INVERSE PCR (Susie)

1. Digest approximately 2 μg of genomic DNA with the appropriate enzyme in a 50 μl reaction. i.e.:

DNA	2 μg
10x buffer	5 μl
spermidine (100mM)	1.5 μl
restriction enzyme (10 U/μl)	2 μl
dH_2O	to 50 μl

2. Clean the digest using a Wizard DNA Clean-up System kit (or other similar method – final volume should be about 50 μl)

3. Self ligate 12.5 μl of the cleaned DNA in a 100 μl reaction. i.e.:

DNA	12.5 μl
10 x T4 DNA ligase buffer	10 μl
T4 DNA ligase	1 unit
dH_2O	to 100 μl

4. Incubate at 12°C overnight.

5. The PCR is based on the Boehringer Mannheim Expand Long Template PCR System protocol. Set up two reactions, one with about 10 μl of ligated DNA and the other with about 20 μl of ligated DNA. A hot-start method is used, so use two 25 μl mixes for each 50 μl reaction, as follows:

Mix 1

ligated DNA (see step 3)	10/20 μl
dNTPs (25 mM)	1.25 μl
each primer (10 μM)	1.5 μl
dH_2O	to 25 μl

Mix 2

	10x buffer 3 (Expand Long Template System kit)	5 μl
	Expand enzyme mix (DNA polymerases)	0.75μl
	dH_2O	19.25 μl

6. Overlay Mix 1 with about 30 μl paraffin oil, then heat the tubes to 94°C, in the first step of the PCR. Once the tubes are at 94°C, add Mix 2, beneath the oil. Then run the PCR:

35 rounds of	94°C	10 secs
	55°C	30 secs
	68°C	2 minutes
then	68°C	10 minutes

7. A nested reaction can then be carried out, either using 1 μl of the first reaction as template, or by running the reaction on a gel, gel-extracting the band of interest and then using this as template. I found the second method gave a clearer, stronger band when the nested PCR products were subsequently run on a gel.