PCR screening of MiMIC RMCE events to determine orientation of GFSTF or Trojan-GAL4 cassette

This is the protocol that we use to screen and verify the stocks we send to the Bloomington Drosophila Stock Center. For details of the insertion collection see:

http://flypush.imgen.bcm.tmc.edu/pscreen/rmce/
Venken et al. (2011) *Nature Methods* 8:737-743
Nagarkar-Jaiswal et al. (2015) *eLife* 4:e05338
Nagarkar-Jaiswal et al. (2015) *eLife* 4:e08469

We recommend screening at least 5 independent candidate events.

Step 1. Genomic DNA extraction

We use the PureLink® Genomic DNA Mini Kit from Thermo Fisher (cat. no. K1820-02 for 250 reactions). All solutions mentioned below (with the exception of EtOH) are provided in the kit.

1. Prepare proteinase K digestion reaction master mix (total 200 µl per reaction.)

    a. Digestion Buffer - 180 µl per reaction (180 x __ candidates)
    b. Proteinase K - 20 µl per reaction (20 x __ candidates)

2. Collect 10 to 15 adult flies for each candidate and homogenize with 200 µl digestion mix. We use polypropylene Kimble™ Kontes™ Pellet Pestle™ tissue grinders (Fisher Scientific cat. no. K749521-1590).

3. Incubate at 55°C for 60 min.

4. To each tube, add 20 µl RNase A. Stand at RT for 2 min.

5. Centrifuge at max for 5 min and transfer supernatant to fresh microcentrifuge tube.

6. To each tube, add:

    a. Lysis/Binding Buffer 200 µl
    b. 100% EtOH 200 µl

7. Vortex.

8. Run lysate through column (using centrifuge or vacuum manifold).

9. Wash with 500 µl Wash Buffer 1. (Spin at max for 1 min. Discard flow through.)

10. Wash with 500 µl Wash Buffer 2. (Spin at max for 1 min. Discard flow through.)

11. Spin once again at max for 1 min and discard flow through.

12. Place column in a collection tube, add 100 µl Elution Buffer and stand at RT for 1 min. Spin at max for 1 min.

Step 2. PCR reaction

1. Prepare PCR master mixes. (20 µl reaction volume each candidate). We are using GoTaq® G2 Green Master Mix (Promega M7823, 1,000 reactions) for high throughput, but you can modify to use your lab's PCR Taq/dNTPs.

Reaction 1		
Template	1.0 µl	Mix:
Primer 1	0.5 µl	µl
Primer 3 or 5	0.5 µl	µl
GoTaq Green	10.0 µl	µl
ddH_2O	8.0 µl	µl

Reaction 2		
Template	1.0 µl	Mix:
Primer 1	0.5 µl	µl
Primer 4 or 6	0.5 µl	µl
GoTaq Green	10.0 µl	µl
ddH_2O	8.0 µl	µl

Reaction 3		
Template	1.0 µl	Mix:
Primer 2	0.5 µl	µl
Primer 3 or 5	0.5 µl	µl
GoTaq Green	10.0 µl	µl
ddH_2O	8.0 µl	µl

Reaction 4		
Template	1.0 µl	Mix:
Primer 2	0.5 µl	µl
Primer 4 or 6	0.5 µl	µl
GoTaq Green	10.0 µl	µl
ddH_2O	8.0 µl	µl

Primer sets:

Primer name		**Sequence**	**Use for**	**Description**
1	**MiLF**	GCGTAAGCTACCTTAATCTCAAGAAGAG	All	Forward primer for Minos
2	**MiLR**	CGCGGCGTAATGTGATTTACTATCATAC	All	Reverse primer for Minos
3	**TagR**	GTGGCTGTTGAAGTTGTACTC	GFSTF	Reverse primer for EGFP
4	**TagF**	GGATGACGGCACCTACAAGAC	GFSTF	Forward primer for EGFP
5	**T2A-R**	GGGTTCTCCTCCACATCGC	TG4	Reverse primer for TG4
6	**GAL4-5F**	AACTGTGCATCGTGCACCATC	TG4	Forward primer for TG4

2. Cycling conditions are as follows (may need to be adjusted for your own Taq).

GFSTF insertions:

94°C	10 min	
94°C	30 sec	|
60°C	30 sec	| 35 cycles
72°C	1 min	|
72°C	10 min	
12°C	hold	

Trojan-GAL4 (TG4) insertions:

94°C	10 min	
94°C	30 sec	|
56°C	30 sec	| 35 cycles
72°C	1 min	|
72°C	10 min	
12°C	hold	

Step 3. Run PCR products on agarose gel

- If gene and original MiMIC insertion are in the same orientation (i.e., either both + or both -), then the successful RMCE should screen positive for PCR reactions 1 and 4 only.

- If gene and original MiMIC insertion are in opposite orientations, then the successful RMCE should screen positive for PCR reactions 2 and 3 only.

See Supplementary Figure 2, Venken et al. (2011) *Nature Methods* 8:737 for an example.