Plasmid constructs

Here we present the general strategy used for plasmid construction. Detailed information regarding each construct and maps are available upon request. **Table 1** is the list of all constructs used in this study together with their general characteristics, provides numeric (**№**) identification for each construct and associates them with the Figures in the Results section. **Table 2** provides information of the primers used in plasmid construction. All critical features in all the constructs used in this study were verified by sequencing.

To obtain our constructs we have used four different backbones of publicly available plasmids: *egfp*-containing *pStinger* and *lacZ*-containing *pPelican* (Barolo *et al.* 2004), *pBluescript* (*SK(+*), Stratagene) and *pLacZ-attB* (Bischof *et al.* 2007). *pStinger*, *pPelican* and *pBluescript* were modified by cloning *attB* sequence from *pTA-attB* plasmid (Groth *et al.* 2004) in the same orientation in each plasmid, immediately upstream to the multi-cloning site (MCS; in case of *pStinger* and *pPelican* it is upstream of the 5' GI, see **A** and **B** in **Table 1**). *pBluescript* was additionally modified by cloning the *e7p7-lacZ* sequence downstream of *attB* (in the MCS) and *3xP3* upstream of *attB* (shown in **Table 1 C**). The latter construct was used as a base to generate all *3xP3*-containing plasmids.

The 7 kb genomic fragment encompassing *E(spl)m7* and *E(spl)m8*, together with their upstream (enhancers and promoters) and downstream (3' UTRs) regulatory regions, was cloned with *XhoI* and *ClaI* restriction enzymes from the R3012 cosmid clone encompassing part of *E(spl)* locus (Delidakis and Artavanis-Tsakonas 1992) into *pPelican-attB*. Due to strong post-transcriptional repression of both of these genes (Lai *et al.* 2005) we have replaced *E(spl)m7* and *E(spl)m8* 3' UTRs with the SV40 (derived from pGL3 vector, Promega) and the *Adh* (derived from the *Ract-HAdh* vector, Swevers *et al.* 1996) poly A terminators, respectively.

Subsequently, unique restriction sites were introduced before the start codons of both genes by mutagenesis to allow cloning of EGFP (derived from *pCRE-d2EGFP* vector, Clonetech) into the ORF of *E(spl)m7* in one construct (*GFPm7-m8*, contruct **№ 1** in Table 1) and of *E(spl)m8* in another (*m7-GFPm8*, construct **№ 2**).

The 'short genomic' constructs (*GFPm7* and *GFPm8*) were generated by the excision of the regulatory and coding portions of the untagged genes from the 'long genomic' constructs, i.e., *GFPm7* (construct **№ 3**) was generated from *GFPm7-m8* by a deletion of the downstream sequences to the *m7*'s (SV40) 3' UTR, and *GFPm8* (construct **№ 4**) was generated from *m7-GFPm8* by a deletion of the *m7*'s 3' UTR and its upstream sequences. The resulting constructs contained 2.1 kb sequence upstream of the start codon of *GFPm7* (which we denote as *e7p7*) and 1.3 kb sequence upstream of the start codon of *GFPm8* (*e8p8*).

The GI-less *e8p8-GFPm8* construct (**№ 5**) was generated by cloning of the *e8p8-GFPm8* module (together with its *Adh 3' UTR*) from *GFPm8* into *pPelican-attB* bearing deletion of both GIs and LacZ. This construct was subsequently used to generate four single-GI versions of *e8p8-GFPm8* by cloning the 5' GI from *pPelican* into 5' (upstream of *e8p8*) or 3' (downstream of *Adh 3' UTR*) positions in forward or reverse orientation (constructs **№ 6-9**).

The *e8p8-lacZ pPelican*-based construct (**№ 10**) was made by cloning PCR-amplified *e8p8* (primers **e8p8F** and **e8p8R**) into the MCS of *pPelican-attB*.

The *e7p7-lacZ pPelican*-based construct (**№ 11**) was made by cloning PCR-amplified *e7p7* (primers **e7p7F** and **e7p7R**) into the MCS of *pPelican*. The *pBluescript* GI-less version of *e7p7-lacZ* (**№ 12**, see also **C** in **Table 1**) was made by *(1)* cloning the entire *e7p7-lacZ* sequence of *pPelican*-based *e7p7-lacZ* (together with its *SV40 3' UTR*) into *pBluescriptSK(+)* in between *KpnI* and *SpeI* restriction sites; *(2)* and subsequent cloning of the DNA fragment containing

3xP3-dsRed and *attB* seqences from *pMinos{3xP3-dsRed}* vector (Berghammer *et al.* 1999; this vector was beforehand modified by us by inserting *attB* sequence in the vicinity of *3xP3-dsRed*) into *KpnI* site of the *pBluescript* such that *attB* site is in between the *3xP3-dsRed* and *e7p7*. The *pBluescript* GI-less version of the *e7p7-lacZ* construct with mini-*white* marker (**№ 13**) was made by the replacement of the *3xP3-dsRed* in the latter construct with *pPelican*'s mini-*white*. The *pBluescript* GI-less version of *e7p7-lacZ-3xP3-dsRed* (**№ 12**) construct was used as a basis to generate *(1)* GI-containg *e7p7-lacZ* constructs by cloning 5' GI from *pPelican* immediately upstream of *e7p7* or/and downstream of *SV40 3' UTR* in a reverse or forward orientation (constructs **№ 14-20**); *(2)* the three *e7p7-lacZ* constructs containing different parts of mini-*white* gene cloned into *SpeI* site (immediately downstream of *SV40 3' UTR*): the 0.24 kb *AfeI*/*EcoRI* fragment encompassing the 5'/promoter region of mini-*white* (*e7p7-lacZ-w5',* construct **№ 21**), the 2.4 kb *AflII*/*EcoRV* fragment encompassing mini-*white*'s gene body (*e7p7-lacZ-wB*, construct **№ 22**) and the 0.9 kb *EcoRV*/*BsrGI* 3' part of the mini-*white* (*e7p7-lacZ-w3'*, construct **№ 23**). The *pBluescript e7p7-lacZ* construct with two GIs in forward orientation (**№ 14**) was used to generate two 'tester' constructs by cloning *e8p8-m8-Adh 3' UTR* module from the EGFP-untagged version of the *GFPm8* construct (*e8p8-m8* tester, **№ 24**) and *pH-gfp-SV40 3' UTR* module from *pHStinger* (*pH-gfp* tester, **№ 25**) immediately downstream of the 3' GI.

The *e7p7-ΔTATA-lacZ* construct (**№ 26**) containing deletion of 20 nt encompassing TATA box was generated by introducing an *EcoRV* restriction site by site-directed mutagenesis upstream of the TATA box of *p7* in *pPelican*-based *e7p7-lacZ* construct (**№ 11**) and subsequent excision of the seqeunce between *EcoRV* and *BstEII*. The intermediate *e7p7-lacZ* construct with introduced *EcoRV* (but without *EcoRV*/*BstEII* deletion; **№ 27**) produced LacZ expression pattern and levels indistinguishable to that of the *e7p7-lacZ* without *EcoRV* and was used as a control transgene (to *e7p7-ΔTATA-lacZ* and *e7p7-ΔDPE-lacZ*) containing wild-type *p7*. The

e7p7-ΔDPE-lacZ (**№ 28**) construct was generated from *pPelican*-based *e7p7-lacZ* by excision of 83 nt containing INR and DPE motifs in between *BstEII* and *StuI* restriction sites. The *e7-lacZ* (promoterless construct, **№ 29**) was generated by an excision of sequence between *EcoRV* and *StuI* from the *e7p7-lacZ* construct with the introduced *EcoRV* site (**№ 27**).

All *pH-gfp* constructs (**№ 30**-**36**) were made based on *pHStinger* (a version of *pStinger* containing *pH* fused to *egfp*; Barolo *et al.* 2000). The *pH-gfp* with two GIs in forward orientation (**№ 30**) was made by inserting *attB* sequence into *pHStinger*. Subsequent deletions of the 3' GI, 5' GI and both GIs from this construct resulted in generation of constructs **№ 31**, **32** and **33**, respectively. The 5' GI^{REV} *pH-gfp* (**№ 34**) was made by cloning GI in reverse orientation into GI-less *pH-gfp*. The 5' GI^{FOR} 3' GI^{REV} *pH-gfp* (**№ 35**) was made by cloning GI in a reverse orientation in place of the 3' GI^{FOR} of GIs^{FOR} *pH-gfp* (**№ 30**). The 5' GI^{REV} 3' GI^{FOR} *pH-gfp* (**№ 36**) was made by cloning GI in a reverse orientation in place of the 5' GI^{FOR} of the GIs^{FOR} *pH-gfp* (**№ 30**).

The 2 kb *BglII-EcoRV e7*-containing fragment derived from the *e7p7-lacZ* construct bearing *EcoRV* site introduced upstream to TATA box of *p7* (**№ 27**) was used to generate *(1)* the *e7* construct (**№ 37**) by replacing *lacZ* in the *pPelican*-attB and *(2)* the *e7pH-gfp* (**№ 38**) by cloning it upstream to *pH* of the GIs^{FOR} *pH-gfp* (**№ 30**). The *e7p7-gfp* construct (**№ 39**) was made by PCR-amplifying the *p7* promoter from *e7p7-lacZ* (**№ 11**) with **p7F** and **p7R** primers and ligating the *NheI/HeaIII*-digested PCR product into *NheI* and *StuI* sites in the *e7pH-gfp* construct (**№ 38**) such that *p7* sequence replaces the sequence of *pH*. The *p7-gfp* (**№ 40**) was based on the *e7p7-gfp* (**№ 39**) by excision of *e7* with *KpnI*. The sequence of *e8* was PCR-amplified with **e8F** and **e8R** primers, its product was cut with *AvrII* and *NheI* and ligated to *XbaI* and *NheI* sites (replacing *e7*) in the *e7* construct (**№ 37**) to generate *e8* construct (**№ 41**). The sequence of *e8*

(as a *MfeI-NheI* fragment) was cloned from *e8* construct (**№ 41**) upstream of *p7* (*EcoRI*/*NheI*) in the *p7-gfp* construct (**№ 40**) to generate *e8p7-gfp* (**№ 42**). The *e7p8-gfp* construct (**№ 43**) was made by PCR-amplifying the *p8* promoter from *e8p8-lacZ* (**№ 10**) with **p8F** and **L5R** primers and ligating the *NheI/HeaIII*-digested PCR product into *NheI/StuI* sites in the *e7pH-gfp* construct (**№ 38**) such that *p8* sequence replaces the sequence of *pH*. The *e8* sequence was cut out from the *e8* construct (**№ 41**) with *MfeI* and *NheI* restriction enzymes and ligated to *EcoRI* and *NheI* sites (such that the *e8* sequence replaces the sequence of *e7*) in the *e7p8-gfp* construct (**№ 43**) to generate *e8p8-gfp* construct (**№ 44**). The *p8-gfp* construct (**№ 45**) was generated by excision of the *e7* sequence with *NaeI* and *NheI* restriction enzymes from the *e7p8-gfp* construct (**№ 43**).

The luc constructs (**№ 46**-**48**) were generated by replacing *pH-gfp* module with the restriction fragment containing *pH-luc* module (derived from the *pGL3-hsp70-luc* construct, gift from M. Monastirioti, IMBB) in the GIs^{FOR} *pH-gfp* (**№ 30**), GIs^{FOR} *e7pH-gfp* (**№ 38**) and GI-less *pH-gfp* (**№ 33**) constructs – resulting in generation of GIs^{FOR} *pH-luc* (**№ 46**), GIs^{FOR} *e7pH-luc* (**№ 47**) and GI-less *pH-luc* (**№ 48**), respectively.

The *'blank' sender* construct (**№ 49**) was made by cloning *(1)* the *HindIII/BamHI*-cut PCR-amplified product of *e7* (primers **e7-2F** and **e7-2R**) into *pLacZ-attB* construct (**D** in **Table 1**) replacing *lacZ* sequence and *(2)* subsequent cloning of the *NheI/BglII*-cut PCR amplicon of *e8* (primers **e8-2F** and **e8-2R**). The *'blank' sender* construct (**№ 49**) was used to generate GI, Fab8 and 1A2 sender constructs (**№ 50**, **51** and **52**, respectively) by cloning the PCR-amplified sequences of GI, Fab8 or 1A2 into the *BglII* site located in between *e7* and *e8*. The 400 bp GI sequence was amplified on the *pPelican* template with **GIF** and **GIR** primers and contains full-length 367 bp GI (see **Figure S7**). The 540 bp Fab8 sequence was PCR-amplified from

Drosophila genome using **Fab8F** and **Fab8R** primers and contains the $F8^{254}$ sequence and part of $F8^{469}$ sequence (as defined in Kyrchanova *et al.* 2008; see **Figure S7**). The 420 bp 1A2 sequence was PCR-amplified from *Drosophila* genome using primers **1A2F** and **1A2R**, and relates to the exact same region defined as 1A2 insulator in Kyrchanova *et al.* 2008a (see **Figure S7**). The WI-less sender constructs, **№ 53** and **54**, were generated from the *'blank' sender* (**№ 49**) and the *GI sender* (**№ 50**) constructs, respectively, by excision of the 341 bp WI-containing fragment between *BsrGI* and *BstBI* sites.

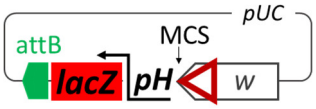
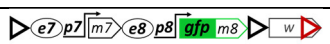
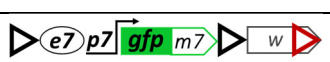
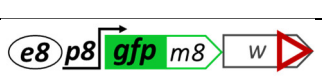
The *'blank' responder* construct (**№ 55**) was made by *(1)* replacing the *pH* in the *pHStinger* with the the *DSCP* promoter (*pD*) derived from *pBPGUw* plasmid (Pfeiffer *et al.* 2008) and *(2)* ligating the resulting *pD-gfp-SV40 3' UTR* module in between the *BglII* and *NheI* sites of *pLacZ-attB,* which already contains the *pH-lacZ-SV40* module (Bischof *et al.* 2007). Subsequently, this construct was used to generate *GI* and *Fab8 responder* constructs (**№ 56** and **57**, respectively) by cloning into the *BglII* site the sequences of GI and Fab8 PCR-amplified with the same sets of primers (i.e., **GIF**/**GIR** and **Fab8F**/**Fab8R**) and in the same orientation as it was for the corresponding *sender* constructs. The 1A2 sequence was amplified with the **1A2F2** and **1A2R** primers and ligated into *EcoRI/BglII* sites of the *'blank' responder* construct (**№ 55**) resulting in generation of the *1A2 responder* construct (**№ 58**). The WI-less *GI responder* (**№ 59**) was generated by a deletion of the 502 bp WI-containing *BstBI/NsiI* fragment from the *GI responder* construct (**№ 56**).

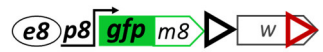
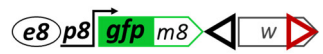
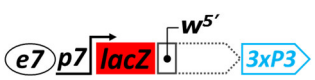
The *HH-1.5*, *HH-2.1*, *HH-5.4*, *HB-1.6*, *BB-1.8*, *BB-3.1* and *BB-2.3* constructs (**№ 60-66**) were generated by cloning 1.5 kb-, 2.1 kb- and 5.4 kb-*HindIII-HindIII*, 1.6 kb-*HindIII-BglII*, 1.8 kb-, 3.1 kb- and 2.3 kb-*BglII-BglII* fragments, respectively, derived from the *R3007* cosmid (Delidakis and Artavanis-Tsakonas 1992) into the MCS of *lacZ*-deficient *pPelican-attB*.

The *BB-5.4*, *BB-1.3*, *BB-7.9*, *BB-1.7*, *BB-07*, *BP-3.2* and *PB-4.7* constructs (**№ 67-73**) were generated by cloning 5.4 kb-, 1.3 kb-, 7.9 kb-, 1.7 kb- and 0.7 kb-*BglII-BglII*, 3.2 kb- and 4.7 kb-*PstI-BglII* fragments, respectively, derived from the *R3012* cosmid (Delidakis and Artavanis-Tsakonas 1992) into the MCS of *lacZ*-deficient *pPelican-attB*.

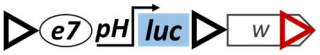
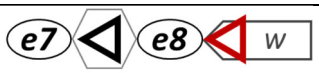
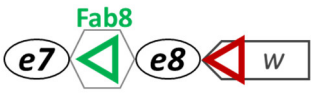
The 0.8 kb sequence of Vestigial Quadrant Enhancer (*vgQ*, Kim *et al.* 1996) was derived from a *pBluescript-vgQ* vector and cloned into the MCS of *lacZ*-deficient *pPelican-attB* to generate the *VGQ* construct (**№ 74**).

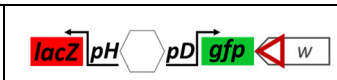
Table 1 List of constructs used in the study

№	schematic	Name (as in text)	Marker gene	Insulators	Backbone	Figures
A		*pStinger-attB*	mini-*white*	WI, GIs^{FOR}	*pStinger*	NA
B		*pPelican-attB*	mini-*white*	WI, GIs^{FOR}	*pPelican*	NA
C		*e7p7-lacZ-attB*	*3xP3*	-	*pBluescript*	NA
D		*pLacZ-attB*	mini-*white*	WI	*pLacZ-attB*	NA
1		*GFPm7-m8*	mini-*white*	WI, GIs^{FOR}	*pPelican*	1
2		*m7-GFPm8*	mini-*white*	WI, GIs^{FOR}	*pPelican*	1
3		*GFP-E(spl)m7*	mini-*white*	WI, GIs^{FOR}	*pPelican*	1
4		*GFP-E(spl)m8*	mini-*white*	WI, GIs^{FOR}	*pPelican*	1, 2, 3, S1
5		*e8p8-GFPm8*	mini-*white*	WI	*pPelican*	3, S1, S10

6		*e8p8-GFPm8*	mini-*white*	WI, 5' GI^{FOR}	*pPelican*	S1, S10
7		*e8p8-GFPm8*	mini-*white*	WI, 5' GI^{REV}	*pPelican*	S10
8		*e8p8-GFPm8*	mini-*white*	WI, 3' GI^{FOR}	*pPelican*	S10
9		*e8p8-GFPm8*	mini-*white*	WI, 3' GI^{REV}	*pPelican*	S10
10		*e8p8-lacZ*	mini-*white*	WI, GIs^{FOR}	*pPelican*	6, S3
11		*e7p7-lacZ*	mini-*white*	WI, GIs^{FOR}	*pPelican*	1, 2, 3, 4
12		*e7p7-lacZ*	*3xP3-dsRed*	-	*pBluescript*	3, S8, S10
13		*e7p7-lacZ*	mini-*white*	WI	*pPelican*	3, 4
14		*e7p7-lacZ*	*3xP3-dsRed*	GIs^{FOR}	*pBluescript*	3, 4, 7, 8, S6, S11
15		*e7p7-lacZ*	*3xP3-dsRed*	5' GI^{FOR}	*pBluescript*	7, 8, S8, S10
16		*e7p7-lacZ*	*3xP3-dsRed*	5' GI^{REV}	*pBluescript*	7, S10
17		*e7p7-lacZ*	*3xP3-dsRed*	3' GI^{FOR}	*pBluescript*	7, S10
18		*e7p7-lacZ*	*3xP3-dsRed*	3' GI^{REV}	*pBluescript*	S10
19		*e7p7-lacZ*	*3xP3-dsRed*	5' GI^{FOR}, 3' GI^{REV}	*pBluescript*	7
20		*e7p7-lacZ*	*3xP3-dsRed*	5' GI^{REV}, 3' GI^{FOR}	*pBluescript*	7
21		*e7p7-lacZ-w5'*	*3xP3-dsRed*	-	*pBluescript*	S1

22		*e7p7-lacZ-wB*	*3xP3-dsRed*	-	*pBluescript*	S1
23		*e7p7-lacZ-w3'*	*3xP3-dsRed*	WI	*pBluescript*	S1
24		*e8p8-m8* tester	*3xP3-dsRed*	GIs^{FOR}	*pBluescript*	8
25		*pH-gfp* tester	*3xP3-dsRed*	GIs^{FOR}	*pBluescript*	8
26		*e7p7-ΔTATA-lacZ*	mini-*white*	WI, GIs^{FOR}	*pPelican*	6, 8, S5
27		*e7p7-lacZ*	mini-*white*	WI, GIs^{FOR}	*pPelican*	6, S5
28		*e7p7-ΔDPE-lacZ*	mini-*white*	WI, GIs^{FOR}	*pPelican*	6, 8, S5
29		*e7-lacZ*	mini-*white*	WI, GIs^{FOR}	*pPelican*	6, 8, S5
30		*pH-gfp*	mini-*white*	WI, GIs^{FOR}	*pStinger*	4, 5, 6, 7, 8, S2, S3, S4, S6, S11
31		*pH-gfp*	mini-*white*	WI, 5' GI^{FOR}	*pStinger*	7, 8, S8
32		*pH-gfp*	mini-*white*	WI, 3' GI^{FOR}	*pStinger*	7
33		*pH-gfp*	mini-*white*	WI	*pStinger*	4, S8
34		*pH-gfp*	mini-*white*	WI, 5' GI^{REV}	*pStinger*	7
35		*pH-gfp*	mini-*white*	WI, 5' GI^{FOR}, 3' GI^{REV}	*pStinger*	7
36		*pH-gfp*	mini-*white*	WI, 5' GI^{REV}, 3' GI^{FOR}	*pStinger*	7
37		*e7*	mini-*white*	WI, GIs^{FOR}	*pPelican*	5, 6, S4

38		*e7pH-gfp*	mini-*white*	WI, GIs^{FOR}	*pStinger*	5, S3, S4
39		*e7p7-gfp*	mini-*white*	WI, GIs^{FOR}	*pStinger*	5, S3
40		*p7-gfp*	mini-*white*	WI, GIs^{FOR}	*pStinger*	5, S3, S5, S11
41		*e8*	mini-*white*	WI, GIs^{FOR}	*pPelican*	5, 6
42		*e8p7-gfp*	mini-*white*	WI, GIs^{FOR}	*pStinger*	5
43		*e7p8-gfp*	mini-*white*	WI, GIs^{FOR}	*pStinger*	5, S3
44		*e8p8-gfp*	mini-*white*	WI, GIs^{FOR}	*pStinger*	5
45		*p8-gfp*	mini-*white*	WI, GIs^{FOR}	*pStinger*	5, S3, S5, S11
46		*pH-luc*	mini-*white*	WI, GIs^{FOR}	*pStinger*	6
47		*e7pH-luc*	mini-*white*	WI, GIs^{FOR}	*pStinger*	6
48		*pH-luc*	mini-*white*	WI	*pStinger*	6
49		*'blank' sender*	mini-*white*	WI	*pLacZ-attB*	9
50		*GI sender*	mini-*white*	WI, GI^{REV}	*pLacZ-attB*	9
51		*Fab8 sender*	mini-*white*	WI, Fab8	*pLacZ-attB*	9
52		*1A2 sender*	mini-*white*	WI, 1A2	*pLacZ-attB*	9
53		*'blank' sender*	mini-*white-dWI*	-	*pLacZ-attB*	9
54		*GI sender*	mini-*white-dWI*	GI^{REV}	*pLacZ-attB*	9

55		*'blank' responder*	mini-*white*	WI	*pLacZ-attB*	9
56		*GI responder*	mini-*white*	WI, GI^{REV}	*pLacZ-attB*	9
57		*Fab8 responder*	mini-*white*	WI, Fab8	*pLacZ-attB*	9
58		*1A2 responder*	mini-*white*	WI, 1A2	*pLacZ-attB*	9
59		*GI responder*	mini-*white-dWI*	GI^{REV}	*pLacZ-attB*	9
60		*HH-1.5*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
61		*HH-2.1*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
62		*HH-5.4*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
63		*HB-1.6*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
64		*BB-1.8*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
65		*BB-3.1*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
66		*BB-2.3*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
67		*BB-5.4*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
68		*BB-1.3*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
69		*BB-7.9*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
70		*BB-1.7*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
71		*BB-07*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2

72		*BP-3.2*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
73		*PB-4.7*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2
74		*VGQ*	mini-*white*	WI, GIs^{FOR}	*pPelican*	S2

Table 2 List of primers

Name	**Sequence (5' to 3')**
e8p8F	CGTCTAGAGGGGAATCTATTTTACAGCACAATCCAATAGGGG
e8p8R	GCGGTACCCGGCTTGTTGCTGCCTGCTCG
e7p7F	CGGCATGCGTCGCCAGAAAAATTGTAACGGCCC
e7p7R	GCCTCGAGGAACTTCTTCGATCTTTCGGAGGAGG
p7F	GAGGCTAGCAGCTATAAAAGCAGCGGTAACC
L5R	TCAGACGATTCATTGGCACC
e8F	CGGCATGCGGGGAATCTATTTTACAGCACAATCCAATAGGGG
e8R	CTGGCTAGCTCCCTGGTCCCTGAAATCC
p8F	GAGGCTAGCGGTATAAAAGGACGGGACCTC
e7-2F	GGGAAGCTTGTCGCCAGAAAAATTGTAACGG
e7-2R	GGCGGATCCGTGCCGCCGCGAGAG
e8-2F	CGTCTAGAGGGGAATCTATTTTACAGCACAATCCAATAGGGG
e8-2R	CCCGCTAGCCGCTCCCTGGTCCCTGAAATCC
GIF	GGAGATCTGCATCACGTAATAAGTGTGCGT
GIR	GGAGATCTGCCGAGCACAATTGATCG
Fab8F	GGAGATCTGGGGAGGGAATTTTCTTCA
Fab8R	GGAGATCTCATCTTCCGTTCATCCGTT
1A2F1	GGCGGATCCACTACCAGGCAAGAAAGTAGGT
1A2F2	GGTGCGAATTCACTACCAGGCAAGAAAGTA
1A2R	GGCGGATCCTATATGCTTCGTCTACCGTTGTG

Literature Cited

Barolo S., L. A. Carver, and J. W. Posakony, 2000 GFP and B-galactosidase transformation vectors for promoter/enhancer analysis in Drosophila. Biotechniques 29: 726–732.

Barolo S., B. Castro, and J. W. Posakony, 2004 New Drosophila transgenic reporters: insulated P-element vectors expressing fast-maturing RFP. Biotechniques 36: 436–40, 442.

Berghammer a J., M. Klingler, and E. a Wimmer, 1999 A universal marker for transgenic insects. Nature 402: 370–371. https://doi.org/10.1038/46463

Bischof J., R. K. Maeda, M. Hediger, F. Karch, and K. Basler, 2007 An optimized transgenesis system for Drosophila using germ-line-specific phiC31 integrases. Proc. Natl. Acad. Sci. U. S. A. 104: 3312–7. https://doi.org/10.1073/pnas.0611511104

Delidakis C., and S. Artavanis-Tsakonas, 1992 The Enhancer of split [E(spl)] locus of Drosophila encodes seven independent helix-loop-helix proteins. Proc. Natl. Acad. Sci. U. S. A. 89: 8731–5. https://doi.org/10.1073/PNAS.89.18.8731

Groth A. C., M. Fish, R. Nusse, and M. P. Calos, 2004 Construction of transgenic Drosophila by using the site-specific integrase from phage phiC31. Genetics 166: 1775–82.

Kim J., A. Sebring, J. J. Esch, M. E. Kraus, K. Vorwerk, *et al.*, 1996 Integration of positional signals and regulation of wing formation and identity by Drosophila vestigial gene. Nature 382: 133–138. https://doi.org/10.1038/382133a0

Kyrchanova O., S. Toshchakov, Y. Podstreshnaya, A. Parshikov, and P. Georgiev, 2008a Functional interaction between the Fab-7 and Fab-8 boundaries and the upstream promoter region in the Drosophila Abd-B gene. Mol. Cell. Biol. 28: 4188–95.

https://doi.org/10.1128/MCB.00229-08

Kyrchanova O., D. Chetverina, O. Maksimenko, A. Kullyev, and P. Georgiev, 2008b Orientation-dependent interaction between Drosophila insulators is a property of this class of regulatory elements. Nucleic Acids Res. 36: 7019–28. https://doi.org/10.1093/nar/gkn781

Lai E. C., B. Tam, and G. M. Rubin, 2005 Pervasive regulation of Drosophila Notch target genes by GY-box-, Brd-box-, and K-box-class microRNAs. Genes Dev. 19: 1067–80. https://doi.org/10.1101/gad.1291905

Pfeiffer B. D., A. Jenett, A. S. Hammonds, T.-T. B. Ngo, S. Misra, *et al.*, 2008 Tools for neuroanatomy and neurogenetics in Drosophila. Proc. Natl. Acad. Sci. U. S. A. 105: 9715–20. https://doi.org/10.1073/pnas.0803697105

Swevers L., L. Cherbas, P. Cherbas, and K. Iatrou, 1996 Bombyx EcR (BmEcR) and Bombyx USP (BmCF1) combine to form a functional ecdysone receptor. Insect Biochem. Mol. Biol. 26: 217–21.