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Versatile P[acman] BAC libraries for transgenesis studies in *Drosophila melanogaster*

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Supplementary figures and text:

Supplementary Figure 1

Supplementary Figure 1. The em7-lacZ Marker Gene. Sequence of the *attB*-P(acman)-Cm^R-BW vector in the region of the cloning site and the *em7-lacZ* marker gene, indicated in blue text. The *em7* promoter is indicated in lower case text, and the truncated *lacZ* coding sequence is indicated in upper case text. The premature stop codon in the *lacZ* allele, the sequences of primers Pac-BW-F and Pac-BW-R and the *Bam*HI cloning site are underlined.

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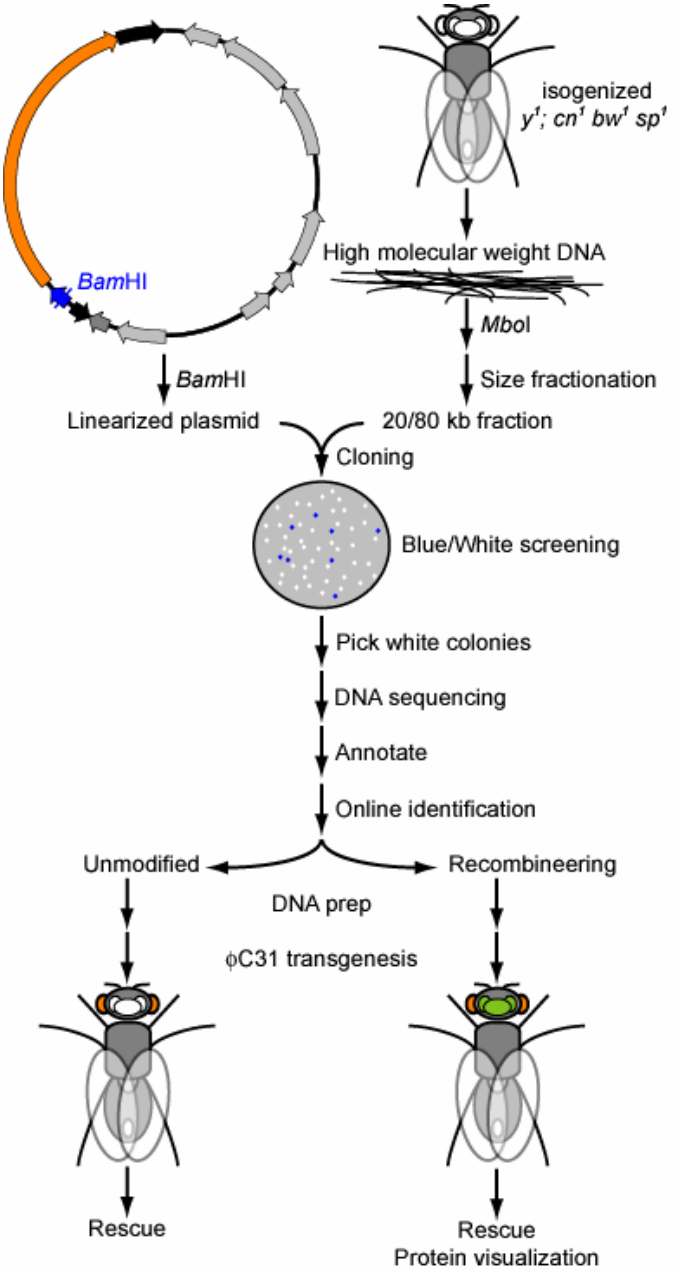
Supplementary Figure 2



Supplementary Figure 2. Overview of the P(acman) BAC System. High molecular weight DNA, isolated from the isogenized *y*^{1}*; cn*^{1} *bw*^{1} *sp*^{1} strain, was partially digested with *Mbo*I and ligated into the *Bam*HI site of the *attB*-P(acman)-Cm^{R}-BW plasmid. White recombinant clones

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were identified after blue/white screening on LB plates using X-Gal. Clones were end-sequenced with primers Pac-BW-F and -R (A), and the sequences were mapped to the reference genome. Clones containing genes of interest are identified in an online genome browser www.pacmanfly.org and obtained from the BACPAC Resources Center. Clones can be modified through recombineering (**Supplementary Fig. 3**), for example by incorporating protein tags such as EGFP. Both unmodified and modified constructs are integrated into the *D. melanogaster* germ line using ΦC31 integrase, and transgenic flies are identified as *white*$^{+}$ (orange eyes) flies. Transgenic flies, containing untagged or tagged constructs, can be tested in mutant rescue experiments (**Tables 1**, **2** and **Supplementary Table 2**). In addition, tagged clones can be used for *in vivo* protein localization using EGFP (green) (**Fig. 2**) or other tags, and in other experiments requiring engineered protein or peptide tags.

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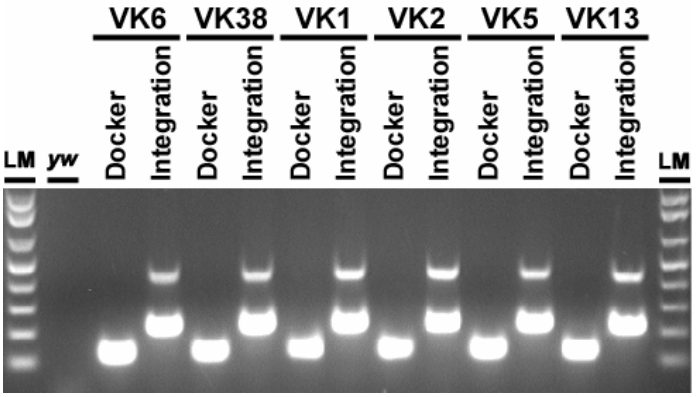
Supplementary Figure 3



Supplementary Figure 3. Verification of ΦC31-Mediated Site-Specific Integration by Multiplex PCR. Integration events were identified by loss of the *attP* (134 bp) PCR product specific for the docking site (Docker), and the appearance of both *attL* (227 bp) and *attR* (454 bp) PCR products specific for the integration event (Integration), as illustrated for six different VK docking sites: VK6 and VK38 on the X chromosome, VK1 and VK2 on chromosome 2, and VK5 and VK13 on chromosome 3. A fly strain containing no docking sites (*y w*) is the negative control. LM, molecular weight marker.

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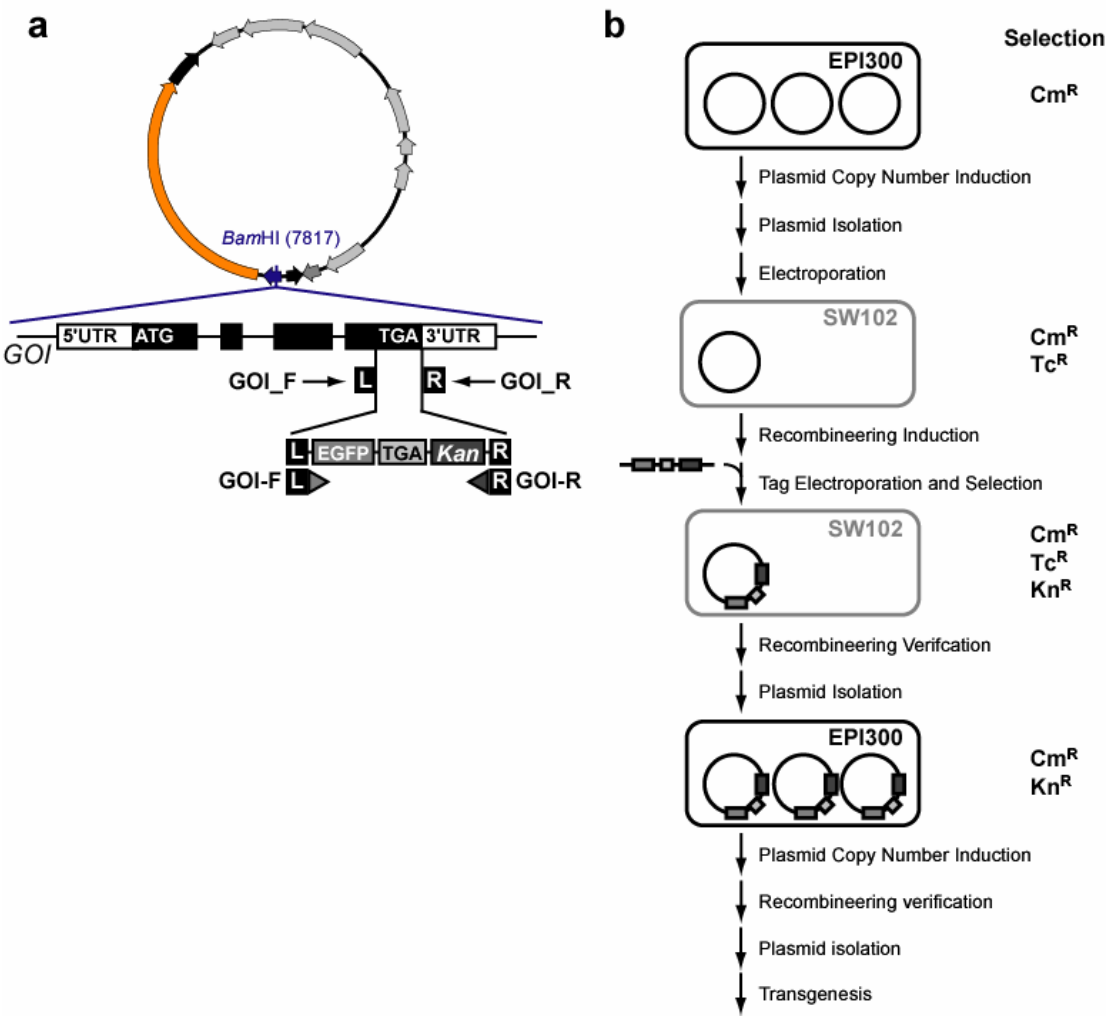
Supplementary Figure 4



Supplementary Figure 4. Overview of the Recombineering Strategy. (**a**) Schematic of a P(acman) BAC clone containing a gene of interest (GOI) and the recombineering strategy. The 5' untranslated region (UTR), start codon (ATG), stop codon (TGA) and 3'UTR are indicated. Exons are illustrated as boxes: UTRs and coding exons are indicated in white and black, respectively. The tag, consisting of EGFP followed by a stop codon and a kanamycin resistance marker (*Kan*), is amplified by PCR using recombineering primers GOI-F and GOI-R

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(**Supplementary Table 5**). Recombineering homology regions are indicated on the left (L) and right (R). Recombineering replaces the endogenous stop codon; a novel stop codon is introduced downstream of the EGFP tag. Verification of correct recombineering is performed using primers GOI_F and GOI_R (**Supplementary Table 6**). (**b**) Flow diagram of the procedure. The plasmid copy number of the P(acman) BAC (Cm^R) is induced in EPI300 cells. Plasmid is isolated and electroporated into the tetracycline resistant (Tc^R) SW102 strain. Recombineering functions are induced, and the strain is electroporated with PCR product encompassing EGFP, kanamycin resistance (Kn^R) and homology arms (A). Potential recombinants are selected (Cm^R, Tc^R, Kn^R) and verified. The recombinant construct is isolated, transformed into EPI300 and selected (Cm^R, Kn^R). The plasmid copy number is induced, the recombination event is verified, and plasmid is isolated for transformation into the fly genome.

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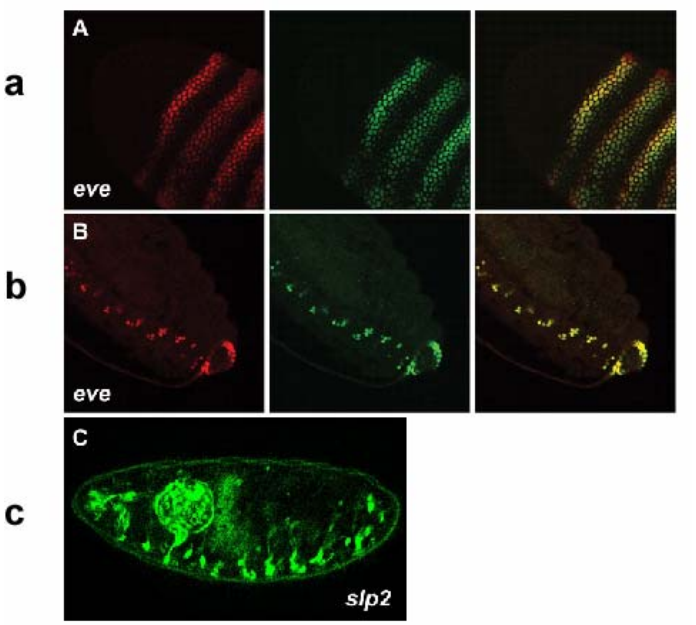
Supplementary Figure 5



Supplementary Figure 5. Embryonic Expression of EGFP Fusion Proteins in Transgenic Flies. (**a, b**) Double labeling with anti-Eve (red) and anti-GFP (green) antibodies confirms proper expression of the transgene at embryonic stage 5 (**a**) and embryonic stage 15 (**b**). (**c**) EGFP fluorescence in a live stage 17 embryo bearing the *sloppy paired 2* fusion construct. Compare to **Fig. 2i**.

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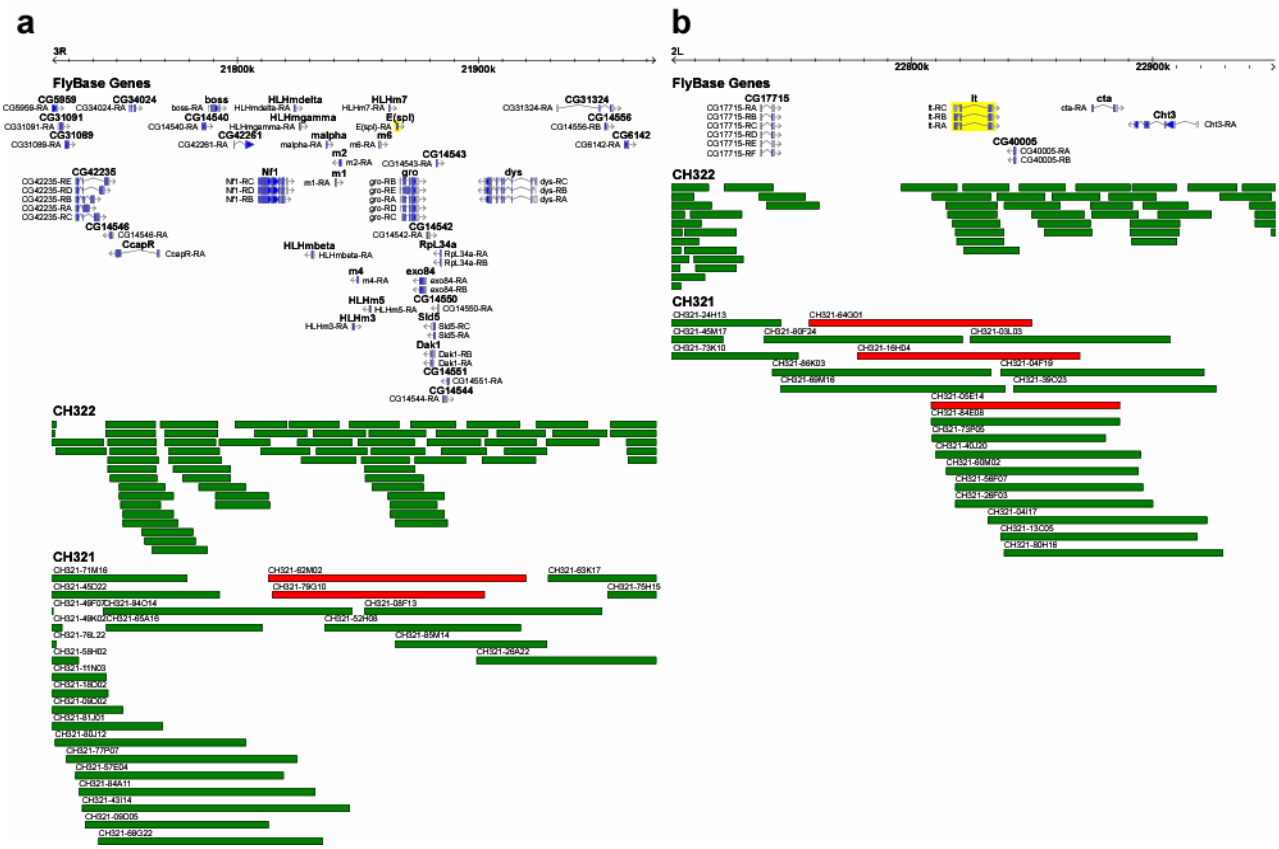
Supplementary Figure 6



Supplementary Figure 6. P(acman) BAC Coverage of a Gene Complex and a Heterochromatic Region. Mapped CHORI-321 and CHORI-322 P(acman) BAC clones (green) are indicated below the FlyBase R5.9 gene annotation. Note that the clone names for the CHORI-322 library are not indicated due to the large size of both genomic regions. (**a**) A 250-kb region surrounding the *E(Spl)* complex on chromosome 3R at polytene division 96F10. Two clones, CH321-62M02 and CH321-79G10, that encompass the entire gene complex are indicated in red. (**b**) A 250-kb region surrounding the *lt* gene (yellow) on chromosome arm 2L at polytene division 40B in heterochromatin. CH321-64G01, CH321-16H04 and CH321-05E14, selected for

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transformation rescue experiments, are indicated in red. CH321-64G01 complemented the lethality of a *lt* mutant; CH321-16H04 and CH321-05E14 did not.

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Supplementary Table 1

Library	Genes Spanned			Genome Coverage	
	+/- 0 kb	+/- 2 kb	+/- 5 kb	X	A
CHORI-321	14,410 (99.3%)	14,362 (98.9%)	14,270 (98.3%)	8.2X	9.3X
CHORI-322	12,898 (88.9%)	12,121 (83.5%)	9,750 (67.2%)	4.3X	5.9X

Supplementary Table 1. P(acman) BAC Coverage of Genes and the Genome. The number and percentage of FlyBase R5.9 gene models spanned by P(acman) clones in the two BAC libraries, including 0 kb, 2 kb and 5 kb of additional sequence at the 5' and 3' ends of each model to account for unannotated transcribed and regulatory sequences, is shown. The redundancy of coverage of the genomic sequences of the X chromosome (X) and the autosomes (A) is shown.

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Supplementary Table 2

Inj	Gene	Clone	Insert	VK#	G0	Tr	%
1-U	***bcd***	*100D18*	18,452	37	47	8	17.0%
1-T					22	2	9.1%
2-U	***hb***	*05M03*	24,093	37	56	0	0.0%
2-T					76	0	0.0%
3-U	***en***	*92I14*	21,093	33	34	9	26.5%
3-T					100	16	16.0%
4-U	***gt***	*05H16*	24,464	33	31	1	3.2%
5-U	***eve***	*103K22*	21,174	33	118	2	1.7%
5-T					35	1	2.9%
6-U	***run***	*115B21*	19,432	33	80	9	11.3%
6-T					21	0	0.0%
7-U	***cad***	*115J21*	21,274	33	59	4	6.8%
7-T					39	6	15.4%
8-U	***tll***	*83J21*	23,996	37	31	1	3.2%
8-T					49	2	4.1%
9-U	***slp2***	*127L06*	21,598	33	57	16	28.1%
9-T					54	3	5.6%
10-U	***Dfd***	*75M21*	20,767	37	56	2	3.6%
10-T					54	1	1.9%
11-U	***Chi***	*91I05*	18,227	33	34	1	2.9%
11-T					114	1	0.9%
12-U	***Bro***	*16P24*	26,146	37	37	9	24.3%
12-T					30	1	3.3%
13-U	***exd***	*35A16*	22,572	33	108	2	1.9%
13-T					90	1	1.1%
14-U	***D***	*169N20*	22,404	37	43	1	2.3%
14-T					52	1	1.9%
15-U	***h***	*135D17*	18,449	37	19	2	10.5%
15-T					20	2	10.0%
16-U	***ems***	*143M02*	19,969	37	46	2	4.3%
16-T					61	5	8.2%
17-U	***kni***	*25A12*	23,149	37	31	4	12.9%
17-T					71	0	0.0%

Supplementary Table 2. Tagging and Transformation of CHORI-322 Clones. Genes spanned by 17 CHORI-322 clones were tagged by recombineering; untagged (U) and tagged (T) constructs are indicated. For each clone, the genomic insert length in bp (Insert), *attP* VK

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docking site used (VK#), number of fertile G0 crosses (G0), number of vials resulting in at least one transgenic animal (Tr) and integration efficiency (%) are indicated. Note that only the gene of interest is indicated; most clones contain more than one gene. Construct 4-T resulted in an aberrant supercoiled plasmid migration pattern after gel electrophoreses and was not injected.

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Supplementary Table 3

Primer name	Sequence
BW-rpsL-Neo-F	AGTTTTATTTTTAATAATTTGCGAGTACGCAACCGGTGGCCTGGTGATGATGGCGGGATC
BW-rpsL-Neo-R	AAAAATGGGTTTTATTAACTTACATACATACTAGAATTCTCAGAAGAACTCGTCAAGAAG
BW-em7-LacZ-F	TAAAGTTTTATTTTTAATAATTTGCGAGTACGCAACCGGTCCTGTTGACAATTAATCATC
em7-LacZ-R	CTTGGCGTAATCATGGTCATGATCTGCCTCCTGGGTTTAG
em7-LacZ-F	CTAAACCCAGGAGGCAGATCATGACCATGATTACGCCAAG
BW-em7-LacZ-R	GCAAAAATGGGTTTTATTAACTTACATACATACTAGAATTCCTATGCGGCATCAGAGCAG
Pac-BW-F	ATCGGCATAGTATATCGGCATAG
Pac-BW-R	GATGTGCTGCAAGGCGATTAAGT
attP-F	AGGTCAGAAGCGGTTTTCGGGAGTAGTG
attP-R	GGTCGTAAGCACCCGCGTACGTGTCCAC
P(acman)-F	ACGCCTGGTTGCTACGCCTGAATAAGTG
P(acman)-R	CCCACGGACATGCTAAGGGTTAATCAAC

Supplementary Table 3. Primers for Cloning and Sequencing (see Online Methods).

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Supplementary Table 4

Primer name	Sequence
bicoid-F	ATCCCCATCGGAACGCCGCGGGCAACTCGCAGTTTGCCTACTGCTTCAATGATTATGATATTCCAACTACTG
bicoid-R	AGTGGTTAACCTAAAGCTAATGAAACTCTCTAACACGCCTCTCATCCAGGTCAGAAGAACTCGTCAAGAAG
hunchback-F	GCGACGGACCCGTCGGCCTCTTCGTTCACATGGCCAGGAATGCTCACTCCGATTATGATATTCCAACTACTG
hunchback-R	ATATGATAATAGTGATAAATAATAATAACAAGGTGATGGTGATGGGGAACTCAGAAGAACTCGTCAAGAAG
engrailed-F	TGACCAAGGAGGAGGAGGAGCTCGAGATGCGCATGAACGGGCAGATCCCCGATTATGATATTCCAACTACTG
engrailed-R	ACGCCCCCGTCAGAAGGAGTAACCCCTTATGGGTAACCATTGGTCAGCGCTCAGAAGAACTCGTCAAGAAG
giant-F	CCCTCAAGGTCCAGCTGGCCGCCTTCACCTCCGCCAAAGTAACCACCGCCGATTATGATATTCCAACTACTG
giant-R	ACATACGATTCGGATCCTCGCGTTCAACGCATCAAGAGAGGAGTGGACCTTCAGAAGAACTCGTCAAGAAG
eve-F	TGATTGCGGAGCCCAAGCCGAAGCTCTTCAAGCCCTACAAGACTGAGGCGGATTATGATATTCCAACTACTG
eve-R	CTTTTGGGGGAGCATGGGGGGGGGGGAGAGAGTGTGTGTGGATCGCGGGCTCAGAAGAACTCGTCAAGAAG
runt-F	CCAAGATCAAGAGCGCCGCCGTGCAGCAGAAGACCGTGTGGCGGCCCTACGATTATGATATTCCAACTACTG
runt-R	TATATCACTTTGTTTTCTTCATTCCTCCAGATTTTTGGGGATCAGATGCCTCAGAAGAACTCGTCAAGAAG
caudal-F	AGCACAGTGCGCAGATGTCCGCTGCGGCGGCAGTGGGCACGCTCTCGATGGATTATGATATTCCAACTACTG
caudal-R	TCCATGTAGTTGTTACTGTCGCCGCTCGCCGCATAACAGGAATGGTCGTGTCAGAAGAACTCGTCAAGAAG
tailless-F	ACATCACCATTGTGCGCCTCATCTCCGACATGTACAGTCAGCGCAAGATCGATTATGATATTCCAACTACTG
tailless-R	ACTTGGAAGGCACTTCGAGTGCGGCGATTAGTCTAGGCTCTACATACTTTTCAGAAGAACTCGTCAAGAAG
slp2-F	CCTCGCCACAGCCTCTTCACAAACCCGTCACCGTAGTCTCCCGCAATAGCGATTATGATATTCCAACTACTG
slp2-R	TATGCTGCTTCTCAAGCCAAAGAATCCTCCGATTCTTCTCGCCAGAATCTTCAGAAGAACTCGTCAAGAAG
Deformed-F	TGACCAATCTTCAGCTACACATCAAGCAGGACTACGATCTGACGGCCCTGGATTATGATATTCCAACTACTG
Deformed-R	GGTATTTAATTTACAATGCTGAAGTCATCTTGAAGATATCCCTGCTGATTTCAGAAGAACTCGTCAAGAAG
Chip-F	ACAACGCGAATATATCTGACATAGATAAAAAGAGCCCCATTGTATCGCAAGATTATGATATTCCAACTACTG
Chip-R	GTATGAAAATACATTTGACAATATAGCGAAATATTATGTTTTATTAAGTTTCAGAAGAACTCGTCAAGAAG
Brother-F	ATCACCATCACAGAGGTGGGCCTGGTCTGCCTAGAGGACCAATGGGATGGGATTATGATATTCCAACTACTG
Brother-R	TTGTTGATTTACTTAGATGTTACTTAAAGCTATGAGGGTAATATATAGCATCAGAAGAACTCGTCAAGAAG
extradenticle-F	ACAACAGCATGGGCGGCTACGACCCAAATCTCCATCAGGATCTAAGCCCCGATTATGATATTCCAACTACTG
extradenticle-R	CAACTGTATGAGGGATTCTCCGGACTGGGAGTGGTTCACAGCCCTGATCCTCAGAAGAACTCGTCAAGAAG
Dichaete-F	TGGATGGTTCCATGGACAGTGCCCTGAGGCGACCGGTTCCGGTGCTCTATGATTATGATATTCCAACTACTG
Dichaete-R	AATGCGTCAACAATTGTACTCTACTCTGTACTCTAACCTAAAACTCGACTTCAGAAGAACTCGTCAAGAAG
hairy-F	TGGTGATCAAGAAGCAGATCAAGGAGGAGGAGCAGCCCTGGCGGCCCTGGGATTATGATATTCCAACTACTG
hairy-R	AGATTCGATAGGGGTGGCTATGCTATATGATATGCATATGCAGACACCCTTCAGAAGAACTCGTCAAGAAG
ems-F	AGATGGACGAGTGTCCCAGCGATGAGGAGCACGAGCTGGACGCCAGCCACGATTATGATATTCCAACTACTG
ems-R	AGGATAAGGAACTCACGGACCGCCGCAGGACTATCTCCTGGCCGCTTCTCTCAGAAGAACTCGTCAAGAAG
knirps-F	CGGTGGCCCACAATGCGGCTAGTGCCATGAGGGGAATATTCGTGTGTGTCGATTATGATATTCCAACTACTG
knirps-R	AACGAGGGTTTTTGGGGCGACTCCTCCCACTTGGTTTTTTCGCCGTGTACTCAGAAGAACTCGTCAAGAAG

Supplementary Table 4. Primers for Recombineering (see Online Methods).

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Supplementary Table 5

Primer name	Sequence
bicoid_F	TCCCTGGGAACCATTTACAC
bicoid_R	GGCGAATCGAACCAATATCA
hunchback_F	AGCGGCTTAATTGGCTTATG
hunchback_R	CCGTGCTCTACACCATTCAC
engrailed_F	TCGCTACGGATGGGTCTTAC
engrailed_R	CGGGCCAAGATCAAGAAGT
giant_F	AAGAAAGTTGAGCCCCCTAAA
giant_R	CGCATCAAGGAGGATGAGAT
even-skipped_F	CTCCACTGACCACCACCAG
even-skipped_R	CAATCTGTACAATCTTCGGGAAT
runt_F	CTACCACGAGAGCGGTCCT
runt_R	CGCTGCCGTTTATCCTTCTA
caudal_F	ATGGCGGCGATGAACATT
caudal_R	GGGAGCACTGAAGGACACAT
tailless_F	TGATGCACAAGGTCTCAAGC
tailless_R	GGCTCGACTCCTGGATATGA
sloppy-paired-2_F	ACCCATCACCACCCACAC
sloppy-paired-2_R	AAGCGTCTAGCGCCTCAGT
Deformed_F	CAATCAAATGGGTCACACGA
Deformed_R	TGCCTGAAGTTCAAGGTCATT
Chip_F	AAGAACAGAAGTATCCTACGGTTTAAT
Chip_R	CTCGAATCCGTGGAGCAT
Brother_F	TGGCTGAGTCCAGGAGAATC
Brother_R	GGAAACTCCTTGTGAAATGAGA
extradenticle_F	CACAGGATTCCATGGGCTAT
extradenticle_R	TCTTGGCGAAGAAGCAAATA
Deformed_F	ATCTGAAGTGCGCGAAGATT
Deformed_R	ACTCTACCCATCCTCGTCCA
hairy_F	ACATCAAGCCATCGGTCATC
hairy_R	GGCGAATGATGTGAAATGTCT
empty-spiracles_F	ATGCAGCAGGAGGATGAGAA
empty-spiracles_R	CCTTGGGATCGCTCTAAGAA

Supplementary Table 5. Primers for PCR and Sequencing. Primers used to verify the corresponding P(acman) BAC by PCR and to sequence the tag junctions following recombineering.

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SUPPLEMENTARY NOTE

Preparation of P(acman) BAC DNA for Microinjection

Selected clones from the library plates were re-streaked on LB plates (12.5 μg/ml Chl), and single colonies were used to produce working glycerol stocks. An aliquot of primary culture was used to innoculate a secondary culture, induce high plasmid copy number, and perform paired end sequencing, as described above.

Two plasmid DNA preparation methods were used; these can be substituted. For CHORI-322 clones, cultures were induced to high plasmid copy number with CopyControl™ Fosmid Autoinduction Solution (Epicentre) in LB (12.5 μg/ml Chl) for 17 hrs at 37°C. Plasmid was isolated using the PureLink™ HiPure Plasmid Kit (Invitrogen) and rehydrated in EB Buffer (Qiagen; 10 mM Tris-Cl, pH 8.5). DNA solutions for microinjection were prepared (100 ng/μl per 10 kb of plasmid length) and stored at 4°C.

For CHORI-321 clones, DNA was prepared using a "double acetate precipitation" procedure followed by CsCl gradient density centrifugation, as described [1] with modifications. A single colony was grown in 3 ml LB (12.5 μg/ml Chl) at 37°C for 8 hrs. A 200 μl aliquot of this starter culture was transferred to 200 ml LB (12.5 μg/ml Chl) with CopyControl™ BAC Autoinduction Solution (Epicentre) and grown for 17 hrs at 37°C. The bacterial pellet was collected and stored at -80°C for at least 1 hr. The pellet was resuspended in 7 ml of 10 mM EDTA (pH 8.0), 14 ml of alkaline lysis solution (0.2N NaOH, 1% SDS) was added, and the sample was incubated at room temperature for 5 min. The sample was neutralized with 10.5 ml of 2M KOAc and incubated on an ice 15 min. The sample was centrifuged, and the supernatant additionally cleared with a 70 μm nylon cell strainer (Becton Dickinson). The supernatant was

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precipitated with 0.6 volumes isopropanol and centrifugation. The pellet was dissolved in 3.6 ml 10:50 TE. After addition of 1.8 ml of 7.5M KOAc, the sample was incubated at -80°C for 30 min. The solution was thawed and centrifuged. The supernatant was precipitated with 2.5 volumes EtOH and centrifugation. The pellet was dissolved in 2.93 ml TE, and 3.4g CsCl and 66.7 μl EtBr (10 mg/ml) were added. The sample was loaded into a 3.5 ml Quick-Seal tube (Beckman) and banded by ultracentrifugation. The supercoiled DNA was isolated, and EtBr was extracted with NaCl-saturated butanol. Finally, the DNA sample was dialyzed using a 3,500 MWCO Slide-a-Lyser dialysis cassette (Pierce) against Injection Buffer (10 mM Tris-HCl pH8.0) at room temperature. O.D. measurements and agarose gel electrophoresis were performed to assess the yield and supercoiled quality of the DNA preparation, respectively. DNA samples were stored at 4°C.

Genetic Complementation Testing

We used the following lethal mutations for complementation experiments: *Hip14*^{1} *FRT80B* [2]; *FRT82B v100*^{1} and *FRT82B v100*^{4} [3]; *n-syb*$^{\Delta F33B}$ (gift from T. Schwarz) [4]; *y*$^{+}$ *Drp1*^{2} *FRT40A* [5]; *w Chc*^{1} (BDSC #4166) and *w Chc*^{4ts} (BDSC #4167) [6]; *y w cv sqh*^{AX3} *cn* and *y w sqh*^{1} *sn FRT101* (gifts from R. Karess) [7]; *eps15*$^{\Delta 29}$ [8]; *dap160*$^{\Delta 1}$ and *dap160*$^{\Delta 2}$ [9]; *FRT82B endo*^{1} [10]; *FRT42D synj*^{1} [11]; *P{GawB}elav*^{C155} *cac*^{HC129} (gift from R. Ordway) [12]; *w*^{1} *shakB*^{15} (BDSC #8132) [13], *shakB*^{20} (BDSC #7478) [14,15] and *y*^{1} *w*^{a} *shakB*^{25} (BDSC #4769) [14]; *FRT42D Dscam*^{20}, *FRT42D Dscam*^{21} and *FRT42D Dscam*^{23} (gifts from D. Schmucker and L. Zipursky [16]; and *lt*^{11} and *Df(2L)C'* (gifts from B. Wakimoto) [17].

All mutations located on the second chromosome and P(acman) clones integrated in the VK37 docking site, indicated by X2, were crossed to the double balancer line *y w/Dp(1;Y)y*$^{+}$*;*

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noc^{Sco}*/CyO; D/TM6B,Tb, Hu, w*$^{+}$ (derived from BDSC #3703). All mutations located on the third chromosome and P(acman) clones integrated in the VK33 docking site, indicated by X3, were crossed to the double balancer line *y w; T(2;3)ap*^{Xa}*/ SM5;TM3, Sb*. From these crosses, males, *y w/Y; X2/ noc*^{Sco}*; TM6B,Tb, Hu, w*$^{+}$*/+* and females, *y w/X;SM5/+; X3/ TM3, Sb*, were crossed. In the next generation, males and females with the genotype, *y w/X/Y; X2/ SM5; X3/ TM6B, Tb, Hu, w*$^{+}$, were crossed. Progeny of this cross were scored for absence of appropriate balancers indicative of rescue of lethality. If homozygous rescue was not obtained, stocks were outcrossed to both the same mutation (control) and other mutations to score for rescue in a transheterozygous condition. For mutations on the X, balanced females were directly crossed to P(acman) clones integrated in VK33. In the next generation, the presence of non-balanced males indicated rescue of lethality.

CH322-55J22 rescued *Hip14*^{1}, *CH322-123J21* rescued *chc*^{1} but not *chc4*^{ts} , *CH322-154I22* rescued *dap160*$^{\Delta 1}$*/dap160*$^{\Delta 2}$, *CH322-83H15* rescued *Drp1*^{2}, *CH322-19L12* rescued *endo*^{1}, *CH322-150F15* rescued *eps15*$^{\Delta 29}$, *CH322-83G13* rescued *n-syb*$^{\Delta F33B}$, *CH322-130G10* rescued *sqh*^{1} and *sqh*^{AX3}, *CH322-188H18* rescued *synj*^{1} and *CH322-119J05* rescued *v100*^{1}*/v100*^{4} (**Table 1**). This is consistent with previous reports using *P* element transformants for *CG6017* [2], *sqh* [7] and *synj* [11]; and P(acman) *P* element transformants for *chc* [18], *dap160* [19], *drp1* [5] and *Eps15* [8]. *CH321-60D21* rescued *l(1)L13*^{HC129}; *CH321-22M14* rescued *Dscam*^{20}, *Dscam*$^{20/21}$, *Dscam*$^{20/23}$; *CH321- 64G01* rescued *lt*^{11}*/Df(2L) C'*, which was not rescued by *CH321- 16H04* and *CH321-05E14* and *CH321- 27E22* rescued *shakB*^{15}, *shakB*^{20} and *shakB*^{25} (**Table 2**). The *Dscam* rescue result is consistent with previously reported tests using P(acman) ΦC31 integrase technology with a 73 kb *Dscam-1* fragment [19] and a 102 kb *Dscam-2* fragment (data not shown).

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DAB Staining and Fluorescence Microscopy

For embryo staining, fly cages containing strains for the tagged constructs (**Supplementary Table 2**) were prepared, as described above for microinjection. We tested the tagged constructs encoding *bcd*, *en*, *eve*, *cad*, *tll*, *slp2*, *Dfd*, *Bro*, *exd*, *D* and *h*. Trangenic flies for tagged *hb*, *gt*, *run* and *kni* were not obtained, and the transgenic flies containing tagged constructs for *Chi* and *ems* were unhealthy. Hence, expression patterns for these were not determined. Embryos were dechrorionated with 50% bleach for 1 min and rinsed with distilled water. Embryos were fixed (2.5 ml 4% formaldehyde in PBS and 2.5 ml n-heptane) for 20 min. The fixative was removed, and 2.5 ml 100% methanol was added to devitellinize embryos with vigorous shaking. Embryos were rinsed 3x with methanol, 2x with 95% ethanol, and stored at -20°C.

Before antibody staining, embryos were rehydrated and rinsed 3x in 1 ml PBT (PBS + 0.2% Tween-20) and blocked for 1 hr at room temperature in 200 µl block buffer (PBT with 4 µl 10 mg/ml BSA and 4 µl NGS). Embryos were subsequently incubated overnight at 4°C on a rocking table in primary antibody diluted in PBT with BSA and NGS, and rinsed 4x over 1 hr using 1 ml PBT. For peroxidase/diaminobenzidine histochemistry, embryos were blocked for 1 hr at room temperature in 200 µl block buffer and subsequently incubated for 1 hr at room temperature in biotinylated secondary antibody (Vector Laboratories). Embryos were rinsed 4x over 1 hr in 1 ml PBT. Subsequently, embryos were incubated in 200 µl avidin-biotin solution (Vector Standard Vectastain kit, Vector Laboratories) for 1 hr at room temperature and rinsed 3x over 30 min in 1 ml PBT. Embryos were transferred to a 24 well plate. A 1:10 dilution of the two solutions of the Metal Enhanced DAB Substrate Kit (Pierce) was prepared, and 1 ml of this dilution was added to each sample. Samples were incubated at room temperature and observed

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under a stereomicroscope until the reaction was complete. DAB solution was removed and samples washed with PBT. Embryos were transferred to a microfuge tube and washed with 1 ml PBT. Samples were rinsed 2x with 1 ml PBS. 200 µl 70% glycerol in PBS was added, and embryos were allowed to settle overnight at 4°C. Embryos were mounted on a microscope slide, covered with a coverslip and sealed with nail polish. Images were captured using a Zeiss AxioImager.Z1 and processed using Adobe Photoshop 7.0. For confocal immunohistochemistry, a similar protocol was performed up to the primary antibody incubation and subsequent wash. Embryos were then blocked for 1 hr with block buffer and incubated with fluorescent secondary antibodies at room temperature while protected from light, then washed 3x with 1 ml PBT over one hr. Stained embryos were mounted in Vectashield (Vector Labs). Images were captured with a Zeiss upright confocal LSM510 microscope, and processed with ImageJ [20] and Adobe Photoshop 7.0.

Primary antibodies used were rabbit anti-GFP (Invitrogen) and guinea pig anti-Eve (a gift from J. Reinitz) [21] both at 1:200 dilution. For DAB stainings, secondary antibodies were biotinylated goat anti-rabbit used at 1:200 dilution (Vector Laboratories). For staining for confocual imaging, secondary antibodies were Alexa488 conjugated goat anti-rabbit (Invitrogen) and Cy3 conjugated goat anti-rat or anti-guinea pig (Jackson ImmunoResearch), all at 1:250 dilutions.

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SUPPLEMENTARY DISCUSSION

The primary BAC libraries used in the *D. melanogaster* genome sequencing projects were produced from genomic DNA fragmented by partial digestion with restriction enzymes that cleave at six base-pair recognition sequences (*Eco*RI, *Hin*dIII) [22,23]. More recently, *D. melanogaster* BAC libraries have been constructed by physical shearing of genomic DNA [24]. Our goal was to produce P(acman) BAC libraries with high densities of fragment endpoints and high depth of mapped clone coverage. We therefore chose partial digestion with a restriction enzyme that cleaves at a four base-pair recognition sequence, *Mbo*I, as a compromise between the lower density of endpoints resulting from restriction digestion and the lower cloning efficiency inherent to the physical shearing approach. In addition, to produce the shorter insert CHORI-322 library, we performed partial digestion at three reaction conditions to further reduce bias in the distribution of fragment end points. A P1 library [25] and a BAC library [22] had previously been made using isoschizomers of *Mbo*I (*Sau*3AI and *Nde*II, respectively). However, no thorough analysis of the distribution of endpoints of cloned fragments in large-insert genomic libraries across the *D. melanogaster* genome has been reported. As we have shown, our strategy produced libraries containing mapped clones that span nearly all annotated *D. melanogaster* genes including their transcriptional regulatory sequences. Where no P(acman) BAC clone with mapped paired ends is available for a region of interest, the partially mapped clones for which only one end is aligned to the genome provide additional options. The location of the unmapped end of such clones can be determined by conventional techniques such as end sequencing and restriction mapping.

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Previously available *D. melanogaster* BAC and P1 libraries were constructed in conventional low-copy cloning vectors. Clones in these libraries are not transformation-ready, and plasmid copy number is not inducible, hampering the efficient isolation of DNA for embryo microinjection. Transformation-ready cosmid libraries were previously constructed for *D. melanogaster* [26,27]. However, cosmids have a limited insert size. The available cosmid libraries are not mapped onto the reference genome and do not support ΦC31 integrase-mediated germ-line transformation. Moreover, the medium-copy number backbone of cosmids interferes with some recombineering paradigms. The new P(acman) libraries do not suffer from these disadvantages. The libraries are mapped onto the reference genome. The vector supports both *P* element transposase-mediated and bacteriophage ΦC31 integrase-mediated germ-line transformation in *D. melanogaster*. The clones can be maintained at low-copy number to ensure plasmid stability and to facilitate recombineering, and can be induced to high-copy number to improve plasmid yield. Hence, the development of mapped BAC libraries in the P(acman) transgenesis system provides a versatile resource for efficient manipulation of individual genes and for genome-wide applications.

The expression patterns illustrated by the transgenic fusion constructs (**Fig. 2**) are similar to those described for the normal expression of the *eve* [28], *D* [29,30], *cad* [31], *Dfd* [32], *tll* [33], *slp2* [34], and *exd* [35] genes and proteins.

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