

File S1

PCR primers

1. Standard PCR primers:

For detection of integrase plasmid; gives 829 bp product from ϕ C31 integrase coding sequence:

act- ϕ C31 F: 5'-GAG CCC GCT GAG TGG TAT GAG C-3'

act- ϕ C31 R: 5'-CGC TAC GCC GCT ACG TCT TCC-3'

for detection of attP, attB, attL, and attR sites:

These primers are based on a published set (VENKEN *et al.* 2006); attA primers are identical to those published previously, and attB primers are altered from the published version to reflect the slightly shorter attB sites used in our targeting vectors

attP-forward: 5'-CTT CAC GTT TTC CCA GGT CAG AAG-3'

attP-reverse: 5'-GTC GCG CTC GCG CGA CTG ACG GTC-3'

attB-F-replace: 5'-GGT CAC GGT CTC GAA GC-3'

attB-R-replace: 5'-GAG AAC CCG CTG ACG CT-3'

flanking primers for Kc167-PP-16F:

16F-forward: 5'-ACA GTG AGC CCT GGG AAT TA-3'

16F-reverse: 5'-TCT TAT CTG GAG TGT CGG AGT G-3'

flanking primers for Kc167-PP-21B:

21B-forward: 5'-CTA TCC TGG CTG CCC AAT TA-3'

21B-reverse: 5'-TCA TGT GGT GCG CTT CTT-3'

flanking primers for Kc167-PP-21D:

21D-forward: 5'-GTT CGG AAT TCA AGA GAC GAA TG-3'

21D-reverse: 5'-GTT GCT GAG AGG TGA GTG AG-3'

flanking primers for Kc167-PP-50Aa:

50Aa-forward: 5'-GTG TGT ATG TGT GTG CTT GC-3'

50Aa-reverse: 5'-GTG ACC CGC TCT TCC ATT TA-3'

flanking primers for Kc167-PP-50Ab:

50Ab-forward: 5'-ACG GGA AAG GAA CGA AAG AG-3'

50Ab-reverse: 5'-CAA ATC CCA GGG TCT AAC CAA-3'

flanking primers for Kc167-PP-52E:

52E-forward: 5'-CGG TTG GTA GGA TTG CTT CT-3'

52E-reverse: 5'-GCG ATT AGA GGA AGT GTC TCA G-3'

flanking primers for Kc167-PP-61C:

61C-forward: 5'-GAT CCC ACT GGC TGC TAT TT-3'
61C-reverse: 5'-GTG GAG GAG GCT CAT TCA TAT T-3'

flanking primers for Kc167-PP-89B:
89B-forward: 5'-CCA TTA GGG TCT TGA GCA CTA T-3'
89B-reverse: 5'-CGC CAT TAT GCA TGA GTA ACA A-3'

flanking primers for Kc167-PP-93E:
93E-forward: 5'-ACA CAC TCG CAG GCA TTT-3'
93E-reverse: 5'-CGC CAG CCC ATT CAT CTA TT-3'

flanking primers for Kc167-PP-99A:
93E-forward: 5'-TCG GTC TTC TAA GCT ACC CTA TC-3'
93E-reverse: 5'-GTT CTC CGT TTC TCC GAT TCT C-3'

flanking primers for Sg4-PP-3A:
3A-forward: 5'-AAA CGC TCC CCA CAA GAG AG-3'
3A-reverse: 5'-CAG TTG ACC CAG AGA GCG AG-3'

flanking primers for Sg4-PP-27F:
27F-forward: 5'-GCC TTT TGC CGA TTT TCG GT-3'
27F-reverse: 5'-TGT CTC CCA TTT GGT GCG AA-3'

flanking primers for Sg4-PP-49B:
49B-forward: 5'-TCA CCA TGA CAA TGG CGG AA-3'
49B-reverse: 5'-CTG GAA AGT GGA AGG CGG AT-3'

flanking primers for Sg4-PP-57B:
57B-forward: 5'-CGA CGA ACT GCA ATG AGC TG-3'
57B-reverse: 5'-ACC TGC AAC CCA AAC TCA CA-3'

flanking primers for Sg4-PP-70F:
70F-forward: 5'-TCT CTT GCC CGT ACG CTT TT-3'
70F-reverse: 5'-AAT GCC GCT CGA CAA CTG TA-3'

flanking primers for Sg4-PP-84E:
84E-forward: 5'-AGC GCC GTG TCT TCT GTT AT-3'
84E-reverse: 5'-GTG TGC TCC CCC TCT CTT TC-3'

2. ddPCR primers:

for EcR standard:

5'-/5HEX/CGGCCGTC/ZEN/CGAGAAGATCACAAT/31ABkFQ/-3' (probe)
5'-CGTAGCGTGTTCGATGTAGTAG-3' (primer 1)

5'-AGGTGGACAACGTCTGAATAC-3' (primer 2)

for attP-GFP copy number:

5'-/56-FAM/TGTTGTAGT/ZEN/TGTACTCCAGCTTGTGCCC/31ABkFQ/-3' (probe)

5'-CTGCTTGTCGGCCATGATATAG-3' (primer 1)

5'-GAAGGGCATCGACTTCAAGG-3' (primer 2)

Venken, K. J., Y. He, R. A. Hoskins and H. J. Bellen, 2006 P[acman]: a BAC transgenic platform for targeted insertion of large DNA fragments in *D. melanogaster*. *Science* 314: 1747-1751.