# DNA extraction from a single fly for PCR

### Introduction:

This procedure is designed to isolate genomic DNA for PCR from single flies.

### Materials:

Squishing buffer: 10 mM Tris-Cl pH 8.2, 1 mM EDTA, 25 mM NaCl

Proteinase K: 20 mg/ml stock (Invitrogen), stored in the freezer

### Preparation:

1. Put each fly to be tested in a 0.5 microfuge tube and label the tube.
2. Thaw the Proteinase K stock and add to a working volume of squishing buffer for 200 µg/ml final Proteinase K.
	1. Note: The squishing buffer can be stored at room temperature, but the Proteinase K must be added fresh each time. Any excess squishing buffer + Proteinase K must be discarded at the end of the day.

### Procedure:

1. Mash each fly for 5 - 10 seconds with a pipette tip containing 50 µl of squishing buffer without depressing the pipette plunger (sufficient liquid escapes from the tip).
2. Expel the remaining squishing buffer into fly mixture.
3. Incubate at 55°C for ½-1 hr
4. Inactivate the Proteinase K by heating to 95 °C for 2-5 minutes. Ensure contact between the tube and the heating block.

### Notes:

* The preparation can be stored at 4 °C for months.
* Typically, 1 µl of the DNA prep is used in a 10-15 µl reaction volume.
* It does not matter if fly parts (wings, bristles, legs) are inadvertently added to the PCR mixture.
* Product will typically start to appear after 24-25 cycles, but 28-30 cycles seems to give maximal yield.
* Increasing the number of flies does not seem to increase the signal significantly, probably due to increasing concentrations of inhibitors.
* There should be no problem scaling up the number of flies screened if the volume is increased proportionately.