INVERSE PCR (Jill)

1. Digest 2.5μg of genomic DNA with restriction enzymes. Stop reaction at 65°C for 20'.

2. Fractionate on a 1% agarose gel to check DNA has been cut.

3. Set up self-ligation reaction:

40μl	10 x DNA Ligase buffer
1.5μl	NEB concentrated DNA ligase
358.5μl	1μg digested DNA plus dH_2O

   Incubate overnight at room temperature. Do not put in more that 1μg of DNA as fragments may ligate to each other rather than to themselves.

4. Add 400μl 25:24:1 phenol:chloroform: IAA. Vortex and spin for 5' in microfuge. Remove aqueous layer to fresh tube.

5. Add 2.5 volumes ethanol and 0.1 volume 3M NaOAc pH4.5. Precipitate at –20°C overnight or at –80°C for 1 hr.

6. Spin in microfuge for 10' at full speed. Wash pellet with 70% ethanol, spin again and drain off supernate.

7. Resuspend pellet in 30μl dH_2O and check concentration on a gel or by OD_{260}

8. Set up PCR with 50ng DNA using extend taq for long products:

37.5 μl	Ligated DNA plus dH_2O
5 μl	10 X PCR buffer
4 μl	25 mM $MgCl_2$
1 μl	10 mM dNTP's
1 μl	Primer 1 (10μM)
1 μl	Primer 2 (10μM)
0.5 μl	Taq DNA Polymerase

50 μl TOTAL

9. Cycle:

94°C (45s)	X 1
94°C (15s), 50°C (30s), 68°C (4′)	X 10
94°C (15s), 50°C (30s), 68°C (4′ plus 5s per cycle)	X 20
72°C (7′)	X 1

10. 25 μl of the final PCR product should be run out on a 1% agarose gel, viewed and blotted for band verification by hybridization. The remaining 25 μl of the PCR reaction can then be used, if positives are forthcoming, to clone and sequence the band of interest.