Preparation of genomic DNA from single flies

For many purposes (e.g. genotyping), genomic DNA of sufficient quantity and quality for PCR can be obtained from a single adult fly via this quick and simple protocol. If needed, high-quality DNA can be isolated in larger quantity from multiple flies using a genomic DNA isolation kit (e.g. Qiagen DNeasy kit).

Squishing buffer (SB) recipe:

component	volume	final conc.
dH_2O	965 ul	--
1M Tris-Cl pH 8.0	10 ul	10 mM
100 mM EDTA	10 ul	1 mM
5M NaCl	5 ul	25 mM
20mg/ml proteinase K	10 ul	0.2 mg/ml
	1 ml	

Procedure:

1. Make fresh SB (recipe above). Adjust volume accordingly: you will need 50ul per fly.
2. Knock out flies and transfer a single adult of the desired genotype into an empty 1.5ml tube.
3. Put the tube on ice for a few minutes to knock out the fly.
4. Aspirate 50ul of SB into a 200ul pipet tip.
5. Squish the fly using the pipet tip, without expelling the 50ul of SB (some of the SB will be drawn out of the tip).
6. Expel the remainder of the SB, and mix.
7. Incubate at room temp for 20 min.
8. Incubate at 95°C for 5 min.
9. Ice, then spin down at max speed for 5 min.

Single-fly genomic DNA preps should be stored at 4°C for up to a few months.
For genotyping, use 2.5ul of this genomic DNA prep as template for a 25ul PCR.

Example PCR conditions (for a 2-kb PCR product):

component	volume
dH_2O	12.4 ul
10x Taq Buffer	2.5 ul
dNTP (10mM)	2.5 ul
oligo 1 (10uM)	2.5 ul
oligo 2 (10uM)	2.5 ul
DNA	2.5 ul
Taq DNA polymerase	0.1 ul
	25 ul

cycling conditions:	
1. 95°C	3:00
2. 95°C	0:30
3. 60°C	3:00
4. 72°C	2:00
5. repeat steps 2-4 29x	
6. 72°C	10:00
7. 4°C	hold