

## Supplementary Methods

### Primers for screening FLP/FRT deletion stocks by PCR

#### Hybrid PCR (Transposon-specific primers facing inward)

w+/w-	left isolate	right isolate	left primer	right primer	fragment size
w-	XP5' plus	WH5' minus	AATGATTTCGCAGTGAAGGCT	GACGCATGATTATCTTTTACGTGAC	1.8kb
w-	WH5' plus	XP5' minus	GACGCATGATTATCTTTTACGTGAC	AATGATTTCGCAGTGAAGGCT	1.8kb
w-	XP5' plus	RB3' plus	AATGATTTCGCAGTGAAGGCT	TGCATTTGCCTTTTCGCCTTAT	1.7kb
w-	RB3' minus	XP5' minus	TGCATTTGCCTTTTCGCCTTAT	AATGATTTCGCAGTGAAGGCT	1.7kb
w+	RB5' plus	WH5' minus	GACGCATGATTATCTTTTACGTGAC	GACGCATGATTATCTTTTACGTGAC	6.1kb
w+	WH5' plus	RB5' minus	GACGCATGATTATCTTTTACGTGAC	GACGCATGATTATCTTTTACGTGAC	6.1kb
w+	RB3' minus	WH3' plus	TGCATTTGCCTTTTCGCCTTAT	TGCATTTGCCTTTTCGCCTTAT	7.3kb
w+	WH3' minus	RB3' plus	TGCATTTGCCTTTTCGCCTTAT	TGCATTTGCCTTTTCGCCTTAT	7.3kb

#### Two-sided PCR (Transposon-specific primers facing outward)

w+/w-	left isolate	right isolate	left primer	right primer	fragment size
w+	XP3' minus	WH3' plus	TACTATTCCTTTCACTCGCACTTATTG	CCTCGATATACAGACCGATAAAAC	variable
w+	XP3' minus	RB5' minus	TACTATTCCTTTCACTCGCACTTATTG	TCCAAGCGGCGACTGAGATG	variable
w+	WH3' minus	XP3' plus	CCTCGATATACAGACCGATAAAAC	TACTATTCCTTTCACTCGCACTTATTG	variable
w+	RB5' plus	XP3' plus	TCCAAGCGGCGACTGAGATG	TACTATTCCTTTCACTCGCACTTATTG	variable
w+	RB5' plus	RB3' plus	TCCAAGCGGCGACTGAGATG	CCTCGATATACAGACCGATAAAAC	variable
w+	WH5' plus	WH3' plus	TCCAAGCGGCGACTGAGATG	CCTCGATATACAGACCGATAAAAC	variable
w+	RB3' minus	RB5' minus	CCTCGATATACAGACCGATAAAAC	TCCAAGCGGCGACTGAGATG	variable
w+	WH3' minus	WH5' minus	CCTCGATATACAGACCGATAAAAC	TCCAAGCGGCGACTGAGATG	variable
w+	XP5' plus	XP3' plus	TTTACTCCAGTCACAGCTTTG	TACTATTCCTTTCACTCGCACTTATTG	variable
w+	XP3' minus	XP5' minus	TACTATTCCTTTCACTCGCACTTATTG	TTTACTCCAGTCACAGCTTTG	variable
w+	RB3' minus	WH3' plus	CCTCGATATACAGACCGATAAAAC	CCTCGATATACAGACCGATAAAAC	variable
w+	WH3' minus	RB3' plus	CCTCGATATACAGACCGATAAAAC	CCTCGATATACAGACCGATAAAAC	variable
w+	WH5' plus	RB5' minus	TCCAAGCGGCGACTGAGATG	TCCAAGCGGCGACTGAGATG	variable
w+	RB5' plus	WH5' minus	TCCAAGCGGCGACTGAGATG	TCCAAGCGGCGACTGAGATG	variable

#### 96-well 5 Fly Genomic Prep

- 1) Place 5 flies per well in a 96-well flat bottom plate on ice.
- 2) Homogenize flies in 100  $\mu$ L Buffer A using Burkard Scientific Multiple Homogenizer or other compatible homogenizer, cover and incubate at 65°C 15 min.
- 3) Quick spin to pull down condensation. Add 100  $\mu$ L 3M KOAc, cover, mix and put on ice for 10 min.
- 4) Spin 20 min @ 2000 RCF.
- 5) Transfer 150  $\mu$ L supernatant with 12 channel pipet with aerosol barrier tips to 96-deep well block.
- 6) Add 90  $\mu$ L cold isopropanol, cover, mix about 1min, incubate @ RT for 5 min.
- 7) Spin for 30 min @ 2000 RCF @ 4°C
- 8) Pour out supernatant then invert on paper towel briefly.
- 9) Add 200  $\mu$ L cold 70% EtOH, cover, spin for 10 min @ 2000 RCF @ 4°C
- 10) Pour out supernatant then invert on paper towel briefly.
- 11) Speed vacuum to dry on medium heat, about 10-15 min (look to make sure all EtOH is gone).
- 12) Add 100  $\mu$ L TE, mix, put in 65°C H<sub>2</sub>O bath 15-20 min to resuspend. Mix again then quick spin to pull down all liquid.
- 13) Transfer to 96-well round bottom plate with a 12 channel pipet using aerosol barrier tips, and seal for storage at -80°C.

#### Buffer A

100 mM TrisHCl 7.6  
100 mM EDTA

100 mM NaCl  
0.5% SDS