Exploiting position effects and the gypsy retrovirus insulator to engineer precisely expressed transgenes

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Supplementary PDF Contents:

Supplementary Table 1
Detailed genomic information for attP docking sites characterized with the UAS::luciferase reporter.

						Genes Neighboring Intergenic Landing Sites	
Site	Chr.	Strand[b]	Cytology	Location	Intragenic Location	Left Gene (Strand) Distance	Right Gene (Strand) Distance
attP1[d]	2R	+	56C1	Intergenic		*sbb*[c] *(-)* 62 bp	*IM23* (-) 45,322 bp
attP2[d]	3L	+	68A4	Intergenic		*CG6310 (-)* 685 bp	*Mocs1* (+) 44 bp
attP3	X	+	19C4	Intergenic		*CG1631* (+) 1,897 bp	*CG15462* (-) 36,196 bp
attP4	X	+	12C6	Intragenic	*5'UTR of Clic*		
attP10	3	-	85D7/92B1	TE[e]			
attP14	2L	+	36A10	Intragenic	*Intron of grp*		
attP16	2R	+	53C4	Intergenic		*CG15711* (-) 22,181 bp	*CG33960* (+) 12,439 bp
attP18	X	+	6C12	Intragenic	*5'UTR of CG14438*		
attP22	2R	-	45D8	Intergenic		*ced-6*[f] (-) 7,143 bp	*CG13952* (+) 4,022 bp
attP23	2R	-	60C7	Intragenic	*Intron of bs*		
attP24[g]	2R	+	42C1	Intergenic		*Or42A* (-) 2054	*Tsp42A* (+) 458
	2R	+	58C1	Intergenic		*CG34205* (-) 5,676 bp	*a* (+) 102 bp
attP29	2L	+	21E2	Intragenic	*5'UTR of drongo*		
attP30	2L	+	29C3	Intragenic	*Intron of Akap200*		
attP32	2R	-	49D6	Intergenic		*CG17574* (-) 806 bp	*bic* (+) 7 bp
attP33	2R	+	50B6	Intergenic		*CG12464* (-) 4,373 bp	*fas* (+) 80 bp
attP40	2L	-	25C7	Intergenic		*CG14035* (+) 4,373 bp	*Msp-300* (+) 9,129 bp
attP52	3R	-	89B11	Intragenic	*5'UTR and intron of gish*[h]		
attP64	3R	+	89B9	Intragenic	*5'UTR and intron of tara*[h]		
attP83	CyO	+	39D3[i]	Intergenic		*nrv3* (+) 11,321 bp	*His1* (+) 15,409 bp
attP88	3L	-	64A12	Intragenic	*5' coding exon of CG1265*		
attP112	3L	+	68C13	Intragenic	*Intron of Mob1*		
attP154	3R	-	97D2	Intergenic		*CG14247 (+) 60,412 bp*	*Tl* (+) 37 bp

[a]Based on Release 5.1 of the *D. melanogaster* genome. [b]Strand is in reference to the chromosome; see Supplementary Figure 1 for representative illustrations. AttP docking sites are denoted on the (+) strand when oriented from the 5'P end to the 3'P end in a left-to-right fashion along the chromosome, and denoted on the (-) strand when in the reverse orientation. Likewise genes are denoted on the (+) strand when oriented 5' to 3' in a left-to-right fashion along the chromosome and they are denoted on the (-) strand when in the reverse orientation. [c]Based on accession AF247562. [d]See reference 22. [e]Transposable Element, RT1-alpha; attP10 maps genetically to chromosome 3'P end, but by iPCR it is unclear if it is in the RT1-alpha element at 85D7 or 92B1. [f]The 5' end of ced-6 is based on RE47146. [g]Genomic PCR indicates that the UAS-luciferase reporter is integrated at 42C1 but not 58C. [h]Due to alternative splicing, the attP site is located in the 5'UTR of some transcripts and in the intron of others. [i]On wild-type chromosome 2 position 39D3 is flanked by *nrv3* and *His1*; we did not determine if this gene order holds on CyO.

Supplementary Table 2
PCR Primers.

Primers used in constructing pCa4B

ID #	Name	Sequence
MM#7	attB_spe	AAGACTAGTGCTGCATCCAACGCGTTGGGAGCTC
MM#8	attB_not	AGGCGGCCGCGAATTAGGCCTTCTAGTGG

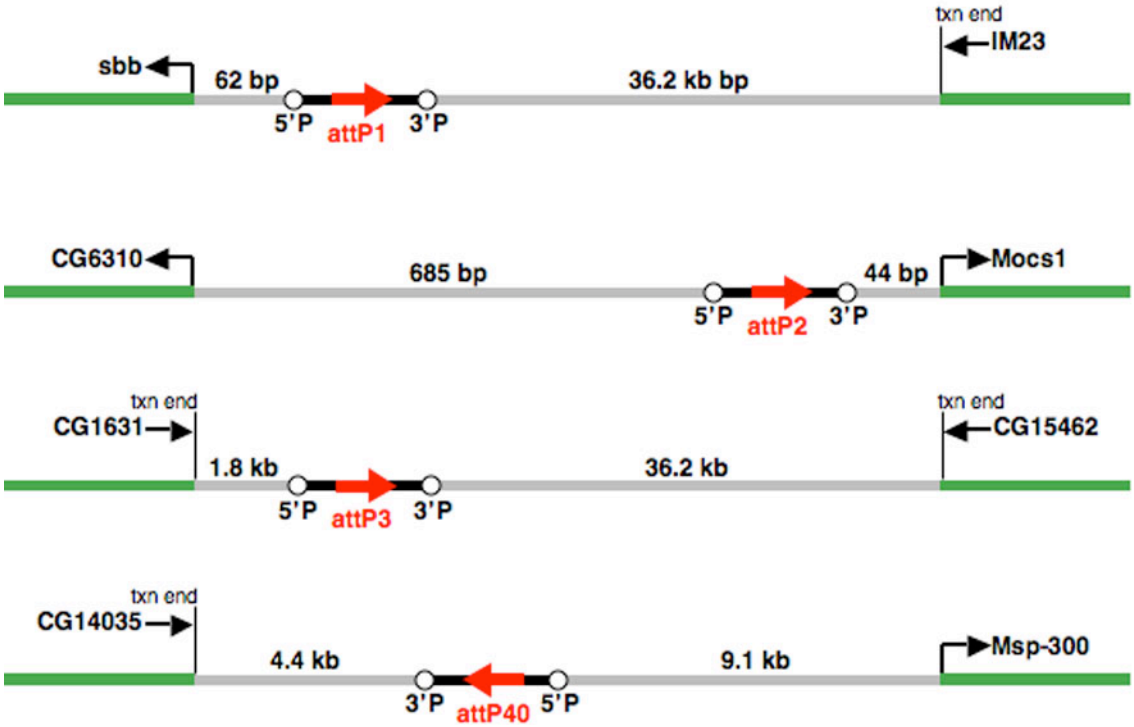
Primers used in constructing pCa4B2G

ID #	Name	Sequence
MM#91	spe_Gypsy	AATACTAGTTGGCCACGTAATAAGTGTGCGTTG
MM#92	xba_Gypsy	AATTCTAGAGTTGTTGGTTGGCACACCACA

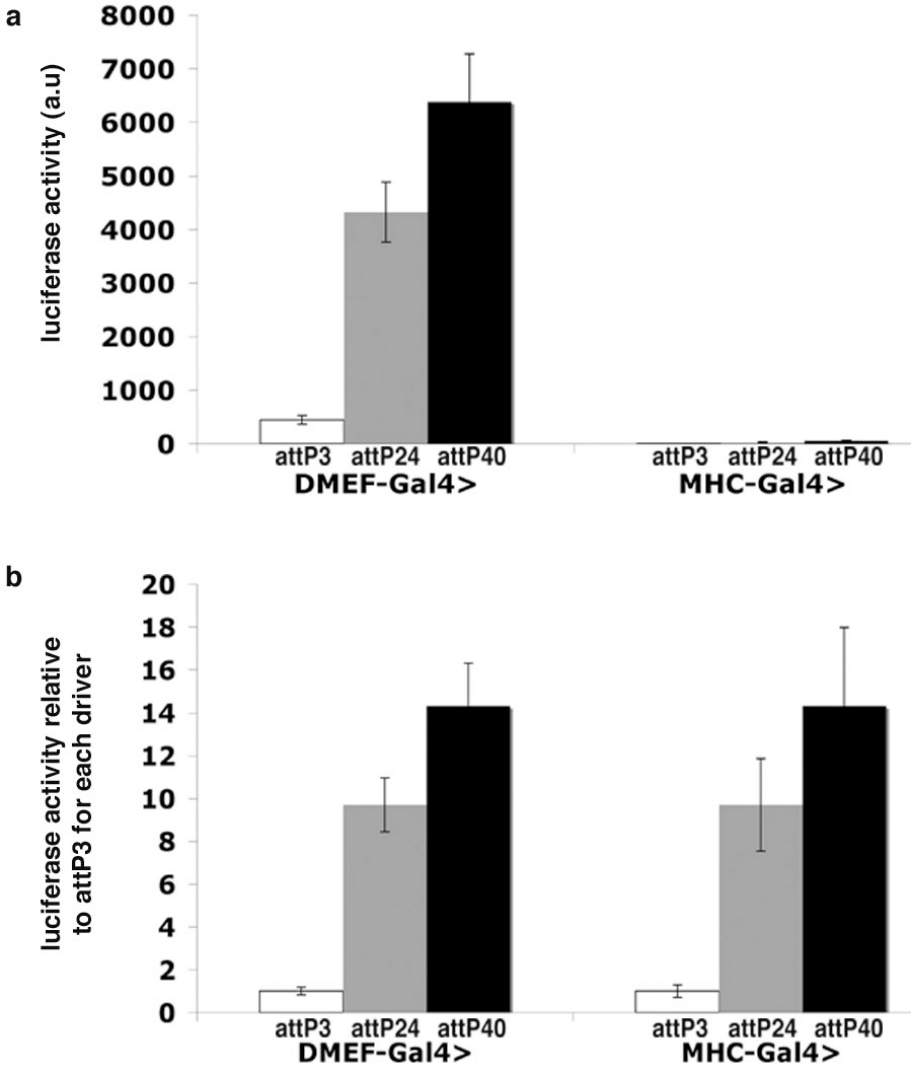
Primers used to verify insertion of attB plasmids into attP landing sites

ID #	Name	Sequence
MM#49	yellow	GGCTTCACGTTTTCCCAGGTCAGAAGCGGT
MM#50	attB	GGCGTAAACCGCTTGGAGCTTCGTCACGA

Supplementary Figure 1
Diagrams of representative attP landing sites in relation to neighboring genes.

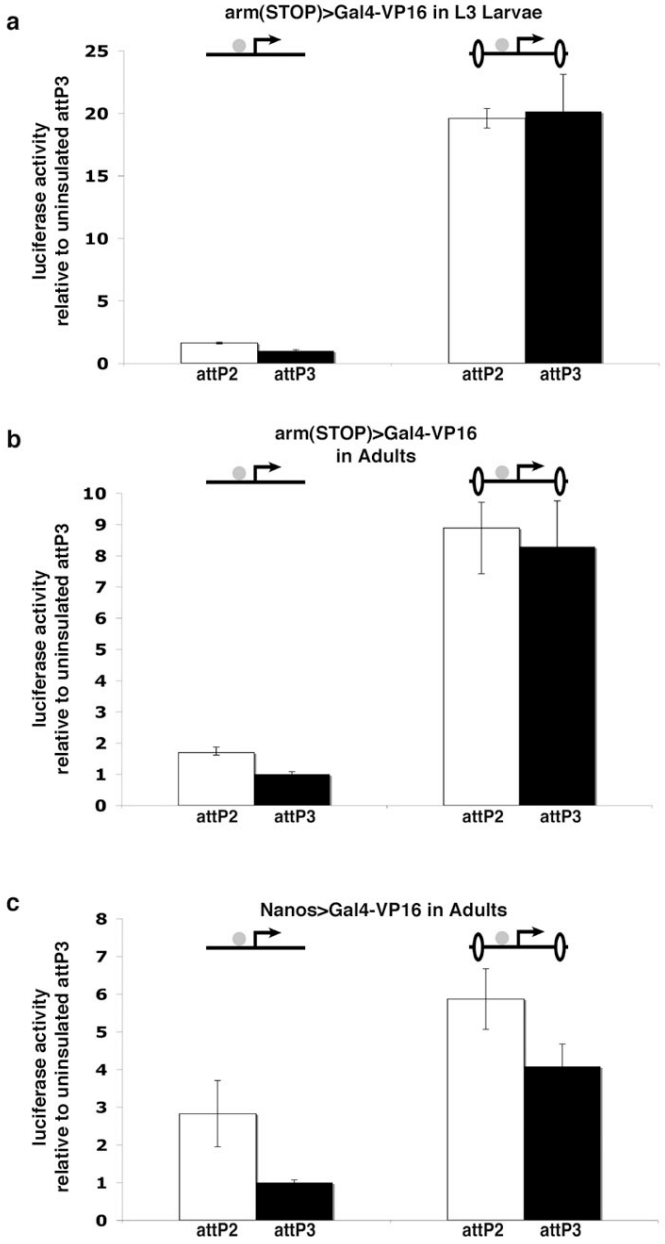


Supplementary Figure 2
Relative differences in inducibility are independent of Gal4 driver strength.



Supplementary Luciferase activity was measured from 5 pools of compound heterozygous female larvae, carrying one copy of the UAS::luciferase transgene and one copy of a muscle-specific driver, either *dMEF2*::Gal4 (left) or *MHC*::Gal4 (right). Luciferase activity was normalized to total protein. Each bar represents the mean and the error bars represent the standard deviation from each of the 5 pools. **(a)** The top panel shows luciferase activity, in arbitrary units (a.u.), obtained with each muscle-specific driver. This data shows that the *dMEF*::Gal4 driver on average induces 130-fold more activity than the *MHC*::Gal4 driver at each tested locus. (b) The lower panel shows the same data, but relative to the activity obtained from attP3 with the dMEF2 driver (left) and with the MHC driver (right). Each bar represents the fold of luciferase activity induced at the specified attP landing site relative to luciferase activity induced from the attP3 site.

Supplementary Figure 3
The boosting effect of the gypsy insulator is observed with the hybrid Gal4-VP16 transcriptional activator.



Levels of luciferase activity were measured from 5 pools of either **(a)** three female larvae each or **(b,c)** three adult females each. Each bar represents the fold of luciferase activity induced at the specified attP landing site relative to luciferase activity induced from the attP3. Bars on the left are from measurement of the un-insulated luciferase transgene, whereas those on the right are from the gypsy-insulated luciferase transgene.
(a,b) Luciferase activity was induced using an *arm* [FRT-STOP-FRT]::Gal4-VP16 driver[1]. This driver was designed to be silent unless crossed to a line carrying the flipase gene, which would remove the FRT-STOP-FRT cassette. However, we found that this line was leaky in the presence of the STOP cassette, resulting in Gal4 expression in a reproducible subset of larval neurons as well in reproducible expression levels in the adult. **(c)** Luciferase activity was induced in adult female ovaries using the *nanos*::Gal4-VP16 driver[2].

References

1. Seugnet, L., Simpson, P. & Haenlin, M. Transcriptional regulation of Notch and Delta: requirement for neuroblast segregation in Drosophila. *Development* **124**, 2015-2025 (1997).
2. Van Doren, M., Williamson, A. L. & Lehmann, R. Regulation of zygotic gene expression in Drosophila primordial germ cells. *Curr Biol* **8**, 243-246 (1998).

Supplementary Data
Genomic DNA sequences flanking each attP docking site, obtained by iPCR with primers to the 5′P and 3′P ends of the attP docking site plasmid, pCARY[1].

>**attP1** 5′P end [insertion position71]
AGTGTTTTATCCCCGCTGTCCGAACGAACCGAAGGAGCAACGGGTACAAAAGC
GCTTAAATTGAGAGGCGAGAG

>**attP2** 5′P end [insertion position 76]
CCGGCAAAAAATTTCAAATGCTGCCTAACACTGGCACTAGAACAAAAGCTTTG
GCGAAAGCTGCGCTGGCAGCTCGTCCATAT

>**attP2** 3′P end [insertion position1]
GTCCATATGATCGTCACTCTCAATTTAGTTTATAAACAACGCTCGCCGGCTGCA
GTTGGAAACGGAGAGCGCATAATCGGCGATATCAATACGTAGGACATAGGAC
CGCCGTCAGCATATGTAATTGCTTATCACAGTCTGGCTGTCCGAAATCGCTCCA
GCGACAACAGATCCCCCAACAACTCGTGCCACCCCCCGCAAATTGCCGGCTTA
CGTAAGCCAATTGTATTCGTTTTAGCTGAGAGAACATCAATCAAAATACAAAT
AACAAAAAACATGCGGCTATTGGCC

>**attP3** 5′P end [insertion position 418]
AACAAAAAAAAAAAAACCGAAACACAGCTGGACAGAACAGAGTGATGACAA
AAAAGATGTCAGTTGGGGATGTCACACACCAGCCGCTGAACAGCTGAACTCTA
AACTATGAACTGCGGGTCGCGTCAAGTTAGGACTTGTCGAGATTTGGCGAGAG
ATTTGCGGGCCACGCACGTATCCGCATCCGCATCCGGTACCCGGTACTGATGT
CAACAAGCTACCACACGCAACTAATTCAAAAAATCCAGCGAACGGATTACGG
CGCCACTGTCCGCATTGCCAGCCGAAGCCGAAGGCGTCCCCAATCGGGTAGCT
CGGCGCTAGCCGAGTCCGTATCCGAAGTAGCCTCGTTGTCCTCTCGTGGCCGCT
CGGCTCAGGCAATCGTTTGACGTCGGTGGCGTTCGGCAGGAACCGGCTGGAG

>**attP3** 3′P end [insertion position 1]
GGGCTGGAGCAGTGGTCCGCAGTCGTTGGTCGCCAGCGGAGTGGTTCGCATGT
GGCCCGGCTGTAAGTGGCACAGGATTATGGATGAGACCCCGCGTCCTGGCGGG
ATTCTCTTTTCGTCCTGCCAGCGGGCATTAACGTGCTGTGCGCTTTATTTAAAA
AAGCAGCTTAACCTGCTTTTGTGACCTTTTCCCTTTTGGCCAACACGTGGCCAA
CGGCTTCACCAGCAATCGCCTCACATTTGTCCACCTCCAACGCCGGCGATGGA
TAGACTGCGTCCTCATCCTGGACCACGCCATGCGTGTTGATAAATGGGTTTTTA
TTTTGATTTTATTCACTGTTTGCCTTGTTTTTGCATATTCATTGACGGGGCTTCG
ACTCCGATTCCGATTGCGATTCGATTCGAATTCCCGTCCGTCCACCTGCTCCTG
CTTCTGCCTCTGCTCTGCTTCTGATC

>**attP04** 5'P end [insertion position 3'P end03'P end]
CCTGGCTTTGCTGCGATTCAACTTCCGACATTGCGATATTGTTTGTTTTTATCGT
TTATTCTCGATATCTTTGATCAGATAGAAGAGTTTTTTCTTTATCCGCGTGACG
AGGTGCGTGCGTGTTCGTTTCCTTTTCGCTTTTGTTGTGCTAATTTCCTTTGCCA
ATACACACACTCACGTACACAAGCTGTAACACACACAGGCAGCACACTCACAC
ACACACAGAGAGACGGAGGGAAGAAGAAGCCGCCTCGCAGTGTTTAAAAAAA
ATATGCCGTTCTGTTTTACGATTTGCGCGCTGCTAAAGTC

>**attP04** 3'P end [insertion position 1]
CTAAAGTCTATTCACGATTGAATTGGTTAGCCGCTGAATAAACCAATCTGCACT
GCGCGAATACACTAACGCCTGCTGTTCAGTGTTACCAGACGGTCCTGCCTTTCG
GCCGG

>**attP04** 5'P end [insertion position 100]
GGCCGATTAAATTTCCCAACCGCGCTTTTCCGCACTCTTTTTCGCGCACGCACC
GAATTTCGCTGGCAAAACGGAATTTAAAATAATAATTATTTTTTCAGCTCGTC

>**attP05** 5'P end [insertion position 43'P end1]
GGCCTATAAATTGATAAAGGGTTTTTTAATTATTAAAGAAGAAGAAAATGTGT
TATATTATCTCGCTGTTAAAAATGTTATCTTTTGATTTGTTCTATTCCATGTTTT
TAGGTATCATATAATATTTTTTTTATTTTTTATATAAAAAAAGCCATACGAATT
GGTCCCACTGTGCGGTTCGCGGTGTTTGTCAGTTAGTCAGTCAGTCGGGTGACT
GTGGCAATCGAAAAGAGTGGACAAAGACTGTGGCGCACAGGGACGGGGGAAT
GGGTTGCTCCGTCCCATGTTGTCCATTCGGTCCCACCGTGCACACAACTCAGCA
CAGCACGGCACAGCAGCAACAAAAGCGCCGCAAAAGGGAACTGGTATTATGG
CATCCCGCTCGCACTTGCACTCCAAGCACGCACACATGCAAGCGCAGACACCT
CGCGACACGAT

>**attP10** 5'P end [insertion position 416]
ACCTCCACAGCTGCACTGCAGTCGCTAGCTTTTGGCGGCGGGGGGCCTGCTTG
CTCACCTCCCCCTCCCTGGATTTGGGTTCCGACTCCTCTTATAGGGGATCTCGA
TAGCCTACCACTCCTCCTAAACGGGTCGTTGGGGTCGGGGGCACTGCCCCCCC
CCTGTTCTGTCCATCAGATATAAAAGGGGGACTTTGGCTTGTTGGTCCCCAGCG
CTCCGTGGGAAATAGAACTAGCTTCAGTTCTTAGGTTATATTTCAAATTTTTGT
TCAACTTAATTAGTTCAAATTTGAATTTGAATTCGAATTTCGCGCCCAACGAGC
GCGCGGCCGAATGACGATCGCACCGCTGATCTGGCAACGCCAGTCTTACCAGC
TCGTCGACTGCGAAAGGTTGGCAATGCTATCAGCTGTATTGTTTGATG

>**attP14** 5'P end [insertion position 160]
GATCTTATAAAAAAATGTGCTAAAACTTTTTGTGTATTTTCTTAATTTTCAATAT
TTTATGCTAGTGAAGTATTTTTGGTTAATATGCAACCACGGTTTTGCTTCTCTTT
TTACTCCAAAACGAGTGCTCGTTTTTGCTCCTCACTCAACTTTATGTCCTTCC

>**attP16** 3'P end [insertion position 1]
CTTCGGCGGCGGCGAAGCAACAACGACGGATTGTTCAGTTTGTCTTGAAATCG
CGACTCGAACGGTCATCGTGTGCGGATGTTAAGCGGGACAGTGGGAAATCGG
AAAAGCGCAGTCAGAAACCGAAACCCAGCTAAAGAAAACTCGTGCAACTCGC
TAGCAGTGCCCACGCAACAAACAAGAATAATAAATGCGAAATATATATGAAA
AACACAGGTTTCAGTTTCAGTGACAAGTGTGCAACATAACTCAGCAGCAGTGA
GGTGAATATACAAATCTCGATC

> **attP16** 3'P end [insertion position 1] (has insert)
CTTGCGGCGGCGGCGAAGCAACAACGACGGATTGTTCAGTTTGTCTTGAAATC
GCGACTCGAACGGTCATCGTGTGCGGATGTTAAGCGGGACAGTGGGAAATCG
GAAAAGCGCAGTCAGAAACCGAAACCCAGCTAAAGAAAACTCGTGCAACTCG
CTAGCAGTGCCCACGCAACAAACAAGAATAATAAATGCGAAATATATATGAA
AAACACAGGTTTCAGTTTCAGTGACAAGTGTGCAACATAACTCAGCAGCAGTG
AGGTGAATATACAAATCTCGATCCCCAAACGACTGTGCAACGCTCTCCAATCC
CCGAATCCAATCAGAATCCCACCTGAGCTGAGCGCTACCTGCCAAGCGTGTTT
CACTTTGCATTACCCGCGCTGACCAGTTGATCCCAACTTGTTTGCAACCCGTTC
TCTTTCGTGCCTCTGACTGCAGAAAAGAGTGCGGATGGCGGAGTGCGGAGTGC
AGCGTGAGGAAGACAAAGGAGTTATTTTTGGCC

>**attP16** 5'P end [insertion position 132] (has insert)
CCGGCTCTCTCCGTCCGCAGTGTGAACAGCCTGGCTGCCGAATATGTATCAAT
GCTATCGATATCCGCATACATCGATACTGCTTCCTTGCACCCTTAATTTGTTTCT
AGGTGTTGGCTGCCAATTGTAGAGTGCAAAT

>**attP18** 5'P end [insertion position 517]
GATCCACCGGTTGAACCACAGACATATTAACACCCACATATCCTTCGCCACCC
ATCCATCTCCCTTTAAAACAGCTTTCATTTGTATATAAATAAATTTTTCACTTTA
TTTTCAAGGCTTGAAGAAAAATCGAAATTCTATTTAAATCCTAATTATAAATAT
AAAAAAAAATCAAGATGCTATCTTTAAAATTGTAATTAATACCAAAATTTCCG
GCTTTTTACCCATAGTGCAACGTACCAGTTTCCTTCAGTATGACCATGTTGCAT
CTAAGCCAAAAAGTTTTGTTTGTCCATAACCATCGATAGCTATCGATAACTAAC
TTAATAAAATAGTTGCATGTATGACAACAACATCAAAGTACGTGGCTTAATTT
CTTTCATTTCTATGCCTTATGGCCAGCTCCTTCGAGTAACATCTACAAAAAAAT
AAGAAAAATTTTCTCCCCGAAACGAAAAACCCTAGTTTTTCCCAACTTTCACTT
ACGACGAAAATCATCGCGGCGAATTGCAGACACGTA

> **attP18** 3'P end [insertion position 1]
GCCTTGGCAGAGTGAGCATTTTGCTCTGCTATTCGTGTCTATCGTATTCGCCGG
CAATTGCGCATTGTGAGACACGGGCATAGTTCCCTCACTTCGGCGTCAACTTTG
GAAGTGTTCGGATC

>**attP22** 3'P end [insertion position 1]
AATAATTGAATTACTCGAAATGTGTGCATGTCCG

>**attP22** 3'P end [insertion position 1]
CATCAGCAGCAACAAGCATTGCAACAACAGCGACGGCGC

>**attP23** 5'P end [insertion position 27]
ATGTGGAATATGGAATATGGGTGTACGGGC

>attP23 5'P end [insertion position 25]
CCTATTTTGTGGAATATTGTTTGTCGTT

>**attP23** 3'P end [insertion position 1]
GTCTGTGCCTAATAGGCAGCCGAGAGAGAGCCGGCTTAAGAGAGTACCGAAG
AGAGCGGCTCGAAGGTGCAAGCGGATC

>**attP24** 5'P end [insertion position 422] ==> chromosomal position 42A1
GGCCAAATATCTTTTTGTTACACCCACTCTAACTTCGTTTAATTGTTTGCCTTGC
CAAATTTTATCGGTATACCAAAAACACTGGTCACGCCTCTCCCCCCCACATTTTC
CTAACGGTCCACTTTTTTTAAACAATTTTTAAAATTTTTCTTTCTATCTATCGATA
TCCCAGAAAAATTATGAAATTTTGCGTTCGCATTCCCACTAGCTGAGTAACGG
GTATTTGATAGTCGGGGAACTTGACTAAGCATTCTCTTTTGTTTTTCAAATACA
GAGGTGGTCAAAAGTATTTACACAACGAACTATTTTTCAATTAGTTGACAATTC
TTGTCAATTAAATTTCATAATTTTTCTGGATATTTTCTGTCGTGGAGCGAAGAG
ACCGCCTCTCCTACCCCGCTCCTCTCGCAGCGCCAAACGACCGTCCCGGG

>**attP24** 3'P end [insertion position 1] ==> chromosomal position 58C
GGAGAGACTCGTTCTCATCTCTCGTTGAGCGACGCGAGCGAGAGAGGCAA
ACTATTTAAGCCACATCGCAAGGCAACTCGTGTTCAATCTTTGTTTTCGT
AGCGCGGCGGTCGCATCGGAGTCGAGAACTCGAAGTGAAGGTGCACGAAG
AATTCGATAAATACAATTAAATAGTTTTGAATATATTGCGGTGATTTTTG
GATTTGCATACCGG

>**attP29** 5'P end [insertion position 107]
GATCGTTTAGGGAATCAAAAATTTTTATCGGGAATGCGAAACTGACGTAATTC
CCCTTCCCCTCTTCTAAGCTTTCCTTTCCTTTTCTTTACGCAATTTTTGGCTTGAC
C

>**attP30** 3'P end [insertion position 1]
GGGTCGTACAAAGACATATCGGGACGTTCTCAACGAGCTCATAAAATTTTACG
GTTCTTGCTGATGATTTTATGCGTTTTGTGCCTCATTATATTTCGTACATATGCG
TGTGATGCCCGATTGTGCGGCGGTTTTGATAGTTTTTGAATGGCAGGCCAAGA
GGAAGAAGATATTTTCAAGTGCGTCTAATTTTACGATC

>**attP30** 3'P end [insertion position 1]
CCACCACCTCTCCAAATATCCGCGCTGTTCGGAACTACCGTTCAGTTTGCGAGC
GTTGGTCGTCAGCAGCGGACGTGCGTCGTGGCGATTTGCAGATTTATATATAC
GTACATTTGGTGGAAAATCGACTTTCGCAACGGAGCGTGCGGCGTGCGTTAAA
GTCTCCGGCATTTCCCCACTAAGTTTTCCTCAACTGGGCGGCTTAGCTTGGCC

>**attP40** 5'P end [insertion position 101]
CCGGTTCGATACTAAACCCAAACCCACTGGCGTTCAATGTGCTACGCCTTGTCC
AGTCCAAAAAGTCGCGAGAGAAGAGCTGTATAGTAATCGTAGTAAAGCTCAG
AG

>**attP40** 3'P end [insertion position 1]
GCTCAGAGTCTGTGCTACCCAATGTGTGTTGGTATTTCGCTTGAAAGACAAGA
GAGAGAGCGAGAGTCTTGGAGCGATGTGGTCGAAAAACCGAGAGAGTATCAG
AGACTCAAAACGGGAGTATACGCAATATTTCGTACCACTTTTGATGGATGTTG
TTTACCACGTACTCTCCGCTTTTCGACGACTGCTTTTGTTGTTGTTTGGGGTCAG
TGGCGCAGTAGCCGGCTATTTTTAGGGGGTGAGGTATTTCTTGGAGAAATATTT
GGGGCTCCACCTAAATGGAACACAATTTTACAAGGTCAACGTTTCCCTAGACT
TTTTCAGAGTTTAAGTACTCCATTGATCTTTCAACTTATAATCAAATATCAGTC
ATACTCCAATAAAAACTTTAATTTGAACAAATATTTCCTATTTTATGCATTTTTC
TTCACTTTAAAAACCACCAGCAATAAAAATCATATACGACG

>**attP52** 5'P end [insertion position 247]
TCTTGCGGCGAGGGGTGCCCTTGCCGCCAATGCGCACCTGCGCCTGCAACTTCT
TCAGCTTTTCCGCGTTCATCTGTTCAATTGAGTACACACAATGGTCGCATCAGT
AAAACGCCAATCGAATGCCGCACTTTCCACTACTCACTTTGATTTTACTAGAAT
TTAGCACAGAGCCTTGCCAGAAAACACGTGCGCCCAGTCGTAGTCAAATGTCA
AAAAGAAATCGCCATGATAAAAATGGCAAAAGGTGTATG

>**attP52** 5'P end [insertion position 276]
TTTTCAACCCCCAATTTTTTTTTTTTTTTTCCCGCCCTTTTTTTTTTTTTTCAACT
TTTGACACACACACACACGCAGCAAAGCGAATAAAAAACGTGGCTGCACTGC
ATTGAAATTTATTTCAAAAGGAAACTGGAGCAAACACAATTTATTCAACGTCA
CAGCGATAAAGCGATTAACGCGTATAACACACACCACAAGTTCTCACAAGTAT
ATGTACTGCACACCCGCAGCGCAGATTTGCGAGAGAGCTGCGATTGCAAAAAC
AATGATACGGCGGAG

>**attP52** 3'P end [insertion position 1]
GCGGCGGAGGAGGAGACGGAATGACAACTGCCGCGCCAAGCGATCGAAAATC
AAGTGAGCTCCGGCGCAAAGAACACACGATGCCGAAGCCAGCGGAAGAGAAG
GAGAGAGCGCAAAATTAAACCCACACGTAGAAAAATTTTAGTAGATCATGAA
GATCAATTTAGGATAACATTCTCAGTAACAAACTAAATTCTTTCTTTTATTTTG
TAATCGGTTCTATGAAATGAAAATGTACATTTTAATTTGAAAGTATACTGGTTT
ATGATTAGTACCTTGACTAGGCTGAATTTTTCTCTGTGTAAGAAAAAGAGAGA
TACAAAGCTTATGAGATTGAGAGAGCGGACTAAAACACTTGTGAGCGTGTGAA
AATGCGGCAATTGAGCAGTTTGTATTTGTATGTGATTATTGTTCATGCCGCTGC
TGGTTGATGTTGTTGTTCCTGTTCTTGTCCCTGTACAGAGAGAAAAGCAACAAG
AACATCTAGCTGCGAGAGCGAGCGACAAGCTGATAATGACGTGCAACAGAGA
CAGATAGCGGGTCATGCGTCAGAGGCC

>**attP64** 3'P end
ACTGCCAGTGTGCGGAGCGAACCAGTCAGCCAACATCTGAGATACAGATACG
ACCGCGC

>**attP83** 5'P end [insertion position 478]
TCAGTTTTGACTTTGTCGCCTACGGTAGACGCAAAGACACTAGAATATTCTAGT
GTCTTTGGTAGACGCCAGTAAACTGTTCATCTTCAAATTACGAAAAATGTGTAC
ATGCAAGTTTTCGTTTTTTTGCCTATCACGTTGCGTCAAATTAATAACTGCGTA
TTCCCTCTTTGCACTATATCTCTGGTCGTTTTCGACAAAATCGAAACCAGTAAA
TTCCCGTACAATAAAGCGGAAGCTCAGCCCTAAAAATTCCGATACAGAAAACA
AGCCGAAAAATATACGCGATAATACTACCTTGATCAACAATGAAGCGCCCGTA
AATAACAACAGTTTCGCTTTGCTGGCAGCCAAGACACTAGAATAACAAGATGC
GTAACGCCATACGATTTTTTGGCACACGATTTTTTCGCCGTGGCTCTAGATGTG
GCTCCAAGCTCTCTCGAATTTTTGTTAGAGAGCGAGAGAGCAAAGAGCGCTAC
AGC

>**attP83** 3'P end [insertion position 1]
GGCTACAGCGAACAGCTCTTTTCAACGCACAAAGTGATAGCAGACATCTGTAT
GTGTGCACACGTATTCACATGCATTGTAAATTTGACAAAATATGCCCTTCACCT
TAGAAGTTCTTTGGCC

>**attP88** 5'P end [insertion position 46]
AGCACTGGGCCGCGCTCCAGGTATTCGGACACCACATCTCCGAGAGCTCCACT

>**attP112** 3'P end [insertion position 1]
CGCACATCTCTACGGTTTTCGACCACTTGTTTGACTGCTTTGGCTTTAGCCCTCT
CTCTTCTACGCTCTCTCGAATCAGCTGAGCGCTCATGTTTTGATGCTC

>**attP112** 5'P end [insertion position 262]
CCCCCCCCTGTTCTGTCCATCAGATATAAAAGGGGGACTTTGGCTTGTTGGTCC
CCAGCGCTCCGTGGGAAATAGAACTAGCTTCAGTTCTTAGGTTATATTTCAAAT
TTTTGTTCAACTTAATTAGTTCAAATTTGAATTTGAATTTGAATTTCGCGCCCA
ACGAGCGCGCGGCCGAATGACGATCGCACCGCTGATCTGGCAACGCCAGTCTT
ACCAGCTCGTCGACTGCGAAAGGTTGGCAATGCTATCAGCTGTATTGTCTGAT
G

>**attP154** 5'P end [insertion position 143]
GGCCCACAACACACGAACGCGGGACTCTGGCTGGGAAAATCAAAAATCCAAC
TGCTACAAGCGCGACAAGCGTGCGTTCACGTCGGCGATTCGGAACAAACTGAA
CTCGACTCGAAAAGAAAGAAGAACTGAGAGATGAAGAGCTGGTAG

References

1. Groth, A. C., Fish, M., Nusse, R. & Calos, M. P. Construction of transgenic Drosophila by using the site-specific integrase from phage phiC31. *Genetics* **166**, 1775-1782 (2004).