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Supplemental Data

The Regulation and Evolution of a Genetic Switch Controlling Sexually Dimorphic Traits in *Drosophila*

Thomas M. Williams, Jane E. Selegue, Thomas Werner, Nicolas Gompel, Artyom Kopp, and Sean B. Carroll

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Reporter Constructs and Transgenic Fly Production

Primer combinations used to PCR amplify the sequences analyzed in the *bab* locus screen are provided in Table S1 and combinations used in truncation analysis to determine the minimal-sufficient sequences of the *D. melanogaster* dimorphic and anterior elements are provided in Table S2. The orthologous *D. willistoni* anterior and dimorphic elements were amplified respectively using the primer combinations: ggtaccTCGAGATGTTGATGGAGAAGGAGA and gctagcCTTGGATGTTTTGAATGGTGACCT; ggcgcgccCACATAAAAATCAGCAACAAASTTGC and cctgcaggGCCACGCCCTYAATCAACTAAGTAA.

Mutations to dimorphic elements were made using the QuickChange Site-Directed Mutagenesis Kit (Stratagene) or by PCR Synthesis by Overlap Extension. The bab1 intron 4 and intron 5 sequences were cloned into the transformation vector RINheXho (Wittkopp et al., 2002), a derivative of the *hsp70-LacZ* CaSpeR plasmid. All additional sequences involved in the *bab* locus screen, truncation analysis, and the *D. wil* anterior and dimorphic elements (Figure 5) were cloned into a modified version of

the P-element based transformation vector SMG2 (Gompel et al., 2005) that additionally includes the endogenous *bab2* promoter (as suggested by J-L Couderc). Wild-type and mutant versions of the *D. mel.* and *D. wil.* dimorphic elements whose activity was quantitatively assessed in the same transgenic insertion site (Figure 3, Figure 4, Figure 6, and Table S3) were cloned into a modified reporter vector called S3aG. This vector contains the *hsp70* promoter upstream of the GFP gene and a 284 base pair *attB* recombination sequence inserted in the *AvrII* restriction enzyme site of the SMG3 p-element transformation vector (gift from B. Prud'homme). This *attB* sequence was PCR amplified from the *attB*-P[acman] plasmid template (Venken et al., 2006) using the primers cctaggTCGACATGCCCGCCGTGACC and cctaggGTCGACGATGTAGGTCACGGTCTC.

The *D. melanogaster yw* strain was used for p-element mediated transgenesis (injection protocol can be obtained at: http://www.molbio.wisc.edu/carroll/methods//methods.html). Site-specific integration of transgenes was accomplished by injecting embryos from flies containing the X-chromosome *attP* docking site VK00046 (Venken et al., 2006) and a genomic ϕC31-integrase source on chromosome II (gift from F. Karch). The standard injection protocol was varied only by the absence of a helper plasmid in the injection mix for site-specific transgenesis. The activity of all reporter constructs except for bab1 intron 4 and intron 5 were imaged for EGFP expression. The intron 4 and transgenes activity were assessed by *lacZ* staining. Briefly, samples were fixed for 10 minutes in 1% glutaraldehyde in 10mM NaH2PO4 pH7.0; 150mM NaCl; 1mM MgCl2; 0.1% Triton-X. Samples were washed and then stained until ready in 0.1% X-Gal in 10mM NaH2PO4

pH7.0; 150mM NaCl; 3.3 mM K4[Fe(CN)6]; 3.3 mM K3[Fe(CN)6].; 0.1% Triton-X. Results relevant to this study are reported in Table S1-S3.

Annotation of the *bab* Noncoding Sequences

Scaled representations of the *bab* locus (Figure S1A) and *bab1* (Figure 1E, Figure 3A, and Figure S1B) and their annotation with the surveyed non-coding sequences were produced using the GenePalette software tool (Rebeiz and Posakony, 2004).

Protein Production and DNA-Binding Analyses

The coding sequence for Bab1 amino acids 273-473 (referred to as Bab1 20 kDa) was amplified by PCR from *Canton*^{S} genomic DNA using the primers gaattcGCTGAGCTGAGGCTCTCCCCA and gcggccgcTTACTAGCCCACTCCACCACCGCCAT. The coding sequence for the first 106 amino acids containing the DNA-binding domain and common to both DSX^{F} and DSX^{M} (referred to here after as DSX DBD) was PCR amplified from a cDNA containing plasmid using the primers ggatccATGGTTTCGGAGGAGAACTGGAA and ctcgagTTACTACACCTCGTGCATGTGCAGCG. Each of these regions were cloned into the pGEX4-T1 protein expression vector (Pharmacia-Amersham) using the restriction enzyme site combinations EcoRI /NotI and BamHI /XhoI respectively.

GST-Bab1 20kDa, GST-Dsx DBD and GST-AbdB HD (Jeong et al., 2006) fusion proteins were purified from the BL21 Lys S *E. coli* strain as described previously (Williams et al., 1995) and purified using glutathione-agarose according to the

manufacture's protocol (Pharmacia-Amersham). DNaseI footprinting reactions and EMSAs were performed in 1X footprinting buffer as previously described (Jeong et al., 2006) with the following modifications; annealed oligonucleotide probes for EMSAs had 5' T-overhangs that were end-filled with [α-^{32}P] dATP using the Klenow fragment (Roche). EMSA binding reactions were performed without poly(dI-dC). PAGE-purified oligonucleotides for EMSAs are listed in Table S4.

Antibody Production

Rabbits were immunized with purified GST-Bab1 20 kDa recombinant protein (21st Century Biochemicals) and collected serum was affinity-purified using a GST-Bab1 20 kDa protein column. GST-reactive antibodies were depleted using a GST-only protein column (Williams et al., 1995).

Sequence Analysis of Orthologous Dimorphic Elements

Sequences for *D. erecta*, *D. willistoni*, *D. virilis*, and *D. grimshawi* were obtained from their respective genome databases. All other sequences were obtained by cloning and sequencing of orthologous sequences using genomic DNA prepared from species stocks obtained from the Tucson *Drosophila* stock center. The primer combination used to PCR amplify the sequences from *D. saltans*, *Z. vittiger*, and *D. tripunctata* were CACATAAAAATCAGCAACAAASTTGC and GCCACGCCCTYAATCAACTAAGTAA, which does not include the binding site 14 region. All other sequences were amplified by the primer combination CACATAAAAATCAGCAACAAASTTGC and CAAAACKGCRCATAAAAMSAAATTACA, which includes the binding site 14 region.

Novel sequences have been deposited in GenBank as follows: *D. melanogaster* *Canton*^{S} strain (EU835207), *D. mauritiana* (EU835206), *D. fuyamai* (EU835205), *D. auraria* (EU835204), *D. obscura* (EU835203), *D. saltans* (EU835202), *Z. ghesquierei* (EU835201), *Z. vittiger* (EU835208), *D. tripunctata* (EU835209). Orthologous sequences were aligned using CHAOS + DIALIGN (Brudno et al., 2004) with subsequent manual alignment in problematic regions.

Measurement of Relative Fluorescence Intensity

The relative fluorescence intensities for GFP-reporter expression in segment A6 were determined as previously described (Jeong et al., 2008) with the following modifications. All transgenic constructs compared were integrated into the same genomic site to eliminate variation due to site of integration. For each transgene, a representative line was chosen for quantification based on a prior qualitative survey of multiple independent lines. All lines were measured on the same confocal microscope over a short time interval to reduce differences in measurements due to the microscope. Using transgenic female pupae containing the wild-type dimorphic element GFP-reporter transgene, the optimal confocal settings were determined as those that gave the brightest image with the fewest saturated pixels in segment A6. Mean fluorescent intensity of segment A6 for each image was calculated as the mean intensity measured for segment A6 minus the mean intensity of segment A3 (background correction). For each construct, the mean value for A6 intensity and standard error of the mean was calculated using images from multiple independent samples from the same transgenic line (n of 2-5, Table S3). The percent of wild-type activity was calculated as:

(experimental A6 mean/A6 mean of wild-type dimorphic element in female pupae) X 100. In our experience using the *attP* line VK00046 (referred to as X-out), we found that the activity and variation between transgenic lines is not consistently or significantly greater than that of individuals from the same line. For example, for three independent lines of the transgenic construct mel 14 KO, the % wild type activity and SEM was determined as 55%±0 (line 1), 56%±1 (line 2) and 54%±1 (line 3), while the between line comparison gave 55.7%±1 (line 1 vs. 2), 54.7%±0 (line 1 vs. 3) and 55.2%±1 (line 2 vs. 3).

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Figure S1. Screen of the *bab* locus for pupal cis-regulatory elements (CREs) active in pupae.

(A) The *D. melanogaster bab* locus consists of the tandemly duplicated genes *bab1* and *bab2* which are located between the adjacent genes *trio* and *CG13912* (red). Numbers and adjacent bars indicate the *bab* locus region surveyed in a given reporter construct. Within the *bab* locus, the only non-coding sequences that were not tested for CRE activity were those that consisted of sequences derived from a *ROO* and a *mdg* transposable element and sequences between the 1st exon of *trio* and the *mdg* element. (B) To scale representation of the informative truncated GFP-reporter constructs tested that identified the minimal sufficient sequences of the dimorphic and anterior elements. The screen of the *bab* locus identified CREs regulating expression in abdominal oenocytes (C; 4); leg tarsal segments (D; 10); abdominal bristle and longitudinal muscle cells (E; 28). The red arrow points to a representative longitudinal muscle expressing GFP. The first 15 kb of the *bab1* intron drove dimorphic reporter expression in the pupal abdominal epidermis, with expression in males limited to segments A2-A4 (F; 17), while females had also expressed the reporter in posterior segments A5-A7 (G; 17). This dimorphic pattern is mediated by the anterior element (32) that drives monomorphic expression in segments A2-A4 (H) and the dimorphic element (23) that drives female-specific expression in A5-A7 (I). Additional information on the numbered GFP-reporter gene constructs can be found in Table S1 and Table S2.

Figure S2. DNaseI footprinting analysis of the dimorphic element identifies multiple sites bound by ABD-B

DNase I footprinting analysis on the *D. melanogaster* dimorphic element determined that of the 14 ABD-B consensus sites, all but sites 9 and 10 were appreciably bound by ABD-B HD protein. Amounts of each protein used were as follows: lane 1, 1000 ng GST only; lane 2, no protein; lane 3, 64 ng ABD-B HD; lane 4, 160 ng ABD-B HD; lane 5, 400 ng ABD-B HD; lane 6, 1,000 ng ABD-B HD. A G+A sequencing ladder is included in lane L. The location of the 14 consensus binding sites are indicated by a black rectangle with the site's number adjacent to it. The additional TTAT motifs (non-TTTAT) are indicated by black rectangles with an adjacent "t".

Figure S3. Direct binding of ABD-B to multiple sites within the dimorphic element.

EMSAs were performed separately on annealed oligonucleotide probes containing the wild-type and mutant (KO) ABD-B putative binding sites 4, 8, 13 and 14 (see text) with increasing amounts of ABD-B HD protein (from left to right: 0 ng, 16 ng, 62 ng, 250 ng, and 1,000 ng). For probes containing the wild-type binding sites 8 (lanes 1-5), 13 (lanes 11-15), 14 (lanes 21-25), and 4 (lanes 31-35), as the amount of ABD-B HD protein increased a correlative increase in the amount of probe bound was observed. Protein binding was strongly reduced when sites 8 (lanes 6-10), and 14 (lanes 26-30) were mutated, and completely abolished when site 4 (lanes 36-40) was mutated. Compared to sites 4, 8 and 14, site 13 (lanes 11-15) was not bound as efficiently by the ABD-B HD and mutation of the site (lanes 16-20) resulted in a smaller decrease in binding.

Figure S4. The Dsx1 site of *D. willistoni* is of the same affinity but opposite polarity to the Dsx1 site of *D. melanogaster*.

EMSAs comparing the ability of DSX to bind annealed oligonucleotide probes containing the *D. mel.* Dsx site 1 (Dsx1 WT; lanes 1-5), a mutated version of Dsx site 1 (Dsx1 KO; lanes 6-10), and the Dsx site 1 of *D. wil.* (Dsx1 wil; lanes 11-15). For each probe, binding reactions were performed using increasing amounts of the DSX DBD protein (from left to right: 0 ng, 16 ng, 62 ng, 250 ng, and 1,000 ng). In contrast to the mutant probe (lanes 6-10) where DSX binding was reduced >16-fold, the *D. wil.* binding site (lanes 11-15) was bound as efficiently by DSX as the *D. mel.* site. Blue arrowhead points to the location on gel of complex between the probe and a single DSX DBD monomer, and the red arrow indicates location of a complex containing probe bound by two monomers. Below the respective lanes for each probe are the sequences of the binding sites tested. Red bases indicated those changed in the Dsx1 KO probe and blue bases are the changes made in the Dsx1 wil probe.

Figure S5. Alignment of orthologous sequences containing dimorphic elements.

Sequences containing the dimorphic elements from 12 species from the genus *Drosophila* were aligned and annotated with relevant features. Species included are: *D. melanogaster* (D. mel), *D. mauritiana* (D. mau), *D. simulans* (D. sim), *D. erecta* (D.ere), *D. fuyamai* (D. fuy), *D. auraria* (D. aur), from the *melanogaster* species group of the subgenus *Sophophora*; *D. obscura* (D. obs), *D. willistoni* (D. wil), *D. saltans* (D. sal) from the *obscura*, *willistoni*, and *saltans* species group of the subgenus *Sophophora*; and the non-Sophophoran species *Zaprionus ghesquierei* (Z. ghe), *D. virilis* (D.vir), *D.*

grimshawi (D. gri). Additionally, the Dsx1 sites of the non-Sophophoran *D. tripunctata* (D. tri), and *Zaprionus vittiger* (Z. vit) were added to the alignment which indicates that the ancestral orientation of this site in the common ancestor of *Sophophora*, and the Dsx site 2 of Z. vit was added to indicate that this site predates the common ancestor of *Sophophora*. The additional TTAT sites in the D. mel dimorphic element are bolded in brown and indicated below the alignment by "t". Regions I, II, III of increased spacing in D. wil are underlined and the region where these sequences were inserted in D. mel are indicated below the alignment by the adjacent "□" below the alignment. Binding sites 1-14 are indicated in bolded red letters and annotated below the alignment. The nucleotides adjacent to site 14 are in blue which confirm the sites orthology in otherwise poorly conserved sequences. Dsx sites 1 and 2 are bolded in blue (yellow when the polarity is inverted) and annotated below the alignment.

Figure S1

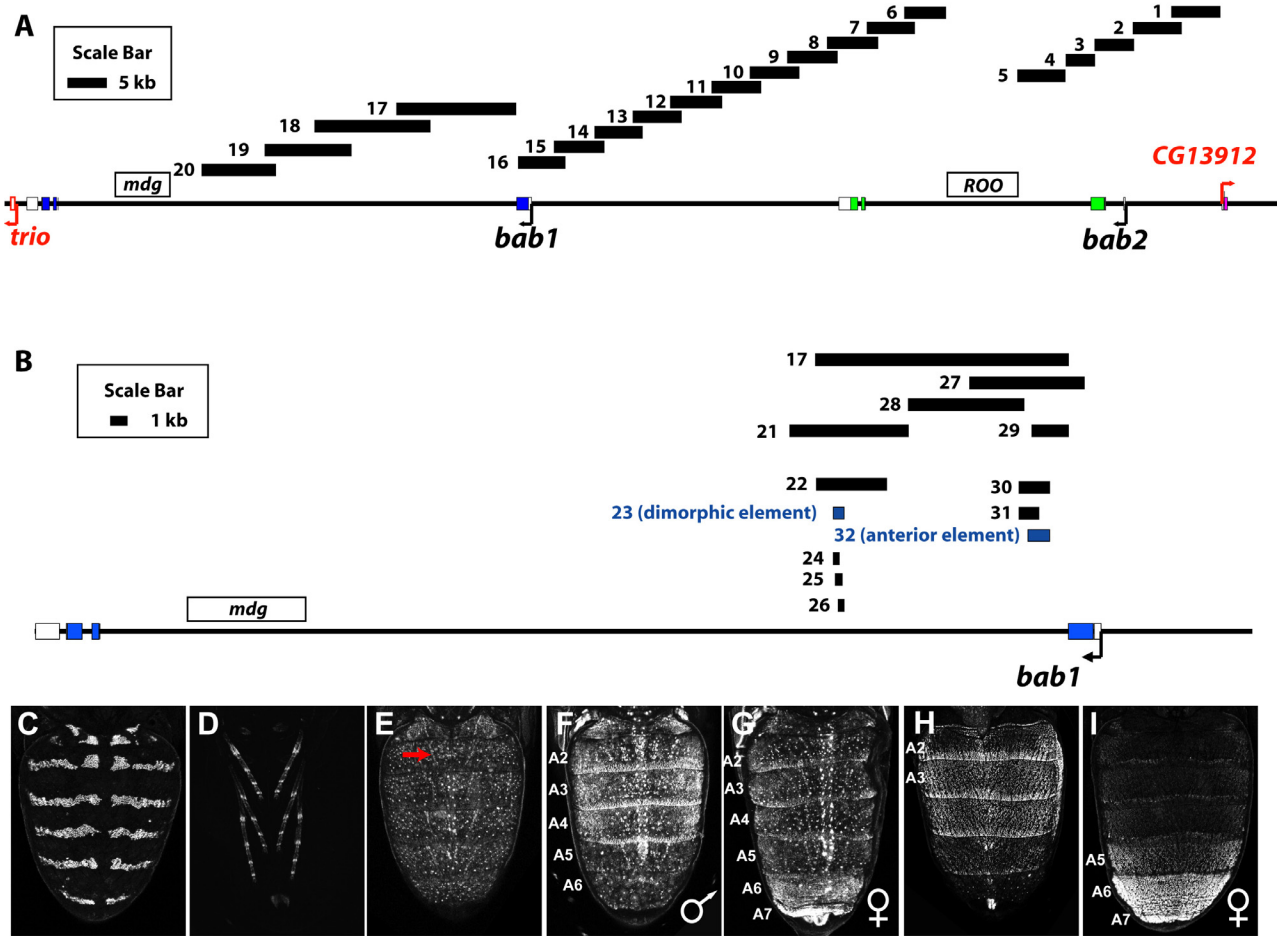
A
Scale Bar
5 kb
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
mdg
ROO
CG13912
trio
bab1
bab2
B
Scale Bar
1 kb
17
27
28
21
29
22
30
23 (dimorphic element)
31
32 (anterior element)
24
25
26
mdg
bab1
C
D
E
F
G
H
I
A2
A3
A4
A5
A6
A7

Figure S2

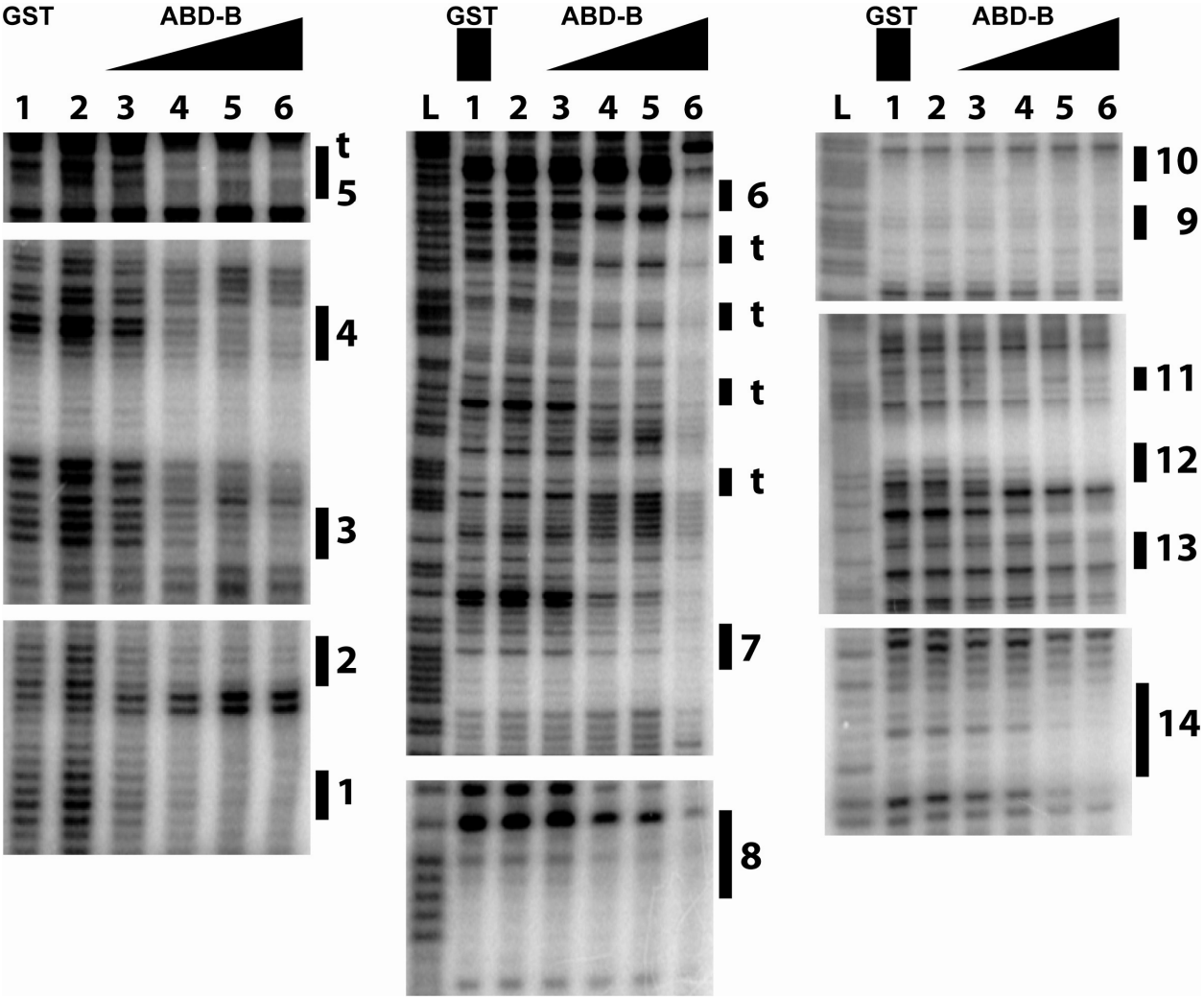
GST
ABD-B
1 2 3 4 5 6
t
5
4
3
2
1
GST
ABD-B
L 1 2 3 4 5 6
6
t
t
t
t
7
8
GST
ABD-B
L 1 2 3 4 5 6
10
9
11
12
13
14

Figure S3

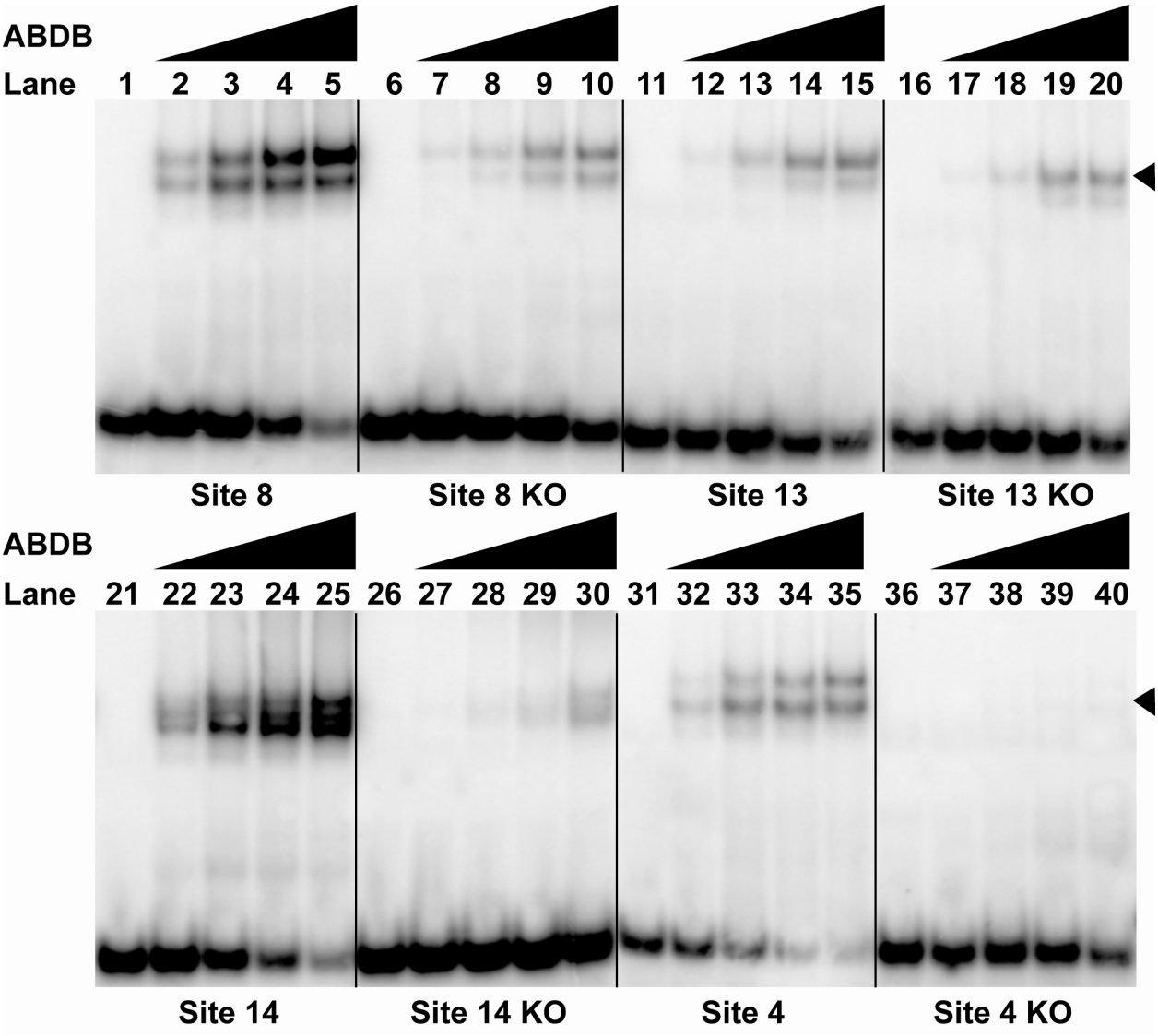
ABDB
Lane 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Site 8
Site 8 KO
Site 13
Site 13 KO
ABDB
Lane 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Site 14
Site 14 KO
Site 4
Site 4 KO

Figure S4

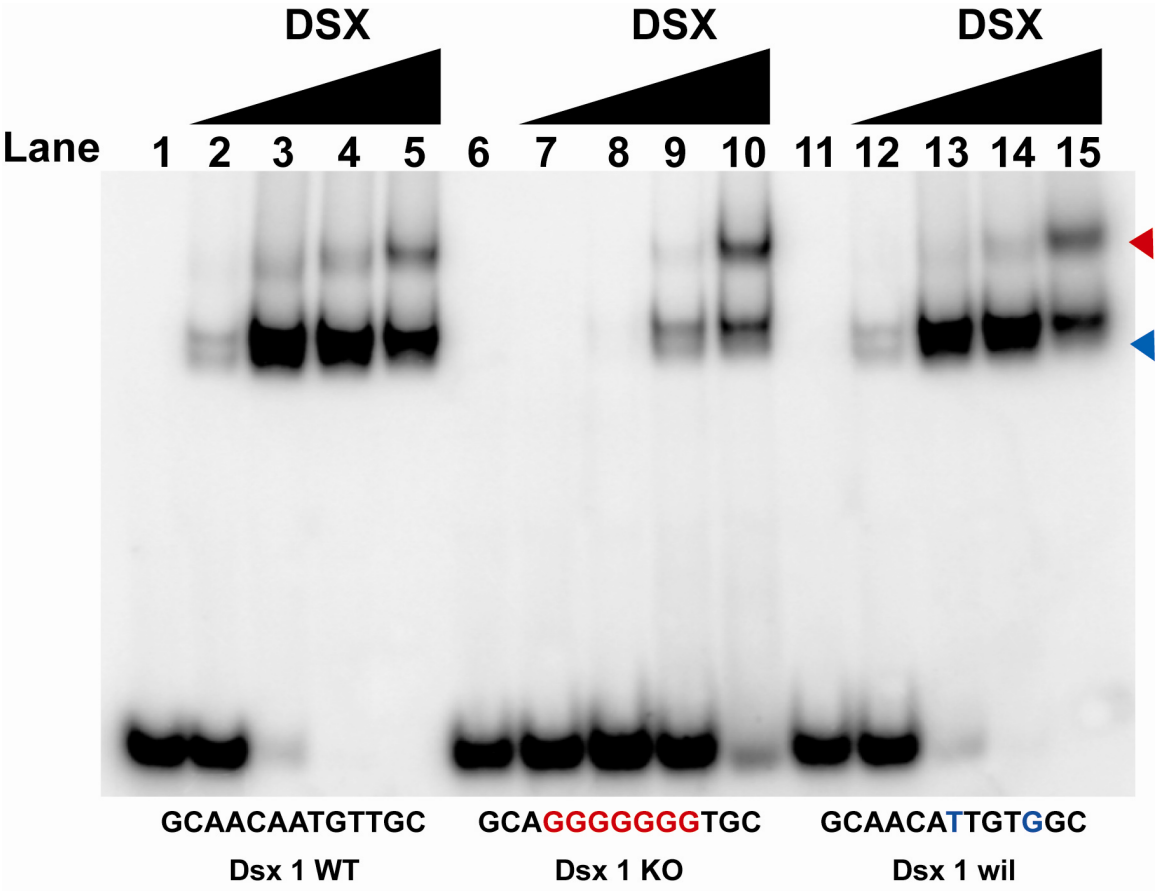
DSX
DSX
DSX
Lane
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
GCAACAATGTTGC
Dsx 1 WT
GCAGGGGGGGTGC
Dsx 1 KO
GCAACATTGTGGC
Dsx 1 wil

Figure S5

```
D.mel     CACATAAAAA TCAGCAACAA AGTTGCTCTG G------CCC CATAAAAGAT
D.mau     CACATAAAAA TCAGCAACAA AGTTGCCCTG G------CCC CATAAAAGAT
D.sim     CACATAAAAA TCAGCAACAA A-TTGCCCTG G------CCC CATAAAAGAT
D.ere     CACATAAAAA TCAGCAACAA ACTTGCCCTG G------TCC CATAAAAAAT
D.fuy     CACATAAAAA TCAGCAACAA AGTTGCCCTG G------CCC CATAAAAAAT
D.aur     CACATAAAAA TCAGCAACAA AGTTGCCCTG G------CCC CATAAAAAAT
D.obs     CACATAAAAA TCAGCAACAA ACTTGCCaac tggaaaaCCC CATAAAAAAT
D.wil     CACATAAAAA TCAGCAACAA ACTTGCGCCT ---------- CATAAAAAAA
D.sal     CACATAAAAA TCAGCAACAA ACTTGCGCCT ---------- CATAAAAAAA
Z.ghe     CACATAAAAA TCAGCAACAA AGTTGCGGCT ---------- CATAAAAAAT
D.vir     CACATAAAAA TCAGCAACAA AGTTGCGGCT ---------- CATAAAAAAT
D.gri     CACATAAAAA TCAGCAACAA AGTTGCGGCT ---------- CATAAAAAAT
          (Dimorphic element wil Fwd Primer)>

D.mel     TGCAAACAAA AACAGAACA- -ACAGAA--- ---------- ----------
D.mau     TGCAAACAAA AACAGAACA- -ACAGAA--- ---------- ----------
D.sim     TGCAAACAAA AACAGA--AC AACAGAA--- ---------- ----------
D.ere     TGCAAACAAA AACAGA--AC aacagaa--- ---------- ----------
D.fuy     TGCAAACAAA AAGAGA--AC Aac--AA--- ---------- ----------
D.aur     TGCTAACAAA aaaggagaAC AACAGAA--- ---------- ----------
D.obs     TGCAtttaca tctgagaaaa aaaaaaacac acacacaaga aataaaagcc
D.wil     ATTGC----- ---------- ---------- ---------- ---ACAAAAA
D.sal     ATTGC----- ---------- ---------- ---------- ---ACAAAAA
Z.ghe     TGCGCACAAA TAAAAAACAA cagcaacacc aacaacaaca aaaACAAAAA
D.vir     TGCGCACAAA TAAAAAACAA caacaa---- ---------- ----------
D.gri     TGCGCACAAA TAAAAAAaaa taaaaaaata acaacaacaa caagaacaag

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     aaatgtaaag aaacgaggtg gagatactct tttaaagggc atttgttatg
D.wil     CA------AA A--------- ---------- ---------- ----------
D.sal     CA------AA A--------- ---------- ---------- ----------
Z.ghe     CA------AA A--------- ---------- ---------- ----------
D.vir     -A------AA A--------- ---------- ---------- ----------
D.gri     aacaacatAA A--------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
```

```
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     gtttgtaggt catatatact gagccattct taatggatgc caaagctaca
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     atcttcacag ttctattagc aaaatatcag aatttgggga ttaatctccc
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     aatttctccc ttaaaattcc tccctttcac tcaggagaac atttttaaca
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     gaacaaaaaa aaaacagtaa ctaattcagc gacaattatt tcgaatgtgt
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------
```

```
D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     gttgcccatg gcacaggtga agccatggcc tatacaaacc cataatcgaa
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----TGGCAT
D.mau     ---------- ---------- ---------- ---------- ----TGGCAT
D.sim     ---------- ---------- ---------- ---------- ----TGGCAT
D.ere     ---------- ---------- ---------- ---------- ----TGGCGT
D.fuy     ---------- ---------- ---------- ---------- ----TGGCAT
D.aur     ---------- ---------- ---------- ---------- ----TGGCAT
D.obs     actcggtaca gggtaccgaa tagatgcaac aacagacaga attgTGGCAT
D.wil     ---------- ---------- ---------- ---------- ----TGGCAT
D.sal     ---------- ---------- ---------- ---------- ----TGGCAT
Z.ghe     ---------- ---------- ---------- ---------- ----TGGAAT
D.vir     ---------- ---------- ---------- ---------- ----TGGCAT
D.gri     ---------- ---------- ---------- ---------- ----TGGCAT

D.mel     GGAATAAAAT TTATATGAAT AACAAAAAGC AGCTAAAGCA ----AGC---
D.mau     GGAATAAAAT TTATATGAAT AACAAAAAGC AGCTAAAGCA ----AGC---
D.sim     GGAATAAAAT TTATATGAAT AACAAAAAGC AGCTAAAGCA ----AGC---
D.ere     GGAATAAAAT TTATATGAAT AACAAAAAGC AGCTACAGCA ----AGC---
D.fuy     GGAATAAAAT TTATATGAAT AACAAAAAGC AGCTAAAaga -AACAGC---
D.aur     GGAATAAAAT TTATATGAAT AACAAAAGCA GCAGTA---- -----GT---
D.obs     GGAATAAAAT TTATATGAAT AACAAAAAac gccaacgaga agcatcagca
D.wil     GAAATAAAAT TTATATGAAT AACAAAAAGG CAGGCA--AA CACCAGG---
D.sal     GAAATAAAAT TTATATGAAT AACAAAAAGG CAGGCA--AA CACCAGC---
Z.ghe     GAAATAAAAT TTATATGAAT AACAACAAaa gcaacaacAA GAACAAG---
D.vir     GAAATAAAAT TTATATGAAT AACAAAAACA GCAGCA---- -----AC---
D.gri     GAAATAAAAT TTATATGAAT AACAAAAGCA acaaaaagca agaacaacta

D.mel     ---------- AGCAACAACA ATAGTTTACT GCCCCGGCTC AGCGGTACAC
D.mau     ---------- AGCAACAACA ACAGTTTACT GCCCCGGCTC AGAGGTACAC
D.sim     ---------- AGCAACAACA ACAGTTTACT GCCCCGGCTC AGAGGTACAC
D.ere     ---------- GGCAACAACA ACAGTTTACT GCCTCGTCTC AGAGGTACAC
D.fuy     ---------- AGCAACAACA ACAGTTTACT GCTCTGGCTC AGCAGTACAC
D.aur     ---------- AGCAACAACA acagTTTACG GCCCTGGCTC AACAGTACAC
D.obs     acgggatggg GGCAAGAACA ACAGTTTACT GCCACGGaaa gtacttccca
D.wil     ---------- AACAACAAA- -CAGTTTACT ACTGCTCAGA AGAGAATGCA
```

```
D.sal     ---------- AACAACAAAA ACAGTTTACT ACTGCTCAGA AGAGAATGCA
Z.ghe     ---------- AGCAAGAACA ACAGTTTACG GCgttgaagg caaaatgatg
D.vir     ---------- AACAACAACA ACAaaaggca caaacagatg aagc------
D.gri     caagaaga-- ---------- ---------- ---------- ----------
D.mel     TGTGCAAAAC G-TTGTACTC CTCCTCAT-- ---------- ----------
D.mau     TGAGCAAAAT GAaTGTACTC TTTTTCAT-- ---------- ----------
D.sim     TGAGCAAAAT GATTGTGCTC CTCCTCAT-- ---------- ----------
D.ere     TGTACGAAAT A-TTCgtact ctcaaaatac aaagtgctct ctaccatcga
D.fuy     TGTGGAAAAT A-TTGatacc attctttttt atatccataa taaaggccaa
D.aur     AGAGagaaaa aatattcacg acttttctta gacaaaatta tattagtttg
D.obs     c---GAGAGA GGGAGAGAGA TATGGAGAGA Gaatgcatct ca--------
D.wil     Acacca---- ---GCAACAA CAACAACAAC AACAGCAACA ACTACAACAA
D.sal     Acaccagcaa acaACAATAA CAACAAGAAC AGCAGCAGTA GAAACAACAA
Z.ghe     gtgaGAGAAA AGGAGAGACA GAGAGAGAGA Gctgagaatg catcgttg--
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     atataaacat agata----- ---------- ---------- ----------
D.fuy     tagagtattt ttactgcatg atagtatttg ggagctcata atttgtaaac
D.aur     atgtagaaaa atttttggtg tttaatatct ttgtatatat atggttttta
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     CAGCAACAgc ---------- --------TT GTTGCAACTA GAGTAGAAGT
D.sal     CAGCAGCAgc agcagcagca gcagcagcTT GTTGCAACTA GAGTAGAAGT
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----AGAAGA

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     tgaaaacaag tttgctttgg ttctttaggg aagaaaaaaa ggagctttta
D.aur     ttttgtaaga aagtggtttt taaagacgca ataaatctta agtcccttt--
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     AGTAGAAAAA GAAGCAGAAG AAGAGAAGTT GCAGCATtcg ctgttgccgc
D.sal     Atgtaaaagt g--------G AAGAAAAGTT GCAGCAT--- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- -AAGAGGAAG AACAGTTTAC TGCTCTGCA- ----------
D.gri     TGGAGAAGAA GAAGATGAAA AACAGTTTAC TGCTCTGCAc tctcggagaa

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
```

```
D.fuy     aatttaaaat atcattgcca ttagaacagg aaaaactact taatatttgt
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ttttgctgcT GCTGCTGC-- ---------- ---------- ----------
D.sal     ---------T GCTGCTGC-- ---------- ---------- ----------
Z.ghe     ---------- ---------- --------C- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     tgcaatgcga aacgttcgtt gcaacaaaC- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     taagccttaa ataaaataaa tacaaattta ttccaatgca aaaatacatg
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----AATAAT
D.mau     ---------- ---------- ---------- ---------- ----ACCAAT
D.sim     ---------- ---------- ---------- ---------- ----ACCAAT
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ttttttttatt caaaaaaagg cttaactaaa ctttctgaac gtgaAACAAT
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ATGAGTA--- ---------- ----TATAGA GTATATAATA TACTATATAT
D.mau     AACaggaagt agtTAATATG AAAGTATAAA GTAAATATCA TACTATATAT
D.sim     AACT------ ---TAATATA AAAGTATAAA GTAAATAACC TACCATATAT
D.ere     AACA------ ---TAATATG AATGTATAAA CTGAAGgact tgcaggcttt
D.fuy     ATTACTA--- ---------- ----ACTAGG GTATGTACTA aatataattt
D.aur     ---------- ---------- -AAATATAAA TAAAATATTA TTCAATATag
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------
```

```
D.mel     CTCcattgat aatttcgatc attttcacct tttaactaat ttatgcccaa
D.mau     CTCTATAGAT AGTTTCATCa ccttTTTTTC ACCTAATTTA TATCCATTA-
D.sim     CTCTATAGAT AGTTTCATCt ----TTTTCA ACCTTATTTA TGTCCATTAa
D.ere     gtaaatatta agtactacat tgtagATTAT ATAGATTATT TCAATTTCTt
D.fuy     gtataaaatc tggccaaaag caatgcaaat tttttgTAGT GTA-------
D.aur     tatttaaatg ttaatacat- -----ATTTT TTATATTATT TTAATTACTa
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ------TAGT ATA-------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     tatagTTGCA ---------- ------T--- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     aa---TTGCA ---------- ------T--- ---------- ----------
D.ere     ttagaaaata tctgtccagc acaaatT--- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     atttgtgttt tacttttcac agaggcagtc agaaaagggc tgccttttag
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     gcaaactttg attaacattt taaggtattt caggaatctt tttaacaaga
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------T TCTCTGAGTG TGCAGTAAGT GCCCCAGAAT
D.mau     ---------- -------AAT TCTCTGAGTG TGCAGTAAGT GCCCCAGAAT
D.sim     ---------- ---------T TCTCTGAGTG TGCAGTAAGT GCCCCAGAAT
D.ere     ---------- ---------T TCTCTATCTG TGCAGTAAGT GCCCCAGAAT
D.fuy     ---------- ---------- ---------- --CAGTAAGT GCCCAAGAAT
D.aur     taaaataata tgctaagAAT TCTTTCAGTG CCAAGTAAGT GCCCgg----
D.obs     ---------- ---------- ---------- ---------- ----------
```

```
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- --CAGTAAGT GCtcggagaa
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     GCGAATGCAT CTCGGGTTCA TCG--GCGGG TCGAGTTTGT TGCAACAACC
D.mau     GCGAATGCAT CTCGGGTTCA TCG--GCGGG TCGAGTTTGT TGCAACACCC
D.sim     GCGAATGCAT CTCGGGTTCA TCG--GCGGG TCGAGTTTGT TGCAACACCC
D.ere     GCGAATGCAT CTCGGGTTCA TCG--GCGGG TCGAGTTTGT TGCAACACcc
D.fuy     GCGAATGCAT CTCGGGTTCA ACG--GCGGG TCGAGTTTGT TGCAt--CAC
D.aur     --GAATGCAT CTCGGGTTCA TCGagGCAGG TCGAGTTTGT TGCAACACAC
D.obs     ---------- ---------- ---------- --GAGTTTGT TGCAACAtc-
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     tgtgcaacgt gcaacgtggt gcaacgtgca gcgtgcaacg tggtgcag--
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     GAAGAaCGAA GAAGTTGCAG CGTGCGTT-- -------CGG CATTAAAATT
D.mau     GAAGAACGAA GAAGTTGCAG CGTGCGTT-- -------CGG CATTAAAATT
D.sim     GAAGAACGAA GAAGTTGCAG CGTGCGTT-- -------CGG CATTAAAATT
D.ere     gaagaa---- ---GTTGCAG CGTGCGTT-- -------CGG CATTAAAATT
D.fuy     C-----CGAA GAACTTGCAG CGTGCGTC-- -------CGG CATTAAAATT
D.aur     C-----CGAA GAAGTTGCAG CATGCGTC-- -------CGG CATTAAAATT
D.obs     --------AA GAACTTGCAA CGTCCGtccg tcctatgCGG CATTAAAATT
D.wil     ---------- ---------- --TGCGTC-- -------CGG CATAAAAATT
D.sal     ---------- ---------- --TGCGTC-- -------CGG CATAAAAATT
Z.ghe     ---------- -AACGTGCAA CGTGCGTC-- -------CGG CATAAAAATT
D.vir     ---------- ---CGTGCAA CGTGCGTC-- -------CGG CATAAAAATT
D.gri     ---------- -AACGTGCAA CGTGCGTC-- -------CGG CATAAAAATT

D.mel     GTGTTTATGC GTGTTCGGTA A--TTTTATA AAAGTTAAAT TAGTTTTAAG
D.mau     GTGTTTATGC GTGTTCGGTA A--TTTTATA AAAGTTAAAT TAGTTTTAAG
D.sim     GTGTTTATGC GTGTTCGGTA A--TTTTATA AAAGTTAAAT TAGTTTTAAG
D.ere     GTGTTTATGC GTGTTCGGTA A--TTTTATA AAAGTTAAAT TAGTTTTAAG
D.fuy     GTGTTTATGC GTGTTTGGTA A--TTTTATA AAAGTTAAAT TAGTTTTAAG
D.aur     GTGTTTATGC GTGTTTGGTA A--TTTTATA AAAGTTAAAT TAGTTTTAAG
D.obs     GTGTTTATGC GTGTTTcggt aatTTTTATA AAAGTTAAAT TAGTTTTAAG
D.wil     GTGTTTATGC GTGTTTGGTA A--TTTTATA AAAGTTAAAT TAGTTTTAAG
D.sal     GTGTTTATGC GTGTTTGGTA A--TTTTATA AAAGTTAAAT TAGTTTTAAG
Z.ghe     GTGTTTATGC GTGTTTGGTA A--TTTTATA AAAGTTAAAT TAGTTTTAAG
D.vir     GTGTTTATGC GTGTTTGGTA A--TTTTATA AAAGTTAAAT TAGTTTTAAG
D.gri     GTGTTTATGC GTGTTTGGTA A--TTTTATA AAAGTTAAAT TAGTTTTAAG
```

```
D.mel     ACCATAAATT CAGCTCACTC TCTCTCtcTC GCTCTTTC-- ----------
D.mau     ACCATAAATT CAGCTCACTC CCTCTC--TC GCTCTTTC-- ----------
D.sim     ACCATAAATT CAGCTCACTC CCTCTC--TC GCTCTTTC-- ----------
D.ere     ACCATAAATT CAGCTCACTC TCtct----- ---------- ----------
D.fuy     ACCATAAATT CAGCGCACTC TCTggcat-- ---------- -------AG-
D.aur     ACCATAAATT CAGCGCACTC TCGCTggcgc agttccccat ggcccgaAG-
D.obs     gccataaatt ccggactcat tctgaggcac tctctaggct gcccctggcc
D.wil     ACCATAAATT GTGCTCCCTC TCAGTCGCAC TCgcagtctg ccccctcgca
D.sal     ACCATAAATT GTGCTCCCTC TCAGTCTCAC ACacttgcac actattgcta
Z.ghe     ACCATAAATT GTGCTtgtgt agtcgctgct cttaccgctg ctgctgttgt
D.vir     ACCATAAATT GTGCCCCCCC Tgccttgccc tgactgcc-- ----------
D.gri     ACCATAAATT GTGCTgttgc ctttgctctg tttatgctct acctaccccc
                                (Dimorphic element mel Fwd Primer)>

D.mel     ---------- ---------- ---------- ------TCT- ----------
D.mau     ---------- ---------- ---------- ------TCT- ----------
D.sim     ---------- ---------- ---------- ------TCT- ----------
D.ere     ---------- -CTCTATCTC GCTCTCGCTC TCGCTCTCT- ----------
D.fuy     ---------- ---------- ---------- ------TCT- ----------
D.aur     ---------- ---------- ---------- ------TCT- ----------
D.obs     tgcccctctg gtccgtctct ctcttgttgc cat------- ----------
D.wil     ttctctctgt ctgacacac- ---------- ---------- ----------
D.sal     tcccccactc aCTCTCTCTC TCTCTCTCTC TCTCTCTgtg tgtgtgttgc
Z.ghe     tgttgttgtt ggagctgcag ttgcagaagt tgttgctgca ttcacccgtt
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     tccccctccc cctctccatc cccatccatc aacct----- ----------

D.mel     ---------- ---------- ----TTGCCA TTTTAACTTT TATTACTCTT
D.mau     ---------- ---------- ----TTGCCA GTTTAACTTT TATTACTCTT
D.sim     ---------- ---------- ----TTGCCA TTTTAACTTT TATTACTCTT
D.ere     ---------- ---------- ----CTGCCA TTTTAACTTT TATTACTCTT
D.fuy     ---------- ---------- ----CTGCCA TTTTAACTTT TATTACTTTT
D.aur     ---------- ---------- ----CTGCCA TTTTAACTTT TATTACTTTT
D.obs     ---------- ---------- ---------- TTTTAACTTT TATTACTTTT
D.wil     ---------- ----TTGCTC TTGCTTGCCA TTTTAACTTT TATTACTTTT
D.sal     tctctttcta tcgtTTGCTC TTGCTTGCCA TTTTAACTTT TATTACTTTT
Z.ghe     tgccccctca tcgtcgtcgt tg--TTGCCA TTTTAACTTT TATTACTTTT
D.vir     ---------- ---------- -TGCCTGCCA TTTTAACTTT TATTACTTTT
D.gri     ---------- ---------- TTGCTGCCCA TTTTAACTTT TATTACTTTT
                                                      1
```

```
D.mel     AATATAAA-- ---AAAGCTG GCTAGATGCG GGC------- ----------
D.mau     AATATAAA-- ---AAAGCTG GCTAGAAGCG GGC------- ----------
D.sim     AATATAAA-- ---AAAGCTG GCTAGAAGCG GGC------- ----------
D.ere     AATATAAAAA A-----GCTG GCTAGAAGCG GGC------- ----------
D.fuy     AATATAAAAA A-----GCTG GATAGAAGCG GGC------- ----------
D.aur     AATATAAAga AAAAAAGGTG GCTAGGAGCA GGC------- ----------
D.obs     AATATAAAAA aacagagaga gagagagaga gggagcgctg gaatccaagg
D.wil     AATATAAAAA A-----GCTG GCTGGGAT-- ---------- ----------
D.sal     AATATAAAAA A-----GCTG GCTGGGAT-- ---------- ----------
Z.ghe     AATATAAAAA A-----GCTG GCGCTGTgtg tagctcgccc aaggagggca
D.vir     AATATAAAAA A-----GCTG GCGCTGggct ggcaaagcag ccgccagttc
D.gri     AATATAAAAA A-----GCTG GCGCTGTtgc aagcaag--- ----------
               2

D.mel     ---------- ---------- --------CA GCT---GTAA AAA--TGCAC
D.mau     ---------- ---------- --------CA GCT---GTAA AAA--TGCAC
D.sim     ---------- ---------- --------CA GCT---GTAA AAA--TGCAC
D.ere     ---------- ---------- --------CA GCT---GTAA AAA--TGCAT
D.fuy     ---------- ---------- --------CA GCT---GTAA AAA--TGCAC
D.aur     ---------- ---------- --------CA GCTgtaGTAA AAA--TGCAC
D.obs     agcagggcat acgaatgtgT GGCAGCAACA GCT---GTAA AAA--TGCAC
D.wil     ---------- ---------T GGGAGCAGGA GCT---GTAA AAA--TGCAC
D.sal     ---------- ---------T GGGAGCAGGA GCT---GTAA AAA--TGCAC
Z.ghe     gcagccaaAG GAGCCGCTTG CCAGCT--GA GCT---GTAA AAC--TGCAC
D.vir     gttcgca--- ---------- -CAGCT--CA GCT---GTAA AACgcTGCAC
D.gri     --------AG GAGCCGCTAG CCAGCT--GA GCT---GTAA AAC--TGCAC
                                                    3

D.mel     GCGGTCATAA AAAGTTGCAG GAGGcat--- ---------- --GTTGCCAG
D.mau     GCGGTCATAA AAAGTTGCAG GAGGCATGTT GCC------- --------AG
D.sim     GCGGTCATAA AAAGTTGCAG GAGGCATGTT GCC------- --------AG
D.ere     GCGGTCATAA AAAGTTGCAG GCGGCAcgtt gcca------ --GTTGCCAG
D.fuy     GCGGTCATAA AAAGTTGCAG GAGGCATGTT GCtggtagcc aaGTTGCCAG
D.aur     GCGGTCATAA AAAGTTGCAG gaggcatcta catcgacgtc cacatccaca
D.obs     GCGGTCATAA AAAGTTGCAG GCAGaggagg caggcagacg aagcggtggc
D.wil     GCGGTCATAA AAAGTTGCAG GCAGGCA--- ---------- ----------
D.sal     GCGGTCATAA AAAGTTGCAG GCAGGCA--- ---------- ----------
Z.ghe     GCTGCCATAA AAAGTTGCAA GCGGCAgc-- ---------- ----------
D.vir     GCTGCCATAA AAAGTTGCAA GCAGCagccg ctgctgctgc gTCACATGTT
D.gri     GCTGCCATAA AAAGTTGCAA GCAGCattt- ---------- -TCACATGTT
                4

D.mel     TTGCCTGCAA CCGGCAACAT TCGC------ ---------- ----------
D.mau     TTGCCTGCAA CCGGCAACAT CCGC------ ---------- ----------
D.sim     TTGCCTGCAA CCGGCAACAT CCGC------ ---------- ----------
D.ere     TTGCCTGCAA CCGGCAACAT CCaC------ ---------- ----------
D.fuy     TTGCCGGTTG CCTGCAACAT CCactga--- ---------- ----------
```

```
D.aur     tcgccatcgg gctggagtcc ccgggatcgg ttggtatgtt ----------
D.obs     agcagaggca cagcacagca caggcggcgg gagaatgttg ccagtgaa--
D.wil     ---------- ---------- ---------- ---------- ---AGCAGG-
D.sal     ---------- ---------- ---------- ---------- ---GGCAaaa
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     GCGCGGCAAC ATTGCCTACt g--------- ---GCAACAT TGCAGCAAG-
D.gri     GCTCGGCAAC ATAGCCTACa actactacta cttGCAACAT TGCATCAAG-

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ---AGGCATG
D.sal     gcaaaagcaa gaagcaagga gggggggggg gcaggcgggc acaAGGCATG
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ---TGGCAAG
D.gri     ---------- ---------- ---------- ---------- ---AAGCAAt

D.mel     ---------- --AGAACAGC A--------- ----GCAACA ----TCGTAA
D.mau     ---------- --ACAACAGC A--------- ----GCAACA ----TCGTAA
D.sim     ---------- --ACAACAGC A--------- ----GCAACA ----TCGTAA
D.ere     ---------- --AGAACAGC A--------- ----GCAACA ----TCGTAA
D.fuy     ---------- ----AACGGC A--------- ----GCAACA ----TCGTAA
D.aur     ---------- --------GC A--------- ----GCAACA ----TCGTAA
D.obs     ---------- --ACAGCAGC A--------- ----GCAACA ----TCGTAA
D.wil     CGGCATGAGG CATGCGGCAT GAGAGATGTT GCTGGCAACA ----TCGTAA
D.sal     ATGATGGGGG CATGAGGCAT GAGAGATGTT GCTGGCAACA ----TCGTAA
Z.ghe     --------GG CAGCAGAAGC A--------- ----GCAGCA GCAggaacgt
D.vir     TGGCAAGAGG CAGCAGCAGC A--------- ----GCAGCA GC--tcGTAA
D.gri     t--------G CAGCAGCAGC A--------- ----GCAGCA GCAaTCGTAA
                                                              5
D.mel     AATAACTTCT TGCTCTGCGG TCTGAGTTTG GCC------- ----------
D.mau     AATAACTTCT TGCTCTGCGG TCTGCATTTG GCC------- ----------
D.sim     AATAACTTCT TGCTCTGCGG TCTGCATTTG GCC------- ----------
D.ere     AATGACTTCT TGCTCTGCGG TCTACGTTTG GTC------- ----------
D.fuy     AATAATTTCT TGCTCTGCGG TCTCCATTTG GCC------- ----------
D.aur     AATAATTTCT TGCTCTGCGG TCTCCGTTTG GCC------- ----------
D.obs     AATAATTTCT TGCAttcgca tcgcgattgc tgtggc---- ----------
D.wil     AATAATTTCT TGCAATCCCA TTTTTTTCGC CTC------- -----TGTTG
D.sal     AATAATTTCT TGCAATCCCA TTTTTTTCGC CTCtgttgct gttggTGTTG
Z.ghe     cacatgttgc tcgcaacata gcctactact ggcaaccggc agtcggcagc
D.vir     AAtgagcgcc agt------- ---------- ---------- ----------
D.gri     AATGACTcaa atgctct--- ---------- ---------- ----------
             t
```

```
D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     TTGCTGCTGC TGCTGCTGCT GCTTCttcat atttttcgtt -TTTGGTT--
D.sal     TTGGTGGTGG TGCTGCTGCT GCTTCttctt cttattattc gTTTCGTT--
Z.ghe     aatcggaggc aacacatcgc atccaaaaga catcatgcaa catgcaatcg
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------
                     region I (+58 bp)

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     taaaccgagt acgcTGCAAC GTTTTGTTCT GCCGTCGGTT TTTTTTATta
D.vir     ---------- ----TGCAGC GTTTTGTTCT GCggctttta ttgtgtg---
D.gri     ---------- --------GC GTTTTGTTCT GCAATTTTAT TTTTTAATac

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     -----TGTT- ---------- ---------- ---------- ----------
D.wil     ---TGTATT- ---------- ---------- ---------- ----------
D.sal     ---TGTGTT- ---------- ---------- ---------- ----------
Z.ghe     gtgTGCAAT- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     tcttgccgaa ggttctattg agaagtctac gactgcataa gacataagac

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
```

```
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     tatatatata tatatataaa tcatctgata gattctgctg tgcatctcag

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     agcatcaact ttgacttgaa atttctcatc gatttttga tttcattttg

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     gaattagcct tggctgcaga gtatcgcaaa gtcgacttgc attgagttgg

D.mel     ---------- ---------- ---------- GCAACAATGT TGCTGCATTT
D.mau     ---------- ---------- ---------- GCAACAATGT TGCTGCATTT
D.sim     ---------- ---------- ---------- GCAACAATGT TGCTGCATTT
D.ere     ---------- ---------- ---------- GCAACAATGT TGCTGCATTT
D.fuy     ---------- ---------- ---------- GCAACAATGT TGCTGCATTT
D.aur     ---------- ---------- ---------- GCAACAATGT TGCCGCATTT
D.obs     ---------- ---------- ---------- GCAACAATGT TGCCACATTC
D.wil     ---------- ---------- ---------- GCAACATTGT GGCAACATTT
D.sal     ---------- ---------- ---------- GCAACATTGT GGCAACATTT
Z.ghe     ---------- ---------- ---------- GTAACATTGT TGCAACATTT
D.vir     ---------- ---------- -------TGC CACACAATGT TGCAACACTT
D.gri     acactgttaa ttgtgatgat agccgctTGC CACACAATGT TGCAtcgcac
Z.vit                                     GTAACAATGT TGCAACATTT
D.tri                                     GTAACAATGT TGCAACATTT
                          (ins I in D. mel)^  Dsx1             6
```

```
D.mel     ATTC---G-- -TATTTATTAT TACA-TTTTA ATGAATAATT CTAATTTATAT
D.mau     ATTC---G-- -TATTATTAT TACA-ATTTA ATGAATAATT CTAATTATAT
D.sim     ATTC---G-- -TATTATTAT TACA-ATTTA ATGAATAATT CTAATTATAT
D.ere     ATTC---G-- -TATTATTAT TACA-TTTTA ATGAATAATT CTAATTATAT
D.fuy     ATTC---G-- -TATTATTAT TACA-TTTTA ATGATTAATT CTAATTATAT
D.aur     ATTC---G-- -TATTATTAT TACA-TTTTA ATGATTAATT CTAATTATAT
D.obs     ATTCCtcG-- -AATTATTAT TACA-TTTTA ATGATTAAaa ttctaatgtg
D.wil     ATACCA-G-- -AATTATTAT TACA-ATTTA ATGATTAATT CTAATGATAT
D.sal     ATACCA-G-- -AATTATTAT TACA-ATTTA ATGATTAATT CTAATGATAT
Z.ghe     ATAC---G-- -AATTATTAT TACAtTTTTA ATGATTAATT CTAATGATAT
D.vir     ATGC---G-- -CATTATTAT TACA-TTTTA ATGATTAATT CTAATGATAT
D.gri     acacttgtgc aAATGATTAT CACA-TTTTA ATGATTAATT CTAATGATAT
                       t  t                    t          t

D.mel     GCAACTTGA- ---------- -----ATAAG C--------- ----------
D.mau     GCGACTTGA- ---------- -----ATAAG G--------- ----------
D.sim     GCGACTTGA- ---------- -----ATAAG G--------- ----------
D.ere     GCAACTTGA- ---------- -----ATAAG Ggccgctgca agagcgtcGA
D.fuy     GCGACTTGA- ---------- -----ATAAG GCCGCtgact gagcgaaa-A
D.aur     GCGACTTGA- ---------- -----ATAAG Gccgccgaat ggccgaatga
D.obs     atgcgaatcg gcagcgacat ggccatggca gaggaaatcc cacatgggaa
D.wil     GCGACATGA- ---------- ---------- ---------- ----------
D.sal     GCGACATGA- ---------- ---------- ---------- ----------
Z.ghe     GCGACATGA- ---------- ---------- ---------- ----------
D.vir     GCGACATGA- ---------- ---------- ---------- ----------
D.gri     GCGACATGA- ---------- ---------- ---------- ----------
                                    t

D.mel     ---------- CCGCCGATGC CAATAAAAAG -CGGCGTGGC AAAGTGGAGT
D.mau     ---------- CCGCAGAAGC CAATAAAAAG CCGGCGTGGC AAAGTGGAGT
D.sim     ---------- CCGCAGAAGC CAATAAAAAG CCGGCGTGGC AAAGTGGAGT
D.ere     ---------- -------AGC CAATAAAAAG -TGCCGGGGC AAAGTGGAGT
D.fuy     ---------- -------AGC CAATAAAAAG -TGCCGAGGC AAAGTGGAGT
D.aur     -gaaatgctc tggcagcgGC CAATAAAAAA TGGCCGGGGC AAAGTGGAGT
D.obs     atactAAATG AAATGGCAGC CAATAAAtgc gccaatccaa agagtgctgc
D.wil     -----AAATG AAATGGCAAC CAATAAAAAG AATCTaccct ttcctaacca
D.sal     -----AAATG AAATGGCAAC CAATAAAAAG AATCTgcctt cccacttttc
Z.ghe     -----AGTTG AAATGGCAAT CGATAAAA-- ---------- ----------
D.vir     -----AAATG AAATGGCAAT CAATAAAA-- ---------- ----------
D.gri     -----AAATG AAATGGCTAT CAATAAAA-- ---------- ----------
                                   7

D.mel     GGACTGGGtt tgtgtggcgc CCCTGCTAGT GGCACATAAA AATTGGC---
D.mau     GGATTGGCga tgtgtggcgc CCCAGCTAGT GGCACATAAA AATTGGC---
D.sim     GGATTGGCga tgtgtggcgc CCCGGCTAGT GGCACATAAA AATTGGC---
D.ere     GGATTTCGga tgtgtggcgc CCCTGCTAGT GGCACATAAA AATTGGC---
D.fuy     GGATTTTGgc cgtgtggcgc CCcgtgCTGC TAGTGGCACA TAAAAATTGG
```

```
D.aur     Gttttttttt tggtcgccgt gtggcgcccc aGGGAggcga CTCGTAGTGG
D.obs     tgtggcgcct ccccgggtgg c------AGC GGCACATAAA AATTGGCGC-
D.wil     catgcaactg ccaggctgaa ggctgctcaa gacagaccga ctgactgact
D.sal     actctacatc aacatctctt cccacatgca acaTGCGACA GCAGCAACAG
Z.ghe     ---------- ---TGCCACA GCA--GCAAA GGCACATAAA AAT
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------
                                                  8

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     C--------- ---------- ---------- ---------- ----------
D.aur     GGCATAAAAA TTGGT----- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     gacaagcagg cagcaggcag gcggcagtaa ----GGCAGT CAGATTGAAG
D.sal     CAGcagcaac accaaaagca aaaggctggc aggcGGCATT CAGATTGAAG
Z.ghe     CAGtgCAGAA CTCTGCTTTA GTTCTTATGA CCgacgatgc cgccgcctgg
D.vir     ----tCGGGC ATTTGCTTTt ttccttattc ctacaactac ttcgtcttct
D.gri     ----tCAGAC ATTTGCTTTA GTTTTTTTCT CCttctgcca tctcactttg
                              region II (+98 bp)

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     GATTCGCTcT TCGGTTTTGT TGGTTTCGGT TTG------- TGGCGCCCCA
D.sal     GATTCGCTgT TCGGTTTTGT CGGTTTCGGT TTG------- TGGCGCCCCA
Z.ghe     cgccgcgggg cagcgtt--- ---------- ---------- ----------
D.vir     tctgctttgt ttc------- ---------- --------TG TGGCGCCACA
D.gri     ttttttattt tttctgttct ttttttccat tcgttGTTTG TGGCGCCACA
```

```
D.mel     ---------- ---------- ---------- ------GCAA GTTAATTGTG
D.mau     ---------- ---------- ---------- ------GCAA GTTAATTGTG
D.sim     ---------- ---------- ---------- ------GCAA GTTAATTGTG
D.ere     ---------- ---------- ---------- ------GCAA GTTAATTGTG
D.fuy     ---------- ---------- ---------- ------GCAA GTTAATTGTG
D.aur     ---------- ---------- ---------- ------GCt- GTTAATTGTG
D.obs     ---------- ---------- ---------- ------ACAA GTTAATTGTG
D.wil     cggaca---- ---------- ---------- ------GCAA GTTAATTGTG
D.sal     cggaca---- ---------- ---------- ------GCAA GTTAATTGTG
Z.ghe     ---------- ---------              TG--GCCCAA GTTAATTGTG
D.vir     GGCAGCGTgc cgcacac--- ---------- TG--GCCCAA GTTAATTGTG
D.gri     GGCAGCGTtc gccatttggg ---------- -G--GCCCAA GTTAATTGTG
                          (ins II in D. mel) ^

D.mel     GTAGTTATTT GCTGT--TTT GCCATTTGGT ------C--- -------ATT
D.mau     GTAGTTATTT GCTGT--TTT GCCATTTGGT ------C--- -------ATT
D.sim     GTAGTTATTT GCTGT--TTT GCCATTTGGT ------C--- -------ATT
D.ere     GTAGTTATTT GCTGT--TTT GCCATTTTGC CATTTggtca ttttgcaATT
D.fuy     GTAGTTATTT GCTGT--TTT GCCATttggc cg-------- --------TT
D.aur     GTAGTTATTT GCTGT--TTT GCCATTTGGC CATTTc---- ----------
D.obs     GTAGTTATTT CTGctggTGC TGCTGCTGCt ttgtggC--- -------ATC
D.wil     GTAGTTATTT GCTAT--TTT GCCAT----- ---------- ---------T
D.sal     GTAGTTATTT GCTAT--TTT GCCAT----- ---------- ---------T
Z.ghe     GTAGTTATTT GTTGT--TGT TGCTGCTGCt cctcctcctg ttgttgtttt
D.vir     GCAGTTATTT GTTGT--Tat atatagagcc aagagctctt ggcct-----
D.gri     GTAGTTATTT CTGttattat tccaaagagt tttgctctac acacagcggg
               t

D.mel     TTACAATTTT ACCATTTCAG CCAC---AAC TTTTCGCACT GCTCCCcccc
D.mau     TTACAATTTT ACCc------ ---------- ---------- -------CCT
D.sim     TTACAATTTT ACCATTTCAG CCAC---AAC TTTTCTCACT GCcacccCCT
D.ere     TTACAATTTC ACCATTCCAG CCAC---AAC TTTTCGCACT GCTCCCttcg
D.fuy     TTACAATTTT ACCATTCT-G CCAC---AAC TTTTCGCATT GCTCCGCTTG
D.aur     --ACAATTTT ACCATTCT-G CCACcacAAC TTTTCACATT GCTCTGGTTG
D.obs     TTACAATTTT ACCATTTCTG CCAC---Aca actttttcga taccaaggca
D.wil     TTACAATTTT ACCACAAGTT TTGCGCATTT ---------- ----------
D.sal     TTACAATTTT ACCACAAGTT TTGCACATTT ---------- ----------
Z.ghe     tgccatagag ttttccacaa tttcttttgc tg-------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     gctgtgtgtg tgtgtgtgtg caacaat--- ---------- ----------
           9      10

D.mel     tttccCAG-- ---------- -----CACAA CAATGTTGCG GCATTCTCGC
D.mau     CTCCACCCCG CCCCTCAACC CAA--CGCAA CAATGTTGCG GCATTCTCGC
D.sim     CTCCACCCCG CCCCTCAACC CAA--CGCAA CAATGTTGCG GCATTCTCGC
D.ere     ---------- --------CC CGA--CGCAA CAATGTTGCA CCATTCTCTC
D.fuy     CCTGGT---- ---------- ------GCAA CAATGTTGCc gcagtcgctg
```

```
D.aur     ---------- ---------- CTCCGCGCAA CAAAGTTGCa tccaagagtt
D.obs     atgtgca--- ---------- ------ACAA CAATGTTGCA GGCGGCAagc
D.wil     ---------- ---CTCAACA TTT--CACAA CAATGTTGCA GGCGTTGgca
D.sal     ---------- ---CTCAACA TTTcaCACAA CAATGTTGCA GGCGTTGctg
Z.ghe     -----CAG-- ---------- -----CCCAA CATTGTTGCA GGCGGCAGTT
D.vir     ---------- ---------- -------CAA CAATGTTGCA GGCGGCTCTT
D.gri     ---------- ---------- ---------- ---------- -------GTT
Z.vit                                ACAA CATTGTTGCA GGCGGCAG
                                        Dsx2

D.mel     AC-------- ---------- ---------- ---------- ----------
D.mau     AC-------- ---------- ---------- ---------- ----------
D.sim     AC-------- ---------- ---------- ---------- ----------
D.ere     AC-------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     agcaaccagc acttgcattc tcgcC----- ---------- ----------
D.wil     ttgtcgctgc tgctgcccca ctcagtcgac gtcagcagca actg------
D.sal     gttgccgcca atgcccgttc tcgttcccgt tcccaatgtc ggcatctgct
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------
                        region III (+57 bp)

D.mel     ---------- ---------- -TTTACGAGG CGTTTTT--- --------TT
D.mau     ---------- ---------- -TTTACGAGG CG----T--- --------TT
D.sim     ---------- ---------- -TTTACGAGG CG----T--- --------TT
D.ere     ---------- ---------- -TTTACGAGG CG-------- -------TTT
D.fuy     ---------C ATTCTCGCAC TTTTACGAGG CG-------- -------TTT
D.aur     gcccTGCCGC ATTCTCGCAC TTTTACGAGG CGTTTTTTTT CcccttaTTT
D.obs     ---------- ----TTGCAC TTTTACGAGG CCTTGTT--- -----TTTCT
D.wil     ------GCTG CATTTTGCAC TTTTACGAGG CATTTTG--- ----TTTTTT
D.sal     gttgctGCTG CATTTTGCAC TTTTACGAGG CATTtctttt ttttTTTTTT
Z.ghe     --GTAAGTTG CATTTGGCGC TTTTACGATC CGTT------ ----------
D.vir     --GCAAcTTG CATTTGACGA TTTTACGAGC CATT------ ----------
D.gri     --GCAAGTTG CATTTGACGC TTTTACGAGC CATT------ ----------
             (ins III in D. mel)^  11

D.mel     TTTATATCAC TTAC------ ---------- -------TTT ACTTAGTTGA
D.mau     TTTATATCAC TTAC------ ---------- -------TTT ACTTAGTTGA
D.sim     TTTATATCAC TTAC------ ---------- -------TTT ACTTAGTTGA
D.ere     TTTATATCAC TT-------- ---------- ---------- ACTTAGTTGA
D.fuy     TTTATATCAC TT-------- ---------- ---------- ACTTAGTTGA
D.aur     TTTATATCAC TTAC------ ---------- ---------- --TTAGTTGA
D.obs     TTTATATCAC Tatgag---- ---------- ------ACTT ACTTAGTTGA
D.wil     TCCTTTTTTT TTATTTTTAT ATGTATTTTT TATATCGCTT ACTTAGTTGA
D.sal     TTTGTTTTTT TTTTTTTTAT ATGga-TTTT TATATCGCTT ACTTAGTTGA
```

```
Z.ghe     TTTATAT--- ---------- ---------- ------GCTT ACTTAGTTGA
D.vir     TTTATAT--- ---------- ---------- ------GCTT ACTTAGTTGA
D.gri     TTTATAT--- ---------- ---------- ------GCTT ACTTAGTTGA
           12                                          13

D.mel     TTAAGGGCGT G--------- GCCGAT-GGG CCAGATAcaT GCTTAGATTT
D.mau     TTAAGGGCGT G--------- GCCGAT-GGG CCAGATA--T GCTTAGATTT
D.sim     TTAAGGGCGT G--------- GCCGAT-GGG CCAGATA--T GCTTAGATTT
D.ere     TTAAGGGCGT G--------- GCCGAT-GGG CCAGATA--T GCTTAGATTT
D.fuy     TTAAGGGCGT G--------- GCCGAT-GGG aaAGATA--T GTTTAGATTT
D.aur     TTAAGGGCGT G--------- GCCGAT-GGG CCAGATA--T GTTTAGATTT
D.obs     TTAAGGGCGT aaagggcgtg GCCAAT-GAG CCAGATA--T tcaaaaggag
D.wil     TTAAGGGCGT G--------- GCCAAT-TGG GCAGATGCTT AGATTCCTAC
D.sal     TTAAGGGCGT G--------- GC-------- ---------- ----------
Z.ghe     TTAAGGGCGT G--------- GCtg----AG GCAGATGTTT AGATTTCTAC
D.vir     TTAAGGGCGT G--------- GCacttgaAG GCAGATGTTT AGATTTTTCG
D.gri     TTAAGGGCGT G--------- GCCccaaTGG GCAGATGTTT AGATTTCGCA

D.mel     GCTCCAGC-- ---------- --------AG TGGGCTGCAT TTTACGACCC
D.mau     GCTCTAGC-- ---------- ---------- --------GT TTTACGACCC
D.sim     GCTCTA---- ---------- ---------- ------GCGT TTTACGACCC
D.ere     GCTCTATGTA TCCcag---- -----AGGAG TGGGCTGCGT TTTACGACCC
D.fuy     GCTCTTTGTA TCCGgacatt ctatcAGGAG TGGGTTGCAT TTTACGACCC
D.aur     GGTCTTTGTA TCCGtcctaa -------GAG TGGGCTGCAT TTTACGAGCC
D.obs     ggaaaggaac gggcttagat ttcagtggaa tggtgtaggc agggattgag
D.wil     aggccaaaat gtaggcgtaa gagaatgtga aacgtgtgtg gcg-------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     caccactaca ---------- GCAACAGCAA CAACAACACT AACAGTTACA
D.vir     CACAGgCTAT GACTACAACA AAAATAAAAA GAAAtacaac aacta-----
D.gri     CACAG-CTAT TACTACAATA ACAACAATAT CAAAAGCAAC AACAACAACA
                                                       14

D.mel     TCAAAACCCG ATCCAAAT-- -----GGAAA ATATGAAAat ac--------
D.mau     TCAAAACCCG AACCAAAT-- -----AGAAA ATATGAAA-- ----------
D.sim     TCAAAACCCG ATCCAAAT-- -----GGAAA ATATGAAA-- ----------
D.ere     TCGAAACCCG ATCGAAtcgg aaaggAGAAA ATATGAAA-- ----------
D.fuy     GCCAAAGCCG ATCAAAAC-- -----GGAAA cagaaga--- AATATGAAAT
D.aur     TCGAAAGGTG ATCGAAAT-- -----Ggcta cggaaagagg AATATCAAAT
D.obs     gggagtaata acatTTTTAT GACCttacag agatacagat acagaaatat
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ACATCTATTG TGGCGTGCAA ATGCCGACGA TGAGTTTTAC GACCAACAGA
D.vir     ---------- TGGCGTGCTA ATGCCCCCGA TGAGTTTTAC GACCg-----
D.gri     ACAACAACTA TGGCGTGCTA ACGCCGCCAA TGAGTTTTAT GGCCAACAAA
                               14                    14
```

```
D.mel     -------GGC TAATCCGCTT ATGAGCACAA CAAAttggtt CACACACTTC
D.mau     ------TGGC TAATCCGCTT ATGAGCACAA CAAAATGTTA CACACACTCC
D.sim     ------TGGC TAATCCGCTT ATGAGCACAA CAAAATGTTA CACACACTCC
D.ere     ------TGGG TAATCCGCTT ATGGGCACAA CAAAAAGATA CACTCACTTC
D.fuy     GGCCTCTGGG TAATCCGCTT ATGGTTATAA CAATATTata gaaatttcga
D.aur     GGGTTCGAGG TAATCCGCTT Acgaaatgag ctccttagaa tcctcacact
D.obs     tggaaggaaa tgaaggagtg cgatccactg aatccgaatc acttttgaat
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     AAATACAACA agaaaacc-- ---------- ---------- ----------
D.vir     --CAAATACA ACAAAAAACA AAAAAAaaag gaaTTTATTT TGAAAA----
D.gri     TACAAATACA ACAAAAGAAA AAAAAAtac- ---TTTATTT TCAAAA----
                  <(dimorphic element mel Rvs primer)
                           t

D.mel     GATCGAAATT ACTTGCGATC GCCATTTGAT TGGTTTCAAT GTATTGCTTT
D.mau     GATCGAAATT ACTTGCAATC GCATTTTAAt gtt------- ---TTACTTT
D.sim     GATCGAAATT ACTTGTGATC GCATTTTAAG TGGTTTCAGT GTATT-CTTT
D.ere     GATCGAAATC ACCTGCGATC GCATTTTAAG TGGCTTCAGT TTATTGATTC
D.fuy     taagatttga aataagatct ttttttgact attaaatggt ttcaatccac
D.aur     gtgatcacag ctgaattatt gccatactta tatctgacat taaaaattct
D.obs     tctgaaaagt agcaagAAGT TGCAAAATTT CTGGTAGCCG CTACAtttca
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ------AAGT TTCGAAATTT GTTGTATGAG CTGCACAAAT
D.vir     ---------- ---------- ---------- --TGTATGTG CAGCACAAAT
D.gri     ---------- ---------- ---------- --TGTtgca- --GCAGAAAT

D.mel     AACTGGCAGG ---------- ---------- ---------- ----------
D.mau     AACTAGCAGG ---------- ---------- ---------- ----------
D.sim     AACTAGCAGA ---------- ---------- ---------- ----------
D.ere     AACTAGCAGG ---------- ---------- ---------- ----------
D.fuy     tgccttttga taatggttaa t--------- ---------- ----------
D.aur     cttgtttacc ttatttttaa tgaccttaat tactgcctta gttataactt
D.obs     ctacctctcc ctcttccatt gtggcttagc agtacctctt tctttatcta
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ATTTTTATCA CAaactagtc aaatgttata gtaactgtta taagtgtgag
D.vir     ATTTTTATTA CACCTACTCT AATACAcaca cacccacacc cacacagata
D.gri     ATTTTTATTA CACCTCCTCT AATACAtttc tcactcacac atgtacatat

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     acataaatgg gactttattt aacaggtttc ggtggaa--- ----------
```

```
D.obs     cggatttctc gaaacttctt taatttcgtg agagttctcc gtcagcgtaa
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     tttcatccaa aaacgaactc tgttcgattc ccattatgct gtcataaatc
D.vir     tatagtatgc catacctagt cctgactttc atttatagcc aga-------
D.gri     ttatatatat atatatatat atatatatgt ttatatatat atatatctat

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ttccattgcc cattcccatt acgaaccaac gaaatgctac gcttcgaggc
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     c--------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ---TACACTT
D.gri     atatatgtat gtacattcac atatcgtata cctggccgtg actTACACAT

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     g--------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     TTGTTATTTA Ttactttacc ataataaaaa gaattatact gaaaacattt
D.gri     TTGTAATTTA Tgagtgaagc catcataaac cgatatacaa agagaaacca

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     catgaaagaa aaataactaa agaaaaaaaa tataataata ataataaatt
D.gri     tataggaagg taccagaagg gcaaagaatt gatgtgcaac taaaacgaat
```

```
D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     atggaaaatt gcaaaggaag cgcttaagaa aaataagtca ataaagtata
D.gri     aaataaaaaa cttgagcaac aacttaaagg ggtaaaatac actaaaaatt

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     cgtgtatata tatagaacta aatgaaaata aaacacatac ttttatattt
D.gri     caattataga atagatgcaa caaataataa agtttttc-- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     acttctttct gaaaaaatac aaattctcgg gcttgttcca tagtatttct
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ------TGTG AATATTGAAA ACATTTTAAT
```

```
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ggagaattgg agaaagattt aaactaTGTA GAAATTGAAC TCATTTTAAT
D.gri     ---------- ---------- ---------- ---------- ---TTGTAAT

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     GAGAAATGAC TAAATTTgtt gttttgcttt tgtttttgtt ttttcttttc
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ttttttaaa tcaatatttt gcaagcctaa aatgcctgct aaacaaaggt
D.gri     TTGAAATAAG TAAATTTaat atattcagaa gaggaaccta ccccaaaaat

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ctactgctaa ttactcctac taaaaaggag tagccaccat gacaaattaa
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     gttttttctt tatatataaa gaatatgttt tcaatataaa tgcatttgta
D.gri     atgttaactc tagcttgaaa attgtgagag atacaattgg tcttatcgat

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     gacattaaat aagcactaac aaagcaatTG AtgTTTACtA CCaaaaattA
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ---------A
D.vir     aatattaaac aataaagtta aaaatccaat tgtacattat tgttgattct
D.gri     ttgcacttat cgatgtaatc aatttcccag acccacacat gttaaaactt
                                                14

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
```

```
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     AAACACAAAA GAATGAAAGA AAAcaaaaac ttttctagca ttacggcgcc
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ATATACAAAA CAATGAAACA GAAacccccc aaaaacccca acagaatttt
D.vir     aagtaaatta attttaaatt caatcatatt tcaattaaat tttattgttg
D.gri     tatctgatct ggacatagtc gaatatatat taaaagtttc aagttctgtt

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- --AGCAA--- ---------- ----------
D.wil     attggcgcca aattgacaaa attttcgggg atttctacat tttttttct
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ctttctttcg cattatcacg aaAGCAG--- ---------- ----------
D.vir     attctggtta agacaaaaga atataaacta catatcttca aaatttaaat
D.gri     ttaccttcca tggaagagta ataaaaacaa aacaaagctt tcttcaatat

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     tttctattga accatctagc agtcacttca actaatagat agt-------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     taacatcaat aattcggaaa taaatatagt taaagaaaaa caaattaaat
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- --TAAGAACT
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
```

```
D.vir     gcaattaatt ttaaatttaa atagctttgt attaaattgc acTAAGAACT
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     AAAGGAATTG AAATTTTGAT acttatagtt tctagtctcg tttaggttaa
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     AAAAAAAGTT AATTATTGTT gcttagttta actaaaacaa atgaaattgc
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ------TGAA CACTTTGTTT ---------- --TTTATCTA ACGATTCTTA
D.mau     ------TGAA CACTTTGTTG ttaccttact atTTAC-CTT ACTATTCTTA
D.sim     ------TGAA CACTTGGTTG ---------- --TTAC-CTT ACTATTCTTA
D.ere     ------TGAA AATCGGGCTT ---------- --TGAG-CTA ACGATTGCCA
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ctaattTGAG CACTTTCTTT ---------- --CTTATCac tgattaagag
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     attacgtatt tagaaaaaca attattcata tatttttt-- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     CTATTTAATA TCCTAGTCAA TTAATGTATT TTCCACTACT TCCATCGATA
D.mau     CTATTTGTTG TCCTAGTCAA TTAATGTATT TTCCAGTACT TCTATCGATA
D.sim     CTATTTGTTG TCCTAGTCAA TTAATGTATT TTCCGGTACT TCCATCGATA
D.ere     CTATTTGTTG TCCAAGTCCC TTAAAGTATT TTCCAGTACT TCTgcgtttt
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     agattgtttt ttaaagtata tcgaatgact ctttagacaa atagtaTTAA
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ------TTAT
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     Tcacagagtt cccatt---- ---------- ---------- ----------
D.mau     TTCCCAACCC C--------- ---------- ---------- ----------
D.sim     TTCCCAACCC A--------- ---------- ---------- ----------
D.ere     ccgtccatcc atattcatgg ccacctttc gaag------ ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
```

```
D.aur     -----ATTTG ATTAGAATGG AAGTTTTAAg ggatttttta ataagccact
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     GTACTATTTA ATTAGTATTT AAATTTTAAa accttaaatc tatctatata
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     GTATTATTTA ATTTATATAT CAATTACatc tttgtattta ctctaaggga
D.gri     ---TTACTTA AGTTTCATAT TAATTACcaa agaatcactt ggtcttcgcc

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     aagaaatttt acaggttact tggttatttg cagtattaac gttggaaacc
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     taagtaaact gatgtataag ttttaatctt aataaagttc ctaaataaag
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     aagttaagtt acctttacct tagggtattt tgagtacgaa ttaggcttgt
D.gri     tttagttggg acagcgacac attgattcaa tatacgctgc agctaactat

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     taaacaattt ttctgttaaa aatat----- ---------- ----ATTTTA
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     gattattcat cgttttctgt attaaaacct gataaaagta aacaATTTTA
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ttggttttct atagcaataa aagaaaccca tcaactat-- ----------
D.gri     tctagggtat gttaaatgcc atttgggatt gtttgtttta ttgtggcacg

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     AACATAATTT AATAAATTta ttaaatagca agattgagag ctcatgaatt
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     GAAACGATTT TATAAATTcg aaactaacac atcctagaat tattgctctc
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     aaa------- ---------- ---------- ---------- ----------
```

```
D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ----TTGGTT ---------- ---------- ---------- ----------
D.aur     ttgcTTGTTT ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     tgcctctttc tctctaattt tacttaaata ctttactgga gaaggaccat
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---TAAAATT GTAAATGTTc agtttccgat
D.aur     ---------- ---------- ---AAATATT TTAAATGTTT Taaaattaaa
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     tggcaattgt aaatcatttg aagAAATATT TTAAGTTTTT Tcagataact
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     taatttctat cagcccattt tataagaaag ttttttttg ctatttgcct
D.aur     ttagaagcat gataaatttt taaataatac cactacgttt taaagccaat
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     aaaactatta tttgagaatt caaaaaaaca tttcttaatc ctacacaaaa
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ttttgttttt caaattaatt tctaggatat attaaacatt ctgtattttt
D.aur     ttaagtgccg attttatttt gtagatttta ttacaaagtc aggttctaaa
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     aaagacttaa tttacattat ttattgaata gagatataag tttatctcac
```

```
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     aaccattctt tcaataaata cttttcttta tagactaatt ----------
D.aur     gtctacaatt ttagttcggt ttaatcacct taactccata accatgccaa
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ttttaagtta tatggacttt atctgttaaa acaataagaa agaatcattc
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     gtgaaatctt tccgctagta tcttataaaa atgttgttct acaaatggtg
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     gctatatggg taagattggt tcctatgaaa atttcaaatg aaaaaagttt
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ttttatttcc caggccttga ggtgata--- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     tatatacata tattcaactg gaataagttt tattgtacaa gtaaaattga
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
```

```
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     aatattggca cgaattgtca caagaacaga tacatatatt ttgcaaacca
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     aaaacaatca aaggatattt cattactact gaaatgaatt atagctgatt
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     aaattgttag ctaaagtttt atttactttt cagaaatcat taattatatt
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     cgtatggggt ataaaaactg atttcttacg tatttcatat gcgttgagat
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
```

```
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ---------- ---------- ---------- ---------- ----------
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     atttttacaa ttgttttgtt tccttctctt tcaacaatgg gtttattcat
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     ------TATT TAAAAAACAA AACAtgttgt atataaaaaa cttcaattta
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ttatagTATT AAAAAAAAAA AACAacaaca aatcgtATAA TGCC------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ------ATAA GACT------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     attttgatat tttaatcaat acttaaaaac cctgaaagca tgtttcaatt
D.aur     ---------- ---------- ---------- ---------- ----------
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- ---------- ---------- ----------
D.sim     ---------- ---------- ---------- ---------- ----------
D.ere     ---------- ---------- ---------- ---------- ----------
D.fuy     aatttgtaac ttagtactac atgaagtgat gTAAAGATAT GTCCGCATCG
D.aur     ---------- ---------- ---------- -TAAAGATAC CACCGCATCG
```

```
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ---TCGCAAA
D.mau     ---------- ---------- ---------- ---TGAG--- ---TCGCAAA
D.sim     ---------- ---------- ---------- ---TGAC--- ---TCCCAAA
D.ere     ---------- ---------- ---------- CCGTGGG--- ---TCCCAAA
D.fuy     ATTTTCCAGA aacccctttc atcccctttc CCTTGAC--- ---ACCCCAA
D.aur     ATTTTCCAGA gagaccccct tgtcagccca cattcccctt aatTCCCTTA
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     GTCACATATT TGTTCTTTTA TAACATGAA- ---CGCGTAC C---------
D.mau     GTCACATATT TGTTCTTTTA TAACGCGAA- ---CGCGTAC C---------
D.sim     GTCACATATT TGTTCTTTTA TA-------- --------Ac gtgaacgagt
D.ere     GTCACATATT TGTTCTTTTA TA-------- --------AC C---------
D.fuy     GCCTCATGTT TGTTATTTTA CAACGTCAca cgtCGCGAAC C---------
D.aur     GCCAAATGTT TGTTATTTTA TAACGTCAA- ---CGCGTcg cgaaccgaga
D.obs     ---------- ---------- ---------- ---------- ----------
D.wil     ---------- ---------- ---------- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ---------- ---------- ---------- ----------
D.vir     ---------- ---------- ---------- ---------- ----------
D.gri     ---------- ---------- ---------- ---------- ----------

D.mel     ------GCGA AGGCCCCATA AAGTGTTCG- ---------- -CAATAAAAT
D.mau     ------GCGA AGGTCCCATA AAGTGTTCG- ---------- -TAATAAAAT
D.sim     accgcgGCGA AGGTCCCATA AAGTGTTCG- ---------- -TAATAAAAT
D.ere     ------GCGA ATGCCCCATA AAGTGTTCG- ---------- -TAATAAAAT
D.fuy     ------GAGA AGGTCTCATA AAGTGTTCG- ---------- -TAATAAAAT
D.aur     aggc------ ---CCCCATA AAGTGTTTG- ---------- -TAATAAAAT
D.obs     ---------- ----CCAATA AAATGTTTTG CTATAAAATa cAATATATAT
D.wil     ---------- ---ACTCATA AAATGTTTC- ---------- -TAATATATT
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ---------- ----CTCATA AAATGTTTGG CAATAAAAT- -------TAT
D.vir     ---------- ---------- -----TTCT- ---------- -AATAAATAT
D.gri     ---------- ---CCTCATA AAGTGTTTG- ---------- -CAATAAAAT

D.mel     ATATTGTGCA ATAGTTA--- ----TAcagc cactcatata cat-------
```

```
D.mau     ATATTGTGCA ATATTTGTGC TATAGTTA-- ---------- ---TACA---
D.sim     ATATTGTGCA ATATTTGTGC TATAGTTA-- ---------- ---TACA---
D.ere     ATATTGCGCA ATATTTGTGC TATAGTTA-- ---------- ---TACA---
D.fuy     ATATTGTaca atatttg-GC TATAGTTA-- ---------- ---TATA---
D.aur     ATATTGTgca atatttt-GC TATAGTTA-- ---------- ---TAGA---
D.obs     TGTTTGTCCA ATATATT--- ----GTTA-- ---------- ---TATA---
D.wil     acgccataat ttttttttt ttgctttgtt ttagttctat tgaTATT---
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ATATTGTGCA ATATATT--- ----AATA-- ---------- ---TATTTTt
D.vir     ATATTGTGCA ATATATA--- ----TATT-- ---------- ---TTTTTTt
D.gri     ATATgcatat atattgtgaa att------- ---------- ----------

D.mel     ---------- ---------- ---------- ---------- ----------
D.mau     ---------- ---------- GCCACTC--- ---------- ----------
D.sim     ---------- ---------- GCCACTC--- ---------- ----------
D.ere     ---------- ---------- GCCACTC--- ---------- ----------
D.fuy     ---------- ---------- GCCACTC--- ---------- ----------
D.aur     ---------- ---------- GCCACTC--- ---------- ----------
D.obs     ---------- ---------- TGCCCCA--- ---------- ----------
D.wil     ---------- ---------- CCCACAA--- ---------- ----------
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     gtgtgcccac tctatataag GCCCCTA--- ---------- ----------
D.vir     tgcccgaacg tatatctcac gggccaaatg tttatcggcc tggcaTATAT
D.gri     ---------- ---------- ---------- ---------- -----TATAT

D.mel     ---TATATAC AATATATATA TATGTGgATG TGTATGTGCA CAACC-----
D.mau     ----ATATAC ATTATATATA CATATGtgt- ---------- ----------
D.sim     ----ATATAC ATTATATATA TATGC----- ---------- ----------
D.ere     ----ATATAC ATTATATACT T------ATG TGTATGTGCG CAACT-----
D.fuy     ----ATATAC ATTATATATA TATGagc--- ---G------ CAACT-----
D.aur     ----ATATAC ATTATATATA TATATAAATg tggG------ CAACT-----
D.obs     ----ATATAC ATTATATAGA CAacgcacac acacacacac acactgcata
D.wil     ----TGGAGA TGTGTATATA C------TTA TGTATATATA TTTAT-----
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     ----ATATCG TTTATATATA TATAT----- ---------- ----------
D.vir     ATAAATATGT ATTATATATA TACGC----- ---------- ----------
D.gri     ATATATATAT ATTATACATA TATCTAAATa aatattaggc caactgttta

D.mel     ---------- ---------- ---------- --ATATAGAT GTGTTGTATA
D.mau     ---------- ---------- ---------- -TATATAGAT GTGTTGTATA
D.sim     ---------- ------ATGT GTGTGTGTAA CTATATAGAT GTGTTGTATA
D.ere     ---------- ---------- ---------- --ATATAGAT G---TGTATA
D.fuy     ---------- ---------- ---------- --ATATAGAT G---TGTATA
D.aur     ---------- ---------- ---------- --ATATAAAT G---TGTATA
D.obs     ctatgtatgt aactattata acggggtaca catatacgca gccccttac
D.wil     ---------- ---------- ---------- --ATATACTT A---TATATA
D.sal     ---------- ---------- ---------- ---------- ----------
```

```
Z.ghe     ---------- ------CTTA TATATGCATA tcttctcttg tttggcatag
D.vir     ---------- ------AATA TATATTTATA agcaagtatg gca-------
D.gri     ttgccattgt gtagatGTGT GTGTGTGTGT TTAAGTAGAT ATGGGAAATG

D.mel     TAAAT--TGC CATC------ --CCATTGCT TATCATCGCC TTTATAGGTA
D.mau     TAAAT--TGC CATC------ --CCATTGCT TATCACCGCC TTTATAGGTA
D.sim     TAAAT--TGC CATC------ --CCATTGCT TATCATCGCC TTTATAGGTA
D.ere     TAAAT--TGC CATA------ --CCATTGCT T---ATCGCC TTTATAGGTA
D.fuy     TATAgaagg- -------CTG TCCCACTGCT T---ATCGCC TTTATAGGTA
D.aur     TATATatTGC CATG------ --CCATTGCT T---ATCGCC TTTATAGGTA
D.obs     tccttccact tttgtttttc cattgcctct tatcgcattt ttgttatttt
D.wil     TAAGT--ATA CATatttCTA TCTTATTGCT T---ATCGCT TTTATTGGTt
D.sal     ---------- ---------- ---------- ---------- ----------
Z.ghe     tgggtagaat tcGTA----- ---------- ---------- ----------
D.vir     --------AA T-GTA----- ---------- ---------- ----------
D.gri     AGAATgagAA T-GTA----- ---------- ---------- ----------

D.mel     G--------- --AATGTAAT TTCTTTTTAT GCGCCGTTTT G
D.mau     G--------- --AATGTAAT TTCGTTTTAT GCGCCGTTTT G
D.sim     G--------- --AATGTAAT TTGGTTTTAT GCGCCGTTTT G
D.ere     G--------- --AATGTAAT TTCGTTTTAT GCGCAGTTTT G
D.fuy     G--------- --AATGTAAT TTCTTTTTAT GTGCAGTTTT G
D.aur     G--------- --AATGTAAT TTCGTTTTAT GCGCAGTTTT G
D.obs     ctataggtag aaAATGTAAT TTCGTTTTAT GCGCAGTTTT G
D.wil     tgaatgtata tT--TGTAAT TTCGTTTTAT GTGCCGTTTT G
D.sal     ---------- ---------- ---------- ---------- -
Z.ghe     ---------- -T--TGTAAT TTGTTTTTAT GCGCAGTTTT G
D.vir     ---------- -T--TGTAAT TTGTTTTTAT GCGCAGTTTT G
D.gri     ---------- -T--CGTAAT TTGTTTTTAT GCGCAGTTTT G
                          <(dimorphic element wil large)
```

Table S1. GFP-reporter screen for pupal cis-regulatory elements in the *D. melanogaster bab* locus

#	Construct Name	Size(bp)	RE Site	Primer Sequence	Abdominal Epidermis Other Expression
1	bab2 -12	6368	KpnI	ggtaccTCATCTTTCCGCTCCGTTAG	None
			KpnI	ggtaccTCTCCCAGTCGAATCCAGTT	
2	bab2 -7	6330	FseI	ggccggccCGAAGCAACACACTCACAA	None
			SbfI	cctgcaggGGAAAACGGAGACCAACACA	
3	bab2 -2	4051	FseI	ggccggccACAATGGTGGAGGTGGTCAT	None
			SbfI	cctgcaggGCCCTCTTACGGCACTTTTA	
4	bab2 +2	3727	NheI	gctagcGTCCCTCTCCACGAGTCATC	None
			SbfI	cctgcaggCATGACCACCTCCACCATT	(Oenocyte)
5	bab2 +7	6108	SbfI	cctgcaggGTGGGGCTTCATGGTCTAAA	None
			FseI	ggccggccGCAGTGACTCGCTGAATTTG	bristle/muscle
6	bab2 +23	5313	SbfI	cctgcaggAGCCGGAGAAGAAAGGCTAC	None
			FseI	ggccggccATTATTAATCAATGATCTGTTACA	
7	bab2 +28	6143	SbfI	cctgcaggTCCACAGCCATAACCTTTTACA	None
			FseI	ggccggccTTTAGACCCTGCCTGGACTG	
8	bab2 +32	6533	SbfI	cctgcaggAGATGGCTAACGGCAGAGTG	None
			FseI	ggccggccCCATAGAAGGGAAACTTTACGA	
9	bab2 +38	6463	SbfI	cctgcaggGTCCGTTCCTCCAGTCCTCT	None
			FseI	ggccggccAGGAACAATGGCTCGAAAGA	
10	bab2 +42	6360	NheI	gctagcTGAGGGGCAAATTATGGAGA	None
			SbfI	cctgcaggGTGGGTGCTGCTGTTCCT	Tarsal Segments
11	bab2 +47	6325	NheI	gctagcAGAAGCGGCCAAACAAAAG	None

		KpnI	ggtaccGCGCCTAACTAGCCAACAAT	Tarsal Segments
12 bab2 +52	6674	SbfI	cctgcaggCTTTGACGATGAGGGGATGT	None
		FseI	ggccggccGAGTCCTGCATCGAGAATCC	
13 bab2 +58	6246	FseI	ggccggccTTCCAGTTCCACTCCCACTC	None
		KpnI	ggtaccGCGGGGACACAGTTAGCA	
14 bab2 +63	6189	SbfI	cctgcaggCCCTTCCACCCTTTCACC	None
		FseI	ggccggccCAAACGCAGGCAAACAATC	
15 bab2 +67	6467	SbfI	cctgcaggTGAATGCACTGGCAGAAAAC	None
		FseI	ggccggccCCCTCTTGATTTCCCATCCT	
16 bab2 +72	6047	SbfI	cctgcaggGAGGGGAAAACGGGAATCT	None
		FseI	ggccggccGCTTCAGCAGCAACAGCAT	
17 bab1 intron 1	15361	AscI	ggcgcgccTGCTTCTGCCGTTCGTCTGGAGT	Male A2-A4
		SbfI	cctgcaggCCGAGAGGAAGAAAGGGTGAGTGA	Female A2-A7
18 bab1 intron 2	14894	AscI	ggcgcgccCAAGTGATAAGTGCTCCAGGGGAATG	Female A5-A7
		SbfI	cctgcaggTCGAGTGCAGGGACAGATGACGAT	
19 bab1 intron 3	11111	KpnI	AAACGCATAAATATAAAAACGCGTTG	None
		KpnI	CATTCTCGGAATTATTCGCGAA	
20 bab1 intron 4	9435	NotI	AAGACGCGATAAGACGCGATT	None
		NotI	GGTAAATATTTTTAATACGCGTCGCAT	

Table S2. Identification of the minimal sequence necessary for dimorphic and anterior CRE activity

#	Construct Name	Size(bp)	RE Site	Primer Sequence	Observed Expression
21	bab1 intron 6	7217	AscI	ggcgcgccCATCGCATCCCCTGCTCGTATCT	Female A5-A7
			SbfI	cctgcaggTCGAGTGCAGGGACAGATGACGAT	(strong)
22	bab1 intron 7	4263	KpnI	ggtaccTTTCTCCGTTTCTACGAACGTGTTC	Female A5-A7
			NheI	gctagcGCAATAAAATAAAAGAACCGATGCGA	(strong)
23	dimorphic element	663	KpnI	ggtaccCTCGCTCTTTCTCTTTGCCATTTT	Female A5-A7
			NheI	gctagcTTGTGCTCATAAGCGGATTAGCGA	(strong)
24	dimorphic element (Left)	382	KpnI	ggtaccCTCGCTCTTTCTCTTTGCCATTTT	Female A6-A7
			NheI	gctagcACTACCACAATTAACTTGCGCCAA	(weak)
25	dimorphic element (Middle)	450	KpnI	ggtaccGCAACATTCGCAGAACAGCAGC	Female A6-A7
			NheI	gctagcCTGCTGGAGCAAATCTAAGCATGT	(weak)
26	dimorphic element (Right)	356	KpnI	ggtaccCGTGGCAAAGTGGAGTGGACTG	Female A6-A7
			NheI	gctagcTTGTGCTCATAAGCGGATTAGCGA	(very weak)
27	bab1 intron 8	6986	AscI	ggcgcgccTGATGACGCCGAGTATGCCGA	monomorphic A2-A4
			SbfI	cctgcaggGTGGAGTTCATGTATCGCGGC	
28	bab1 intron 9	7067	AscI	ggcgcgccTCGTCATCTGTCCCTGCACTCG	bristle/muscle
			SbfI	cctgcaggGTAATCAATGCCACATCCCGC	
29	bab1 intron 10	2237	KpnI	ggtaccGGACCACGACGACTGACACTTATC	monomorphic A2-A4
			NheI	gctagcCCGAGAGGAAGAAAGGGTGAGTGA	
30	bab1 intron 11	1916	KpnI	ggtaccTCCTCTTTCCCGATTCCTCACCT	monomorphic A2-A4
			NheI	gctagcACTGCGACTGCATTAGCACCGA	
31	bab1 intron 12	1256	KpnI	ggtaccTCCTCTTTCCCGATTCCTCACCT	No Activity
			NheI	gctagcTTCTCCTCATTTCCCCATCACCG	

32	anterior element	1357	KpnI NheI	ggtaccGTGAACTGATCGAGAAGCTGGAGAG gctagcACTGCGACTGCATTAGCACCGA	monomorphic A2-A4

Table S3. Summary of the Activity of Mutant Dimorphic Elements

Construct	Description	% Activity Female/Male
mel	(wild-type)	100±2%(4)/20±3%(4)
mel/+	(transgene heterozygote)	59±2%(5)/ND
mel TTAT KO	(15 TTAT sites mutated)	9±0%(3)/ND
mel TTTAT KO	(7 TTTAT sites mutated)	19±2%(3)/ND
mel TTTAC KO	(7 TTTAC sites mutated)	26±3%(3)/ND
mel 9,10 KO	(sites 9 & 10 mutated)	110±7%(3)/ND
mel 11-13KO	(sites 11-13 mutated)	79±4%(3)/ND
mel 9-12,14 KO	(sites 9-12 &14 mutated)	26±6%(3)/ND
mel 6,7,9-11,13,14 KO	(sites 9-11, 13 &14 mutated)	30±4%(3)/ND
mel Dsx1 KO	(Dsx site 1 mutated)	23±2%(3)/29±3%(3)
mel Dsx2 KO	(Dsx site 2 mutated)	34±3%(3)/21±3%(4)
mel Dsx1,2 KO	(Dsx sites 1 & 2 mutated)	24±1%(2)/53±3%(3)
mel 8 KO	(site 8 mutated)	78±5%(2)/ND
mel 13 KO	(site 13 mutated)	104±4%(4)/ND
mel 14 KO	(site 14 mutated)	55±0%(3)/ND
mel Dsx1 wil	(Dsx site 1 converted to D. wil site)	87±2%(4)/ND
mel Dsx1 wil 8,13 KO	(Dsx1 wil conversion + sites 8 &13 mutated)	66±3%(3)/ND
wil	(D. wil dimorphic element)	1±1%(2)/ND
wil Dsx1 WT	(D. wil Dsx site 1 converted to D. mel)	34±3%(3)/ND
mel ins I	(58 bp of D. wil added to region I of D. mel)	62±3%(3)/ND
mel ins II	(98 bp of D. wil added to region II of D. mel)	41±3%(3)/ND
mel ins III	(57 bp of D. wil added to region III of D. mel)	137±3%(3)/ND
mel ins I-III	(58,98 and 57bp added to region I-III of D. mel)	44±4%(3)/ND

NOTES: For each construct, the mean for A6 intensity and standard error of the mean was determined using images from multiple independent samples from the same transgenic line. The percent of wild-type activity was calculated as: (construct A6 mean/A6 mean of wild-type dimorphic element in female pupae) X 100 and reported above with the standard error of the mean (± SEM) and number of replicates (N).

Table S4. Oligonucleotide sequences used in EMSAs

Binding Site	Orientation	Sequence
Dsx 1	Top	TTTTGGCCGCAACAATGTTGCTGCATTTA
	Bottom	TTAAATGCAGCAACATTGTTGCGGCCAAA
Dsx 1 KO	Top	TTTTGGCCGCAGGGGGCGTGCTGCATTTA
	Bottom	TTAAATGCAGCACGCCCCCTGCGGCCAAA
Dsx 2	Top	TTTCCCAGCACAACAATGTTGCGGCATTC
	Bottom	TGAATGCCGCAACATTGTTGTGCTGGGAA
Dsx 2 KO	Top	TTTCCCAGCACAGGGGGCGTGCGGCATTC
	Bottom	TGAATGCCGCACGCCCCCTGTGCTGGGAA
Dsx 1 wil	Top	TTTTGGCCGCAACATTGTGGCTGCATTTA
	Bottom	TTAAATGCAGCCACAATGTTGCGGCCAAA
Site 1,2	Top	TTTTAACTTTTATTACTCTTAATATAAAAAAGCT
	Bottom	TAGCTTTTTTATATTAAGAGTAATAAAAGTTAAA
Site 4	Top	TTGCACGCGGTCATAAAAAGTTGCAGGA
	Bottom	TTCCTGCAACTTTTTATGACCGCGTGCA
Site 4 KO	Top	TTGCACGCGGTCgccgAAAGTTGCAGGA
	Bottom	TTCCTGCAACTTTcggcGACCGCGTGCA
Site 8	Top	TGCTAGTGGCACATAAAAATTGGCGCAA
	Bottom	TTTGCGCCAATTTTTATGTGCCACTAGC
Site 8 KO	Top	TGCTAGTGGCACgccgAAATTGGCGCAA
	Bottom	TTTGCGCCAATTTcggcGTGCCACTAGC
Site 11,12	Top	TTCGCACTTTACGAGGCGTTTTTTTTTTATATCACT
	Bottom	TAGTGATATAAAAAAAAAACGCCTCGTAAAGTGCGA
Site 11,12 [m11]	Top	TTCGCACTcggCGAGGCGTTTTTTTTTTATATCACT
	Bottom	TAGTGATATAAAAAAAAAACGCCTCGccgAGTGCGA
Site 11,12 [m12]	Top	TTCGCACTTTACGAGGCGTTTTTTTTcggcATCACT
	Bottom	TAGTGATgccgAAAAAAAACGCCTCGTAAAGTGCGA
Site 13	Top	TTATCACTTACTTTACTTAGTTGATTA
	Bottom	TTAATCAACTAAGTAAAGTAAGTGATA
Site 13 KO	Top	TTATCACTTACTcggCTTAGTTGATTA
	Bottom	TTAATCAACTAAGccgAGTAAGTGATA
Site 14	Top	TGTGGGCTGCATTTTACGACCCTCAAAA
	Bottom	TTTTTGAGGGTCGTAAAATGCAGCCCAC
Site 14 KO	Top	TGTGGGCTGCATTcggCGACCCTCAAAA
	Bottom	TTTTTGAGGGTCGccgAATGCAGCCCAC