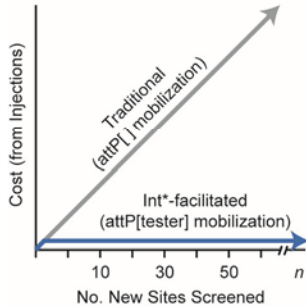


A. Steps to isolate new landing sites.

Gen.	attP[] mobilization	Int*-facilitated
1	Mobilize	Mobilize
2	Isolate new insertions	Isolate new insertions
3	Balance	Balance
4	Expand	Screen
5	Integrate reporter	Excise reporter
6	Isolate transformants	
7	Screen	

B. Cost comparison between methods



C. Crossing scheme to isolate new candidate landing site insertions of P{attP[R11C05-lexA]}.

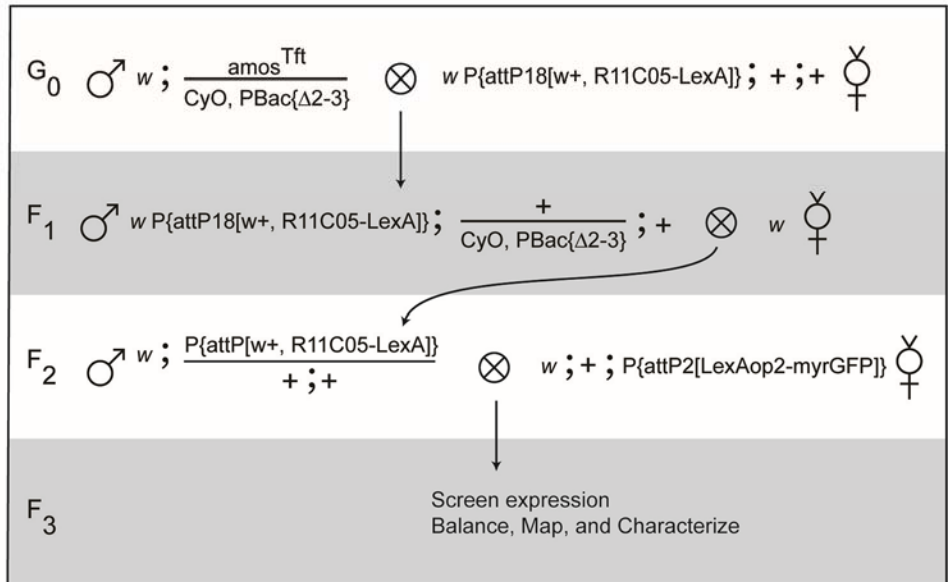


Figure S2. Characterization of Int*-facilitated landing site isolation.

Figure S2 Characterization of Int*-facilitated landing site isolation. (A) Comparison of steps required to isolate new landing sites via traditional or Int*-facilitated methods: For either method, the first three steps are identical. However, the Int*-facilitated method allows new candidate landing sites to be screened as early as the fourth generation. This eliminates the need to maintain and expand lines that will later be rejected, thereby significantly reducing the amount of fly work required to characterize new candidate landing sites. (B) Illustration of the theoretical cost differential for assessing n new landing sites by traditional vs. Int*-facilitated methods: Because the traditional method requires a separate injection for each site tested, total injection cost increases linearly with the number of sites assessed. In contrast, Int*-facilitated landing site isolation requires no injections once the tester transgene has been integrated, resulting in a flat cost curve. (C) Crossing scheme to isolate new, autosomal candidate landing site insertions: To mobilize $P\{CaryP\}attP18[R11C05-lexA]$ off the X chromosome, males bearing P transposase on *CyO* ($PBac{\Delta 2-3}$; BL#8201) were crossed to virgins homozygous for $P\{CaryP\}attP18[R11C05-LexA]$ to produce dysgenic males, which were crossed to w^{1118} virgins. The Cy^+ , *mini-white*⁺ male progeny of this cross represent new insertions of the P-element. (Note: the mobile element can be followed by the mini-white associated with the R11C05-lexA tester or by the mini-yellow allele that is part of the $P\{CaryP\}attP[]$ landing site, though in practice we found it more convenient to track mini-white.)