INVERSE PCR (Tom)

1. Digest approx. 30-50 μg of genomic DNA and fractionate on an 0.8% agarose gel.

2. Excise appropriately sized fraction and purify with Geneclean (Bio101) according to manufacturer's instructions. Elute twice with 20 μl dH_2O. Final concentration should be approximately 10 $ng\mu l^{-1}$.

3. Set up self-ligation with 10 ng of cleaned DNA:

DNA	1 μl
5 x ligase buffer	10 μl
BRL low. conc. ligase	1 μl
dH_2O	38 μl

4. Incubate overnight at 4°C.

5. Add 50 μl 1 x TE buffer to ligation, heat kill at 65°C for 10 minutes, and purify with a Qiagen spin column to remove remaining salts. Elute in 50 μl dH_2O.

6. Set up first round PCR, adding primers last:

purified ligation	10 μl
10 x buffer	5 μl
DMSO	2 μl
dNTPs (10 mM)	1 μl
Taq	2.5 units
each primer (25 μM)	1 μl
$MgCl_2$ (25 mM)	3 μl
dH_2O	27 μl

7. Run PCR:

Step 1	94°C	2 minutes

Step 2	94oC	45 seconds
Step 3	55°C	1 minute
Step 4	72°C	2 minutes
Step 5	repeat step 2-5, 25 times	
Step 6	72°C	10 minutes

8. Add 1 μl of PCR product to 200 μl dH_2O, and set up a second round PCR
   with nested primers (repeat steps 6 and 7).

9. Run out 10 μl of PCR products to identify and confirm sizes of bands.

10. Gel purify and clone fragments.

10 x buffer
500 mM Tris pH9.1
140 mM $(NH_4)_2SO_4$