Single Fly Genomic DNA Prep

From Georg Dietzl in Barry Dickson's Lab, IMP Vienna 12/2002

SQUISHING BUFFER (SB)
10 mM Tris-HCl pH8
1 mM EDTA
25 mM NaCl
200 g/ml Proteinase K

1. 200 g/ml Proteinase K are added to the squishing buffer (SB) freshly from 20X stock every day.
2. Place 1-2 (I always take 2) flies in a 0.5 ml tube (I use 96 microwell plates from MJ Research since I usually do a larger number of preps at once). Mash the flies for 5-10 sec with a yellow pipette tip containing 50 l of SB, without expelling any liquid (sufficient liquid escapes from the tip). Then expel the remaining SB. (If you use 96 microwell plates you can use a 100 or 300 l 8-channel pipette for mashing the flies. This works really well and is much faster for a larger number of samples)
3. Incubate at 37^{o}C for 30 min
4. Inactivate the Proteinase K by heating to 95^{o}C for 3 min.
5. Spin down the solid junk briefly and pipette the supernatant to a new tube / microwell plate.
6. That's it

Vosshall Lab Modifications and Notes:

(1) Use Disposable Kontes Pellet Pestle for grinding. These come with RNase/DNase free 1.5 ml microcentrifuge tubes that we use to freeze the 2 flies. Packaged separately but included in the catalog number below are RNase/DNase free disposable pestle grinders that are very effective at grinding flies.

Fisher Scientific Catalog Number: K749520-0090 2003 list price: $88.30/100 tubes + pestles

(2) Make a 10mg/ml Roche Proteinase K stock solution in 1XPBS and freeze in small aliquots at -20^{o}C. Immediately before use, add 20ul of this Proteinase K stock solution to 1 ml of SB

(3) Use 1 ul of this crude DNA prep for PCR reactions.