This is based on the procedure of :

Greg Gloor and William Engels, from the early days of PCR

We have developed a simple method for the rapid and

reproducible isolation of DNA from single flies for amplification

by the polymerase chain reaction (PCR) (Saiki et al, Science 239:

487), and direct sequencing by asymmetric PCR (Gyllensten and

Erlich, Proc. Nat. Acad. Sci. 85: 7652).

The simplicity of this

procedure means that the problem of contamination with other

amplified or cloned DNA is greatly reduced. Sufficient DNA is

obtained from one fly for a minimum of 50 PCR analyses, and the

DNA is stable for at least one month in the refrigerator. A

simple modification of this technique allows the isolation of DNA

suitable for use in inverse PCR (Ochman et al, Genetics 120:

621-623). These methods substantially reduce the time involved in

DNA isolation, and among other uses, allows the PCR to be used to

monitor the segregation of an allele for which there is no

phenotype or transposition of an unmarked P element (Engels et

al. Cell 62: 515-525).

A. DNA PREPARATION PROTOCOL:

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 diluted

fresh from a frozen stock each day.

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[o]C (or room temp.) for 20-30 minutes.

4. Inactivate the Proteinase K by heating to 95[o]C for 1-2

minutes.

NOTES:This preparation can be stored at 4[o]C for months. We

typically use 1 ul of the DNA prep in a 10-15 ul reaction volume.

It does not matter if fly parts (wings, bristles, legs) are

inadvertantly 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.

B. DNA PREPS FROM MANY INDIVIDUAL FLIES

A similar method can be used with 96-well (8 x 12) micro plates

to prepare DNA from a large number of individual flies. Up to 80

flies can be tested with a single plate.

1. Place one anesthetized or frozen fly in each well. All rows

(A-H) can be utilized, but leave the first and last columns (1

and 12) empty to ensure complete heating in step 3.

2. Add 50 ul of SB to each well and macerate each fly with a

toothpick for 5-10 seconds. Then cover the plate with an

adhesive-backed strip to prevent evaporation and contamination.

Incubate at room temperature as before.

3. Use two standard-size heating blocks (9.5 x 7.5 cm) pre-heated

to 95[o]C to inactivate the Proteinase K. Sandwich the micro

plate, with its adhesive lid still in place, between the two

heating blocks. The lower block should be inverted so that the

tube holes are facing downward and the flat surface is touching

the bottom of the micro plate. After 2-3 minutes remove the micro

plate and set it on the bench top with the upper hot block still

on top. This upper block will gradually cool to room temperature,

preventing condensation on the underside of the adhesive.

NOTES: It is helpful to tape a piece of waxed paper over the open

micro plate while the PCR tubes are being set up. That way the

DNA samples can be drawn from each well by poking the pipet tip

through the waxed paper. This procedure reduces the possibility

of contamination and helps to keep track of which wells have been

used.

C. INVERSE PCR

PMSF (phenylmethylsulfonylfluoride) can be used instead of the

95[o]C treatment to inactivate the Proteinase K if the DNA preps

are to be used for inverse PCR or other methods that require

double-stranded DNA.

1.Add 1 ul of 0.1 M PMSF to the fly prep following step 3 of

protocol A above. Then heat the mixture to 65[o]C for 10 - 15

minutes to denature any proteins not inactivated by the

proteinase.

2.Add 4 ul of fly supernatant to 16 ul of 1.25X NdeII buffer

(125 mM Tris-HCl pH 7.6, 12.5 mM MgCl[2], 188 mM NaCl, 1.25 mM

DTT). Add 0.5 ul of NdeII (BRL) and incubate at room temp. for 15

min. Inactivate the enzyme by heating to 65[o]C for 15 min.

3. Take 3 ul of digested DNA and add to 7 ul of ligation mix (5

mM MgCl2, 20 mM DTT, 0.8 mM ATP). Add 0.5 ul T4 DNA ligase (NEB)

and incubate at room temperature for 20-30 min. Inactivate the

enzyme by heating to 95[o]C for 2-3 min; this also serves to nick

the DNA.

NOTES:For the first attempt with a new insertion, we recommend

using the following conditions: denature at 94[o]C for 45 sec.,

anneal at 60[o]C for 45 sec., extend at 72[o]C for 4 min; try

30 - 35 cycles. - The restriction enzyme appears to be the most

critical component in this protocol. The enzyme must be specific

under conditions of very low DNA concentration. Sau3A1, for

example, is too promiscuous and digests at several sites in

addition to its canonical restriction sequence. The protocol

should work with other enzymes. The protocol has been used for a

combined inverse/asymmetric PCR procedure to get DNA sequences

flanking P element insertions.