

**Inverse PCR from p[SuPoR-P] (BDGDP KG lines) in the Featherstone lab**

1. Isolate genomic DNA from 20-30 flies using Gentra PureGene DNA isolation kit. Instead of the kit's "DNA Hydration Solution", resuspend DNA in 60 ul H<sub>2</sub>O.
2. Genomic DNA digestion:
  - a) 20 ul DNA solution (of the 60ul from step 1)
  - b) 5 ul restriction buffer
  - c) 23 ul H<sub>2</sub>O
  - d) 2 ul HpaII restriction enzyme
  - e) Incubate 2.5h at 37°C.
  - f) Heat inactivate enzyme: 20 minutes at 65°C.
3. Ligation:
  - a) Entire 50ul digest reaction (from step 2)
  - b) 40ul of 10x ligation buffer
  - c) 310ul H<sub>2</sub>O
  - d) 2ul T4 DNA ligase
  - e) Incubate overnight at 4°C.
4. PCR:
  - a) 37ul H<sub>2</sub>O
  - b) 5ul ligation reaction (from step 3)
  - c) 1ul of forward/reverse primer\* mix (0.5ul each at ~0.33ug/ul)
  - d) 5ul 10X PCR buffer
  - e) 1ul dNTP mix (10mM each dNTP)
  - f) 1ul Taq polymerase

\*For 3' end inverse PCR, use primers:

3.rev.hpa2 (TTGCCACTTGCTCATACGTC)  
Pry4 (CAA TCA TAT CGC TGT CTC ACT CA)

For 5' end inverse PCR, use primers:

Plac1 (CACCCAAGGCTCTGCTCCCACAAT)  
Pwht1 (GTAACGCTAATCACTCCGAACAGGTCACA)

For 3' iPCR:

95°C, 5 min

95°C, 30 sec -- 55°C, 1 min -- 68°C, 2 min (35 cycles)

72°C, 10 min

For 5' iPCR:

95°C, 5 min

95°C, 30 sec --60°C, 1 min -- 68°C, 2 min (35 cycles)

72°C, 10 min

5. Use Qiaquick PCR purification kit (for now; might switch) to purify PCR product.

Dilute product appropriately in H<sub>2</sub>O for sequencing (for details see: [www.rrc.uic.edu/SERVICES/DNAS/dna\\_sequencing\\_policies\\_and\\_procedures.html](http://www.rrc.uic.edu/SERVICES/DNAS/dna_sequencing_policies_and_procedures.html)).

Sequencing primers:

Sequence 3' iPCR products with oligo 3.SUP.seq1 (TAT CGC TGT CTC ACT CAG)

Sequence 5' iPCR products with oligo 5.SUP.seq1 (TCC AGT CAC AGC TTT GCA GC)

Protocol based on: <http://flypush.imgen.bcm.tmc.edu/pscreen/faq.html> (Bellen lab)