Single fly DNA prep for PCR

1) Place one fly in a 0.5 ml tube and mash the fly for 5 - 10 seconds with a pipette tip containing 50 µl of SB, without expelling any liquid (sufficient liquid escapes from the tip). Then expel the remaining SB.
2) Incubate at 25-37 C (or room temp.) for 20-30 minutes.
3) Inactivate the Proteinase K by heating to 95 C for 1-2 minutes.

   85° C heat inactivation allows longer fragments to be amplified -- up to more than 9 kb with long PCR

This preparation can be stored at 4 C for months.

We typically use 1 µl of the DNA prep 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

Squishing Buffer:

Tris-HCl pH = 8.2	10 mM
EDTA	1 mM
NaCl	25 mM
Proteinase K	200 µg.mL^{-1} ***(Add just before use)***