From: Single-Fly DNA Preps for PCR

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1. The squishing buffer (SB) is 10 mM Tris-Cl pH 8.2, 1 mM EDTA, 25 mM NaCl and 200 ug/ml Proteinase K, with the enzyme added fresh.
2. Place one fly in a 0.5 ml tube and mash the fly for 5-10 seconds with a pipette tip containing 50 ul of SB, without expelling any liquid (sufficient liquid escapes from the tip). Then expel the remaining SB.
3. Incubate at 25-37^{0}C (or room temp.) for 20-30 minutes.
4. Inactivate the Proteinase K by heating to 95^{0}C for 1-2 minutes.

It is not necessary to remove fly parts for PCR. This preparation can be stored at 4^{0}C for months.

My notes:
-Incubate at 37^{0}C for 30 minutes & then boil for 2 minutes.
-Mash 5 flies in 200 ul of SB plus Proteinase K.
-Alternatively add 2 ul of SB (5ul for 5 flies), mash with just the tip, and then add the remaining amount of SB. (Depending on the pipettor, the tip sometimes doesn't stay on well during mashing step.)

Squishing buffer minus proteinase K (100 ml)
1 ml Tris pH 8.2
200 ul EDTA pH 8.0
500 ul 5 M NaCl

Make cocktail for appropriate number of tubes [e.g. 1 ml SB + 20 ul proteinase K, 20 mg/ml] prior to squishing.

Squish Plus Prep

Steps 1-3 are as described above.

Optional: Add additional squish buffer without proteinase K. (I add 50 ul to the single fly preps to make the phenol extraction easier.)

Add a mixture of Phenol/Sevag (1:1) equal to the final squish volume.
Vortex 45 to 60 seconds; centrifuge for 3-5 minutes; save aqueous top layer

Sevag extract 1X with equal volume.
Vortex 30 seconds; centrifuge for 1 minute; save aqueous top layer

Special Caution: Phenol (corrosive, toxic), chloroform/sevag (anesthetic, toxic)- work in hood, wear proper PPE, dispose waste in proper hazardous waste containers.