



B.

attP
 GTAGTGCCCCAACTGGGGTAACCT **TTG** AGTTCTCTCAGTTGGGGGCGTAG

attP18 ...GA GTAGTGCCCCAACTGGGGTAACCT **TTG** AGTTCTCTCAGTTGGGGGCGTAG GG...

JK22C ...GA GTAGTGCCCCAACTGGGGTAACCT **TTG** AGTTCTCTCAGTTGGGGGCGTAG GG...

JK65C ...GA GTAGTGCCCCAACTGGGGTAACCT **TTG** AGTTCTCTCAGTTGGGGGCGTAG GG...

JK66B ...GA GTAGTGCCCCAACTGGGGTAACCT **TTG** AGTTCTCTCAGTTGGGGGCGTAG GG...

JK73A ...GA GTAGTGCCCCAACTGGGGTAACCT **TTG** AGTTCTCTCAGTTGGGGGCGTAG GG...

C.

	Location	Insertion
JK22C	2L: 22C3	5' - <i>GlyP</i> <i>CG4259</i> - 3'
JK64A	3L: 64A12	5' UTR of <i>CG1309</i>
JK65C	3L: 65C3	5' UTR of <i>dikar</i>
JK66B	3L: 66B4	5' - <i>Ect4</i> <i>CR32360</i> - 3'
JK73A	3L: 73A9	5' - <i>Smn</i> <i>nxf2</i> - 3'
JK80B	3R: 80B1	5' UTR of <i>CG33170</i>
JK87B	3R: 87B9	5' UTR of <i>CG5196</i>

Figure S5. Sequencing and genomic location of new landing sites.

Figure S5 Molecular characterization of new landing sites. (A) Genomic PCR verification of R11C05-LexA excision. Top – The presence of R11C05-LexA was probed using primers that bind in the LexA coding sequence. Controls that harbor the transgene (lanes 3 and 5) showed amplification, but not native attP18 or the new landing sites following treatment with Int* (lanes 4 and 6-9). Bottom – Amplification of a region containing attP corroborates the absence of the R11C05-LexA transgene. Amplification was successful in samples with an intact attP, but fails in samples with an integrated transgene. Arrows indicate bands that were gel-extracted and sequenced. (B) To confirm the integrity of reconstituted attPs in new landing sites, the PCR products indicated in (A) were sequenced and compared to wild-type attP. The cross-over nucleotides where recombination occurs are indicated in bold. (C) Genomic location of new landing sites: Landing sites were mapped to the genome using splinkerette PCR (Potter and Luo 2010).