

### 7.1.3 Mth212/D151X mutants creation and verification

#### Primers for Mth212/D151X (X: N, A, S) mutant creation (2.1.3.1):

##### D151N

212\_D151N\_FOR (DNA, 27mer) 5'-GATTATATGTGGG AAC TTCAACACAGC-3'  
 212\_D151N\_REV (DNA, 27mer) 5'-GCTGTGTTGAA GTT CCCACATATAATC-3'

##### D151A

212\_D151A\_FOR (DNA, 27mer) 5'-GATTATATGTGGG GCA TTCAACACAGC-3'  
 212\_D151A\_REV (DNA, 27mer) 5'-GCTGTGTTGAA TGC CCCACATATAATC-3'

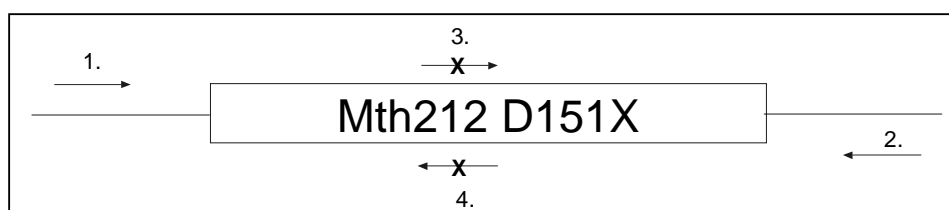
##### D151S

212\_D151S\_FOR (DNA, 27mer) 5'-GATTATATGTGGG TCA TTCAACACAGC-3'  
 212\_D151S\_REV (DNA, 27mer) 5'-GCTGTGTTGAA TGA CCCACATATAATC-3'

#### Sequencing primers for pET-vector ( 2.1.3.1):

PETS1\_LO (18mer) 5'(d)ATAGGGGAATTGTGAGCG 3'

PETS1\_UP (18mer) 5'(d)CAGCAGCCAACTCAGCTT 3'



**Figure 25:** Schematic representation of Mth212 gene (black rectangle), gene flanking regions originated from pET\_B001 vector (black lines on the left and right sides from the gene) and primers (black arrows) used for sequence analysis and site-directed mutagenesis, where 1. and 2. indicate sequencing primers for pET-vector and 3. and 4. the mutagenic primers (listed above).

All chromatogram files are stored in ,ab1' format on attached CD and can be downloaded using Chromas Software attached as well.

**6.2.3.1 Chromatogram files of Mth212/D151N mutant (clones 2,4):**

Sequence Name	Date	Clone N	Primer
26_B04_Shcoma_7_eHS	17.08.2005	2	PETS1_LO
21_E03_Schoma_2_eHS	17.08.2005	2	PETS1_UP

Sequence Name	Date	Clone N	Primer
28_D04_Schoma_9_eHS	17.08.2005	4	PETS1_LO
23_G03_Schoma_4_eHS	17.08.2005	4	PETS1_UP

**6.2.3.2 Chromatogram files of Mth212/D151A mutant (clones 1,3,4):**

Sequence Name	Date	Clone N	Primer
C02_a1-plo.scf	14.02.2006	1	PETS1_LO
E01_a1-pup.scf	14.02.2006	1	PETS1_UP

Sequence Name	Date	Clone N	Primer
D02_a3-plo.scf	14.02.2006	3	PETS1_LO
F01_a3-pup.scf	14.02.2006	3	PETS1_UP

Sequence Name	Date	Clone N	Primer
E02_a4-plo.scf	14.02.2006	4	PETS1_LO
G01_a4-pup.scf	14.02.2006	4	PETS1_UP

**6.2.3.3 Chromatogram files of Mth212/D151S mutant (clones 1,2,3):**

Sequence Name	Date	Clone N	Primer
F02_s1-plo.scf	14.02.2006	1	PETS1_LO
H01_s1-pup.scf	14.02.2006	1	PETS1_UP

Sequence Name	Date	Clone N	Primer
G02_s2-plo.scf	14.02.2006	2	PETS1_LO
A02_s2-pup.scf	14.02.2006	2	PETS1_UP

Sequence Name	Date	Clone N	Primer
H02_s3-plo.scf	14.02.2006	3	PETS1_LO
B02_s3-pup.scf	14.02.2006	3	PETS1_UP