F53 .....
Ph_gbx_5B05 .....
Ph_gbx_3B03 .....
Ph_gbx_3B03 .....
Ph_gbx_3R11 .....
Ph_gbx_3R12 .....
Ph_gbxvsP105 .....
Ph_gbxvsP111 .....
Ph_gbxvsP112 .....
Ph_gbxvsP108 .....
Ph_gbxvsP110 .....
Ph_gbxvsP106 .....
Ph_gbxvsP109 .....
Ph_gbx_S802 .....
Consensus .....
301 |-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
Ph_gbx_ref  QTVATLTLSESDIHTLILSCVQKQFMRWHRWHRWPKRNLVSSSGLVGGTNGKGGSGWITWYFHWMLSLKSNRDLKXNGSRGSGKSG
Ph_gbx_08F58 .....
Ph_gbx_08F52 .....
Ph_gbx_08F04 .....
Ph_gbx_08F05 .....
Ph_gbx_08F07 .....
Ph_gbx_08F56 .....
Ph_gbx_08F02 .....
Ph_gbx_08F01 .....
Ph_gbx_08F57 .....
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Ph_gbx_3B03 .....
Ph_gbx_3R11 .....
Ph_gbx_3R12 .....
Ph_gbxvsP105 .....
Ph_gbxvsP111 .....
Ph_gbxvsP112 .....
Ph_gbxvsP108 .....
Ph_gbxvsP110 .....
Ph_gbxvsP106 .....
Ph_gbxvsP109 .....
Ph_gbx_S802 .....
Consensus .....
401 |-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
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Ph_gbx_08F58 .....
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Ph_gbxvsP112 .....
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Ph_gbxvsP109 .....
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Consensus .....
501 |-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
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Ph_gbx_08F52 .....
Ph_gbx_08F04 .....
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Ph_gbx_08F07 .....
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Ph_gbx_3R11 .....
Ph_gbx_3R12 .....
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Ph_gbxvsP106 .....
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Ph_gbx_S802 .....
Consensus .....
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General aspects:

- (i) All sequences are in FASTA format.
- (ii) [bp] indicates nucleic acid sequences; [aa] indicates amino acid sequences
- (iii) Comments are preceded by an apostrophe (')
- (iv) All sequences without oligonucleotide sequence are indicated (e.g. "w/o 1913, 1919"). In these cases, only novel Parhyale sequence is presented.

Parhyale hawaiensis kni1 terminology:

- (i) In general, sequence denotation begins with: ">Ph_kni1_"
- (ii) The above (i) is followed by:
 - "vsdP", in case sequence originates from vsdPCR (5.2.3.1)
 - "5R" or "3R", in case sequence derives from 5' or 3' RACE, respectively
 - "ORF" for coherent ORF sequences
 - "cDNA" for complete cDNA sequences

 - "ref" if reference sequence is presented
 - "ZNFd" if Zinc finger domain (encoding) fragment is presented
 - "KNID" if knirps box/ knirps domain is presented
 - "PIDLS" if PIDLS motif (encoding fragment) is presented
- (iii) The above (ii) is followed by clone id.

Parhyale hawaiensis kni1 synopsis:

cDNA: 2228 bp
5'UTR: 698 bp (1-698)
ORF: 1194 bp (699-1892), 397 aa
3'UTR: 336 bp (1893-2228)
ZNFd: 198 bp (729-926), 66 aa
KNID: 57 bp (945-1001), 19 aa
PIDLS: 36 bp (1716-1751), 12 aa

>Ph_kni1_ZNFd [bp]
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>Ph_kni1_PIDLS [bp]
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>Ph_kni1_ref [bp]
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FEQDAPIDLSVK

>Ph_kni1_ref [aa]
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SVPVDLTSNA&

[PhD_A2_Parhyale_goi_sequences]

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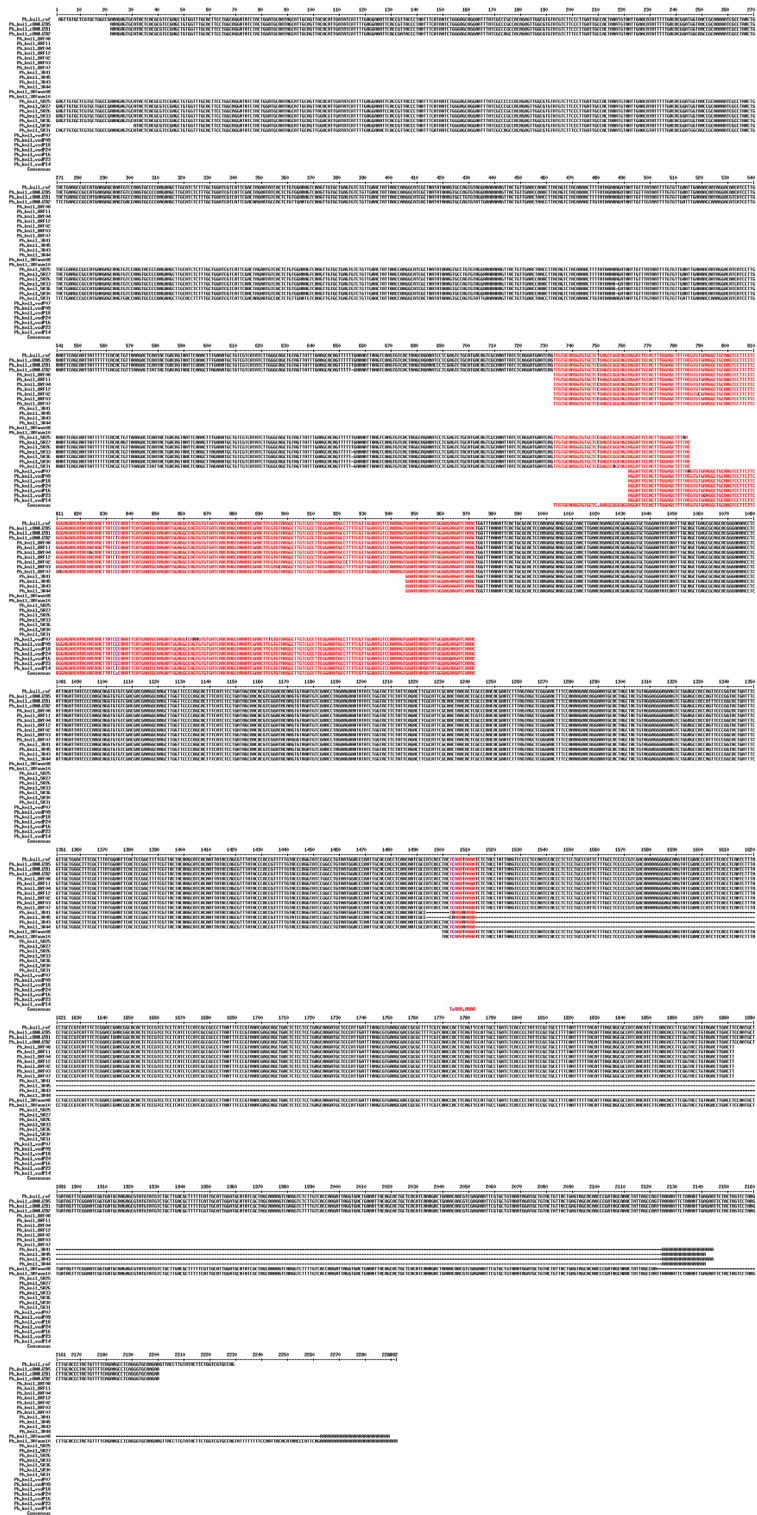
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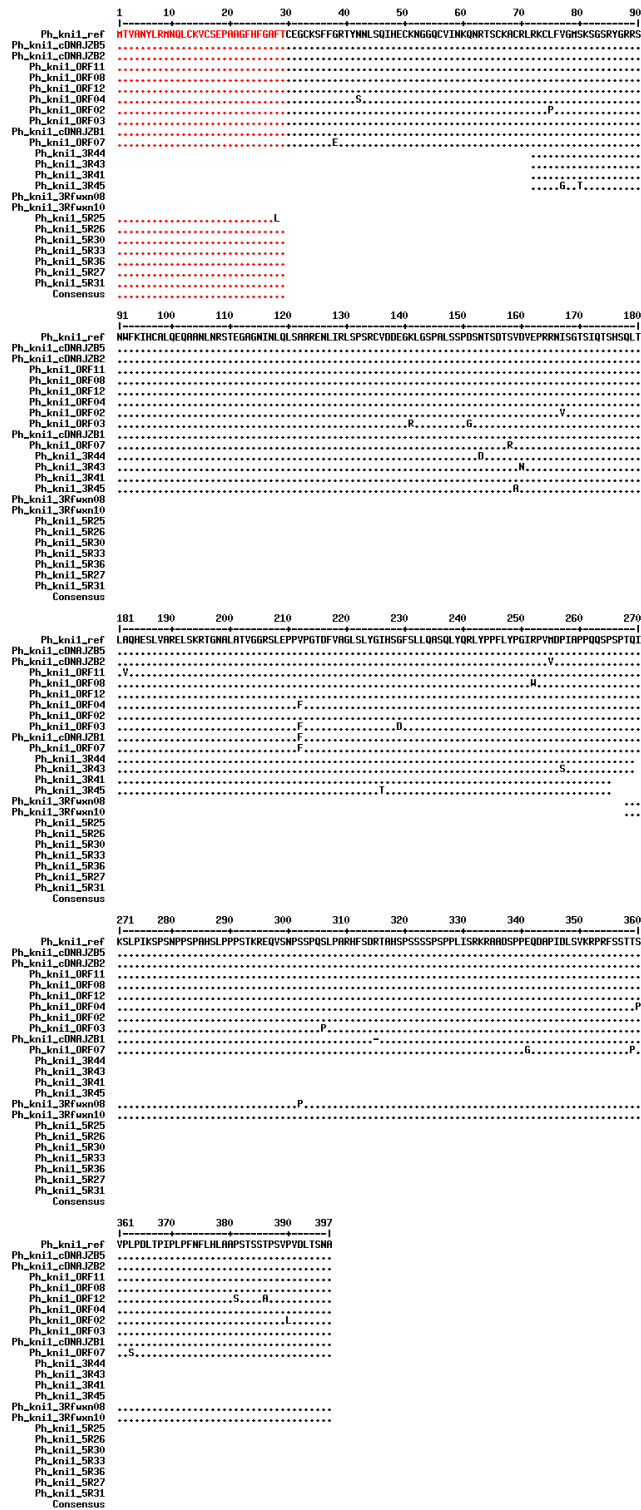


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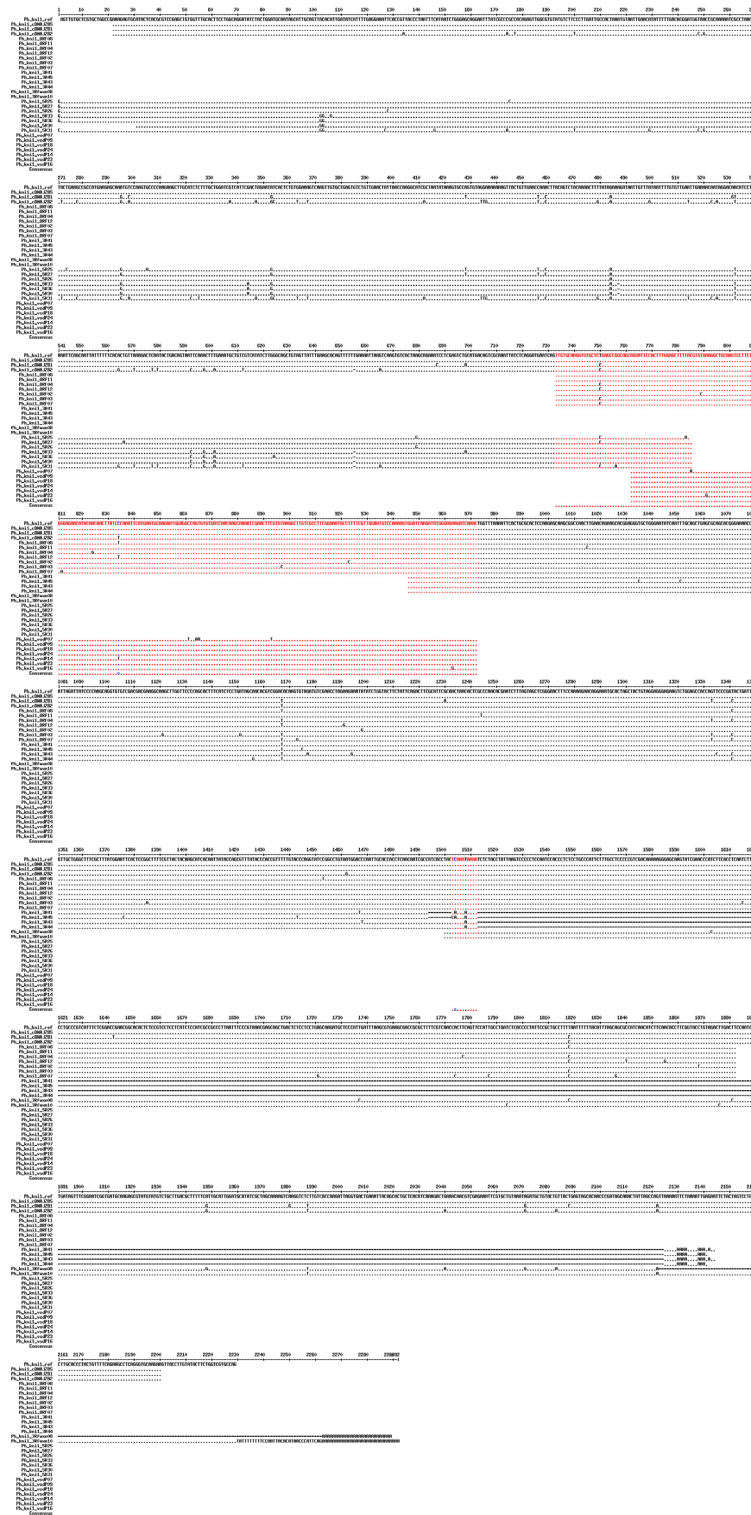
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General aspects:

- (i) All sequences are in FASTA format.
- (ii) [bp] indicates nucleic acid sequences; [aa] indicates amino acid sequences
- (iii) Comments are preceded by an apostrophe (')
- (iv) All sequences without oligonucleotide sequence are indicated (e.g. "w/o 1813, 1816"). In these cases, only novel Parhyale sequence is presented.

Parhyale hawaiiensis kni2 terminology:

- (i) In general, sequence denotation begins with: ">Ph_kni2_"
- (ii) The above (i) is followed by:

"vsdP",	in case sequence originates from vsdPCR (5.2.3.1)
"5R" or "3R",	in case sequence derives from 5' or 3' RACE, respectively
"ORF"	for coherent ORF sequences
"cDNA"	for complete cDNA sequences
"ref"	if reference sequence is presented
"ZNFD"	if Zinc finger domain (encoding) fragment is presented
"KNID"	if knirps box/ knirps domain is presented
"PIDLS"	if PIDLS motif (encoding fragment) is presented
- (iii) The above (ii) is followed by clone id.

Parhyale hawaiiensis kni2 synopsis:

synopsis:
 cDNA: 2656 bp
 5'UTR: 244 bp (1-244)
 ORF: 2079 bp (245-2323), 692 aa
 3'UTR: 333 bp (2324-2656)
 ZNFD: 198 bp (251-448), 66 aa
 KNID: 57 bp (467-523), 19 aa
 PIDLS: 36 bp (1967-2002), 12 aa

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>Ph_kni2_5R03 [aa]
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>Ph_kni2_5R04 [aa]
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>Ph_kni2_5R06 [aa]
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>Ph_kni2_3R13 [aa]
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ERRIQEETLNALRRGVGMGPGSELEATYREQILEALRRSHNMQPPRLDPSAVQTLRRSPPIAEPVRRIGAEYERVRGVSAEAEIRMNNSPPYDWDPRLQEAAPVFEASVVKSESPHSSKVALSVSVST
SQEVDSSSSNQIKIECSRSETETGSKSTPEVKSERRSVGNRSVSLTPKQASARLQDETGA
EERLSFLKSASKISANNATRDGSVAVNSFHGIPGLFPHRSSRDDILYGLPLNNNIFPFASYPPVWPSILPFPFLAKIYPPPPSSPAVSGAPISLDGFKSPLYLPTLRLNTVLPNTLPDVTKEDSSNK
KRILDAILQVQRESACGPRSPGLGSLPPGVTLALSPLQHVTTSTSTLPMVTATTTTSTAPGEQPIDLTVRRKRMAKRIMYQPNESDPQEGMEDEYEQEQKDDADPKEESILKDEEVLEEEFPVDERVS
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>Ph_kni2_3R16 [aa]

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ERRIQEETLNALRRGVGMPGSELEATYREQILEALRRSHNMQSPRLLDPSAVQTLRRSPPIAEPVRRIGA EYERVRGVSAAEAEIRMNNSNPPYDWDPRLQEA PVFSEASVVKSESPHSSKVALSVSVSTS
QEVDSSSSNQIKIECSRSETE TGSKSTPEVKSERRSVGNRSPVSLTPKQASARLQDET GAPMTEEERLSFLKSASKISANNTATR DGS AVNSFHGIPGLFPHRSSRDD ILYGPLN NNI FPFASYPPV
WPSILPFP SLAKIYPPPPFSSPAVSGAPISLDGFKSPLYLPTELRNTVLPNTLPDVTKEDSSNKKRILDAILQVQRESACGPRSP LGG LSPPGVTLALS LPQHVT TSTSTLPMVTATTTTSTAPGEQPI
DLT

>Ph_Kni2_3R17 [aa]

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ERRIQEETLNALRRGVGMPGSELEATYREQILEALRRSHNMQSPRLLDPSA
LTVRRKRKMAKRDI MYQPNESDLQEGMEDEYDQE QKDDADPKEESILKDEEVLEEE PVDERVSK EILVKGTSEIGDLKTSLECAQSLPEPRFEVPMKIMKLETAGDGLIT

>Ph_Kni2_3R20 [aa]

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RRIQEETLNALRRGVGMPGSELEATYREQILEALRRSHNMQSPRLLDPSAVQTLRRSPPIAEPVRRIGA EYERVRGVSAAEAEIRMNNSNPPYDWDPRLQEA PVFSEASVVKSESPHSSKVALSVSVCTSQ
EVDSSSNQIKIECSRSETE TGSKSTPEVKSERRSVGNRSPVSLTPKQASARLQDET GAPMTEEERLSFLKSASKISANNTATR DGS AVNSFHGIPGLFPHRSSRDD ILYGPLN NNI FPFASYPPV
PSILPFP SLAKIYPPPPFSSPAVSGALISLDGFKSPLYLPTELRNTVLPNTLPDVTKEDSSNKKRILDAILQVQRESACGPRSP LGG LSPPGVTLALS LPQHVT TSTSTLPMVTATTTTSTAPGEQPID
LT

>Ph_Kni2_3R21 [aa]

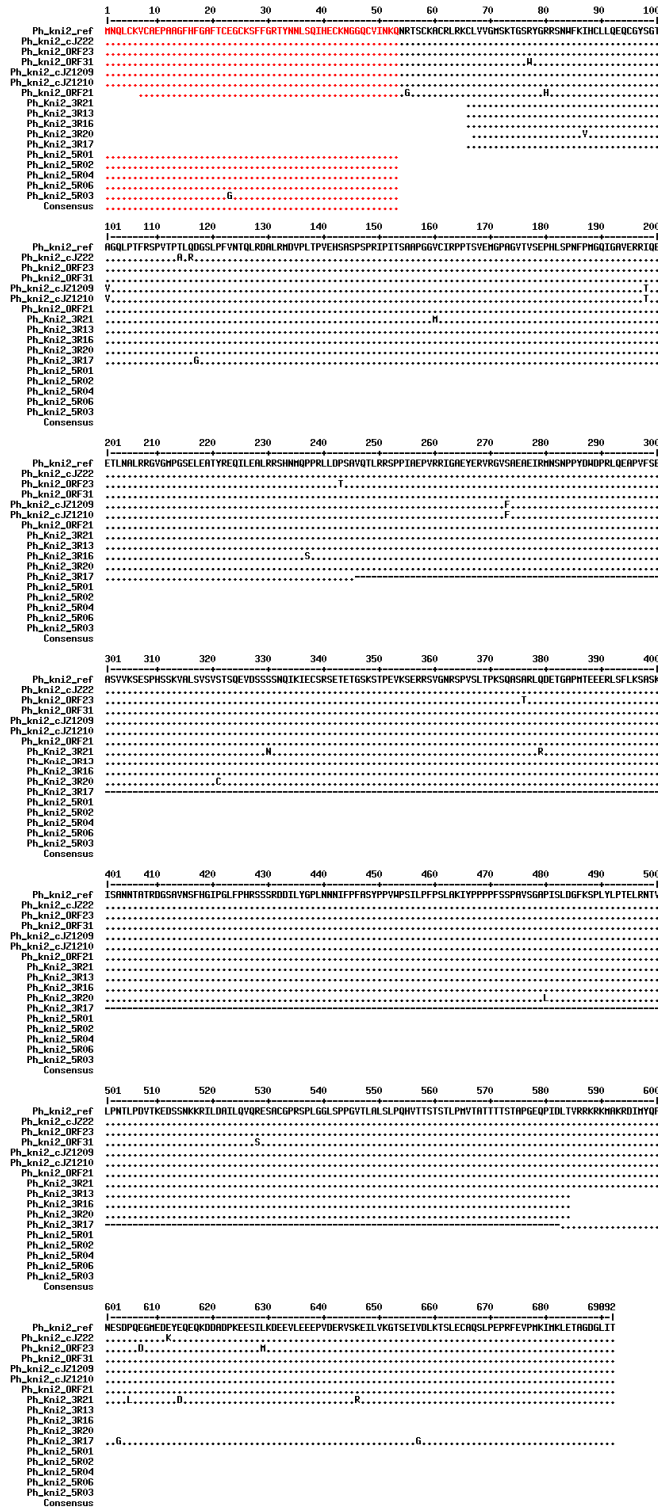
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ERRIQEETLNALRRGVGMPGSELEATYREQILEALRRSHNMQSPRLLDPSAVQTLRRSPPIAEPVRRIGA EYERVRGVSAAEAEIRMNNSNPPYDWDPRLQEA PVFSEASVVKSESPHSSKVALSVSVSTS
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DLTVRRKRKMAKRDI MYQPNESDLQEGMEDEYDQE QKDDADPKEESILKDEEVLEEE PVDERVSK EILVKGTSEIVDLKTSLECAQSLPEPRFEVPMKIMKLETAGDGLIT

[PhD_A2_Parhyale_goi_sequences]

[PhD_A2.12_Ph_kni2]

[PhD_A2.12.2_Ph_kni2_alignment]

“difference_alignment_Ph_kni2_all_aa_sequences.gif”



[PhD_A3_list_of_sequence_clones]

“PhD_A3_List_of_Parhyale_goi_sequence_clones.pdf”

A3 List of *Parhyale hawaiiensis* gene of interest sequence clones

Table A1: List of *Parhyale hawaiiensis* gene of interest sequence clones. Shown are, from left to right: *Parhyale* gene of interest, name of clone, PCR reaction from which the clone derived, used oligonucleotides, length of the clone sequence (without oligonucleotide sequences), number of nucleotide exchanges compared to corresponding reference sequence (5.3.3.2), relative difference in nucleotide sequence, number of polymorphic nucleotide exchanges among all nucleotide exchanges, remaining non-polymorphic nucleotide exchanges and relative amount of non-polymorphic nucleotide exchanges. The last number was used to assess if a specific sequence in fact derived from the corresponding gene locus (or from the locus of a paralogous gene, 5.3.3.3).

<i>P. h. gene o. i.</i>	clone name	PCR reaction	<i>oligo- nucleotides</i>	length	<i>n. e.</i>	<i>rel. n. e.</i>	<i>p.</i>	<i>n.-p. n. e.</i>	<i>rel. n.-p. n. e.</i>
<i>otd1</i>	Ph_otd1_vsdP_QW12	vsdPCR	<i>fw_QRR, re_QVW</i>	109	1	0,9	0	1	0,9
<i>otd1</i>	Ph_otd1_vsdP_Phad04	vsdPCR	<i>fw_QRR, re_QVW</i>	109	1	0,9	0	1	0,9
<i>otd1</i>	Ph_otd1_vsdP_Phad05	vsdPCR	<i>fw_QRR, re_QVW</i>	109	3	2,8	3	0	0
<i>otd1</i>	Ph_otd1_5R01	1-step 5' RACE	<i>1005</i>	605	11	1,8	4	7	1,2
<i>otd1</i>	Ph_otd1_5R03	1-step 5' RACE	<i>1005</i>	605	8	1,3	6	2	0,3
<i>otd1</i>	Ph_otd1_5R04	1-step 5' RACE	<i>1005</i>	605	2	0,3	1	1	0,2
<i>otd1</i>	Ph_otd1_3R09	1-step 3' RACE	<i>1000</i>	1423	4	0,3	1	3	0,2
<i>otd1</i>	Ph_otd1_3R11	1-step 3' RACE	<i>1000</i>	1440	3	0,2	0	3	0,2
<i>otd1</i>	Ph_otd1_3R12	1-step 3' RACE	<i>1000</i>	1432	2	0,1	0	2	0,1
<i>otd1</i>	Ph_otd1_ORF17	ORF isolation via IdPCR	<i>1014, 1015</i>	1132	18	1,6	17	1	0,1
<i>otd1</i>	Ph_otd1_ORF21	ORF isolation via IdPCR	<i>1014, 1015</i>	1132	4	0,4	1	3	0,3
<i>otd1</i>	Ph_otd1_ORF201	ORF isolation via IdPCR	<i>1014, 1015</i>	1132	15	1,3	11	4	0,4
<i>otd1</i>	Ph_otd1_ORFs01	ORF isolation via IdPCR	<i>1014, 1015</i>	1132	3	0,3	1	2	0,2
<i>otd1</i>	Ph_otd1_ORFs02	ORF isolation via IdPCR	<i>1014, 1015</i>	1132	2	0,2	0	2	0,2
<i>otd1</i>	Ph_otd1_ORFs06	ORF isolation via IdPCR	<i>1014, 1015</i>	1132	21	1,9	16	5	0,4
<i>otd1</i>	Ph_otd1_cDNA25	cDNA isolation via IdPCR	<i>1013, 1016</i>	1950	5	0,3	0	5	0,3
<i>otd1</i>	Ph_otd1_cDNA26	cDNA isolation via IdPCR	<i>1013, 1016</i>	1941	35	1,8	31	4	0,2
<i>otd1</i>	Ph_otd1_cDNA27	cDNA isolation via IdPCR	<i>1013, 1016</i>	1955	16	0,8	13	3	0,2
<i>otd1</i>	Ph_otd1_cDNA29	cDNA isolation via IdPCR	<i>1013, 1016</i>	2170	46	2,1	24	22	1
<i>otd1</i>	Ph_otd1_cDNA34	cDNA isolation via IdPCR	<i>1013, 1016</i>	2165	6	0,3	2	4	0,2
<i>otd2</i>	Ph_otd2_vsdP_Pha104	vsdPCR	<i>fw_QRR, re_QVW</i>	109	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>
<i>otd2</i>	Ph_otd2_vsdP_Pha105	vsdPCR	<i>fw_QRR, re_QVW</i>	109	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>
<i>otd2</i>	Ph_otd2_5Rn143	2-step 5' RACE	<i>1105, 1107</i>	566	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>
<i>otd2</i>	Ph_otd2_5Rn164	1-step 5' RACE	<i>1105, 1107</i>	542	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>

<i>otd2</i>	Ph_otd2_5Rn167	1-step 5' RACE	1105, 1107	570	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	n. a.
<i>otd2</i>	Ph_otd2_5Rn169	1-step 5' RACE	1105, 1107	221	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	n. a.
<i>otd2</i>	Ph_otd2_5Rn171	1-step 5' RACE	1105, 1107	339	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	n. a.
<i>otd2</i>	Ph_otd2_5Rn200	1-step 5' RACE	1105, 1107	957	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	n. a.
<i>otd2</i>	Ph_otd2_5Rn203	1-step 5' RACE	1105, 1107	957	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	n. a.
<i>otd2</i>	Ph_otd2_5Rn204	1-step 5' RACE	1105, 1107	957	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	n. a.
<i>otd2</i>	Ph_otd2_3Rn81	2-step 3' RACE	1100, 1101	3255	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	n. a.
<i>otd2</i>	Ph_otd2_3Rn83	2-step 3' RACE	1100, 1101	3260	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	n. a.
<i>hbn</i>	Ph_hbn_vsdPms078	vsdPCR	<i>fw_QRR, re_QVW</i>	109	3	2,8	3	0	0
<i>hbn</i>	Ph_hbn_vsdPms093	vsdPCR	<i>fw_QRR, re_QVW</i>	109	3	2,8	3	0	0
<i>hbn</i>	Ph_hbn_vsdPms109	vsdPCR	<i>fw_QRR, re_QVW</i>	109	3	2,8	3	0	0
<i>hbn</i>	Ph_hbn_5RHH51	1-step 5' RACE	1505	796	5	0,6	4	1	0,1
<i>hbn</i>	Ph_hbn_5RHH53	1-step 5' RACE	1505	796	5	0,6	4	1	0,1
<i>hbn</i>	Ph_hbn_5RHH54	1-step 5' RACE	1505	798	15	1,9	6	9	1,1
<i>hbn</i>	Ph_hbn_5Rn25	2-step 5' RACE	1506, 1507	731	0	0	0	0	0
<i>hbn</i>	Ph_hbn_5Rn26	2-step 5' RACE	1506, 1507	731	0	0	0	0	0
<i>hbn</i>	Ph_hbn_5Rn28	2-step 5' RACE	1506, 1507	731	1	0,1	0	1	0,1
<i>hbn</i>	Ph_hbn_3RHH313	2-step 3' RACE	1500, 1501	628	2	0,3	1	1	0,2
<i>hbn</i>	Ph_hbn_3RHH314	2-step 3' RACE	1500, 1501	628	3	0,5	1	2	0,3
<i>hbn</i>	Ph_hbn_3RHH315	2-step 3' RACE	1500, 1501	628	4	0,6	1	3	0,5
<i>hbn</i>	Ph_hbn_3RHH336	2-step 3' extension RACE	1503, 1504	609	6	1	2	4	0,7
<i>hbn</i>	Ph_hbn_3RHH356	2-step 3' extension RACE	1503, 1504	721	0	0	0	0	0
<i>hbn</i>	Ph_hbn_3RHH357	2-step 3' extension RACE	1503, 1504	1311	0	0	0	0	0
<i>hbn</i>	Ph_hbn_3RHH358	2-step 3' extension RACE	1503, 1504	710	1	0,1	0	1	0,1
<i>hbn</i>	Ph_hbn_3RHH361	2-step 3' extension RACE	1503, 1504	1471	5	0,3	3	2	0,1
<i>hbn</i>	Ph_hbn_3RHH363	2-step 3' extension RACE	1503, 1504	709	5	0,7	3	2	0,3
<i>hbn</i>	Ph_hbn_3RHH366	2-step 3' extension RACE	1503, 1504	713	3	0,4	3	0	0
<i>hbn</i>	Ph_hbn_ORF01	ORF isolation via IdPCR	1514, 1515	1519	5	0,3	4	1	0,1
<i>hbn</i>	Ph_hbn_ORF02	ORF isolation via IdPCR	1514, 1515	1519	6	0,4	4	2	0,1
<i>hbn</i>	Ph_hbn_ORF05	ORF isolation via IdPCR	1514, 1515	1519	5	0,3	4	1	0,1
<i>hbn</i>	Ph_hbn_cDNA01	cDNA isolation via IdPCR	1512, 1516	2559	1	0	0	1	0
<i>hbn</i>	Ph_hbn_cDNA05	cDNA isolation via IdPCR	1512, 1516	2559	0	0	0	0	0
<i>hbn</i>	Ph_hbn_cDNA11	cDNA isolation via IdPCR	1512, 1516	2558	5	0,2	0	5	0,2
<i>al1</i>	Ph_al1_vsdP_Phad16	vsdPCR	<i>fw_QRR, re_QVW</i>	109	0	0	0	0	0
<i>al1</i>	Ph_al1_vsdP_Pha092	vsdPCR	<i>fw_QRR, re_QVW</i>	109	0	0	0	0	0
<i>al1</i>	Ph_al1_vsdP_Pha097	vsdPCR	<i>fw_QRR, re_QVW</i>	109	0	0	0	0	0
<i>al1</i>	Ph_al1_vsdP_Pha102	vsdPCR	<i>fw_QRR, re_QVW</i>	109	0	0	0	0	0
<i>al1</i>	Ph_al1_5Rn2	2-step 5' RACE	1207, 1208	845	16	1,9	15	1	0,1
<i>al1</i>	Ph_al1_5Rn3	2-step 5' RACE	1207, 1208	843	16	1,9	14	2	0,2
<i>al1</i>	Ph_al1_5Rn4	2-step 5' RACE	1207, 1208	845	16	1,9	15	1	0,1
<i>al1</i>	Ph_al1_5Rn118	2-step 5' RACE	1207, 1208	843	0	0	0	0	0

<i>al1</i>	Ph_al1_5Rn119	2-step 5' RACE	1207, 1208	844	0	0	0	0	0
<i>al1</i>	Ph_al1_3Rn5	2-step 3' RACE	1202, 1203	932	1	0,1	0	1	0,1
<i>al1</i>	Ph_al1_3Rn7	2-step 3' RACE	1202, 1203	930	19	2	4	15	1,6
<i>al1</i>	Ph_al1_3Rn8	2-step 3' RACE	1202, 1203	930	1	0,1	0	1	0,1
<i>al1</i>	Ph_al1_ORF13	ORF isolation via IdPCR	1214, 1215	1466	33	2,3	33	0	0
<i>al1</i>	Ph_al1_ORF14	ORF isolation via IdPCR	1214, 1215	1466	35	2,4	33	2	0,1
<i>al1</i>	Ph_al1_ORF15	ORF isolation via IdPCR	1214, 1215	1466	33	2,3	33	0	0
<i>al1</i>	Ph_al1_ORF16	ORF isolation via IdPCR	1214, 1215	1466	4	0,3	0	4	0,3
<i>al1</i>	Ph_al1_ORF17	ORF isolation via IdPCR	1214, 1215	1466	3	0,2	0	3	0,2
<i>al1</i>	Ph_al1_ORF18	ORF isolation via IdPCR	1214, 1215	1466	34	2,3	33	1	0,1
<i>al1</i>	Ph_al1_cDNA01	cDNA isolation via IdPCR	1213, 1216	1752	1	0,1	0	1	0,1
<i>al1</i>	Ph_al1_cDNA04	cDNA isolation via IdPCR	1213, 1216	1752	4	0,2	0	4	0,2
<i>al1</i>	Ph_al1_cDNA05	cDNA isolation via IdPCR	1213, 1216	1752	3	0,2	0	3	0,2
<i>al2</i>	Ph_al2_vsdP_Pha089	vsdPCR	<i>fw_QRR, re_QVW</i>	109	0	0	0	0	0
<i>al2</i>	Ph_al2_vsdP_Pha111	vsdPCR	<i>fw_QRR, re_QVW</i>	109	2	1,8	2	0	0
<i>al2</i>	Ph_al2_5Rg1	1-step 5' RACE	1305	403	5	1,2	5	0	0
<i>al2</i>	Ph_al2_5Rp09	1-step 5' RACE	1305	1559	28	1,8	26	2	0,1
<i>al2</i>	Ph_al2_5Rp11	1-step 5' RACE	1305	1560	31	2	27	4	0,3
<i>al2</i>	Ph_al2_5Rp13	1-step 5' RACE	1305	1559	27	1,7	27	0	0
<i>al2</i>	Ph_al2_5Rn09	2-step 5' RACE	1305, 1306	1520	18	1,2	17	1	0,1
<i>al2</i>	Ph_al2_5Rn11	2-step 5' RACE	1305, 1306	1520	17	1,1	17	0	0
<i>al2</i>	Ph_al2_5Rn12	2-step 5' RACE	1305, 1306	1519	19	1,3	17	2	0,1
<i>al2</i>	Ph_al2_3Rn227	2-step 3' RACE	1300, 1301	590	0	0	0	0	0
<i>al2</i>	Ph_al2_3Rfwx01	2-step 3' extension RACE	1302, 1304	463	1	0,2	0	1	0,2
<i>al2</i>	Ph_al2_3Rfwx05	2-step 3' extension RACE	1302, 1304	559	1	0,2	0	1	0,2
<i>al2</i>	Ph_al2_ORF03	ORF isolation via IdPCR	1314, 1315	1707	14	0,8	11	3	0,2
<i>al2</i>	Ph_al2_ORF04	ORF isolation via IdPCR	1314, 1315	1707	16	0,9	11	5	0,3
<i>al2</i>	Ph_al2_ORF05	ORF isolation via IdPCR	1314, 1315	1707	16	0,9	0	16	0,9
<i>pby1</i>	Ph_pby1_vsdP_PhQW1	vsdPCR	<i>fw_QRR, re_QVW</i>	109	1	0,9	1	0	0
<i>pby1</i>	Ph_pby1_vsdP_Phad02	vsdPCR	<i>fw_QRR, re_QVW</i>	109	1	0,9	1	0	0
<i>pby1</i>	Ph_pby1_vsdP_Phad06	vsdPCR	<i>fw_QRR, re_QVW</i>	109	2	1,8	1	1	0,9
<i>pby1</i>	Ph_pby1_vsdP_Phad08	vsdPCR	<i>fw_QRR, re_QVW</i>	109	2	1,8	1	1	0,9
<i>pby1</i>	Ph_pby1_vsdP_Phad14	vsdPCR	<i>fw_QRR, re_QVW</i>	109	3	2,8	1	2	1,8
<i>pby1</i>	Ph_pby1_5R0101	2-step 5' RACE	1605, 1607	764	8	1	5	3	0,4
<i>pby1</i>	Ph_pby1_5n54	2-step 5' RACE	1606, 1607	764	7	0,9	0	7	0,9
<i>pby1</i>	Ph_pby1_5n55	2-step 5' RACE	1606, 1607	764	1	0,1	0	1	0,1
<i>pby1</i> <i>A</i>	Ph_pby1_3R2219	2-step 3' RACE	1601, 1602	1308	2	0,2	0	2	0,2
<i>pby1</i> <i>A</i>	Ph_pby1_3Rf27	2-step 3' RACE	1601, 1602	1304	10	0,8	7	3	0,2
<i>pby1</i> <i>A</i>	Ph_pby1_3Rf29	2-step 3' RACE	1601, 1602	1304	31	2,4	10	21	1,6
<i>pby1</i> <i>A</i>	Ph_pby1A_ORF09	ORF isolation via IdPCR	1614, 1616	1599	18	1,1	14	4	0,3
<i>pby1</i> <i>A</i>	Ph_pby1A_ORF13	ORF isolation via IdPCR	1614, 1616	1599	24	1,5	16	8	0,5

<i>pby1</i> _A	Ph_pby1A_ORF16	ORF isolation via ldPCR	1614, 1616	1599	1	0,1	0	1	0,1
<i>pby1</i> _B	Ph_pby1_3R1413	2-step 3' RACE	1601, 1602	951	5	0,5	3	2	0,2
<i>pby1</i> _B	Ph_pby1_3Rf21	2-step 3' RACE	1601, 1602	950	10	1,1	3	7	0,7
<i>pby1</i> _B	Ph_pby1_3Rf316	2-step 3' RACE	1601, 1602	928	5	0,5	3	2	0,2
<i>pby1</i> _B	Ph_pby1B_ORF01	ORF isolation via ldPCR	1614, 1615	1132	1	0,1	0	1	0,1
<i>pby1</i> _B	Ph_pby1B_ORF03	ORF isolation via ldPCR	1614, 1615	1132	13	1,1	7	6	0,5
<i>pby1</i> _B	Ph_pby1B_ORF05	ORF isolation via ldPCR	1614, 1615	1132	5	0,4	1	4	0,4
<i>pby2</i>	Ph_pby2_vsdP_Phad08_1	vsdPCR	<i>fw_QRR, re_QVW</i>	109	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	<i>n. a.</i>	n. a.
<i>awh</i>	Ph_awh_vsdP10	vsdPCR	<i>fw_QRR, re_QVW</i>	109	0	0	0	0	0
<i>awh</i>	Ph_awh_vsdP15	vsdPCR	<i>fw_QRR, re_QVW</i>	109	1	0,9	0	1	0,9
<i>awh</i>	Ph_awh5RCR1	2-step 5' RACE	1405, 1406	2089	24	1,1	15	9	0,4
<i>awh</i>	Ph_awh5RCR2	2-step 5' RACE	1405, 1406	2020	14	0,7	14	0	0
<i>awh</i>	Ph_awh5RCR5	2-step 5' RACE	1405, 1406	2021	16	0,8	14	2	0,1
<i>awh</i>	Ph_awh5RCR6	2-step 5' RACE	1405, 1406	2087	23	1,1	20	3	0,1
<i>awh</i>	Ph_awh_5Rn121	2-step 5' RACE	1405, 1406	1861	60	3,2	60	0	0
<i>awh</i>	Ph_awh_5Rn122	2-step 5' RACE	1405, 1406	1957	60	3,1	49	11	0,6
<i>awh</i>	Ph_awh_5Rn150	2-step 5' RACE	1405, 1406	1813	54	3	51	3	0,2
<i>awh</i>	Ph_awh_3Rn21	2-step 3' RACE	1400, 1401	1522	0	0	0	0	0
<i>awh</i>	Ph_awh_3Rn22	2-step 3' RACE	1400, 1401	1377	8	0,6	8	0	0
<i>awh</i>	Ph_awh_3Rn78	2-step 3' RACE	1400, 1401	1521	3	0,2	1	2	0,1
<i>awh</i>	Ph_awh_3RCR07	2-step 3' RACE	1400, 1401	1571	32	2	24	8	0,5
<i>awh</i>	Ph_awh_3RCR09	2-step 3' RACE	1400, 1401	1532	35	2,3	32	3	0,2
<i>awh</i>	Ph_awh_3RCR10	2-step 3' RACE	1400, 1401	1535	26	1,7	19	7	0,5
<i>awh</i>	Ph_awh_ORF20	ORF isolation via ldPCR	1414, 1415	1058	5	0,5	0	5	0,5
<i>awh</i>	Ph_awh_ORF21	ORF isolation via ldPCR	1414, 1415	1058	0	0	0	0	0
<i>awh</i>	Ph_awh_ORF22	ORF isolation via ldPCR	1414, 1415	1058	1	0,1	0	1	0,1
<i>six3</i>	Ph_six3_vsdP13	vsdPCR	<i>fw_QAM, re_PTQ</i>	217	5	2,3	5	0	0
<i>six3</i>	Ph_six3_vsdP17	vsdPCR	<i>fw_QAM, re_PTQ</i>	217	6	2,8	5	1	0,5
<i>six3</i>	Ph_six3_vsdP19	vsdPCR	<i>fw_QAM, re_PTQ</i>	217	1	0,5	1	0	0
<i>six3</i>	Ph_six3_5R01	2-step 5' RACE	2005, 2006	496	4	0,8	1	3	0,6
<i>six3</i>	Ph_six3_5R02	2-step 5' RACE	2005, 2006	562	6	1,1	4	2	0,4
<i>six3</i>	Ph_six3_5R04	2-step 5' RACE	2005, 2006	495	0	0	0	0	0
<i>six3</i>	Ph_six3_5R05	2-step 5' RACE	2005, 2006	540	6	1,1	4	2	0,4
<i>six3</i>	Ph_six3_5R06	2-step 5' RACE	2005, 2006	562	0	0	0	0	0
<i>six3</i>	Ph_six3_5R16	2-step 5' extension RACE	2006, 2007	490	2	0,4	0	2	0,4
<i>six3</i>	Ph_six3_5R17	2-step 5' extension RACE	2006, 2007	490	4	0,8	3	1	0,2
<i>six3</i>	Ph_six3_5R18	2-step 5' extension RACE	2006, 2007	490	4	0,8	3	1	0,2
<i>six3</i>	Ph_six3_5R19	2-step 5' extension RACE	2006, 2008	76	2	2,6	0	2	2,6
<i>six3</i>	Ph_six3_5R23	2-step 5' extension RACE	2006, 2008	76	0	0	0	0	0
<i>six3</i>	Ph_six3_5R24	2-step 5' extension RACE	2006, 2008	76	0	0	0	0	0
<i>six3</i>	Ph_six3_3R08	2-step 3' RACE	2000, 2001	3067	19	0,6	16	3	0,1

six3	Ph_six3_3R09	2-step 3' RACE	2000, 2001	3070	19	0,6	16	3	0,1
six3	Ph_six3_3R10	2-step 3' RACE	2000, 2001	3070	19	0,6	16	3	0,1
six3	Ph_six3_ORF10	ORF isolation via IdPCR	2014, 2015	1638	3	0,2	0	3	0,2
six3	Ph_six3_ORF11	ORF isolation via IdPCR	2014, 2015	1638	4	0,2	0	4	0,2
six3	Ph_six3_ORF12	ORF isolation via IdPCR	2014, 2015	1638	1	0,1	0	1	0,1
six3	Ph_six3_cDNA09	cDNA isolation via IdPCR	2013, 2016	3572	3	0,1	0	3	0,1
six3	Ph_six3_cDNA13	cDNA isolation via IdPCR	2013, 2016	3572	10	0,3	0	10	0,3
six4	Ph_six4_vsdP14	vsdPCR	<i>fw_QAM, re_PTQ</i>	217	0	0	0	0	0
six4	Ph_six4_vsdP16	vsdPCR	<i>fw_QAM, re_PTQ</i>	217	0	0	0	0	0
six4	Ph_six4_vsdP21	vsdPCR	<i>fw_QAM, re_PTQ</i>	217	0	0	0	0	0
six4	Ph_six4_5R13	2-step 5' RACE	2105, 2106	1642	1	0,1	0	1	0,1
six4	Ph_six4_5R14	2-step 5' RACE	2105, 2106	1564	6	0,4	0	6	0,4
six4	Ph_six4_5R15	2-step 5' RACE	2105, 2106	1647	50	3	0	50	3
six4	Ph_six4_3R19	2-step 3' RACE	2100	1179	2	0,2	0	2	0,2
six4	Ph_six4_3R20	2-step 3' RACE	2100	1164	2	0,2	0	2	0,2
six4	Ph_six4_3R24	2-step 3' RACE	2100, 2101	1092	2	0,2	0	2	0,2
six4	Ph_six4_ORF14	ORF isolation via IdPCR	2114, 2115	1556	2	0,1	0	2	0,1
six4	Ph_six4_ORF15	ORF isolation via IdPCR	2114, 2115	1556	2	0,1	0	2	0,1
six4	Ph_six4_ORF16	ORF isolation via IdPCR	2114, 2115	1556	1	0,1	0	1	0,1
gbx	Ph_gbxvsdP105	vsdPCR	<i>fw_AFT, re_KIW</i>	100	0	0	0	0	0
gbx	Ph_gbxvsdP106	vsdPCR	<i>fw_AFT, re_KIW</i>	100	0	0	0	0	0
gbx	Ph_gbxvsdP108	vsdPCR	<i>fw_AFT, re_KIW</i>	100	0	0	0	0	0
gbx	Ph_gbxvsdP109	vsdPCR	<i>fw_AFT, re_KIW</i>	100	1	1	0	1	1
gbx	Ph_gbxvsdP110	vsdPCR	<i>fw_AFT, re_KIW</i>	100	0	0	0	0	0
gbx	Ph_gbxvsdP111	vsdPCR	<i>fw_AFT, re_KIW</i>	100	0	0	0	0	0
gbx	Ph_gbxvsdP112	vsdPCR	<i>fw_AFT, re_KIW</i>	100	0	0	0	0	0
gbx	Ph_gbx_5R02	2-step 5' RACE	2305, 2306	1408	2	0,1	0	2	0,1
gbx	Ph_gbx_5R03	2-step 5' RACE	2305, 2306	1409	3	0,2	0	3	0,2
gbx	Ph_gbx_5R05	2-step 5' RACE	2305, 2306	1409	3	0,2	1	2	0,1
gbx	Ph_gbx_3R03	2-step 3' RACE	2301, 2303	1674	4	0,2	1	3	0,2
gbx	Ph_gbx_3R11	2-step 3' RACE	2301, 2303	2026	8	0,4	1	7	0,3
gbx	Ph_gbx_3R12	2-step 3' RACE	2301, 2303	2021	3	0,1	1	2	0,1
gbx	Ph_gbx_ORF01	ORF isolation via IdPCR	2314, 2315	1722	4	0,2	0	4	0,2
gbx	Ph_gbx_ORF02	ORF isolation via IdPCR	2314, 2315	1722	5	0,3	0	5	0,3
gbx	Ph_gbx_ORF03	ORF isolation via IdPCR	2314, 2315	1725	41	2,4	1	40	2,3
gbx	Ph_gbx_ORF04	ORF isolation via IdPCR	2314, 2315	1722	2	0,1	0	2	0,1
gbx	Ph_gbx_ORF05	ORF isolation via IdPCR	2314, 2315	1722	3	0,2	0	3	0,2
gbx	Ph_gbx_ORF07	ORF isolation via IdPCR	2314, 2315	1722	3	0,2	0	3	0,2
gbx	Ph_gbx_ORF52	ORF isolation via IdPCR	2314, 2315	1722	3	0,2	0	3	0,2
gbx	Ph_gbx_ORF53	ORF isolation via IdPCR	2314, 2315	1722	2	0,1	0	2	0,1
gbx	Ph_gbx_ORF55	ORF isolation via IdPCR	2314, 2315	1722	4	0,2	0	4	0,2

<i>gbx</i>	Ph_gbx_ORF56	ORF isolation via ldPCR	2314, 2315	1722	2	0,1	0	2	0,1
<i>gbx</i>	Ph_gbx_ORF57	ORF isolation via ldPCR	2314, 2315	1722	2	0,1	0	2	0,1
<i>gbx</i>	Ph_gbx_ORF58	ORF isolation via ldPCR	2314, 2315	1722	0	0	0	0	0
<i>kni1</i>	Ph_kni1_vsdP07	vsdPCR	<i>fw_CKV, re_LLQ</i>	211	5	2,4	0	5	2,4
<i>kni1</i>	Ph_kni1_vsdP09	vsdPCR	<i>fw_CKV, re_LLQ</i>	211	0	0	0	0	0
<i>kni1</i>	Ph_kni1_vsdP14	vsdPCR	<i>fw_CKV, re_LLQ</i>	211	1	0,5	1	0	0
<i>kni1</i>	Ph_kni1_vsdP16	vsdPCR	<i>fw_CKV, re_LLQ</i>	211	1	0,5	0	1	0,5
<i>kni1</i>	Ph_kni1_vsdP18	vsdPCR	<i>fw_CKV, re_LLQ</i>	211	0	0	0	0	0
<i>kni1</i>	Ph_kni1_vsdP23	vsdPCR	<i>fw_CKV, re_LLQ</i>	211	1	0,5	0	1	0,5
<i>kni1</i>	Ph_kni1_vsdP24	vsdPCR	<i>fw_CKV, re_LLQ</i>	211	0	0	0	0	0
<i>kni1</i>	Ph_kni1_5R25	2-step 5' RACE	1905, 1906	784	13	1,7	16	4	0,5
<i>kni1</i>	Ph_kni1_5R26	2-step 5' RACE	1905, 1906	785	3	0,4	16	1	0,1
<i>kni1</i>	Ph_kni1_5R27	2-step 5' RACE	1905, 1906	785	9	1,1	16	1	0,1
<i>kni1</i>	Ph_kni1_5R30	2-step 5' RACE	1905, 1906	753	11	1,5	16	0	0
<i>kni1</i>	Ph_kni1_5R31	2-step 5' RACE	1905, 1906	784	44	5,6	16	32	4,1
<i>kni1</i>	Ph_kni1_5R33	2-step 5' RACE	1905, 1906	783	13	1,7	16	2	0,3
<i>kni1</i>	Ph_kni1_5R36	2-step 5' RACE	1905, 1906	783	12	1,5	16	1	0,1
<i>kni1</i>	Ph_kni1_3R41	2-step 3' RACE	1900, 1901	548	2	0,4	2	1	0,2
<i>kni1</i>	Ph_kni1_3R43	2-step 3' RACE	1900, 1901	559	6	1,1	2	4	0,7
<i>kni1</i>	Ph_kni1_3R44	2-step 3' RACE	1900, 1901	559	3	0,5	2	1	0,2
<i>kni1</i>	Ph_kni1_3R45	2-step 3' RACE	1900, 1901	548	6	1,1	2	5	0,9
<i>kni1</i>	Ph_kni1_3Rfwxn08	2-step 3' extension RACE	1903, 1904	621	8	1,3	5	3	0,5
<i>kni1</i>	Ph_kni1_3Rfwxn10	2-step 3' extension RACE	1903, 1904	763	3	0,4	5	2	0,3
<i>kni1</i>	Ph_kni1_ORF02	ORF isolation via ldPCR	1914, 1915	1150	4	0,3	6	4	0,3
<i>kni1</i>	Ph_kni1_ORF03	ORF isolation via ldPCR	1914, 1915	1150	10	0,9	6	5	0,4
<i>kni1</i>	Ph_kni1_ORF04	ORF isolation via ldPCR	1914, 1915	1150	7	0,6	6	2	0,2
<i>kni1</i>	Ph_kni1_ORF07	ORF isolation via ldPCR	1914, 1915	1150	11	1	6	6	0,5
<i>kni1</i>	Ph_kni1_ORF08	ORF isolation via ldPCR	1914, 1915	1150	5	0,4	6	1	0,1
<i>kni1</i>	Ph_kni1_ORF11	ORF isolation via ldPCR	1914, 1915	1150	2	0,2	6	2	0,2
<i>kni1</i>	Ph_kni1_ORF12	ORF isolation via ldPCR	1914, 1915	1150	7	0,6	6	3	0,3
<i>kni1</i>	Ph_kni1_cDNAJZB1	cDNA isolation via ldPCR	1913, 1919	2179	24	1,1	17	7	0,3
<i>kni1</i>	Ph_kni1_cDNAJZB2	cDNA isolation via ldPCR	1913, 1919	2178	50	2,3	46	4	0,2
<i>kni1</i>	Ph_kni1_cDNAJZB5	cDNA isolation via ldPCR	1913, 1919	2179	0	0	0	0	0
<i>kni2</i>	Ph_kni2_vsdP01	vsdPCR	<i>fw_CKV, re_LLQ</i>	211	0	0	0	0	0
<i>kni2</i>	Ph_kni2_vsdP02	vsdPCR	<i>fw_CKV, re_LLQ</i>	211	0	0	0	0	0
<i>kni2</i>	Ph_kni2_vsdP03	vsdPCR	<i>fw_CKV, re_LLQ</i>	211	1	0,5	0	1	0,5
<i>kni2</i>	Ph_kni2_vsdP21	vsdPCR	<i>fw_CKV, re_LLQ</i>	211	0	0	0	0	0
<i>kni2</i>	Ph_kni2_5R01	2-step 5' RACE	1805, 1806	405	0	0	3	0	0
<i>kni2</i>	Ph_kni2_5R02	2-step 5' RACE	1805, 1806	405	3	0,7	3	0	0
<i>kni2</i>	Ph_kni2_5R03	2-step 5' RACE	1805, 1806	405	6	1,5	3	3	0,7
<i>kni2</i>	Ph_kni2_5R04	2-step 5' RACE	1805, 1806	405	2	0,5	3	2	0,5

<i>kni2</i>	Ph_kni2_5R06	2-step 5' RACE	1805, 1806	405	0	0	3	0	0
<i>kni2</i>	Ph_Kni2_3R13	2-step 3' RACE	1800, 1801	1528	2	0,1	0	2	0,1
<i>kni2</i>	Ph_Kni2_3R16	2-step 3' RACE	1800, 1801	1528	1	0,1	0	1	0,1
<i>kni2</i>	Ph_Kni2_3R17	2-step 3' RACE	1800, 1801	1202	6	0,5	0	6	0,5
<i>kni2</i>	Ph_Kni2_3R20	2-step 3' RACE	1800, 1801	1528	5	0,3	0	5	0,3
<i>kni2</i>	Ph_Kni2_3R21	2-step 3' RACE	1800, 1801	2218	7	0,3	0	7	0,3
<i>kni2</i>	Ph_kni2_ORF21	ORF isolation via IdPCR	1814, 1815	2018	4	0,2	1	3	0,1
<i>kni2</i>	Ph_kni2_ORF23	ORF isolation via IdPCR	1814, 1815	2018	10	0,5	0	10	0,5
<i>kni2</i>	Ph_kni2_ORF31	ORF isolation via IdPCR	1814, 1815	2018	3	0,1	0	3	0,1
<i>kni2</i>	Ph_kni2_cJZ1209	cDNA isolation via IdPCR	1860, 1817	2578	3	0,1	3	0	0
<i>kni2</i>	Ph_kni2_cJZ1210	cDNA isolation via IdPCR	1860, 1817	2578	3	0,1	3	0	0
<i>kni2</i>	Ph_kni2_cJZ22	cDNA isolation via IdPCR	1813, 1816	2548	18	0,7	4	14	0,5

[PhD_A4_oligonucleotides]

“PhD_A4.1_degenerate_oligonucleotides.pdf”

A4 Oligonucleotides

A4.1 Degenerate Oligonucleotides

Table A2: List of degenerate oligonucleotides. Shown are: the name of the degenerate oligonucleotide (left), its nucleic acid sequence from 5' to 3' in degenerate nucleic acid code (centre) and the protein sequence it encodes (right).

oligonucleotide	5'-3' sequence	corresponding protein sequence
fw_AFT	MGIMGIMGIACIGCITTYAC	RRRTAFT
fw_CKV	TGYAARGTNTGYGGNGARCCNGCNGC	CKVCGEPAA
fw_QAM	CARGCIATGTGGYTIGARGCICAYTA	QAMWLEAHY
fw_QRR	CAGMGMGGGARMGIACIACITTYAC	QRRERTTFT
re_KIW	AACCADATYTTIACYTGIAC	EVQVKIW
re_LLQ	TGNARNARRCARTGDATYTTTRAACCA	WFKIHCLLQ
re_PTQ	TTYTTRAACCARTTICCIACYTGIGTIGG	PTQVGNWFKN
re_QVW	GCCCKCKRTTYTTRAACCAIACYTG	QVWFKNRRA

[PhD_A4_oligonucleotides]

“PhD_A4.2_specific_oligonucleotides.pdf”

A4.2 Specific oligonucleotides

Table A3: List of specific oligonucleotides. Shown are the identification number of the specific oligonucleotide (left) and its nucleic acid sequence from 5' to 3' (right).

oligonucleotide	5'-3' sequence
1000	CGGCAAGACGCGGTATCCCGACATCTTC
1005	CTGGTAGGTTGATTTTGATCGCAACTTCCTCTCTC
1013	AGTTGTGCGAACGTAAGCTTGTCGAATACG
1014	GATGCACATGAACGGAGATCCCATGGCCTACC
1015	TTGAAGGGCGTATCTATAAGAATGCCGTTC
1016	TCGCAAGTAGAAAAGGTCAAACATATCGTAACAGTG
1030	ACTGGTGC GGCCGCATACCCCTTTCGACGAACGTGG
1031	ACTGGTCCATGGCATAACCCCTTTCGACGAACGTGG
1032	ACTGGTGGTACCCACGTGAAGGTCATTACCAGTTCAGCGATTG
1033	ACTACCGAGGATCCGTATAACGAGGTCATTACCAGTTCAGCGATTG
1100	GCGCAACTGGACATCCTGGAGACCC
1101	GGAAGTTGCGATCAAGATCAATCTCCCTGAG
1105	GATGTCGGGATAACCGCTCTTGCCG
1107	CAGGGTCTCCAGGATGTCCAGTTGCGC
1202	GCGTTCTCCAGAACACATTACCCAGATGTC
1203	GGCAATGAAAATAGGTCTCACGGAAGCC
1207	GGCTTCCGTGAGACCTATTTTCATGCC
1208	GACATCTGGGTAATGTGTTCTGGAGAACGC
1213	GGAATTTTCACTGTTCCCTTTACCACGTTTACTG
1214	TATGGTGAATTTTCAGCGAACTCGCTGTGCAGGG
1215	TTCATGTTTTGATCTCTCTTTTGGGTGAACATCGCG
1216	GGGGAAGTGTTTTCTTTGATGTTTAGTCTAACCAGC
1300	CCGGATGTGTTTACCAGGGAGGAGTTGGC
1301	GAAGATAGGCCTGACGGAGGCACGC
1302	CTTCCCTCAGCTTATCCTCGACCAGCGCTACC
1304	AGCGCAACTGTCACACCGATCCAAAATAGACCC
1305	GCGTGCCTCCGTCAGGCCTATCTTC
1306	CACATCCGGATAATGAGTCCTGGAAAACAC
1314	AATGGGAATTCTGGAAGAGTGCAGCGTC

oligonucleotide	5'-3' sequence
1315	CTCACGCTGTAATTTCCGCCAGCTTTTCG
1400	ACGAGCAGCTGCAGGTGCTCCAGGC
1401	CCCTGACAGCCAGGACCTGGAGCGC
1405	CTTGCTCAGCCCCGTGAGCTGTGCG
1406	GCGCTCCAGGTCCTGGCTGTCAGGG
1414	GATGATGGCCAGCGCAACAGTTCTGTTGCC
1415	GTCATGCTTCTCCAGGCATGAGCGTCATAAGCG
1500	CGTTCCAACCTCCACCAGCTGGAGCG
1501	CCCAATATCCTGACGTCTTCACTCGAGAGG
1503	ATTCCGGTTTAGCCACAACGGCACCTC
1504	CGTTTCCGCACACAATGTTGCCGTTATC
1505	CGCGTGCCCTCGGATAGGTCAAGGTTGAGTG
1506	GCCAACTCCTCTCGAGTGAAGACGTCAG
1507	CTCGAACGCCCGCTCCAGCTGGTGG
1512	TTTACTGAGTCGAGGAGATACTTACCAAAGTG
1514	TTGACTGGTGACATAAACTCTAGACCG
1515	CGCCTGACACGTCACAACAATCG
1516	GTATGTCATGGGGTTTATCCCTTGATCACGAT
1601	GGAAATTCTGGAGCAGGAGTTCTGCAGC
1602	CGCGAGCACCTGGCAGAGAGGACTG
1605	CGCGAGTCGTAATGTCCGGGTATTGGTTGC
1606	GCCTCAGTGAGCCCAGTCCTCTCTGC
1607	GCTGCAGAACTCCTGCTCCAGAATTTCC
1614	AATGGAGTGCATGTATAGCTTTAGGCCTCAG
1615	ACTAATAAGAAGCTTCCGGGGGATTCCCGC
1616	CTCAAGCGAGGCCACGGACATCGG
1800	GGAGCATTCACTTGCGAAGGATGCAAGAGT
1801	TGTCTAGTTGTGGGCATGTCTAAAACGGGG
1805	TTAGACCTGCGGCCGTACCGTGACCCCG
1806	TGAGACGACAGGCCTTGACGAAGTCCTG
1813	TCACTGTTCCCTTTACCACGTTTACTG
1814	GATGAACCAACTGTGCAAGGTCTGTGCTGAAC
1815	GTCAGGTGATGAGGCCATCACCAGCAGTTTC
1816	CAACAGTTAGACTTAACTACTCAATTCAGTCCTTAC
1817	GGAAATGAATTTATAAGAAATAGCTGCTAAGC
1860	CCACGTTTTACTGGACAAATTCGG
1864	CGTCAACACTCAGTTGAGGGACGC
1865	CTCCGTAGTGCATTGAGAGTTTCTTCC
1900	GGAGCTTTTACGTGTGAAGGCTGCAAGTCC
1901	CGGAAATGCCTTTTCGTTGGAATGTCCAAAAGT

oligonucleotide	5'-3' sequence
1903	CACTCCGGCTTTTCGTTACTACAAGCATCAC
1904	CAATTGCACCACCTCAACAATCGCCATCACC
1905	CTTGATCCACTTTTGGACATTCCAACGAAAAGG
1906	CTCCCGAAGAAGGACTTGCAGCCTTCACAC
1913	AGTTGTGCTCGTGCTGGCCG
1914	CATGACAGTCGCAAATTATCTCAGGATGAATCAG
1915	TGCATCACCGATTCCGAAACTATCAAGCATTGG
1918	GGCTAATAGTTTGTCTATCGGGTTGTG
2000	CTTTGGGACCCGTCGACAAGTACCGCGTCAG
2001	GAGCAGAAGTCCCCTGTTTCAAGGAACGCACG
2005	GCCAGTTCCCCTTCTGCTGGGGTTGGGTAC
2006	AGGGACCGCGTGCCTTCTTGAACAGTGGGAC
2007	TTCCTGACCGGTACTIONTGTGACGGGTCCC
2008	GACAGCCCCGCCAGGAGCGCCATG
2013	AGTGCTGCAAGCGCCACCGACAC
2014	GACCTTCTGCGGCCCTGCAGCCCAAC
2015	CGAGAGCTAATAGGAGACAATAAAAAATGTTGAG
2016	GGATCCGAATGGCAACGTTCCGGC
2100	TGCCGTCGACAAGTACCGCATAACGCAAG
2101	ACCATCTATTGCTTCAAAGAGAAGTCCAGGATG
2105	GTGTGAGGCCGGTCTGGCGGGAC
2106	CTTCTCATCAGGGGTCCGGTAGCGGTTT
2114	AGTTCATGATTGAACTTTGTTTTTTAAATGTATCAAG
2115	CTCCATGAAACTGTCCCAATCGATGGTACGTG
2301	CGCAGAGCACCGCGGCACCGGCAG
2303	CCAAACGAAGAAATATCTGACGCTGAGCGAG
2305	TCACTGAGGTGCAGTGTCTGAGCAATGTGC
2306	ACCGCTCGCTCAGCGTCAGATATTTCTTCG
2314	AGTGCGCATTCGATCATTGTTTCATCCCAGG
2315	AGTGCACGATGATATTCTTTATCACCAATCTGTTT
6114	ACTGGTCATATGCGGCCGTGACTCTAGATCATAATCAG
6115	TACCGACGTACGGCGGCCGCTTAAGATACATTGATGAGTTTGGAC
6950	CTCAAGGCGCGCCTTCCGCGGTTACTGTAACCGCAGGGGCAAAAG
6951	GAATACCATGGTTGTGGATTGTTCTGTGAAC
8100	ACTGGTGCGGCCGCATGGCCTCCTCCGAGGACGTC
8101	ACTGGTCCATGGATGGCCTCCTCCGAGGACGTC
8104	TACCGAGGTACCCTACAGGAACAGGTGGTGGCG
8105	ACTACCGAGGATCCCTACAGGAACAGGTGGTGGCG
8250	GTTAATTAAGCATGGTGTAGCAAGGGCGAGGAGCTGTTACCGGTGAGTACCAGTATTAAA
8251	ATCTGAGCTCGACCAGGATGGGCACCACACCTGAGAGGACGAGAACAAAGGAAAC

oligonucleotide	5'-3' sequence
8258	AGATGAGCTCGACGGCGACGTAAACGGCC
8259	CATCCTGCAGGTTACTTGTACAGCTCG
9600	ACTACCGAGGATCCAAGCACAGATGCTTCG TTCAG
9601	ACTGGTGGTACCCGAACCCAGAGTCCCGC
9700	CGCGCCGAATTCGTTTAAACACGTGCGTACGGGCCACGCGTCCATGGCCGG
9701	CCATGGACGCGTGGGCCCGTACGCACGTGTTTAAACGAATTCGG

"PhD_A4.3_siRNA_molecules.pdf"

A4.3 siRNA

Table A4: List of siRNA molecules. Shown are, from left to right: the *Parhyale* gene sequence from which a siRNA molecule was derived, its name, the 5' to 3' sequence, the corresponding position within the respective cDNA and the mRNA region in which the position lay. siRNA molecules were synthesized as STEALTH siRNA duplexes. Here, only the nucleic acid sequence in orientation of the mRNA/cDNA is shown.

<i>Ph kni1</i>	si1	CAGTCGCAAATTATCTCAGGATGAA	703-727	5' ORF
<i>Ph kni1</i>	si2	GGCCAGTGTGTGATCAACAAGCAAA	858-882	5' ORF encoding Zinc Finger domain
<i>Ph kni1</i>	si8	ACCTCAATCTTTACCTGCCCGTCAT	1607-1631	3' ORF
<i>Ph six3</i>	si4	CGTGCCCTTGTTGCTTTCCACTTAG	339-363	5' ORF encoding Six domain
<i>Ph six3</i>	si6	GACGCAGATACTGCCTTGAAGTGCA	1014-1038	3' ORF
<i>Ph six3</i>	si9	GGCACTTCTTGGAGCACCAAATCTT	1137-1161	3' ORF

[PhD_A5_plasmid_sequences]

"pSL_shuttle.txt"

>pSL_shuttle 'pSL_Phsp70_SV40 clone# fcl00+16

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>Ph_hsp70 'amplified via PCR (6950/6951), using pSL_Hsp70_En2 as template (courtesy of Anastassios Pavlopoulos)

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>SV40polyA 'amplified via PCR (6114/6115), using pMi[3xP3_EGFP] as template (Horn C and Wimmer EA)

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ATATG

>pSLfal180fa 'Horn C and Wimmer EA, 1999

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TGATGTTACAGTGGGCCATCGCCCTGATAGAGGTTTTTCCGCTTTCAGCTTGGAGTCCAGCTTCTTAAATAGTGGACTTGTTCACAACTGGAACAACCTCAACCTATCTCGTCTATTC
TTTTGATTTAAGGATTTTCCGATTTCCGCTATTGGTTAAAAATAGCTGATTTAACAAAATTTAACCGGAAATTTAACAAAATTTAACGCTTCAATTTAG

"PhD_A6.1_List_of_species.pdf"

A6 Phylogeny database

A6.1 List of species included in phylogenetic analyses

Table A5: List of species included in phylogenetic analyses. The taxonomic classification of the species was adopted from the NCBI taxonomy database (Sayers et al., 2009; Benson et al., 2010; see below). Shown are (from left to right): common name, scientific name, phylum, class and order (or, if no order is classified, family) to which the species belongs and taxonomy identification number.

common name	scientific name	phylum	class	order (family)	taxonomy ID
pea aphid	<i>Acyrtosiphon pisum</i>	Arthropoda	Insecta	Hemiptera	7029
mosquito	<i>Anopheles gambiae</i>	Arthropoda	Insecta	Diptera	7165
western honey bee	<i>Apis mellifera</i>	Arthropoda	Insecta	Hymenoptera	7460
nematode	<i>Caenorhabditis elegans</i>	Nematoda	Chromadorea	Rhabditida	6239
sea louse	<i>Caligus rogercresseyi</i>	Arthropoda	Maxillopoda	Siphonostomatoidea	217165
white-tufted-ear marmoset	<i>Callithrix jacchus</i>	Chordata	Mammalia	Primates	9483
Mediterranean fruit fly	<i>Ceratitis capitata</i>	Arthropoda	Insecta	Diptera	7213
zebra fish	<i>Danio rerio</i>	Chordata	Actinopterygii	Cypriniformes	7955
fruit fly	<i>Drosophila grimshawi</i>	Arthropoda	Insecta	Diptera	7222
vinegar fly	<i>Drosophila melanogaster</i>	Arthropoda	Insecta	Diptera	7227
fruit fly	<i>Drosophila mojavensis</i>	Arthropoda	Insecta	Diptera	7230
fruit fly	<i>Drosophila pseudoobscura</i>	Arthropoda	Insecta	Diptera	7237
fruit fly	<i>Drosophila virilis</i>	Arthropoda	Insecta	Diptera	7244
velvet worm	<i>Euperipatoides kanangrensis</i>	Onychophora	n. c.	Peripatopsidae (family)	488523
Asian lady beetle	<i>Harmonia axyridis</i>	Arthropoda	Insecta	Coleoptera	115357
deer tick	<i>Ixodes scapularis</i>	Arthropoda	Arachnida	Ixodida	6945
house mouse	<i>Mus musculus</i>	Chordata	Mammalia	Rodentia	10090
parasitoid wasp	<i>Nasonia vitripennis</i>	Arthropoda	Insecta	Hymenoptera	7425
starlet sea anemone	<i>Nematostella vectensis</i>	Cnidaria	Anthozoa	Actiniaria	45351

milkweed bug	<i>Oncopeltus fasciatus</i>	Arthropoda	Insecta	Hemiptera	7536
beach hopper	<i>Parhyale hawaiiensis</i>	Arthropoda	Malacostraca	Amphipoda	317513
human body louse	<i>Pediculus humanus corporis</i>	Arthropoda	Insecta	Phthiraptera	121224
Dumeril's clam worm	<i>Platynereis dumerilii</i>	Annelida	Polychaeta	Phyllodocida	6359
Acorn worm	<i>Saccoglossus kowalevskii</i>	Hemichordata	Enteropneustata	Harrimaniidae (family)	10224
centipede	<i>Strigamia maritima</i>	Arthropoda	Chilopoda	Geophilomorpha	126957
red flour beetle	<i>Tribolium castaneum</i>	Arthropoda	Insecta	Coleoptera	7070
parasitic nematode	<i>Trichinella spiralis</i>	Nematoda	Enoplea	Trichocephalida	6334
African clawed frog	<i>Xenopus laevis</i>	Chordata	Amphibia	Anura	8355

In acknowledgment of:

Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J. and Sayers, E. W. (2010) 'GenBank', *Nucleic acids research* 38(Database issue): D46-51.

Sayers, E. W., Barrett, T., Benson, D. A., Bryant, S. H., Canese, K., Chetvernin, V., Church, D. M., DiCuccio, M., Edgar, R., Federhen, S. et al. (2009) 'Database resources of the National Center for Biotechnology Information', *Nucleic acids research* 37(Database issue): D5-15.

"PhD_A6.2_List_of_non-Parhyale_sequences.pdf"

A6.2 List of non-*Parhyale* protein sequences included in phylogenetic analyses

Table A6: List of non-*Parhyale* protein sequences included in phylogenetic analyses. Shown are (from left to right): name tag of protein sequences used in the phylogenetic calculations in this work, (homologous) gene the respective protein sequence derives from, species the protein sequence was isolated from and NCBI reference sequence number, reflecting accession number and version of the sequence.

name used in phylogeny	gene/homolog	species	reference
Agam_AWH	<i>arrowhead</i>	<i>Anopheles gambiae</i>	XM_316578.4
Agam_OPTIX	<i>optix</i>	<i>Anopheles gambiae</i>	XM_559661.2
Agam_SIX4	<i>six4</i>	<i>Anopheles gambiae</i>	XM_309580.4
Agam_SO	<i>sine oculis</i>	<i>Anopheles gambiae</i>	XM_320814.4
Agam_UNPG	<i>unplugged</i>	<i>Anopheles gambiae</i>	XM_308835.4
Amel_AWH	<i>arrowhead</i>	<i>Apis mellifera</i>	XM_001121365.1
Amel_EG	<i>eagle</i>	<i>Apis mellifera</i>	XM_001120662.1
Amel_KNI	<i>knirps</i>	<i>Apis mellifera</i>	XM_001120531.1
Amel_KNRL	<i>knirps related</i>	<i>Apis mellifera</i>	XM_395932.2
Amel_SIX2	<i>six2</i>	<i>Apis mellifera</i>	XM_396811.3
Amel_SIX3	<i>six3</i>	<i>Apis mellifera</i>	XM_623761.2
Amel_SIX4	<i>six4</i>	<i>Apis mellifera</i>	XM_001120698.1
Cele_H-32	<i>ceh-32</i>	<i>Caenorhabditis elegans</i>	NM_073557.3
Cele_H-33	<i>ceh-33</i>	<i>Caenorhabditis elegans</i>	NM_072019.1
Cele_H-34	<i>ceh-34</i>	<i>Caenorhabditis elegans</i>	NM_072018.5
Cele_LIM-4	<i>lim-4</i>	<i>Caenorhabditis elegans</i>	NM_076268.1
Crog_KNI	<i>knirps</i>	<i>Caligus rogercresseyi</i>	ACO11525.1
Dmel_AL	<i>aristless</i>	<i>Drosophila melanogaster</i>	NM_164382.1
Dmel_AP	<i>apterous</i>	<i>Drosophila melanogaster</i>	NM_165445.3
Dmel_AWH	<i>arrowhead</i>	<i>Drosophila melanogaster</i>	NM_168042.1
Dmel_B-H1	<i>BarH1</i>	<i>Drosophila melanogaster</i>	NM_078663.2
Dmel_CG11085	<i>CG11085</i>	<i>Drosophila melanogaster</i>	NM_132587.3
Dmel_CG32105	<i>CG32105</i>	<i>Drosophila melanogaster</i>	NM_168497.1
Dmel_CG34340	<i>CG34340</i>	<i>Drosophila melanogaster</i>	NM_001103605.1
Dmel_CG34367	<i>CG34367</i>	<i>Drosophila melanogaster</i>	NM_001103670.2
Dmel_CG4328	<i>CG4328</i>	<i>Drosophila melanogaster</i>	NM_140310.2
Dmel_CG9876	<i>CG9876</i>	<i>Drosophila melanogaster</i>	NM_137912.1
Dmel_DR	<i>drop</i>	<i>Drosophila melanogaster</i>	NM_057976.2
Dmel_EG	<i>eagle/egon</i>	<i>Drosophila melanogaster</i>	NM_079482.3
Dmel_ERR	<i>estrogen-related receptor</i>	<i>Drosophila melanogaster</i>	NM_139926.2
Dmel_EXEX	<i>extra-extra</i>	<i>Drosophila melanogaster</i>	NM_139907.2

Dmel_EY	<i>eyeless</i>	<i>Drosophila melanogaster</i>	NM_166789.1
Dmel_GSB	<i>gooseberry</i>	<i>Drosophila melanogaster</i>	NM_079139.2
Dmel_GSB-N	<i>gooseberry-neuro</i>	<i>Drosophila melanogaster</i>	NM_079138.1
Dmel_GSC	<i>goosecoid</i>	<i>Drosophila melanogaster</i>	NM_001144290.1
Dmel_HBN	<i>homeobrain</i>	<i>Drosophila melanogaster</i>	NM_176240.1
Dmel_KNI	<i>knirps</i>	<i>Drosophila melanogaster</i>	NM_079463.2
Dmel_KNRL	<i>knirps related</i>	<i>Drosophila melanogaster</i>	NM_176374.1
Dmel_LIM1	<i>Lim1</i>	<i>Drosophila melanogaster</i>	NM_132277.2
Dmel_LIM3	<i>Lim3</i>	<i>Drosophila melanogaster</i>	NM_165277.1
Dmel_MU	<i>munster</i>	<i>Drosophila melanogaster</i>	NM_057982.3
Dmel_OPTIX	<i>optix</i>	<i>Drosophila melanogaster</i>	NM_079956.2
Dmel_OTP	<i>orthopedia</i>	<i>Drosophila melanogaster</i>	NM_001103918.2
Dmel_PHDP	<i>Putative homeodomain protein</i>	<i>Drosophila melanogaster</i>	NM_079110.2
Dmel_PRDA	<i>paired, isoform A</i>	<i>Drosophila melanogaster</i>	NM_078832.2
Dmel_PRDB	<i>paired, isoform B</i>	<i>Drosophila melanogaster</i>	NM_164990.2
Dmel_REPO	<i>reverse polarity</i>	<i>Drosophila melanogaster</i>	NM_057678.3
Dmel_RX	<i>retinal homeobox</i>	<i>Drosophila melanogaster</i>	NM_166413.2
Dmel_SIX4	<i>six4</i>	<i>Drosophila melanogaster</i>	NM_140999.2
Dmel_SLOU	<i>slouch</i>	<i>Drosophila melanogaster</i>	NM_057309.2
Dmel_SO	<i>sine oculis</i>	<i>Drosophila melanogaster</i>	NM_057385.4
Dmel_SV	<i>shaven</i>	<i>Drosophila melanogaster</i>	NM_079894.3
Dmel_TOY	<i>twin of eyeless</i>	<i>Drosophila melanogaster</i>	NM_079899.3
Dmel_TUP	<i>tailup/islet</i>	<i>Drosophila melanogaster</i>	NM_057427.2
Dmel_UNPG	<i>unplugged</i>	<i>Drosophila melanogaster</i>	NM_057798.2
Dpse_SIX4	<i>six4</i>	<i>Drosophila pseudoobscura</i>	XM_001352978.2
Dpse_AWH	<i>arrowhead</i>	<i>Drosophila pseudoobscura</i>	XM_001353513.2
Dpse_EG	<i>eagle</i>	<i>Drosophila pseudoobscura</i>	XM_001353471.2
Dpse_KNI	<i>knirps</i>	<i>Drosophila pseudoobscura</i>	XM_001352966.2
Dpse_KNRL	<i>knirps related</i>	<i>Drosophila pseudoobscura</i>	XM_001352963.2
Dpse_OPTIX	<i>optix</i>	<i>Drosophila pseudoobscura</i>	XM_001360625.2
Dpse_SO	<i>sine oculis</i>	<i>Drosophila pseudoobscura</i>	XM_001360175.2
Dpse_UNPG	<i>unplugged</i>	<i>Drosophila pseudoobscura</i>	XM_001361091.1
Drer_Gbx1	<i>gastrulation brain homeobox 1</i>	<i>Danio rerio</i>	NM_174861.2
Drer_Gbx2	<i>gastrulation brain homeobox 2</i>	<i>Danio rerio</i>	NM_152964.1
Drer_Lhx6	<i>lhx6</i>	<i>Danio rerio</i>	NM_001004015.1
Drer_Lhx8a	<i>lhx8a</i>	<i>Danio rerio</i>	NM_001003980.1
Drer_Six1a	<i>sine oculis homeobox homolog 1a</i>	<i>Danio rerio</i>	NM_001009904.1
Drer_Six1b	<i>sine oculis homeobox homolog 1b</i>	<i>Danio rerio</i>	NM_207095.1
Drer_Six2.1	<i>sine oculis homeobox homolog 2.1</i>	<i>Danio rerio</i>	NM_131783.2
Drer_Six3a	<i>sine oculis homeobox homolog 3a</i>	<i>Danio rerio</i>	NM_131362.1
Drer_Six3b	<i>sine oculis homeobox homolog 3b</i>	<i>Danio rerio</i>	NM_131363.1
Drer_Six4.1	<i>sine oculis homeobox homolog 4.1</i>	<i>Danio rerio</i>	NM_131717.1
Drer_Six4.2	<i>sine oculis homeobox homolog 4.2</i>	<i>Danio rerio</i>	NM_131718.1
Drer_Six4.3	<i>sine oculis homeobox homolog 4.3</i>	<i>Danio rerio</i>	NM_131720.1
Drer_Six6a	<i>sine oculis-related homeobox 6a</i>	<i>Danio rerio</i>	NM_201105.1
Drer_Six6b	<i>sine oculis-related homeobox 6b</i>	<i>Danio rerio</i>	NM_001020585.1
Drer_Six7	<i>sine oculis homeobox homolog 7</i>	<i>Danio rerio</i>	NM_131354.2
Drer_Six9	<i>sine oculis homeobox homolog 9</i>	<i>Danio rerio</i>	NM_001130608.1
Drer_Zgc_195096	<i>zgc:195096</i>	<i>Danio rerio</i>	NM_001128734.1

Dvir_AWH	<i>arrowhead</i>	<i>Drosophila virilis</i>	XM_002046523.1
Dvir_EG	<i>eagle</i>	<i>Drosophila virilis</i>	XM_002048604.1
Dvir_KNI	<i>knirps</i>	<i>Drosophila virilis</i>	XM_002048059.1
Dvir_KNRL	<i>knirps related</i>	<i>Drosophila virilis</i>	XM_002048061.1
Dvir_OPTIX	<i>optix</i>	<i>Drosophila virilis</i>	XM_002050350.1
Dvir_SIX4	<i>six4</i>	<i>Drosophila virilis</i>	XM_002048047.1
Dvir_SO	<i>sine oculis</i>	<i>Drosophila virilis</i>	XM_002049752.1
Dvir_UNPG	<i>unplugged</i>	<i>Drosophila virilis</i>	XM_002059265.1
Isca_Xvent-2B	<i>xvent-2B</i>	<i>Ixodes scapularis</i>	XM_002404613.1
Mmus_Alx1	<i>ALX homeobox 1</i>	<i>Mus musculus</i>	NM_172553.4
Mmus_Alx3	<i>aristaless-like homeobox 3</i>	<i>Mus musculus</i>	NM_007441.2
Mmus_Alx4	<i>aristaless-like homeobox 4</i>	<i>Mus musculus</i>	NM_007442.2
Mmus_Arx	<i>aristaless related homeobox</i>	<i>Mus musculus</i>	NM_007492.3
Mmus_Esr1	<i>estrogen receptor 1 (alpha)</i>	<i>Mus musculus</i>	NM_007956.4
Mmus_Esr2	<i>estrogen receptor 2 (beta)</i>	<i>Mus musculus</i>	NM_207707.1
Mmus_Gbx1	<i>gastrulation brain homeobox 1</i>	<i>Mus musculus</i>	NM_015739.2
Mmus_Gbx2	<i>gastrulation brain homeobox 2</i>	<i>Mus musculus</i>	NM_010262.3
Mmus_Gsc	<i>gooseoid homeobox</i>	<i>Mus musculus</i>	NM_010351.1
Mmus_Gsc2	<i>gooseoid homeobox 2</i>	<i>Mus musculus</i>	NM_029469.1
Mmus_Isl1	<i>ISL1 transcription factor, LIM/homeodomain</i>	<i>Mus musculus</i>	NM_021459.4
Mmus_Isl2	<i>insulin related protein 2</i>	<i>Mus musculus</i>	NM_027397.3
Mmus_Lhx1	<i>LIM homeobox protein 1</i>	<i>Mus musculus</i>	NM_008498.2
Mmus_Lhx2	<i>LIM homeobox protein 2</i>	<i>Mus musculus</i>	NM_010710.3
Mmus_Lhx3	<i>LIM homeobox protein 3</i>	<i>Mus musculus</i>	NM_001039653.1
Mmus_Lhx4	<i>LIM homeobox protein 4</i>	<i>Mus musculus</i>	NM_010712.2
Mmus_Lhx5	<i>LIM homeobox protein 5</i>	<i>Mus musculus</i>	NM_008499.5
Mmus_Lhx6	<i>LIM homeobox protein 6</i>	<i>Mus musculus</i>	NM_001083125.1
Mmus_Lhx8	<i>LIM homeobox protein 8</i>	<i>Mus musculus</i>	NM_010713.2
Mmus_Lhx9	<i>LIM homeobox protein 9</i>	<i>Mus musculus</i>	NM_001042577.1
Mmus_Lmx1a	<i>LIM homeobox transcription factor 1 alpha</i>	<i>Mus musculus</i>	NM_033652.5
Mmus_Lmx1b	<i>LIM homeobox transcription factor 1 beta</i>	<i>Mus musculus</i>	NM_010725.2
Mmus_Meox1	<i>mesenchyme homeobox 1</i>	<i>Mus musculus</i>	NM_010791.3
Mmus_Meox2	<i>mesenchyme homeobox 2</i>	<i>Mus musculus</i>	NM_008584.3
Mmus_Mnx1	<i>motor neuron and pancreas homeobox 1</i>	<i>Mus musculus</i>	NM_019944.2
Mmus_Nkx1-2	<i>NK1 transcription factor related, locus 2</i>	<i>Mus musculus</i>	NM_009123.2
Mmus_Otp	<i>orthopedia</i>	<i>Mus musculus</i>	NM_011021.3
Mmus_Pax2	<i>paired box gene 2</i>	<i>Mus musculus</i>	NM_011037.3
Mmus_Pax3	<i>paired box gene 3</i>	<i>Mus musculus</i>	NM_008781.4
Mmus_Pax6	<i>paired box gene 6</i>	<i>Mus musculus</i>	NM_013627.4
Mmus_Pax7	<i>paired box gene 7</i>	<i>Mus musculus</i>	NM_011039.2
Mmus_Phox2a	<i>paired-like homeobox 2a</i>	<i>Mus musculus</i>	NM_008887.2
Mmus_Phox2b	<i>paired-like homeobox 2b</i>	<i>Mus musculus</i>	NM_008888.3
Mmus_Prx1	<i>paired related homeobox protein-like 1</i>	<i>Mus musculus</i>	NM_001001796.4
Mmus_Rax	<i>retina and anterior neural fold homeobox</i>	<i>Mus musculus</i>	NM_013833.2
Mmus_Six1	<i>sine oculis-related homeobox 1 homolog</i>	<i>Mus musculus</i>	NM_009189.3
Mmus_Six2	<i>sine oculis-related homeobox 2 homolog</i>	<i>Mus musculus</i>	NM_011380.2
Mmus_Six3	<i>sine oculis-related homeobox 3 homolog</i>	<i>Mus musculus</i>	NM_011381.4
Mmus_Six4	<i>sine oculis-related homeobox 4 homolog</i>	<i>Mus musculus</i>	NM_011382.2
Mmus_Six5	<i>sine oculis-related homeobox 5 homolog</i>	<i>Mus musculus</i>	NM_011383.1

Mmus_Six6	<i>sine oculis-related homeobox 6 homolog</i>	<i>Mus musculus</i>	NM_011384.4
Mmus_Uncx	<i>UNC homeobox</i>	<i>Mus musculus</i>	NM_013702.3
Nvec_GBX	<i>gbx</i>	<i>Nematostella vectensis</i>	ABF61779.1
Nvec_SIX3	<i>six3</i>	<i>Nematostella vectensis</i>	XM_001625109.1
Nvec_SIX4.1	<i>six4.1</i>	<i>Nematostella vectensis</i>	XM_001623084.1
Nvec_SIX4.2	<i>six4.2</i>	<i>Nematostella vectensis</i>	XM_001634946.1
Nvec_SO	<i>sine oculis</i>	<i>Nematostella vectensis</i>	XM_001626384.1
Nvit_KNI	<i>knirps</i>	<i>Nasonia vitripennis</i>	XM_001604919.1
Nvit_UNPG	<i>unplugged</i>	<i>Nasonia vitripennis</i>	XP_001602252.1
Ofas_KNI1	<i>knirps 1</i>	<i>Oncopeltus fasciatus</i>	Jonas Schwirz
Ofas_KNI2	<i>knirps 2</i>	<i>Oncopeltus fasciatus</i>	Jonas Schwirz
Pdum_GBX	<i>gbx</i>	<i>Platynereis dumerilii</i>	AJ505024.1
Phum_GBX1	<i>Homeobox protein GBX-1, putative, mRNA</i>	<i>Pediculus humanus</i>	XM_002423021.1
Tcas_KNI	<i>knirps</i>	<i>Tribolium castaneum</i>	NM_001128495.1
Tcas_AWH	<i>arrowhead</i>	<i>Tribolium castaneum</i>	XM_966109.1
Tcas_EG	<i>eagle</i>	<i>Tribolium castaneum</i>	NM_001114367.1
Tcas_OPTIX	<i>optix/six3</i>	<i>Tribolium castaneum</i>	NM_001113467.1
Tcas_SIX4	<i>six4</i>	<i>Tribolium castaneum</i>	EFA01346.1
Tcas_SO	<i>sine oculis</i>	<i>Tribolium castaneum</i>	XM_967074.2
Tcas_UNPG	<i>unplugged</i>	<i>Tribolium castaneum</i>	XM_969836.1

[PhD_A6_phylogeny_database]

[PhD_A6.3_phylogeny_ClustalW2_alignments]

"alignment_aristaless-group.txt"

Alignment of the HD protein sequences of various aristaless-group genes from different species. This was done using the ClustalW2 algorithm (Larkin et al., 2007). For details and references regarding the species' protein sequences, see Appendix (A7).

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Phaw_HBN      -GTDLEKPRKQVRRSRTTFTTFLHQLERAFKTYQPDVFTREELALNLDLSEARVQVWFQNRRAKWRKREKAMGRES-
Dmel_HBN      -DLSDMERPRK-VRRSRTTFTTFLHQLERAFKTYQPDVFTREDLAMLRLDLSEARVQVWFQNRRAKWRKREKFMNQDKAG-
Dmel_RX       -GQDDNCAKHKHRRNRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREKSESLRGL-
Mmus_Rax      -LSEEEPPKKHRRNRTTFTTFLHQLERAFKTYQPDVFTREELAGKVNLPVSRVQVWFQNRRAKWRKREKLEVS-
Dmel_REPO     -KKKGDPNGIKKKTRTFTTFLHQLERAFKTYQPDVFTREELAIKLNLSERVQVWFQNRRAKWRKREKHEPPRK-TGYIK
Mmus_Uncx     -DPDKESPGCKRRRTNFTTFLHQLERAFKTYQPDVFTREELALNLDLSEARVQVWFQNRRAKWRKREKTKKGPGR-
Dmel_OTP      -NNNNSMQQDQKRHRTRFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREKKT-
Mmus_Otp      -QAGQQQQQKQKRHRTRFTTFLHQLERAFKTYQPDVFTREELALNLDLSEARVQVWFQNRRAKWRKREKKT-
Dmel_CG34367  -PLDTSLVNTKQRRNRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Mmus_Al4      -KTDSESNKGGKRRNRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Mmus_Al1      -KCDNSVSSSKRRHRRTTFTTFLHQLERAFKTYQPDVFTREELALNLDLSEARVQVWFQNRRAKWRKREK-
Mmus_Al3      -SMELAKTKSKRRNRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Phaw_AL1      -GDEVTPNKKKQRRYRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Phaw_AL2      -GDTAGPTKRKQRRYRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Dmel_AL       -EADYAP-KRKQRRYRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Mmus_Arx      -DSEEGLLKRRQRRYRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Dmel_MU       --MDKGTMKRRQRRYRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Dmel_CG34340 -IHDETFVRRKQRRNRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Mmus_Prx11    -DFDDGFLRRKQRRNRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Mmus_Phox2b   -DHGGLNKKRRQRRYRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Mmus_Phox2a   -EPSGLHEKRRQRRYRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Dmel_PHDP     -DGLSLTDKSKQRRYRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Dmel_CG9876   -PAGGGHHSRPRNRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Mmus_Gsc      -QLLNQLHCRKRRHRRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Mmus_Gsc2     -GTSFGPQRRTRRRRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
Dmel_GSC      -HLHGPPPKRRHRRTTFTTFLHQLERAFKTYQPDVFTREELAMKVNLPVSRVQVWFQNRRAKWRKREK-
```

[PhD_A6_phylogeny_database]

[PhD_A6.3_phylogeny_ClustalW2_alignments]

“alignment_Arrowhead-Lhx68_proteins.txt”

Alignment of the protein sequence fractions of various arrowhead and Lhx6/8 genes from different species, encompassing LIM1, LIM2 and HD domains.

This was done using the ClustalW2 algorithm (Larkin et al., 2007).

For details and references regarding the species' protein sequences, see Appendix (A7).

```
Dmel_AWH      TELRSCAACGEPISDRFFLEVGGCSWHAHCLRCCMCMPLDRQQSCFIRERQVYCK-----
ADYSKNFGAKSKCCRGISASDWRVRRARELVFHLACFACDQCGRQLSTGEQFALMDDRVLCKAHYLETVEGGTSSDEGCDGDGYHKS-----
KTKRVRTTFTEEQQLVQLANFQIDSNPDGQDLERIASVTGLSKRVTQVWFQNSRARQKKHHI-AGKNKIREPEGSS-----FARHINLQLTYSFQ
Dpse_AWH      TELRSCAACGEPISDRFFLEVGGCSWHAHCLRCCMCMPLDRQQSCFIRERQVYCK-----
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Dvir_AWH      -----MCMCPLDRQQSCFIRERQVYCK-----
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Agam_AWH      KELRSCTACGEPISDQFLLDVGGCSWHAHCLRCCICHTPLDHQPSCFLRERQIYCK-----
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Tcas_AWH      TEYRLCSACGEPISDKFLEEVSGRSWHARCLRCCVCQLQDRQPSCFIRDRAIYCK-----
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Amel_AWH      ANVMCEGCGGERVRETRVLCVGGRTWHSRCLRCFACARPLHDQHSFCFLKGMRLYCR-----
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Phaw_AWH      PTTESCAGCARIITDRFLLRVNCLSWHQSLRCCVCQLALDRQPSCFIRDQNIYCK-----
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SKRVRTTFTEEQQLVQLANFQIDSNPDGQDLERIAQITGLSKRVTQVWFQNSRARQKKHMTSKKHQGLERGLHDITG-----SLARTIDLHFMYSTR
Cele_LIM-4    AVIVICTQCQHQIQDKFFLSIDGRNYHENCLQCSTCENPLSN--
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KTKRVRTTFTEEQQLVQLANFQIDSNPDGADLEKIASMTGLSKRVTQVWFQNSRARQKKHHQ-KSEGDNGDSQRS-----VGPSSPSQKSDSS
Mmus_Lhx6     AGKNICSSCGLEILDRLYLLKVNLIWHVRCLECSVCRTSLRQNSCYIKNKEIFCK-----
MDYFSRFGTKCARCGRIYASDWRVRRARGNAYHLACFACFCKRQLSTGEEFGLVEEKVLCRIHYDTMIENLKRAAENGLTLEGAVPSEQDS-
QPKPAKRARTSFTAELQVMAQFAQDNNPDAQTLQKLADMTGLSRRVIQVWFQNCRARHKKHT-PQHP--VPPSGAPPTR-----LFSALSDDIHYSFP
Dreer_Lhx6    TGKNVCASCGLEILDRLYLLKVNLIWHVRCLECSVCRTSLRQHSYCYIKNKEIFCK-----
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QPKPAKRARTSFTAELQVMAQFAQDNNPDAQTLQKLADMTGLSRRVIQVWFQNCRARHKKHT-PQHN--GPPQAHFQAR-----MPPSLPDELHYSFP
Mmus_Lhx8     PGKVCSSCGLEIVDKYLLKVNLCVHVRCLSCSVCRTSLGRHTSCYIKDKDIFCK-----
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Dreer_Lhx8a   -EKAICTSCGTEIVDKYLLKVNLCVHVRCLSCSVCRTSLGRHISYIKEKEIFCK-----
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-----ASS-PELDEPIDLSLRS-----
Dvir_EG MNQLCKVCGEPAAGFHGFAFTCEGCKSFPGRTYN--NIPAIAGCKHNGDCVINKNRTACKACRLRCKLLVGMSSKSGSRYGRRSNWFKIHCLLQEQQGGGGGAGTGS-----
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-----SAGDPTDDGTS-----VLSATTSTIRKAMRQSSSAYAGSSEADIAEQQR-----
-----RHKELLDIFRSHSEPLYSSFTPFT-LPPALMASSMPPLPIFKDQFKAELLFPA-----
-----PATSPAEEDPIDLSLRS-----
Tcas_EG MNQLCKVCGEPAAGFHGFAFTCEGCKSFPGRTYN--NISSISECKNNGECVINKNRTACKACRLRCKLLVGMSSKSGSRYGRRSNWFKIHCLLQEQQAG-----
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-----SADPDE-----LRSSAFSYIKPSSERDYFSVKKLPSPADSECFDRRK-----
-----FISALVNAPSVSPASLPSISPSGGFLPKWLPLQGFSDPSAWRDLWARGPVTT-----
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-HLLTNGG--AADELTKRFYLDVAVLKSQQQ-SPP-PTTKLP--PHSKQDYSISALVTPNSESG---REVVKSRQNEEDDEARADGIDG-----
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Dvir_KNRL MNQTCVCGEPAAGFHGFAFTCEGCKSFPGRTYN--
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-----VASPLQEDPIDLSMKT-----
Ame1_KNRL MNQTCVCGEPAAGFHGFAFTCEGCKSFPGRTYN--NLSSISECKNNGECVINKNRTACKACRLRCKLLVGMSSKSGSRYGRRSNWFKIHCLLQEQQ-----
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---NVQTRQSSNESEIGETYTKRFYLDVAVLKSQRTQEGSTAATKE-----
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-----PSPDSPIDLSLKG-----
Mmus_Esr1 ETRYCAVCNDYASGYHYGVMSCEGCKAFAFKRSIQG---
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-----IRHMSNKGMEHLYNMKC-----
Mmus_Esr2 DAHFCAVCSYASGYHYGVMSCEGCKAFAFKRSIQG---HNDYICPATNCTIDKNRRKSCQACRLRCKYEVGMKGGIRKDRRGGRLMKHRQDDLEGRNEMGASGDMRAANLWPS-----
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-----TSGHTPRVKELLNLSLSPQLVLTLEAEPPNVLSR-----
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SSR-KLTHLLNAVTDALVWVVISKSGISSQQQSVRLANLLMLLSH-----
-----VRHISNKGMEHLLSMKC-----
Dmel_ERR LRLRLVCGDVASGFHYGVASCEACKAFAFKRTIQG---NIEYTCPANNECEINKRRRACQACRFQKCLLMGLMKEGVRLDRVGRGRQKYRRNPFVSNYQT-----

MQLLYQSNNTSLCDVKILEVLNSYEPDALSQTPPPQVHTTSITNDEASSSSGSIKLESSVVTNGTCIFQNNNNNDPNEILSVLSDIYDKELVSVIGWAKQIPG-----
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-----DTIINSLNDVVYLLRH-----

[PhD_A6_phylogeny_database]

[PhD_A6.3_phylogeny_ClustalW2_alignments]

“alignment_paired-Pax-group.txt”

Alignment of the complete protein sequences of various paired/Pax-group genes from Parhyale hawaiiensis, Drosophila melanogaster and mouse.

This was done using the ClustalW2 algorithm (Larkin et al., 2007).

For details and references regarding the species' protein sequences, see Appendix (A7).

```
Dmel_TOY -----
MMLTTEHIMHGHPHSS-----VQSTLFGCSTAGHSGINQLGGVYVNGRPLPDSTRQKIVELAHSGARPCDISRILQ-----
VSGNCVSKILGRYYETGSIKPAIRIGGSKPR--VATTVPVQKIADYKRECPISFAWEIRDRLLESEQVCNSDNI PSVSSINRVLRLNLAQKEQQAQ-----
-----QQNESVYEKLRMFNGQTGG-----
WAWYPSNTTHTAHLTPAASVVTSPANLS-----GQADRDDVQKRELQFSVEVSHTNSHDSTS-DGNSEHNSG---DEDSQMRLLRKRKLRNRTSFSNEQIDSLEK-----
-----EFERTHYPDVFARERLADKIGLPEARIQVWFSNRRAKWRREKMRTRRSADTVDGSGRTSTA-----NNSPGTTASSSVATSNSTPGI-----
-----VNSAINVAERTSSALVSNLSPEASN-----GPTVLGGEANTHTTSSESPPL---QPAAPRLPLNSG-----
--FNTMYSSIQPIATMAENYNSL--GSMTPSCLQQRD--AYPYMFHDPLSLGSPYVSA-----HHRNTACN-----
-----PSAAHQPPQHGVIYTNSSPMPSS-----NTGVISAGVSVFVQISTQNV-----SDLTGSNYWPRLQ-----
Mmus_Pax6 -----
MQNSHSGVNQLGGVFNVRPLPDSTRQKIVELAHSGARPCDISRILQTHADAKVQVLDNENVSNGCVSKILGRYYETGSIKPAIRIGGSKPR---
VATPEVVSQIAQYKRECPISFAWEIRDRLLESEQVCNTNDNI PSVSSINRVLRLNLAQKEQQAQ-----
ADGMYDKLRMLNCGQTGS-----WGTRPG-----WYPGTSVP-----GQPTQDGCQQQ--
-----EGGENTNSIS-NGED-----SDEAQMRLLRKRKLRNRTSFTQEIEALEK-----
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VYQPIQPPTTPVSSFTSG-----SMLG-RTDTALTNTYS-----ALP-----
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-----PPHMQTHMSQPMGTSGT-----TSTGLISPGVSVFVQVPG-----SEPDMSQYWPRLQ-----
Dmel_EY -----
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SYFATYYHLTDDDECHSGVNQLGGVFNVRPLPDSTRQKIVELAHSGARPCDISRILQ-----VSGNCVSKILGRYYETGSIKPAIRIGGSKPR---
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Phaw_PBY1A -----
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Phaw_PBY1B -----
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-DGPGFLFPMQQAVALN-MSFSD-----GDEPEN-----YSG-----
-NPPEASY-----
Dmel_PRDA -----
VSHGCVSKILNRYQETGSIKPAIRIGGSKPR---IATPEIENRIEYKRSPPGMFSWEIREKLIREGVCDRSTAPVSAISRLVRGRDAPLDNDMSSA-----
-----SGSPAGDGTKASSSCGSDVSGGHHNKGKPSDEDISDC-----ESEPGIALKRRQRRCRTTFSASQLELER-----
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TETETHQMPNRPNESPNESVSAFQQLPPTPNSLSAVVSGAGVTSSSGANSADPQSLANASAGSEELSAALKVESVDLIAASQSQLYGGWSSMQALRPNAPLSPEDSLNSTSSTQALDVTAHQMFHP
YQHTPQYASY-----PAGHAHSHHGHPHAPHHPHPQYAGAHPHYPPSSAHFMPQNFNAAAFPPSPKVNYYTTPPPQFPYPSWY-----
Dmel_GSB-N -----
MDMSSANSRLP-----LFAGYP-----FQCGQGRVNLGGVFNVRPLPNNIRLKIIVEMAASGVRPCVISRQLR-----
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VSHGCVSKILNRYQETGSIRPGVIGGSKPK---VTSPEIETRIDELRKNPISFWEIREKLIKEGFAD---PPSTSSISRLRGSDR-----

-----GS---EDGRKDYTING---ILGGR-----DSDISDT-----ESEFGIPLKRRKRRSRTTFTAEQLEALER-----
AFSRTQYPDVYTREELAQTALTEARIQWFSNRRLRRLKHK---SGGNSGLSPMNS---GSSNVGVVGLSGATAP-LGYGPLG-----
VQSMGQSPAPGTTATGAGMNDGV---HHAHAPSS---HHAATAAAAHHTQMGGYDL---VQSAHQHGF---GGFAQP-----
-GHFGSQNYHQDYSKLTIIDFSK---LTDASVSKISPS-LHLSDNYSKLEAP-----SNWSQAA-----YHAAANYN-----
-----AHVAQHQLNDYAAAAAHGNPASAYSHPLPTQGQAKYWS-----
Phaw_PBY2
MTIRP-----IFTGYP---FQQGQRVNQLGGVFINGRPLPNHRLKIVEMAAAGVRPCVISRQLR-----
VSHGCVSKILNRYQETGSIRPGVIGGSKPK---ISAPDIEKKIDYKKNPGAFSWEIRDKLVKDGIVDKDAPSSISSRIIRGGGGGGGGGGV-----

-----TGRREDDPRKDHSDIG--ILAEDG-----SDVSDT-----ESEPGLTLKRRKRRSRTTFTAEQLEILER-----
SFEKTQYPDVYTREELAQKARLTEARVQWFSNRRLRRLKHK---LNSQQLATLTPMAPSAASAAAAAASAAAAAPYMAHHQYP-----
AQMCDPSSAMTASAAHAGATSWQGAATCYDYASATLS---GVDHSSLMSTMASMPYAMGGAPDGG-----WG---AAATKS-----
AMGSWCATGMAAPTGFELGGQLTGLQLSLLPAQSYNVSAHAHATFN-ASVSSPSVP-----TSSPDKPQ-----TQAATASSV-----
NGASSGQASVTPHTQAAAVHAHVAASYPSPYSFQADPSSIMCGRVH-----
Dmel_gsb
MAVSALNMT-----YFGYP---FQQGQRVNQLGGVFINGRPLPNHRRQIVEMAAAGVRPCVISRQLR-----
VSHGCVSKILNRYQETGSIRPGVIGGSKPK---VATPDIESRIEELKQSQPGIFSWEIRAKLIEAGVCKQNPASVSSISRLRGGSSGSG-----

-----TSHSIDG--ILGGAGS--VGSEDESD-----DAEPSVLKRRKRRSRTTFTSNDQIDALER-----
IFARTQYPDVYTREELAQSTGLTEARVQWFSNRRLRRLKHK---LNTQVPSFAP---TSTSGATPTTSAAPAPNMGMMSLYS-----
SQSWPSSGAYENHAAYGG-----SVASMPASS-----TSGTSS-----
AAHSPVQQAQPG---TGSEFMTSTYGVSSNATYPSAAYSMPQTP-----ATSAEQLR-----SQFASAA-----
ASGSHHPSTWDSYNFAGSFFPPASAGNHISGYHHQVDKSSMMTTAPTYPYFGF-----
Mmus_Pax7
MAALPQAVPRMRRPGPQN-----YPRGFPLEVSTPLGQGRVNQLGGVFINGRPLPNHIRHKIVEMAAHGHIRPCVISRQLR-----
VSHGCVSKILCRYQETGSIRPGAIGGSKPKQ---VATPDVEKKIEEYKRENPGMFSWEIRDLLKDGHCDRSTVPSVSSISRLRIRKFKGKEDDEEGD-----

-----KKEEDGEKAKHSIDG--ILGDKGNR---LDEGSDV-----ESEPDLPLKRRKRRSRTTFTAEQLEELEK-----
AFERTHYPDIYTREELAQRKLTARVQWFSNRRLRRLKHK---AGANQLAANHLPLGGFPPTGMPTLPPYQLPDSYPTTISQ-----
DGGSTVHRPQLPPTMHQGG---LAAAAAADTSSAYGARHS-----FSSYSDS-----
FMNFGAPSNMHPN-VSNGLSQVMSILSNPFAVPPQADFSISPLHGGSDSASSIS---ASCQRRA-----DSIKPGDS-----
-LPTSQSYCPPTYSTTGYSDVPVAGYQSYQGVAVDYLAKNVLSLQRRMKLGEHSAVLGLLPVETGQAY-----
Mmus_Pax3
MTLGAVERMRRPGPQN-----YPRSGFPLEVSTPLGQGRVNQLGGVFINGRPLPNHIRHKIVEMAAHGHIRPCVISRQLR-----
VSHGCVSKILCRYQETGSIRPGAIGGSKPKQ---VTPDVEKKIEEYKRENPGMFSWEIRDLLKDAVCDNRNTPVSVSSISRLRIRKFKGGEEDADLER-----

-----KEAESEKAKHSIDG--ILSERASAP--QSDGSDI-----DSEPDLPLKRRKRRSRTTFTAEQLEELER-----
AFERTHYPDIYTREELAQRKLTARVQWFSNRRLRRLKHK---AGANQLMAFNHLPLGGFPPTAMPTLPTYSYQPTSIQAV-----
SDPSSVHRPQLPPTVHQST---IPSNADSSAYCLPSTRHG-----FSSYSDS-----
FVPPSGPSNPMNPTIGNGLSPQVMGLLTHGGVPHQPQTDYALSPLTGGLEPTTVS---ASCQRRL-----EHMKNVDS-----
-LPTSQPYCPPTYSTAGYSMDPVTGYQYQYQ---SKPWF-----
Dmel_sv
MLIMDIQTSTHHIHLGTHLQHRILHPLHLSHQEETLNTSTGQLEHDSQHLQHHLLTHQQQDVSPTLHNLQNRTRTGDLSLTTINTNQNHGHQHLGSGSNMYTSSQMEDKSKANKYDEYSRRLSNI
SDANTTPSANNFITQSQGLEWITAMNDIQNAEDSHSSQSGISGDGGGVNQLGGVFVNGRPLPDVVRQRIVELAHNGVRPCDISRQLR-----
VSHGCVSKILSRYYETGSKFAGVIGGSKPK---VATPPVVDIAIANYKRENPTMFAWEIRDLLAEIACSQDNVPSVSSINRVRNKAEEKAKHVHHHQ-----

HHVSQSLGGGHIAESVDSSTGTIGEPPPTSNSSANSVNTNVSASVHASIPTSGTDSVQVSVGHINANSNETHINSTAEQRTTYSINGLLGIQHGHSHNNNNNSVNNNNNTSSCKRRIEA
HDENHDNTNIHSDNDGKQRMTSYSDQLYTNISWKGKWCIKDDHKLALGNLTAAGNCPATYEEASNGFSTTPISSGATASGNDTSMLYD-----
SITTIQTSQSIYPAIGFSIGTSLPLVISMHEMKSANSIQEQTVPPFYTALAF-----DGNYSMTSLENCSSLVQGEHIVMPESSDSNTLCPISTRIVPDITETNSTR-----
VKEPLTNSDGCSEDDNKEPEKSNSSQSDHIASPHLHHIGEEQLRGNRRSNLNASTHPSLLIPLQPSGGSSLLNINPENRSDVLELNLNNSVGLYSTPTVLPSPFNHYAGCSVVPVGS-----
-----YAYNP-AYTQYGGAYGSYGTGSGLINSYYSYEGTQSPHLDLRSPLVATRANSLASASPGSGSACTKSESSDIFLA-----
Mmus_Pax2
MDMCKADPFSAMHR-----HGGVNQLGGVFVNGRPLPDVVRQRIVELAHQGVVRPCDISRQLR-----
VSHGCVSKILGRYYETGSIKPGVIGGSKPK---VATPKVVDKIAEYKRNPTMFAWEIRDLLAEGICDNDTPVSVSSINRIIRTK-----

-----VQQPFHPTPDGAG---TGVTAPG---HTIVPST-----ASPPVSSASNDPVGSYINGILGIPR-----
-----SNGEKREVEVYTD---AHIRGG---GGLHLVWTLRDVSE-----GSPVNGDSQS-----GVDSLR-----
KHLRADTFTQQQLEALDRVFER-----PSYPDVQASEHIKSEQNEYSLP-----ALTPGLDEVKSS-----LSASANPELGSNVSGTQYVVT-----
-----GRDMTSTTLPGYPHVPPTGQ-----GSYPTSLAG-----MVPGSE-----
FSGNPSYHPQYATYNEAWRFSN-PALLSSPYYS---AAPRGSAPAAAAAYDRH-----

[PhD_A6_phylogeny_database]

[PhD_A6.3_phylogeny_ClustalW2_alignments]

“alignment_sine-oculis-Six-family.txt”

Alignment of the entire protein sequences of various sine oculis/Six-group genes from different species. This was done using the ClustalW2 algorithm (Larkin et al., 2007). For details and references regarding the species' protein sequences, see Appendix (A7).

```
Phaw_SIX3 -----MALLAG-----LSAYSAAALGPAPHGAPGVLPS-----
-----SNVSLPSLSFTATQVAAVCETLE-----
ESGDIERLGRFLWSLPVAHP----
HLKDLNKHEAVIRARALVAFHLGNFRELYSLIECNRFTRASYPRLQALWLESHYQEAERLRGRPLGPDVKYRVRKFFPFPRTIWDGEQKSHCFKERTRSLLEAYLQDPYPNPSKRELAQATTLTPTQ
VGNWFKNRRQRDRAAAIAKN-----RLHQQ-----
SSSISSSAKLETGPRSPGADDEDLNPGPSPTPFEDDVSEDEVSLGQQSPLSKLQGPPLAFAFDGVIITGDPQHGGSKGPLDDLSKAD-----
TALKCSSAGSSFSLSEGAASDTSNSAFSSPYPTSLAAGTSWSTKSSSQE-----IFLPASVPVLPDRLRFRI-----
NPDGDHSALRLPLFGALFSPFASALPRLPLAFPHLSTWSQYAPFPTISTFGDSTYHRSPAPLSIDSLISPARNKREAPPSPSPHTVTSKTEPEDPGIADL--
TTAQGDGSPSLRSPVAKRLHTSPGDTISDQQPRPHIFQPIKLEFAPSTETS-----
-----
Nvec_SIX3 -----MFAP-----
-----LPALSFSAHQIAVCETLE-----
ESGDVERLARFLWSLPVAPG----
TLEALGKHESVLRARAIIVAFHMGNFRDLYHILETHRFTRSHAKLQAMWLEAHYQEAERLRGRPLGPDVKYRVRKFFPLPRTIWDGEQKTHCFKERTRSLLEWYLQDPYPNPTKRELAQATGLTPTQ
VGNWFKNRRQRDRAAAIAKN-----R-----
-----
Drer_Six6b -----MFQLPILNFSPPQVAGVCETLE----
ESGDIERLGRFLWSLPVAPA----
ACEVLRNRESVLRARAIIVAFHTGNFRELYHILENHKFTKESHAKLQALWLESHYQEAELRGRPLGPDVKYRVRKFFPLPRTIWDGEQKTHCFKERTRHLLEWYLQDPYPNPSKRELAQATGLTPTQ
VGNWFKNRRQRDRAAAIAKN-----RLQQQ-----VLSNGS-----
VRSLSGE-----DGAVDRLGNASSPEVLSLSSK-----AATS AISITSSDSE-----CDI-----
-----
Drer_Six6a -----MFQLPILNFSPPQVAGVCETLE----
ESGDVERLGRFLWSLPVAPS----
ACDVLGKNESVLRARAVVAFHAGNFRDLYHILENHKFTKDSHAKLQALWLEAHYQEAELRGRPLGPDVKYRVRKFFPLPRTIWDGEQKTHCFKERTRHLLEWYLQDPYPNPSKRELAQATGLTPTQ
VGNWFKNRRQRDRAAAIAKN-----RLQQQ-----VLSGGS-----
VRSLGDD-----DTVDRLGPASSPEVLSLSSK-----AATS AISITSSDSE-----CDI-----
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Mmus_Six6 -----MFQLPILNFSPPQVAGVCETLE----
ESGDVERLGRFLWSLPVAPA----
ACEALNKNESVLRARAIIVAFHGGNYRELYHILENHKFTKESHAKLQALWLEAHYQEAELRGRPLGPDVKYRVRKFFPLPRTIWDGEQKTHCFKERTRHLLEWYLQDPYPNPSKRELAQATGLTPTQ
VGNWFKNRRQRDRAAAIAKN-----RLQQQ-----VLSQGF-----
GRVLRSEG-----EGTPEVLGVASSPAASLSSK-----AATS AISITSSDSE-----CDI-----
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Drer_Six3a -----MVFRSP-----LELYPSHFFLPNFADR---PLLASSAP-----STRSPEDLSMFQLPTLNFSPEQVAVVCETLE----
ETGDIERLGRFLWSLPVAPG----
ACEAINKHESILRARAVVAFHTGNFRDLYHILENHKFTKDSHGKQAMWLEAHYQEAELRGRPLGPDVKYRVRKFFPLPRTIWDGEQKTHCFKERTRSLLEWYLQDPYPNPSKRELAQATGLTPTQ
VGNWFKNRRQRDRAAAIAKN-----RLQHQ-----AIGQNG-----
MRSLSSEG-----CAPRSSAESPTAASPTTSVSSMTE---RVDTGTSILSVTSSDSE-----CDV-----
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Mmus_Six3 -----MVFRSP-----LDLYSSHFLFPNFADSHHCSLLASSGGGSGASGGGGGAGGGGGRAGGGGAGGAGG-----
-----GSGGGGSRAPPEELSMFQLPTLNFSPEQVAVVCETLE-----
ETGDIERLGRFLWSLPVAPG----
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VGNWFKNRRQRDRAAAIAKN-----RLQHQ-----AIGPSG-----
MRS LAEPG-----CPTHGSAESPSTAASPTTSVSSLTE---RADTGT SILSVTSSDSE-----CDV-----
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Drer_Six3b -----MVFRSP-----LELYPSHFLFPNFADR---PLLAGSIP-----RARSPEDLPMFQLPTLNFSAEQVAVVCETLE----
ETGDIERLGRFLWSLPVAPG----
ACDAINKHESIQRARAVVAYHTGFSFRELYHILETHKFTKDSHGKQAMWLEAHYQEAELRGRPLGPDVKYRVRKFFPLPRTIWDGEQKTHCFKERTRGLLEWYLQDPYPNPSKRELAQATGLTPTQ
VGNWFKNRRQRDRAAAIAKN-----RLQHH-----GLGQSS-----
LRSMSEG-----CTPHSSAESPCAASPTTSVSSMNE---RGDGGT-ILSVTSSDSD-----FDV-----
```


Agam_OPTIX -----PVQPHP-----ILAPS-----
-----PLFALPTLNFATSQVATVCTLE-----
ESGDIERLARFLWSLVAHP----
NVTELDSEAVLRARAIVAYHTGHFRELYSILERHKFTKTSHGKQAMWLEAHYHEAEKLRGRPLGVPDKYRVRKKFPLPRTIWDGEQKTHCFKERTSRLLREWYLQDPYPNPTKKRELAQATGLTPTQ
VGNWFKNRRQRDRAAAANK-----R-----

Amel_SIX3 -----MSAVAPAAGTGATAPSANGPIVPA-----
-----PIFALPTLSFTVNOQVATVCTLE-----
ESGDIERLARFLWSLVAHP----
NIQELNQSEAVLRARAIVAFHSGHYRELYAILERHKFTKDSHGKQAMWLEAHYHEAEKLRGRPLGVPDKYRVRKKFPLPRTIWDGEQKTHCFKERTSRLLREWYLQDPYPNPGKKRELAATGLTPTQ
VGNWFKNRRQRDRAAAANK-----RMQQQ-----SGTGNCNT-----
RRALSPGPGGSDSESDISLGGTSPASPPSPPTTQSPVPLGGLPLPPPNSLFRFDPSSQSQ-----
FRFNHATAFKFPPTGFRFGPHSPQHNPISGGGIFRLTETSFPQAVGPRGDLGSRLLPDLPLPPRLHPHEGLLHHPHVQHQQLPERISDGSRLSDGGISRLSDLSLSEAHSPPL-
MATNLVGTPLRVAVSPH--TPSSRGSPPPPASQP--TPQSQSQGLIRVATPPPPNPKPPIHRPFSPA-----

Tcas_OPTIX -----MALGLGSLSSGPGSGNSETSSSPGISVPIPVAPAPLVN-----
-----PMFALPTLNFVAQVAACVCTLE-----
ESGDIERLARFLWSLVAHP----
NIGELNKNEAVLRARAIVCFHSGHYRELYSILESNKFSKSSHHKQEIWQLAHYHEAEKLRGRALGVPDKYRVRKKFPPWPRTIWDGEQKTHCFKERTSRLLREWYLQDPYPNPTKKRELAQATGLTPTQ
VGNWFKNRRQRDRAAAANK-----RMQQQLAAQLAHGGGGR-----
QPPLSPTP--SDS-DSDISLGAHSP--PISSPGPMRFSNS-----PTPITSRFRFGP-----HSPG-PMPP-QFRFGNHSP----PGFRQDRKSR--NSSPIN-
VDTTSNYNNNSAVN-----LRLTSNESPIDVDSQEKSHSPIRVDSSPPMNLRIHSDNTKIETPLALRLTSSHQLTTPRIQVASPH--RMAAPPSPPHSLHLG-TPHG-----
GIVRIAPPSPSNP--PVLHRPFSPPRLT-----

Dmel_OPTIX -----MAVG-----PTEGKQPPSESFSPTHHQIAPS-----
-----PILAVPTLAFSAAQVEIVCKTLE-----
DSGDIERLARFLWSLVAHP----
NMHEILNCEAVLRARAVVAVHGNFRELAYAIENHKFTKASYGKQAMWLEAHYIEAEKLRGRSLGVPDKYRVRKKFPLPPTIWDGEQKTHCFKERTSRLLREWYLQDPYPNPTKKRELAQATGLNPTQ
VGNWFKNRRQRDRAAAANK-----RIQHS-----QNSGGMGCRSRR-----
ADGAASPTPSDSDSDISLGTSPVSSSLQLQHSFGSTSNGANDEESLSVDDDKPRDLGSLPLPLSLPLASPTHTPPQLPPG--
YGGGAGAGPGGLTGPGLPFPKLDAAATSLFSAGCYLQSFNLKEMSQQFPIQPIVLRPH--PQLPQSLALNGASGGPP-LHHPAYAAAYSVECVGGHPPHPPKLRINSPEKLNSTAVAAAAA--
-VGGGGN-----QHHEPTTGYHHSQMLLHRFPSTSPELKHSAPET-----

Dpse_OPTIX -----MAVG-----PTEGKQPPSESFSPTHHQIAPS-----
-----PILAVPTLAFSAAQVEIVCKTLE-----
DSGDIERLARFLWSLVAHP----
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VGNWFKNRRQRDRAAAANK-----RLQHN-----QNS-GMGCRSRR-----
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ASPGLTPPQLPHGGVVFVGGAGGGGGPPLPGGCLPFPKLDAAATSLFSAGCYLQSFNLKEMSQQFPIQPIVLRPH--PQLPQSLALNGASGGPP-LHHPAYAAAYSVECVGGHPPHPPKLRINSPEKLNSTAVAAAAA--
VECVPSHAQQHPPPKLRINSPEKLNSTAVAAAAAAGAGSGGGGANPGAV--NHHHEATTGYHHSQMLLHRFPSTSPELKHSAPET-----

Dvir_OPTIX -----MAVG-----PTEGKQPPSESFSPTHHQIAPS-----
-----PILAVPTLAFSAAQVEIVCKTLE-----
DSGDIERLARFLWSLVAHP----
NMHEILNCEAVLRARAVVAVHGNFRELAYAIENHKFTKASYGKQAMWLEAHYIEAEKLRGRSLGVPDKYRVRKKFPLPPTIWDGEQKTHCFKERTSRLLREWYLQDPYPNPTKKRELAQATGLNPTQ
VGNWFKNRRQRDRAAAANK-----RIQHN-----QNSGGMGCRSRR-----
ADGAASPTPSDSDSDISLGTSPVSSSLQLQHSFGSTSNGANDEESLSVDDDKPRDLGSLPLPLSLPLASPTHTPPQLPPG--
AGCLPFPKLDAAASSLFNAGCYLQSFNLKEMSQQFPIQPIVLRPH--PQLPQSLALNGASGGAPP-LHHPAYAAAYSVECG--
VVAPPPPPKLRINSPEKLNSTAVAAAAAAGAGAAHEISSGNNTTTTTTGYHHSQMLLHRFPSTSPELKHSAPET-----

Drer_Six7 -----MFPLPMFTPEQVARVCENLE-----
ETGDIERLGRFLWSLPAVPGS--
AGELLNRHESVMRARALVAFHGGNFEALYQILQSHRFTRESHAKLQDLWLDHAHYREAERLGRPLGVPVEKYRIRKKFPLPRTIWDGEQKTHCFKERTSRLLREWYLQDPYPNPSRKRHLAQATGLTPTQ
VGNWFKNRRQRDRAAAANK-----RLQQD-----
SSLLPSG-----SSPECSSEHNPLLRGSSPR-----HPDSPGNSDCSSGPR-----
-----GTGASTPDISVSSDSEFES-----

Cele_H-32 -----MFTPEQFTKVMSQLGNFSQLGQMFQPGNVAMLQALQANGASSTPSLFPAMPVSVIPS-----
-----LAAPSSPTTSLNLTADQIVKTCQLE-----
TDGDVDGLFRMCTIPPQKT-----
QEVAGNEAFLRARALVCFHSHFRELAYAIENHKFSKYPKHPKQEMWHEAHYREQEKNGKSLCAVDKYRVRKKYPMPTIWDGEQKTHCFKERTSRLLREWYLKDPYPNPKKKELANATGLTQMVG
NWFKNRRQRDRAAAANK-----KQNIIG-----VELKKT-----
SDMSDDDDFEDSMTDPSPIDEPKDLSKSHIPKLSPTLLP-----KMATPFDMFAAAA-----
PLMMLNLNPFALYMQFHNFNTMRNPQIDEEENSETVEVEADIEPPKRSKLSIDEILNLIKSEVSPSQ-----
CSPCSNESLSPKRAVKTVEVKDEDEAAEEDSRVKSSETSEDPKHSSPKSTTSQSE-----

Cele_H-34 -----MQQSYNPQNS-----

LTATTSYSEQEIVCICESLFNEGLQGRTEQLANFIYNLP-----
QCYQVMEVSLKAQALVYFTTQNMWMLKLECSKFSPHNHTVLQNLWLDHAHYKEAAKTDRDELAVCKYRIRKKNPPNPTIWDGEBEINYCFKSKSRNVLDRDAYKKQYPSVEDKRRLAQQTELSIIQVS
NWFKNRRQRDRARAAGQLD-----RSSAR-----
SNDSDDG-----SSGCEKPMNIDSPAP-----PPLPSTFDLQPYPS-----
-----PYTFAPHCDFSYIQNL-----

Dmel_SO -----MLQHPATDFYDLAANAALVLTARHTPPYSPTGLSGSVALHNNNNNSST-----NANNSTLIDIMAHNGG-----GAGGGLHLN-----
SSSNGG-----GGG-----GVVSGG-----GSGGRENLPFGFTQEQVACVCEVLQ-----

QAGNIERLGRFLWSLPQCDKQ-----
LNESVLKAKAVAFHRGQYKELYRLEHHHFSQNHAKLQALWLKHAHYVEAEKLRGRPLGAVGKYRVRKFFLPRTIWDGEETSFCFKEKSRVLRDWSYHNPYSPREKRLAEATGLTTTQVSNWFK
NRRQRDRAAEHKD-----GSTDQHLDSSSDSEMEGSMPLPSQSAHQ-----QQQQQQQHSPGNSS-----
GNNG-----LHQQLQHVAAEQGLQHHPQP-----HPASNIANVAATKS-----
SGGGGGGVS-----AAAAQMQMPPLTAAVAY-----SHLHSVGMAMPMTA-----MYDMGEYQHL-----

Dpse_SO-----MLQHPATDFYDLAANAVALTARHTPPYSPTGLSGSVALLNNNNNNSSSTGNSNNNNNNLDIMTHNGV----
VVGAVGAGGLHLTGG-----SNG-----GVTGGG--
ASGGRENLPSPFGTQEQQVACVCEVLQ---QAGNIERLGRFLWSLPQCDKQ-----
LNESVLKAKAVAFHRGQYKELYRLEHHHFSQNHAKLQALWLKHAHYVEAEKLRGRPLGAVGKYRVRKFFLPRTIWDGEETSFCFKEKSRVLRDWSYHNPYSPREKRLAEATGLTTTQVSNWFK
NRRQRDRAAEHKD-----GSTDQHLDSSSDSEMEGSMPLPSQSAHQ-----QQQQQ--SP-----
NNNG-----LHQQLQHVAAEQSLQHHPQH-----HPAATGPTSVVSKTG-----
HSGAGGGGGGGGGGG-----AATATQMMPPLSAAVAY-----SHLHSVGMAMPMT-----MYDMGEYQHL-----

Dvir_SO-----MLQHPATDFYDLAANAVALTARHTPPYSPTGLSGSVALLNNNNNSTSN-----
NNNNLDMLTHNGGGVGLGGSVTATGLHLSGG-----
GGGSRNGFGAGGGGSAATAGRETLPSFGTQEQQVACVCEVLQ---QAGNIERLGRFLWSLPQCDKQ-----
LNESVLKAKAVAFHRGQYKELYRLEHHHFSQNHAKLQALWLKHAHYVEAEKLRGRPLGAVGKYRVRKFFLPRTIWDGEETSFCFKEKSRVLRDWSYHNPYSPREKRLAEATGLTTTQVSNWFK
NRRQRDRAAEHKD-----GSTDQHLDSSSDSEMEGNMSSQSAQQQHAATHSHSHSQQQQSPSSGNNN-----
INNNNSNGNS-----NSLHQHQQLQHVAAEQSLQHHPQH-----HAAS-----AAAASKS-----
SAAAA-----AVAVASQMPPLSAAVAY-----SHLHSVGMAMPMT-----DMGEYQHL-----

Agam_SO-----MLQHPSTDYELSS-----VAIKQTPPTFSQSGGGGGVGDGGTGG-----DADLELIAQNGS-----
-----AADRESLPSFGTQEQQVACVCEVLQ-----
QAGNIERLGRFLWSLPQCDKQ-----
LHESVLKAKAVAFHRGNFKELYRLEHHQYSPHNHAKLQALWLKHAHYVEAEKLRGRPLGAVGKYRVRKFFLPRTIWDGEETSFCFKEKSRVLRDWSYHNPYSPREKRLAEATGLTTTQVSNWFK
NRRQRDRAAEHKD-----GESDKQHLDSSSDSEMEGSMPLASMASNG-----VGGPGVLHAT-----
TGGG-----MHLGTSGLTSAASGGV-----TAGVLGGTIVGPG-----
AGGGVGL-----GSGKSTSIGSLSAAY-----SHFHNVMGSMPLY-----DIGDYQHL-----

Nvec_SO-----MLPSFSFTPEQQVACVCEVLQ-----
QSGDIERLGRFLWSLPECETIQ-----
KNESVLKAKAVAFHRGQYKELYRLEHHHFSQNHAKLQALWLKHAHYVEAEKLRGRPLGAVGKYRVRKFFLPRTIWDGEETSFCFKEKSRVLRDWSYHNPYSPREKRLAEATGLTTTQVSNWFK
NRRQRDRAAEAKIR-----

Tcas_SO-----MDEAINRECLKFALRTRESAMLSAGLYGVSG--PVTGTP-----PYSPPP-PARHSP-----
-----LPSFGTQEQQVACVCEVLQ-----
QAGNIERLGRFLWSLPACDKLH-----
NNESVLKAKAVAFHRGNFKELYRLEHHQYSPHNHAKLQALWLKHAHYVEAEKLRGRPLGAVGKYRVRKFFLPRTIWDGEETSFCFKEKSRVLRDWSYHNPYSPREKRLAEATGLTTTQVSNWFK
NRRQRDRAAEHK-----DSG-----QNSDKP-----
-----HLDSSGDSSES-----E-----HKLSTYTPSGAVG-----PPQLAP-----
-----YG-----GLGAPSLG-----QPLYQAPGGL-----LHDYQAL-----

Amel_SIX2-----MAYGTTNGGAMGPPSSGTDVLSGTDYSPQGTPTPLTGHSAQVVEAT-----TYGPTGFPASYSPOD-----
-----SPASSAGGSGSNGQLSSFGTQEQQVACVCEVLQ-----
QAGNIERLGRFLWSLPACTRLH-----
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SVSSPHSYHQHGSTL---SHPTPTHMQHDTGSESGDDKRNH-----HQLQHHLQVGAHGT-----
GLVHTATLPPPPSAQQKSLDYMYQYQDYLITPTSADLQKSLPHTATSMQGAITAPSMGGLSPMGPSTMLPNTMQSVNTMNTSMNGIGMGAYQMGSMGMBTSHGSHFNGLVLGDYHSL---

Drer_Six1b-----MSMLPSFGTQEQQVACVCEVLQ-----
QGNLERLGRFLWSLPACDHLH-----
KNESVLKAKAVAFHRGNFKELYRLEHHHFSQNHAKLQALWLKHAHYVEAEKLRGRPLGAVGKYRVRKFFLPRTIWDGEETSFCFKEKSRVLRDWSYHNPYSPREKRLAEATGLTTTQVSNWFK
NRRQRDRAAEAKE-----RENNENNNTGANKQQLSPLDGG-----
-----KSLMSSSEDEFSPQSPDQ-----NS-----VLLQGNM-----SHPGA-----
-----SAYSMTGLGAAQSVHG-----MQGHPHQLDQSLGFLT-----SSLVDLGS-----

Mmus_Six1-----MSMLPSFGTQEQQVACVCEVLQ-----
QGNLERLGRFLWSLPACDHLH-----
KNESVLKAKAVAFHRGNFKELYRLEHHHFSQNHAKLQALWLKHAHYVEAEKLRGRPLGAVGKYRVRKFFLPRTIWDGEETSFCFKEKSRVLRDWSYHNPYSPREKRLAEATGLTTTQVSNWFK
NRRQRDRAAEAKE-----RENNENNNTGANKQQLSPLDGG-----
-----KPLMSSSEDEFSPQSPDQ-----NS-----VLLQGNM-----GHARS-----
-----SNYSPLGLTASQPSHG-----LQAHQHLQDQSLGFLT-----SSLVDLGS-----

Drer_Six1a-----MSMLPSFGTQEQQVACVCEVLQ-----
QGNLERLGRFLWSLPACDHLH-----
KNESVLKAKAVAFHRGNFKELYRLEHHHFSQNHAKLQALWLKHAHYVEAEKLRGRPLGAVGKYRVRKFFLPRTIWDGEETSFCFKEKSRVLRDWSYHNPYSPREKRLAEATGLTTTQVSNWFK
NRRQRDRAAEAKE-----RENNENNNTGANKQQLSPLDGG-----
-----KSLMSSSEDEFSPQSPDQ-----SS-----VLLQGNM-----NNPGA-----
-----PAYMPGLGAPHLHG-----MQGHPHQIQDQSLGFLT-----SSLVDLGS-----

Drer_Six2.1-----MSMLPTFGTQEQQVACVCEVLQ-----

QGGNIERLGRFLWSLPACEHLH-----
KNESVLKAKAVVAFHRGNFRELYKILESHQFSPHNHFKLQQLWLKHAHYIEAEKLRGRPLGAVGKYRVRKRFPLPRSIWDGEETSICYFKEKSRVLRWYTHNPYSPREKRELAETGLTTTQVSNWFK
NRRQRDRAAEAKE-----RENNENSNTNSHNPLTSSMNGN-----
-----KITILGSSDDDKTPSGTDPH-----TSSSPALLLTSNS-----GLQSL-----
-----HGLAPPPGSAIPVPS-----VDSVHHHSLHDTILNPFMS-----SNLVDLGS-----

Mmus_Six2 -----MSMLPTFGFTQEQVACVCEVLQ-----
QGGNIERLGRFLWSLPACEHLH-----
KNESVLKAKAVVAFHRGNFRELYKILESHQFSPHNHFKLQQLWLKHAHYIEAEKLRGRPLGAVGKYRVRKRFPLPRSIWDGEETSICYFKEKSRVLRWYAHNPYSPREKRELAETGLTTTQVSNWFK
NRRQRDRAAEAKE-----RENSSENSSSSHNPLASSLNGSG-----
-----KSVLGSSEDEKTPSGTDPH-----SSSPALLLSPPPP-----PGLPSL-----
-----HSLGHPGSAVFPVPGG-----GGADPLQHHSLQDSILNPFMS-----ANLVDLGS-----

Drer_Zgc_195096 -----MSMPPTFGFTQEQVACVCEVLQ-----
QGGNIERLGRFLWSLPACEHLH-----
KNESVLKAKAVVAFHRGNFRELYKILESHQFSPHNHFKLQQLWLKHAHYIEAEKLRGRPLGAVGKYRVRKRFPLPRSIWDGEETSICYFKEKSRVLRWYAHNPYSPREKRELAETGLTTTQVSNWFK
NRRQRDRAAEAKE-----REN-EGANPNHNLPTSHVNE-----
-----KSLCESSDDDKSPAGTDPH-----TNMSPAILMPSSS-----GLPPL-----
-----HSPAPPGPSASISDT-----QSHHHFSMHDGLLNPM-----ASLVELGS-----

Drer_Six9 -----MAMGFSPEQVACVCEVL-----
QSGMDRLSFLCPLSISTSSNMYMGFQSQSSEVLKARAFAVHRCRFTLEYALLEGNVFSPRSHPLQLWLRAHYMAELQGRPLGAVGKYRIRKRFPLPRTIWDGEETSICYFKEKSRVLRWY
CRKPYSPREKRDAAATGLTATQVSNWFKNRRQRDRAAATSRQ-----GTSAG-----
-----AFLSSDEEISPPGSPR-----TLFSCSQQLSAHP-----
-----PPLRHLPAPHY-----

Mmus_Six5 -----MATS PAEPSAGPAARGEAAAATEEQEEEARQLLQTLQAAEGEAAAAG-----AGDAAAADSGS-----
-----PSGPGSPRETVEVPTGLRFSPEQVACVCEALL-----
QAGHAGRLSRFLGALPPAERLR-----
GSDPVLRARALVAFQGEYAEYLQLESRPFAAHHAFQDLYLRARYHEAERARGRALGAVDKYRRLKRFPLKTIWDGEETVYCFKERSRAALKACYRGNRYTPDEKRRLATLGLSLTQVSNWFK
NRRQRDRGTGGG-APCKSESDGNPTTEDESSRSPEDLERGVASMAAEPAQSSIPLAGATSPATCPASSSILVNGSFLA-----
ASSPPAVLLNGSPVIINSLALGENSSLGPLLLTGGAPQPQPSLQGV-----SEAKNSLVLDPTGE-----
VRLDEAQSEAPETKGVHGTGEEIPGALPQVVPVPPPTFPLTPGAVPAVAAPQVPLSPSSGYPTGLSPTSPRLNLPQVVPTSQVVTLQAVGVLQLLAAGPGSPVKVAAAAGPTNVHLINSVGV
ALQPSSTAPGNLLANPVSGSIVTGVAVQQGKIILTATPPTSMVSVQLPPAPSLALPLKQEPATVPEGALPVGSPPTLPEGHTLGPISSTQPLPPASVVTSGTSLPFSDDSSGLSSFSAPLPEGL
MLSPAAPVWPAGLELSTGVEGLGTOHTVLRPLPDPDQGLLLGATTGTVEDEGLEABAKVLTQLQSVVVEEPLLE-----
Mmus_Six4 -----MSSSPTQGIASAADIKQENGEMESA SEQE AHREAVAGAAAGLSPAPAPFPLEPGDAAAASRVSRREGAAAAGAADQVQLH-----
-----SELLGRHQHAAAQPLAFSPDHVACVCEALQ-----
QGGNLDRLARFLWSLPQSDLLR-----
GNESILKARALVAFHQIYPELYSILESHSFPESANHPQLQWLKARYTEAERARGRPLGAVDKYRRLKRFPLPRTIWDGEETVYCFKERSRNALKELYKQNRYPSPAERHLAKITGLSLTQVSNWFK
NRRQRDRNPSETQSK-----SESDGNPSTEDESSKGHEDLSHP-----LSGASDG-----
VTNLSL---SSHVEP-VYMQQIGNAKI-SLSSSGVLLNGSLVPASTSPVFLNGN-
SFIQGHNGVILNGLNVGNTQIVSNLPPKMSNIVNGIAMTDILGSTQDVKEFKVLQSSAVNSAATTSYSPSAPVSPGLIPCTEVKREGIQTVAQDGGSV-
VTFTTVPQINQYIVQIPNSGANGQFLNGSIGFSPQLQPPVSVAAASQGNLSVTPSTSDGSTFTSEPATVQHGKFLSPLTPSAVVYVTPNSQTVGAVKQEGLERGLVFSQMLPWNHSAQVNASLSEN
LSCSGLHLPTSSILVNVSAAHGFSLTPTPLTLLNPLDLESQVPSAVASKCTVSSVNTNYATLQNCSLIPGQDLGSGMTQAALGEIVTAEQVSHASTAVHQDFVREQLRVLQSVPIKENFLQ
NSENKATNMLMMLDSKSKYVLDGMVEAGCEDLGTDKKELAKLQTVQLEDMDQL
Nvec_SIX4.1 -----DQVACVCDALR-----
QAGDIERLGRFLWSLPDLDL-----
GSEVLKARALVAFHRGRYREVYNIETNEFDPSSHELLQCLWYKHAHYEAEKLRGRPLGAVDKYRIRKRFPLPRTIWDGEETVYCFKERAALKDCYEQNKYPTPQEKRLIAKQTNLTKQVSNWFK
NRRQRDRIPSNKR-----

Drer_Six4.2 -----MSVSSS---EVTVPDGIKEN---VKYTELESSTDSQE-HVKLLTLDSP-----VATGMRVQQTSPAR---KMPTM-----
-----DSLFAHSSPAVTATS-LAFSPEQVACVCEALQ-----
QGGNVDRLARFLWSLPQSDLLR-----
GNESILRAQALVAFHQARYQELYSILESHSFPSPCHSALQDLWYKARYTEAERARGRPLGAVDKYRRLKRFPLPRTIWDGEETVYCFKERSRNALKDLYKQNRYPSPAERHLAKITGLSLTQVSNWFK
NRRQRDRNPSETQSK-----VSESDGNHSTEDESSKQESLSCP-----
MSTCADGPPVNTALPLPCSGSLDAGVIVQVADSR-T-SISPPSVIFNGVSVHSPAS-VFHNGTSPFLSSTGHVLFSGMNLG-LQSLACRP---TDVEMDGAQE---KGGTADPALAYSSFHCS-VN---
---GTDVEVKEEDLR---KDVSVQDQAPSIGSAFISITPSNGLQIDSYSLVTEGNK-----VVLPQLTVSSSPSSVTQGVVDCS-----SPVCQERLQTLSEPSSTLYSLGN-
IHTLSAVKKEPLEP-----PGGFLYHLGYS-----SDPTHSSPLLAQTDLP-----TEFRG-----
---HTDHMTSSVNGDFLCAVS-----EGGKGE-----MEEE-----EEKQLTKLKTVHLEEMTDL
Drer_Six4.1 -----MCCLKPR-GVTYQDAFKMSSSSSEVISADEIKRESRG---SVKLSVLDP-----AELPME-----
-----NAELLDLDCSPAS---LAFSPEQVACVCEALM-----
QGGNVDRLARFLWSLPQSDLLR-----
GNESILKQAIVAFHHARYQELYSILESHSFPSPNHSLLQDMWYKARYTEAERARGRPLGAVDKYRRLKRYLPRTIWDGEETVYCFKERSRNALKDMYKRNRYSPAERHLAKMTGLSLTQVSNWFK
NRRQRDRNPSEAOQSK-----SESDGNHSTEDESSKQEDLSRP-----
LSSGSSSVPHGTAPPTVYSDGGTTVIQIGDVKMPSHGGGMSYNGDLASVNAH---YNSGAYGHSHSNALNGLGATGGHFLTDP-----QVSHHSHQERLLGGSSVYAPPS-
MGVSETASGKIDTMTQTLVSNGEDGVIS-----SVVSFAS---APSTPSTTSGGLHLLSYSVN-----LPGNESTMGLPPLMLPSS-----
TSSTHGSAPLGNVSDSSQHSSTLYSQ-TVKQEPDVL-----PGSYAYP-----SDMPLDQGSNLGYTAS-----ATVSTLNSDVBHHA
Cele_H-33 -----MQLN-----
-----SSSFPHHFTCDTTR---YSEEQVACICEALS---N---
DARKLSQFVWTVLERDEMR-----
NNQYILKAQAFAPLAFHNSNFKELYRIESHHPFAEHHLPQEWLNAHYHEAEKIRGRQLGAVGKYRIRKRYLPRTIWDGEETSICYFRDKSRVLLRDWYCRNSYSPREKRELAETHLTVTQVSNWFK
NRRQRDRAG-----VPEPKDCLKDISEEDLKLIRKT-----ASKLSNS-----
-----FHNPSDLSSYSAAAAATFPG-----FYMNYNDMMIG-----AGTSYQSL-----

Drer_Six4.3 -----MASLSLESTEQTENGPKEAQNENAKVKEETDPEVSEQLLQS-----
-----FQNSALSFTSDQVACLCEALL-----

QAGNVDRWLRFATIPPSADLLR-----
GNETLKAQALVAFHRDEFKELYAILDSDHDFPHSNHGFLQDLYLKARYKEAERSRGRSLGAVDKYRLRKKFPLPKTIWDGEETVYCFKEKSRNALKECYINRYPTPAEKKNLAKVTGLSLTQVSNWFK
NRQRDRTPSGTNSK-----
SESDGNHSTEDASKGDLEDTDKPAAQETGSSNASLISVPGAPCGTGQVLVNSPGGFLTSPHPLLNGSPILSGAGTGVINGLTLSDGHTVTLSPVTANAPLLLNGAQVISKDERGISDLEAQGSLT
TVVLNPAQSLTSTIPLSLSEDTKSSN-----
VSPDLFISLPEALKNTDNTHPVPANSISLPTIISTVSISSTSLSSGILAPNSISASIPGVSIVSSVVPVLPQTPPEIVVLGKAEPLSSTCSSFSSPQVLSLPQVPSIQGVVPSQLMPPSGTTVSS
RPQLVFPVSPVNPQLSQVSIPOFPTQTFHMGPRLAQAQPPQNGSVTLNTSSALTLQIADGQVTVLTPQLGNESSNLASLPQIQTLSTLGTQVPISSPTQVVPISQTKDSSQMVPLSLPQLMPVSSITGTTG
TFSFPQVVPATPSLISPSHGGAQIILTSAGTGLSVAQGPRLSPIGPPQSVPTGTIPGVQLLNSGVIKLPTASPGNILLAGGIGSSPILSVQNGKLIITIPAGIQFASMPVKVSPDSSVSSNSLDSQ
ISTVHSLNQAPPELLIARNSQTL
Nvec_Six4.2
-----MPFSMEQIISVCECLQ-----
NSGNIERLARFLWSLPKDSEIHE-----
ACETILVAKAVVAFHQNNFKELYSILESRRKQFSEHEKLCQLWRTAHYEAERVRGRPLGAVGKYRVRKKFPLPKTIWDGEETVYCFKEKSRNALKECYINRYPTPAEKKNLAKVTGLSLTQVSNWFK
NKRQRVRAAEIRKR-----

Phaw_SIX4 --
MKTEHPRPSSAESYGSFISIPDGLSTPNPRLPHYSSMNSSSSVANFNSLNFQSTASNLCSRFSKNDYTPPPHIGMGMSPMSSQSSVTTPLSSSPLSPSEPLRNIITNPHLHGSAASPFHYGAL
AAMTSGSTAPCAVQSSTSSCTNINSLSKSPAIKPQPTSPHIATVTPPEPHHRESPSLSTPKKTPSSASPSPPSSPVKTSQFNKEQIDCICEVLI---QARMMDKLSKFLNNLPASHFATD-----
-
AVSEVILRAKVEVAFKAGNYKEVYNILEATSFHANYHTHLQGMWYKAHYKEAERVRQRPLGAVDKYRIRKKYPLPKTIWDGEETVYCFKEKSRNALKDCYRQNRYPTEDEKKTLSRQTLTGLTLTQVSNWF
KNRRQRDRNPAPRPDLLLG-----
-----AHMDHGGGLAMDHVHPDMRAMYHAKMAAHAYDCKMGT-----
-----GVHPAYDPNNVMCMRMRGRADAILHHLPNVAMGSPYSSYDLQVSAATHGNSQDMREDKWTAAAGVQ-----

Ame1_SIX4 -----MPD-----SSDQLSSPN-----SECNSQIGVMHRNTSAGQFDDSS-----GLSLG-----
MPGLALHTHQSNTLSASN-----GGGSLQY-----PQELTTCSSANTSTNIGNIG-----
ITASNYPDQISCMCKALS-----QRQDIEKLTLLWSLPPGELFR-----
RDEVNLIARATVAFHRGAYHELYSILESHPFSSDRHPELQGMWYKSHYREAEKIRGRPLGAVDKYRIRKKYPLPKTIWDGEETVYCFKEKSRNALKDCYRQNRYPTEDEKKTLSRQTLTGLTLTQVSNWF
NRQRDRTPQTRT-----

Dme1_SIX4 MFDKNLDGNLNSVSIIGDLDSTSSGGTSSDHS---
AVHQDNLSMAYGSLFPLNPGYRGNLSCKTVLQDKFAPYEGVEKDHLELRRFQDITNDYDKSPPTASTTPTHYPLNSIIFENGSSGN-LGDLNNGTKTD-LCAGLQSG-GGLGNN-AGSG---
-----GHLISNL---TAHNMSAVSFPIDAKMLQFSTQIQMCEALQ---QKGDIEKLTFLCSSLPPSEFFK-----
TNESVLRARAMVAYNLGQFHELYNLETHCFSIKYHVLDLQNLWFKAHYKEAERVRGRPLGAVDKYRIRKKYPLPKTIWDGEETVYCFKEKSRNALKDCYRQNRYPTEDEKKTLSRQTLTGLTLTQVSNWF
NRQRDRTPQ-QRPDIMS-----
-----VLPVG---QLDNGFPFRMFN-----
-----APSYYPETIFNGQ-----

Dpse_SIX4 MFEKNMNTNLSASISGDLSTSSGGTSSDHS---
AAQDNLSMAYGSLFPLNPGYRGNLSCKTVLQDKFAPYEGVEKDHLELRRFQDITNDYDKSPPTASTTPTHYPLNSIIFENGSSGN-LGDLNNGTKTD-LCAGLQSG-GGLGNN-AGSG---
-----GHLISNL---TAGHNMSAVSFPIDAKMLQFSTQIQMCEALQ---QKGDIEKLTFLCSSLPPSEFFK-----
TNESVLRARAMVAYNLGQFHELYNLETHCFSIKYHVLDLQNLWFKAHYKEAERVRGRPLGAVDKYRIRKKYPLPKTIWDGEETVYCFKEKSRNALKDCYRQNRYPTEDEKKTLSRQTLTGLTLTQVSNWF
NRQRDRTPQ-QRSDIMS-----
-----VLPVG---QLDNGFPFRMFN-----
-----APSYYPETIFNGQ-----

Dvir_SIX4 MFEKNMNTNLSVINGDLSTSSAGTASDHS---AVQHDNISFPMAYGSLFPLNAGYRGNISCKTVLQDKFAPYETSEKD-
LIERRFQDITNDYDKSPPTASTTPTHYPLNSIIFENG---NSSLSDLNNGTKTDALCAGLQRAPTSTLSGTGASSG---GHLISNL--
TAHNMSAVSFPIDAKMLQFSTQIQMCEALQ---QKGDIEKLTFLCSSLPPSEFFK-----
TNESVLRARAMVAYNLGQFHELYNLETHCFSMKYHVLDLQNLWFKAHYKEAERVRGRPLGAVDKYRIRKKYPLPKTIWDGEETVYCFKEKSRNALKDCYRQNRYPTEDEKKTLSRQTLTGLTLTQVSNWF
NRQRDRTPQ-QRTDLM-----
-----LPVG---QLDANFRPFRMFN-----
-----APSYYPESIFNGQ-----

Agam_SIX4 -----TDNNNS-----YLQSLQ-----PPPHPPQFSS--
-----AASG-----GHPLG-----SSNGPIG-PDLERKCFSSPEQIQMCEALQ-----
QEGDLEKLATFLWSLSPDLIS-----
GNESLLRARALVAYHRGLYHELYAVLESHPFKYHADLQALWFKAHYREAEKVRGRPLGAVDKYRIRKKYPLPKTIWDGEETVYCFKEKSRNALKDCYRQNRYPTEDEKKTLSRQTLTGLTLTQVSNWF
NRQRDRTPQSSRPDLMM-----
-----SVLPVTPDQMDGS-YQRLFN-----
-----VANYGPAHGYHANDMYAVQ-----

Tcas_SIX4 -----MEVDATSSSTNSDNNNGFCQNLNYNENLFFTTNGKLDSSFFNSDKSARKNGENEKVARFNCYKDDFS-----
GNNVLSKSDVKNRVSLSVANY-----KKVDVNSENRKNLIDFN-----KKNMNDN-----
SILNNVDSYGGKIGFSPEQVQCMCEALH---QRGDIERLATFLWSLPPSELLR-----
GNESILRARAAVAFYRGSYHELYSILESHPFNQRWHAELQTLWFKAHYREAEKVRGRPLGAVDKYRIRKKYPLPKTIWDGEETVYCFKEKSRNALKECYARNRYPTDEKRALAKRTGLTLTQVSNWF
NRQRDRTPQPRPELILGN-----
-----MSLNQSGMMSAHQSGSLDMVAFQ-----
-----AASKLFDNSNCGVASCYSYQVP-----

[PhD_A6_phylogeny_database]

[PhD_A6.3_phylogeny_ClustalW2_alignments]

“alignment_Unplugged-Gbx-proteins.txt”

Alignment of protein sequence fractions of various unplugged/gbx genes from different species, encompassing the HD domain and VPIPV motifs.

This was done using the ClustalW2 algorithm (Larkin et al., 2007).

For details and references regarding the species' protein sequences, see Appendix (A7).

```
Dvir_unpg          SNSSKSRRRRTAFTSEQLLELELEREFHAKKYLSLTERSQIATSLKLVSEVQVKIWFQNRRAKWKRVKAGLTS HGLG---RNGSASGT-----
KIVVPIPVHVNRFVRSQHQQLEKMC LSG
Dpse_unpg          SSSSKSRRRRTAFTSEQLLELELEREFHAKKYLSLTERSQIATSLKLVSEVQVKIWFQNRRAKWKRVKAGLTS HGLG---RNGSASGT-----
KIVVPIPVHVNRFVRSQHQQLEKMC LSG
Dmel_unpg          SNSSKSRRRRTAFTSEQLLELELEREFHAKKYLSLTERSQIATSLKLVSEVQVKIWFQNRRAKWKRVKAGLTS HGLG---RNGTTSGT-----
KIVVPIPVHVNRFVRSQHQQLEKMC LSG
Agam_unpg          AENSKSRRRRTAFTSEQLLELELEREFHAKKYLSLTERSQIATSLKLVSEVQVKIWFQNRRAKWKRVKAGLNS HGLGN--RNAGSGSGT-----
TTGTANKIIVVPIPVHVNRFVRSQHQQMEKMNLV G
Mmus_gbx2         TSTGKNRRRRTAFTSEQLLELEKEFEHCKKYLSLTERSQIAHALKLVSEVQVKIWFQNRRAKWKRVKAGNANSKT-----GEP SRN-----
PKIVVPIPVHVS RFAIRSQHQQLEQ-ARP-
Dre_r_gbx2        TSTGKNRRRRTAFTSEQLLELEKEFEHCKKYLSLTERSQIAHALKLVSEVQVKIWFQNRRAKWKRVKAGNANSKT-----GEP SRN-----
PKIVVPIPVHVS RFAIRSQHQQLEQ-ARP-
Mmus_gbx1         TPGGKSRRRRTAFTSEQLLELEKEFEHCKKYLSLTERSQIAHALKLVSEVQVKIWFQNRRAKWKRIKAGNVSSRS-----GEP VRN-----
PKIVVPIPVHVNRFVRSQHQQMEQGARP-
Dre_r_gbx1        GSAGKSRRRRTAFTSEQLLELEKEFEHCKKYLSLTERSQIAHALKLVSEVQVKIWFQNRRAKWKRIKAGNVNRS-----GEP VRN-----
PKIVVPIPVHVNRFVRSQHQQIEPGSRP-
Pdum_gbx          SGGGKSRRRRTAFTSEQLLELEKEFEHCKKYLSLTERSQIAHNLKLVSEVQVKIWFQNRRAKWKRVKAGY INGRAPTTTSPGEQNNK-----
PKIIVPIPVHVNRM AIRSQHQQLEERSGRLI
Nvit_unpg         GAGSKARRRRTAFTSEQLLELELEREFHAKKYLSLTERS SHIAHALKLVSEVQVKIWFQNRRAKWKRVKAGLTS GGAGN--
PAARHSGGGSSAGGHQSNGGGPRIVVPIPVHVSRLAVRSHHHLEKCPQPA
Tcas_unpg         GTGTKARRRRTAFTSEQLLELELEREFHAKKYLSLTERSQIASALRLSEVQVKIWFQNRRAKWKRVKAGL GAG-----PHQPKSGG-----QQKS----
KLVVPIPVHVNRFVRSQHQQLEALGDL
Phaw_gbx          GTGSKNRRRRTAFTSEQLMELELEREFQTKKYLTLSERSHIAQTLHLSEVQVKIWFQNRRAKWKRVKAGLVGS-----SGGGLVGG----
TNKGGAGGGHKIIVPIPVHVNRLSNLSQAHQIEKCRGSA
Isca_Xvent-2B    GVTSLQRRRRTAFSSEQLLELEKEFEHCKKYLSLTERSQIAHALQLTSEVQVKIWFQNRRAKWKRVKAGL TAGR-----GPHSHP-----
DAPRIVVPIPVHVNVALRNQDDAKGYVLSLA
Nvec_gbx         LSSASRRRRTAFTSKQLLQLELEREFHNKKYVLSLEERSVIATNLNLTSEVQVKIWFQNRRAKWKRVRS-LSHPAHG-----NAKKINV-----
PKLVVPIPIHG NRFAVMNQANAL-----
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[PhD_A6_phylogeny_database]

[PhD_A6.3_phylogeny_ClustalW2_alignments]

"alignment_Unplugged-Gbx-similar_proteins.txt"

Alignment of the complete protein sequences of various unplugged/gbx and similar genes from different species. This was done using the ClustalW2 algorithm (Larkin et al., 2007). For details and references regarding the species' protein sequences, see Appendix (A7).

```
Mmus_Gbx1 -----
MSPEGDTNPPARPLPPYERESAEPHCAEPAPRRHLGPRARSEPCVSARGARRGPGSAMQRAAGGAPGGSSGGSPGAAPSIDSLIGPPP-PRSGHLLTYGPMFMPYRPLVLP-----
QALAPAPLP-----AGLPPLAPLASFAGRLSNTFCAGLGQAVPSMVALTTALP-SFAEPPDAYYGPPELLAAAAASTASRSNPEPAARR-----
-----TDGALDAEELLPAEKVTEPPPPPPHSETFPPSLPAEGKVYSSDEEKLEPPAGDPAGSEQEEEGS-----
GGDESDSFLDSSAGGPGALLGPKPKLKGSPGTGAEEG--TPVATG-----
VTFPGGKRRRRRTAFTSEQLLELEKEFHCKKYLSTERSQIAHALKLVSEVQKWFQNRRAKWKRIK-----AGNVSSRSGE-----PVRNPKIVVPIPVHVNRFVRSQHQMQGARP-----
-----

Mmus_Gbx2 -----MS-----AAFPPS-----
LMMQRPLG-----SS-----TAFSIDSLIGSPQPSPGHFVYTYGPMFMPYRPPVLPPLPPPPPPALPQAALQ-----PALPPAHPHHQIP-
SLPTGFCSSLAQGMALTSMLATLPGGFSASP-----QHQAARAKFAQPLPGGGNFD-----
KAEALQADAEDGKAFLAKEGSLLAFAAEAVQASLVGAVRGQKDESKVE---DDPKGKEE-----SFSLESVDVYSSDD--NLPQQTAKHEEDPGHALEETPQSGGAAG-----
STTSTGKNRRRTAFTSEQLLELEKEFHCKKYLSTERSQIAHALKLVSEVQKWFQNRRAKWKRIK-----AGNANSKTGE-----PSRNPKIVVPIPVHVSRAFAIRSQHQLEQ-ARF-----
-----

Dmel_UNPG -----
MERRALLQNGEIGTMESPTRLASKPPKPFIESLIANQTPATATPPSPPEERDQEQEAEQEQELSARAMVASSALGLTQFPLYNFWLHGFAQNHERLTHLIAGGCYLPSSPAG-HPAAQPPQA---
-----QAQPQPPPPHPTHALEKQLPPTLPHPLDTRFLPFNPAAGVAPTDLSYRRLAELMNDYVHLSVHARLQHMA-----
-----AAGRMHEDQANPGMAQLQEPFPQAHS SPAKSGSHSPMEPALDVGMEDEFECSGSDCSDSLSTMS-----
PRNYNGEMDKSRNGAYTNSDSEDCSDEGAQSRHEGGMGKDSQG-----
NGSSNSKRRRTAFTSEQLLELEKEFHAKKYLSTERSQIATSLKLVSEVQKWFQNRRAKWKRIK-----AGLTSHGLGRNG-----
TTSGTKIVVPIPVHVNRFVRSQHQLEKMLCSGPKPDLRKKLSAEIAGGFEKFSGNTASSPSGGPVGLGVGVGVGLVSTPLSLRSIY-----
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Mmus_Meox1 -----MDPVANSCVRNP-----QPPAPVWGC-----
LRNPHSEDSASGLSHYPTFPFSFHQKSDFPATAAYPD---FSASCLAATPHSLPRTERIFNEQHPAFQPTDW-----
HFP-----ISEAGQRLNLGP-AGSAREMGAGSPLVDGTAGLG-----EDCMVLGTIAN-----ETEKK-SRRKKERSDNQNGG-----
GKPEGSSKARKERTAFTKEQLELEAEFAHNNYLRLRRIEYIAVNLDSLSEVQKWFQNRRAKWKRIK-----GGQ-----PVSPQEQD-----REDGDSAAASP-----
SSE-----

Mmus_Meox2 -----MEHPLFGCLRSP-----HATAQGLHP-----FSQSSLALHGRSDHMSYP-----
ELSTSSSSCIAGYPNEEGMFPASQHRHGHSHHHHHHHHQHQALQSNW-----
HLPQMSPPSAAARHSLCLQPDGSGPPELGSPPVLCNSSSLG-----SSTPTGAACAPGDYGRQALSADVEKRSKSRKSDSDSQE-GN-----
YKSEVNSKPRKERTAFTKEQIRELEAEFAHNNYLRLRRIEYIAVNLDLTERQKWFQNRRAKWKRIK-----GGQGAAREK-----
ELVNVKGTLLPSELGIGAATLQQTGDSLANEDSRDSDHSEHAHL-----

Phaw_GBX -----
MLASSEMTCDSPPKSPPTTKMGPNSKSFAMEYLLTISGQDKNDCNRKSMKTSAASTLLSRETDTHPNHLPAHMLDISRQLSQTQISQNLALPHLYNSCLLTGG---MASVGLPPF-----
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ALQSRHSFAFQLSSSTSSCSGSHSELNGLSSGRNGSALEAHLGHTKEVTEQLKSMRNGGRTSTTSSTSLQCYFQNHERISP
Mmus_Mnx1 -----EATAAPGDR-----
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Dmel_EXEX -----MQSSKSLIRDLLG-----
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Dmel_CG11085 -----
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SGRKPRRRRTAFTHAQLAYLERKFRQKYLSDVADRSDVAETLNLSETQVKTYQNRRTKWKRN-----
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Dmel_B-H1 -----
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STSDMSGSLKQKARTAFTHQLQLEKSFERQKYLVSQERQELAHKLDLSDCVKTYQNRRTKWKQRTAVGLELLAEAGNFAAFQRLYGGSPYLGAWPYAAAAGAAGATPHTNIDIYYRQAAAA
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Mmus_Nkx1-2 -----
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-----RAESSCAKPRRARTAFTEYQLVALENKFRATRYLSVCERLNALSLTETQVKIWFQNRRTKWKQN-----
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Dmel_SLOU
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Dmel_DR -----
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RLSNFS-----VASLLADTRPTPPNQAADGPQNLTSAAATSPISQASSTPPPPASAAAQVPANTFHPAAVAHHAHLLQAHAHAHAHQ-----

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[PhD_A7_abbreviations]

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A7 Abbreviations

cDNA	complementary DNA
DsRed	<i>Discosoma</i> species red fluorescent protein
dsRNA	double-strand RNA
EGFP	enhanced green fluorescent protein
hp	hairpin
mRNA	messenger RNA
siRNA	small interfering RNA
ORF	open reading frame
RNAi	RNA interference
UTR	untranslated region
WT	wild-type