

Single fly genomic DNA preparation

This is a short protocol for the isolation of genomic DNA from a single fly for PCR applications. Of the DNA yield, 1-5 μ l were used to perform PCR reactions.

Squishing Buffer (SB):

10mM Tris-HCl pH8.2
1mM EDTA
25mM NaCl
200 μ g/ml Proteinase K (store 4mg/ml stock solution in -80°C and add 1/20 to the SB just before use)

1. Add Proteinase K to the Squishing Buffer (SB) freshly from the stock.
2. Place each single fly in wells of a 96 well plate and mash the fly for 5-10 seconds with the tips of a multi-pipette containing 50 μ l of SB each, without expelling any liquid (sufficient liquid escapes from the tip). Expel the remaining SB.
3. Incubate at 37°C for 30 minutes.
4. Inactivate the Proteinase K by heating to 95°C for 5 minutes. It is convenient to use the PCR machine.
5. Add 50 μ l of TE or H₂O. Use 1 μ l for PLP-PCR (~200bp), or 2-5 μ l for normal PCR (~1kb).