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$_2$O. Final concentration should be approximately 10 ngµl$^{-1}$.

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$_2$O  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$_2$O.

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$_2$O  27 µl

7. Run PCR:

   Step 1  94°C  2 minutes
Step 2 94°C 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 dH₂O, 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 pH 9.1
140 mM (NH₄)₂SO₄