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P[acman]: A BAC Transgenic Platform for Targeted Insertion of Large DNA Fragments in *D. melanogaster*

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We describe a transgenesis platform for *Drosophila melanogaster* which integrates three recently-developed technologies: a conditionally amplifiable bacterial artificial chromosome (BAC), recombineering, and ϕC31-mediated transgenesis. The BAC is maintained at low-copy number, facilitating plasmid maintenance and recombineering, but induced to high-copy number for plasmid isolation. Recombineering allows gap repair and mutagenesis in bacteria. Gap repair efficiently retrieves DNA fragments up to 133 kb from P1 or BAC clones. ϕC31-mediated transgenesis integrates these large DNA fragments at specific sites in the genome, allowing rescue of lethal mutations in the corresponding genes. This transgenesis platform should greatly facilitate structure-function analyses of most *Drosophila* genes.

Drosophila is an important model organism to study biology and disease, and new tools are continually developed to facilitate this research (*1*, *2*). A major advance was the development of *P*-element-mediated transformation following injection of plasmids into *Drosophila* embryos (*3*). Hence, *P*-element vectors have been engineered for numerous applications (*4*). However, *P*-element-mediated transformation has a number of limitations: inability to clone large DNA fragments in available *P*-element vectors, difficulties in manipulating large DNA fragments, inability to transform large DNA fragments into the fly genome, and failure to target DNA to specific sites in the genome.

Cloning of large DNA fragments in high-copy-number plasmids, such as typical *P*-element vectors, is inefficient because large fragments are unstable at high-copy number in bacteria. Hence, low-copy-number vectors, including P1 (*5*) and Bacterial Artificial Chromosome (BAC) (*6*) vectors were developed to stably maintain large cloned DNA fragments. Unfortunately, low-copy-number vectors hamper sequencing, embryo injection, and other manipulations requiring large amounts of plasmid DNA. An elegant solution is a conditionally amplifiable plasmid which has two origins of replication (*ori*): *oriS* for low-copy propagation, typical for P1 and BAC vectors, and *oriV* which can be experimentally induced to high copy (*7*). Hence, the introduction of conditionally amplifiable BAC features in fly transformation vectors is a first key step to manipulate large DNA fragments for *Drosophila*.

Cloning of large DNA fragments is limited by conventional methods that rely on restriction enzymes and DNA ligases, hampering analyses of large genes and gene complexes. Recently, efficient *in vivo* cloning technologies utilizing enhanced and regulated recombination systems, commonly known as recombineering, have been developed (*8*). Recombineering greatly facilitates the retrieval of DNA fragments through gap-repair and their subsequent site-directed mutagenesis. Since recombineering is based on homologous recombination, restriction enzymes and DNA ligases are not required. Recombineering is widely employed by mouse geneticists to generate transgenic and knock-out constructs. Recombineering-mediated mutagenesis is much more efficiently with low-copy plasmids (*8*). Hence, using recombineering in a conditionally amplifiable BAC should greatly facilitate gap-repair of large DNA fragment and subsequent mutagenesis at low-copy number.

P-element-mediated transformation is limited by DNA size, precluding the study of large genes (>40kb) and gene complexes. In addition, more than 75% of *P*-elements insert in regulatory elements of genes (*9*), often disrupting genes in subtle ways (*10*). Moreover, *P*-elements are subject to position effects, the effect of a local chromosomal environment on the levels or patterns of transgene expression. This necessitates the generation and characterization of several transgenes for each DNA construct studied. Hence, site-specific integration would greatly facilitate structure-function analysis of transgenes, permitting direct comparison of differently-mutagenized DNA fragments integrated at the same site in the genome. Recently, site-specific integration using the integrase of bacteriophage ϕC31 has been

demonstrated in *Drosophila* (*11*). ϕC31-integrase mediates recombination between an engineered 'docking' site, containing a phage attachment or *attP* site, in the fly genome, and a bacterial attachment or *attB* site in the injected plasmid. Three pseudo *attP* docking sites were identified within the *Drosophila* genome, potentially bypassing desired integration events in engineered *attP* sites. Fortunately, they did not seem receptive for *attB* plasmids, since all integration events were in the desired *attP* sites (*11*). Thus, recovery of large DNA fragments by gap-repair into a low-copy plasmid containing an *attB* site followed by ϕC31-mediated transformation might allow the integration of any DNA fragment into any engineered *attP* 'docking' site dispersed throughout the fly genome.

Here, we describe new vectors that overcome the limitations associated with *P*-element-mediated transgenesis. We developed P/phiC31 artificial chromosome for manipulation or P[acman], a conditionally amplifiable BAC vector that contains recognition sites for both *P*-transposase-(*3*) and ϕC31-mediated integration (*11*). P[acman] permits recombineering-mediated cloning of any genomic DNA fragment from *Drosophila* P1 or BAC clones (*12-15*) and enables transformation of large DNA fragments into the fly genome. The ability to easily manipulate these DNA fragments using recombineering and introduce them into specific sites in the fly genome will greatly facilitate and accelerate *in vivo* genetic manipulations of *Drosophila*.

Results

Construction of a BAC transgenesis vector for Drosophila. The motivation to construct P[acman] grew from our inability to clone 29 and 39 kb DNA fragments in existing *P*-element vectors for transformation rescue of mutations in the gene *senseless2* (*16*). We were unable to identify restriction sites for cloning the entire gene. Moreover, recombineering-mediated gap-repair in existing high-copy *P*-element vectors was unsuccessful. This was consistent with published data demonstrating that gap-repair into high-copy or medium-copy plasmids has an upper size limit of 25 and 80 kb, respectively (*17*). Since high-copy *P*-elements have a substantial size, gap-repair is limited to fragments of about 20 kb. We hypothesized that a low-copy plasmid might alleviate size limitations and improve the stability of large DNA fragments. Hence, we added *P*-element components to a chloramphenicol-resistant, conditionally amplifiable BAC (*7*), resulting in P[acman]-Cm^R (Fig. 1A). The *P*-element components include the *5'P* and *3'P* termini required for *P*–transposase-mediated integration, a multiple cloning site, and the *white*$^+$ marker. The conditionally amplifiable BAC contains two origins of replication, *oriS* and *oriV* (*7*): *oriS* keeps P[acman] at low-copy number for stability of large cloned inserts and efficient recombineering whereas *oriV* permits copy number induction for high-yield DNA preparation for sequencing and embryo injections. We created transgenic flies containing P[acman]-Cm^R using *P*-transposase-mediated integration. To facilitate cloning by gap repair from a variety of sources, we also replaced the ampicillin resistance marker into P[acman]-Cm^R, resulting in P[acman]-Ap^R. Both plasmids can be used to clone any DNA fragment from a variety of donor vectors.

Recombineering-mediated gap repair into P[acman]. P[acman] was used to retrieve fragments by gap repair. For each gap repair, we designed four primer sets (fig. S1). Two homology arms, located at either end of the DNA fragment were cloned into P[acman] (Fig. 1B). Linearization between both homology arms and subsequent transformation of the linearized construct into recombineering-competent bacteria containing the necessary genomic clone allows retrieval of the DNA fragment by gap-repair. Gap repair was performed using two similar strategies, relying on *Red* recombination functions (fig. S2) (*17*, *18*). Both strategies were used to retrieve DNA fragments ranging from 9.4 to 39 kb, from different donor plasmids (Table 1). Colony PCR screening identified correct recombination events at both junctions after gap repair. After plasmid copy number induction, DNA fingerprinting and sequencing demonstrated that the desired fragment was obtained. Hence, the methodology is reliable and large DNA fragments can easily be retrieved.

To test the functionality of the gap-repaired constructs, we used *P*-mediated transposition. Although germline transformation of the constructs was efficient with small inserts, the efficiency dropped for fragments over 20 kb. Transgenic flies containing the gap-repaired fragments in P[acman] were crossed to flies carrying mutations in the corresponding genes. 8 out of 9 fragments tested fully rescued the lethality or visible phenotype associated with the mutations, in homozygous or trans-heterozygous condition (Table 1). One fragment (Sec8-L) did not rescue although a smaller fragment encompassing the same gene did (see below).

ϕC31-mediated integration of P[acman]. Since *P*-transposase-mediated transformation has size limitations, we explored the possibility of using ϕC31-mediated integration to integrate larger constructs at specific sites within the genome. To combine the power of recombineering with ϕC31, we equipped P[acman] with an *attB* site resulting in *attB*-P[acman]. To create genomic *attP* 'docking' sites we introduced an *attP* site and *yellow*$^+$ into a minimal *piggyBac* transposon (*19*) (*piggyBac-yellow*$^+$*-attP*) and integrated and remobilized this *piggyBac* in the *Drosophila* genome using *piggyBac* transposase. We isolated 34 homozygous viable insertions and determined the exact genomic location (Fig. 2A and table S1). Since *piggyBac* has a more random distribution than *P*-transposase (*9*), *attB*-P[acman] should integrate into both gene-poor and -rich regions.

To integrate *attB*-P[acman], we co-injected circular plasmid DNA and mRNA encoding ϕC31-integrase (*11*) into embryos carrying *piggyBac-yellow*$^+$*-attP* (Fig. 2B). Integration results in flies with a "yellow$^+$" body color and "white$^+$" eye color phenotype. Since the *attB* site is upstream of the MCS and *white*$^+$ is downstream of the MCS, only transgenic flies that are "yellow$^+$ white$^+$" should have undergone an integration event containing most of the injected DNA including the cloned insert (Fig. 2B). Hence, successful integration events can be genetically traced. Moreover, since both transposons (*P*-element and *piggyBac*) are maintained after integration, the inserted DNA can be remobilized using the respective transposases.

We tested whether different *attP* docking sites are equally receptive for *attB*-P[acman]-Ap^R. We focused on seven docking sites: two each on chromosomes X, 2 and 3, and one on chromosome 4. As shown in table S2, all but one of these docking sites are receptive with similar integration efficiencies of 20-30%. We occasionally obtain efficiencies as high as 60%, similar to a previous report (*11*). However, in general we observed higher survival rate than Groth et al. (*11*). In our hands, such efficiencies are higher than *P*-transposase-mediated integration of similar-sized constructs. Since ϕC31-mediated-integration is site-specific, only a single insertion is needed, and the injection procedure can therefore be downscaled to 50 embryos or less. Integration of *attB*-P[acman]-Ap^R in the same docking site leads to the same *white*$^+$ expression level (fig. S3). This is convenient, as rare events that were not integrated in the proper site exhibited a different eye color and were therefore distinguished from true integration events. Integration of *attB*-P[acman]-Ap^R in a single docking site on chromosome 2 consistently causes a patchy red eye phenotype (fig. S3D) indicating position effect variegation of *white*$^+$ expression. This was not observed for the *yellow*$^+$ marker present in the same 'docking' site. Moreover, insertion of *attB*-P[acman]-Ap^R into different docking sites resulted in different eye color phenotypes indicating position effects on the expression level of the *white*$^+$ marker (fig. S3). The main difference between ϕC31- and *P*-element-mediated integration is that position effects with the ϕC31 system are predictable, allowing for the selection of different but defined expression levels.

To identify correct integration events, we developed PCR assays specific for the *attP*, *attL* and *attR* sites (Fig. 2C). Correct integration events were identified by the loss of the *attP* PCR product (specific for the original docking site) and the appearance of *attL* and *attR* PCR products (specific for the integration event). PCR analysis indicates that all but one of the insertions is correctly integrated. Moreover, correct integration events in homozygous viable docking sites maintain homozygous viability, demonstrating that insertion does not detrimentally affect the local chromosomal environment. In numerous transformation experiments, we recovered only one event that converted a homozygous viable docking site into a recessive lethal locus. PCR analysis demonstrated that this event did not result in correct integration (see above). In conclusion, ϕC31-mediated integration is very efficient.

Cloning of large DNA fragments into attB-P[acman]. To clone large DNA fragments into *attB*-P[acman]-Ap^R, we performed recombineering-mediated gap repair, as described above. We retrieved fragments up to 102 kb in length from single P1 or BAC clones (Table 2). Multiple genes and gene complexes, including *bancal*, *Dscam*, *teashirt* and the *Bearded* and *Enhancer of Spl* complexes, were cloned into *attB*-P[acman]-Ap^R (Table 2). Unfortunately, some large genes are not contained within a single BAC (*15*). We therefore decided to reconstitute large genes through serial gap repair from two overlapping BAC clones into *attB*-P[acman]-Ap^R: In step 1, the smaller part is retrieved from one BAC, which is followed by step 2, retrieving the remainder of the gene (Fig. 3 and fig. S4). This two-step procedure was successful in retrieving a 133 kb fragment encompassing the *Tenascin-major* gene (Table 2).

ϕC31-mediated integration of large gap-repaired fragments. To obtain transgenic flies with *attB*-P[acman]-Ap^R constructs, we can use either *P*-transposase or ϕC31-integrase. We first tested *P*-transposition for three small fragments. Transgenic animals were obtained for all three (Table 2). Since *P*-transposase-mediated integration is inefficient for fragments larger than 20 kb, we switched to ϕC31-mediated integration. Several injection rounds suggested that the molar DNA concentration was critical. We empirically established that 75 ng/µl of DNA is the lower limit for *attB*-P[acman]-Ap^R (~13 kb). Therefore, large (75-135 kb) supercoiled plasmid DNA (300 -750 ng/µl) was coinjected with ϕC31 mRNA (250-500 ng/µl) (*11*). As shown in Table 2, we obtained transgenic animals for most constructs, including 73, 76, 86, and 133 kb fragments. In these cases the transformation efficiency was about 10% for medium (15-50 kb) and 2 to 4% for large plasmids (>50 kb). PCR analysis confirmed that all constructs integrated correctly. For two transgenes, 18 and 102 kb, we did not obtain transformants, illustrating that optimization for certain constructs might be required. Note that different gap-repaired fragments inserted in the same docking site differentially affect *white*$^+$ expression, suggesting that DNA context is important. Finally, multiple fragments fully rescue two independent lethal mutations, in homozygous or trans-heterozygous condition, in each of the four corresponding genes or gene complexes tested (Table 2). These data show that gap-repair permits the cloning of BAC-sized fragments, large transgenes can be integrated site-specifically in the *Drosophila* genome using the ϕC31, and mutations in

essential genes and entire gene complexes can be rescued by large transgenic fragments.

Discussion

P[acman] provides numerous improvements when compared to current strategies for *Drosophila* transgenesis. First, DNA constructs of more than 100 kb can be retrieved from genomic P1 and BAC clones using recombineering-mediated gap repair. Fragments are retrieved into a plasmid fitted with an inducible *oriV* replication origin that allows easy preparation of large quantities of DNA for sequencing and *Drosophila* transgenesis. The retrieved fragments do not need to be re-sequenced since they are directly retrieved from the genomic clone without PCR amplification. Second, unlike *P*-transposase, ϕC31-integrase enables the integration of large fragments into the *Drosophila* genome. Since ϕC31-integrase catalyzes recombination between two ectopic attachment sites (*attB* and *attP*), transgenes are integrated at specific docking sites in the fly genome. This largely eliminates the problem of position effects, a highly desirable feature when comparing different mutagenized constructs derived from the same transgene for structure-function analysis. Finally, site-directed mutagenesis via recombineering is much more efficient in low-copy plasmids such as P[acman].

We were able to clone fragments as large as 102 kb from single BACs. Only one report documents gap repair of a similarly sized fragment from one BAC (*20*). In both cases, gap repair was successful because of the use of a low-copy vector. Indeed, gap repair into high-copy or medium-copy plasmids has an upper size limit of 25 kb and 80 kb, respectively (*17*). We also reconstituted one of the largest *Drosophila* genes, using serial gap repair, resulting in reconstitution of a 133 kb fragment. One recent report demonstrated a variation of seamless recombination of two large DNA fragments (*20*). The methodology described here may facilitate the cloning of even larger genomic fragments into P[acman], which may not be contained within single BAC clones in the genome tiling path set (*15*).

The numerous docking sites (Fig. 2A and table S1) created in this work will have to be characterized in more detail to determine the expression levels of different genes that are inserted in the same site. It will also be important to determine if adjacent enhancers or regulatory elements influence gene expression in each of the docking sites in order to identify sites that are as “neutral” as possible, a requirement important for the study of regulatory elements. Neighboring genome environment may also become important when overexpression or RNAi transgenes are inserted. We feel that *piggyBac-y+-attP* insertions in intergenic regions may be the best candidates for these applications although this will have to be determined experimentally.

The circular ϕC31 bacteriophage genome is integrated into the linear genome of its host, *Streptomyces lividans*, by ϕC31-integrase (*21*). The genome of ϕC31 is about 41.5 kb, which is larger than standard high-copy plasmids. This suggested that ϕC31-integrase would be useful to integrate circular DNA molecules up to 41.5 kb and potentially larger into the *Drosophila* genome. Indeed, we integrated fragments up to ~146 kb at defined sites in the genome, which was previously impossible. Large gap-repaired fragments should complement many molecularly-defined deficiencies (*22*) and subsequent mutagenesis should permit analysis of genes within the deletion, obviating the need to recover mutations using conventional genetic screens or imprecise excision of mapped *P*-elements. This approach also opens the road for clonal analysis of any gene that maps close to centromeric heterochromatin since transgenic constructs for these genes can be recombined onto an *FRT*-containing chromosome. Finally, recombineering will allow the integration of any peptide or protein tag into the genomic rescue constructs to study protein localization and function *in vivo*.

In conclusion, the proposed methodology opens up a wide variety of experimental manipulations that were previously impossible or difficult to perform in *Drosophila*. Moreover, it is expected that it should be possible to adapt this methodology to other model organisms since *piggyBac* transposes in many species, including mammals (*23*) and ϕC31 is operational in many species including mammalian cells (*24*).

References and Notes

1. K. J. Venken, H. J. Bellen, *Nat. Rev. Genet.* **6**, 167 (2005).
2. K. A. Matthews, T. C. Kaufman, W. M. Gelbart, *Nat. Rev. Genet.* **6**, 179 (2005).
3. G. M. Rubin, A. C. Spradling, *Science* **218**, 348 (1982).
4. E. Ryder, S. Russell, *Brief. Funct. Genomic. Proteomic.* **2**, 57 (2003).
5. N. Sternberg, *Proc. Natl. Acad. Sci. U. S. A* **87**, 103 (1990).
6. H. Shizuya *et al.*, *Proc. Natl. Acad. Sci. U. S. A* **89**, 8794 (1992).
7. J. Wild, Z. Hradecna, W. Szybalski, *Genome Res.* **12**, 1434 (2002).
8. N. G. Copeland, N. A. Jenkins, Court DL, *Nat. Rev. Genet.* **2**, 769 (2001).
9. H. J. Bellen *et al.*, *Genetics* **167**, 761 (2004).
10. K. K. Norga *et al.*, *Curr. Biol.* **13**, 1388 (2003).
11. A. C. Groth, M. Fish, R. Nusse, M. P. Calos, *Genetics* **166**, 1775 (2004).
12. W. Kimmerly *et al.*, *Genome Res.* **6**, 414 (1996).
13. R. A. Hoskins *et al.*, *Science* **287**, 2271 (2000).
14. M. D. Adams *et al.*, *Science* **287**, 2185 (2000).
15. S. E. Celniker *et al.*, *Genome Biol.* **3**, RESEARCH0079 (2002).

16. H. Jafar-Nejad, H. J. Bellen, *Mol. Cell Biol.* **24**, 8803 (2004).
17. E. C. Lee *et al.*, *Genomics* **73**, 56 (2001).
18. D. L. Court *et al.*, *Gene* **315**, 63 (2003).
19. X. Li, N. Lobo, C. A. Bauser, M. J. Fraser, Jr., *Mol. Genet. Genomics* **266**, 190 (2001).
20. G. Kotzamanis, C. Huxley, *BMC. Biotechnol.* **4**, 1 (2004).
21. H. M. Thorpe, M. C. Smith, *Proc. Natl. Acad. Sci. U. S. A* **95**, 5505 (1998).
22. A. L. Parks *et al.*, *Nat. Genet.* **36**, 288 (2004).
23. H. F. Xu *et al.*, *Mol. Genet. Genomics* **276**, 31 (2006).
24. D. A. Sorrell, A. F. Kolb, *Biotechnol. Adv.* **23**, 431 (2005).
25. P. Verstreken *et al.*, *Neuron* **47**, 365 (2005).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/1134426/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 to S4
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Fig. 1. P[acman]: BAC transgenesis for *Drosophila*. (**A**) P[acman] contains *P*-element transposase sites (*3'P* and *5'P*), *white*$^{+}$ and a multiple cloning site (MCS). This *P*-element is inserted in the conditionally amplifiable BAC, containing a low-copy origin of replication (*oriS*) and a copy inducible origin of replication (*oriV*). (**B**) P[acman] is linearized between both homology arms (LA and RA) and transformed into recombineering bacteria containing P1 or BAC clones Integration into the germ line of *white*$^{-}$ flies is mediated by *P*-element medicated transformation.

Fig. 2. P[acman] transgenesis in *Drosophila* using the ϕC31 system. **(A**) The *piggyBac-y*$^{+}$*-attP* 'docking' element was transformed or remobilized in the *Drosophila* genome to obtain multiple *attP* docking lines (VK lines). Locations are indicated on a schematic representation of the polytene chromosomes. White triangles represent insertions between, and black triangles within, annotated genes. VK line numbers (table S1) are indicated within the triangles. (**B**) *attB*-P[acman] can integrate at an *attP* docking site in the fly genome. (**C**) Correct integration events in docking sites are PCR positive for the *attR* and *attL* assays, whereas original docking sites are PCR positive for the *attP* assay. *yw* was serves as negative control. Two docking sites on chromosome X (VK6 and VK38), 2 (VK1 and VK2) and 3 (VK5 and VK13) were used. LM, length marker.

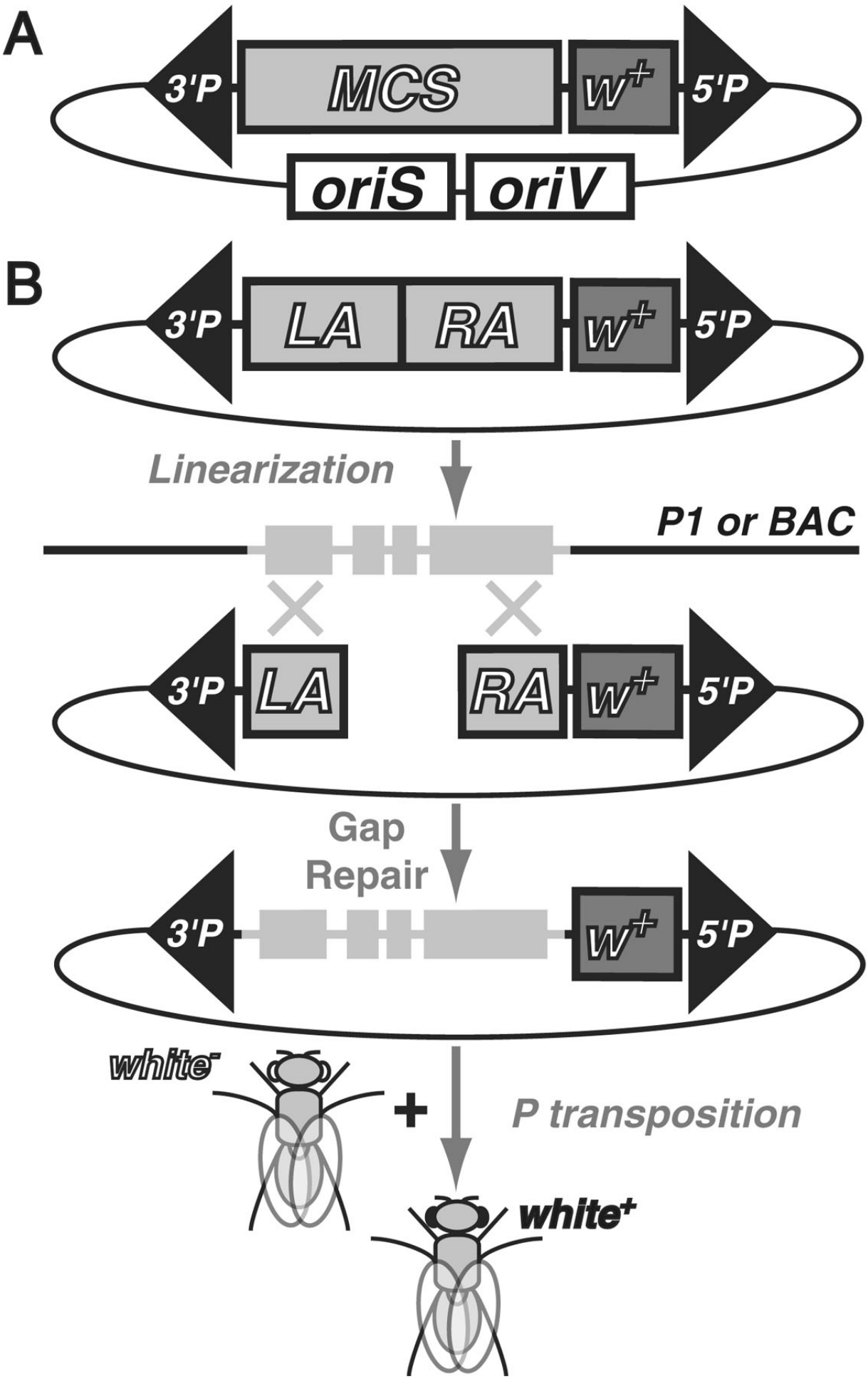
Fig. 3. Reconstitution of large fragments from two overlapping BACs by serial gap-repair. Three homology arms are designed: LA1, RA1 and RA2, located at the left end, the region of overlap of the two BACs, and right end of the desired DNA fragment, respectively. During step 1, the construct is linearized between LA1 and RA1, and the left segment of the gene is obtained from BAC#1, resulting in LA2. In step 2, the construct is linearized between LA2 and RA2, and the remaining segment of the gene is obtained from BAC#2 to reconstitute the entire gene.

Table 1. Retrieval of genes in P[acman]. Genomic fragments containing genes of interest retrieved by gap-repair into P[acman]-Cm^R or -Ap^R. Donor plasmids, with clone coordinates, are P1, BACs, or ampicillin-modified BACs (BAC-Ap^R). Mutations in the corresponding genes were lethal or showed a phenotype. Rescue was obtained for most genes using *P*-element mediated transformation. NA, not applicable. [1]Verstreken *et al*., 2005 (*25*).

Table 1: Recombineering in P[acman]							
Gene	**Construct**	**Size**	**Donor**	**Clone**	**P[acman]**	**Mutation**	**Rescue**
sens2	sens2-22	22.3 kb	P1	DS05421	Cm^R	NA	NA
sens2	sens2-29	28.9 kb	P1	DS05421	Cm^R	NA	NA
sens2	sens2-39	38.9 kb	P1	DS05421	Cm^R	NA	NA
CG10805	CG10805-S	9.7 kb	P1	DS05421	Cm^R	Lethal	Yes
CG10805	CG10805-L	14.7 kb	P1	DS05421	Cm^R	Lethal	Yes
dap160	dap160	10.9 kb	P1	DS02919	Cm^R	Lethal	Yes
sens	sens-S	12.1 kb	BAC-Ap^R	BACR17E13	Cm^R	Lethal	Yes
sens	sens-L	18.1 kb	BAC-Ap^R	BACR17E13	Cm^R	Lethal	Yes
Dalpha7	Da7	29.4 kb	BAC-Ap^R	BACR02B03	Cm^R	Phenotype	Yes
Drp	Drp	9.4 kb	BAC	BACR30P05	Ap^R	Lethal	Yes[1]
Sec8	Sec8-L	12 kb	BAC	BACR02L23	Ap^R	Lethal	No
Eps15	Eps15-L	11.9 kb	BAC	BACR27P17	Ap^R	Lethal	Yes

Table 2. Retrieval of genes in *attB*-P[acman]. Genome fragments containing genes of interest retrieved by gap-repair into *attB*-P[acman]-Ap^R. Donor fragments are from P1 or BACs, or PCR. Clone coordinates of the donor clones are indicated. Mutations in all corresponding genes are lethal. Rescue is indicated for *P*-element or ϕC31-mediated transformation. ND, not determined; NA, not applicable.

Table 2: Recombineering in *attB*-P[acman]								
Gene	**Construct**	**Size**	**Donor**	**Clone**	**Transgenics**			
					P	**Rescue**	**phiC31**	**Rescue**
Sec6	Sec6-S	2.8 kb	PCR	BACR27L09	Yes	No	Yes	ND
miR-4	miR-4	4.5 kb	BAC	BACR02J10	ND	NA	ND	NA
Sec8	Sec8-S	4.9 kb	BAC	BACR02L23	Yes	Yes	ND	NA
Eps15	Eps15-S	10.8 kb	BAC	BACR3B7	ND	NA	Yes	ND
Sec6	Sec6-L	11.5 kb	BAC	BACR27L09	Yes	ND	Yes	ND
sens	sens-L	18.1 kb	BAC	BACR17E13	ND	NA	No	NA
miR-9a	miR-9a	20.1 kb	BAC	BACR01D04	ND	NA	Yes	ND
Tsh	Tsh-1	28.4 kb	BAC	BACR03L08	ND	NA	Yes	Yes
grp	grp	29.8 kb	P1	DS00592	ND	NA	Yes	ND
Brd-C	Brd-C	37.2 kb	BAC	BACR01H12	ND	NA	ND	NA
bancal	bancal	39.5 kb	BAC	BACR33D17	ND	NA	ND	NA
Dscam	Dscam-1	73.3 kb	BAC	BACR26B18	ND	NA	Yes	Yes
E(Spl)-C	E(Spl)-C	77.7 kb	BAC	BACR13F13	ND	NA	Yes	Yes
Tsh	Tsh-2	86.4 kb	BAC	BACR03L08	ND	NA	Yes	Yes
Dscam	Dscam-2	102.3 kb	BAC	BACR26B18	ND	NA	No	NA
ten-m	ten-m	20 kb	BAC	BACR02D04	NA	NA	NA	NA
ten-m	ten-m	133 kb	BAC	BACR22C11	ND	NA	Yes	Yes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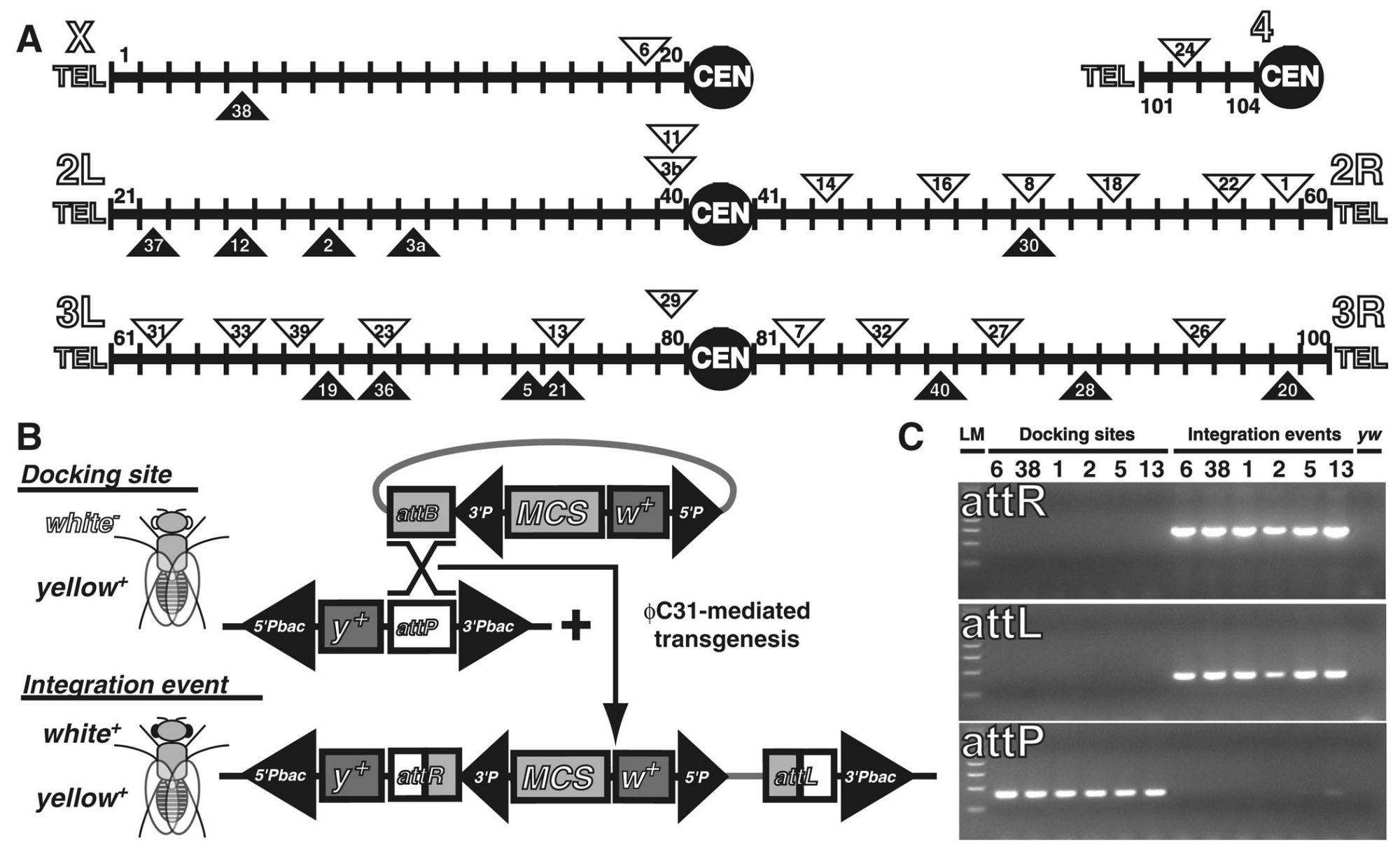
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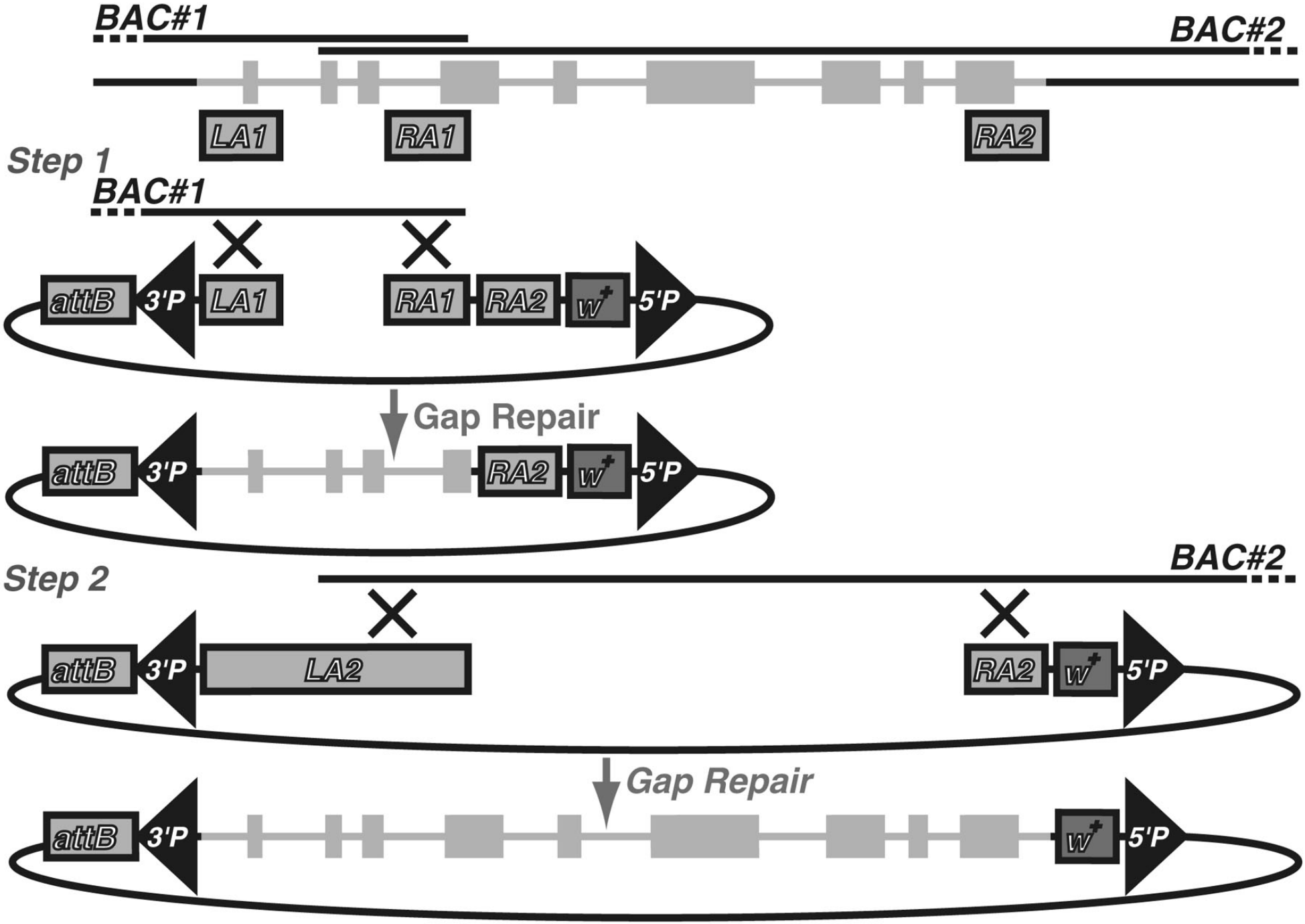
26
100
3R
TEL
19
36
5
21
40
28
20
B
Docking site
white-
yellow+
attB
3'P
MCS
w+
5'P
5'Pbac
y+
attP
3'Pbac
+
ϕC31-mediated
transgenesis
Integration event
white+
yellow+
5'Pbac
y+
attR
3'P
MCS
w+
5'P
attL
3'Pbac
C
LM
Docking sites
Integration events
yw
6 38 1 2 5 13 6 38 1 2 5 13
attR
attL
attP


BAC#1
BAC#2
LA1
RA1
RA2
Step 1
BAC#1
attB
3'P
LA1
RA1
RA2
w+
5'P
Gap Repair
attB
3'P
RA2
w+
5'P
Step 2
BAC#2
attB
3'P
LA2
RA2
w+
5'P
Gap Repair
attB
3'P
w+
5'P