

Inverse PCR from p[GT1] in the Featherstone lab

1. Isolate genomic DNA from 20-30 flies using Genra PureGene DNA isolation kit. Instead of the kit's "DNA Hydration Solution", resuspend DNA in 60 ul H₂O.
2. Genomic DNA digestion:
 - a) 20 ul DNA solution (of the 60ul from step 1)
 - b) 5 ul restriction buffer
 - c) 23 ul H₂O
 - d) 2 ul HinP1 I (or Sau3A I) 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 H₂O
 - d) 2ul T4 DNA ligase
 - e) Incubate overnight at 4°C.
4. PCR:
 - a) 37ul H₂O
 - 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:

Pry1 (CCT TAG CAT GTC CGT GGG GTT TGA AT)

Pry4 (CAA TCA TAT CGC TGT CTC ACT CA)

For 5' end inverse PCR, use primers:

pGT1.5a (CCG CAC GTA AGG GTT AAT G)

pGT1.5d (GAA GTT AAG CGT CTC CAG G)

95°C, 5 min

95°C, 30 sec -- 55°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 H₂O for sequencing (for details see:
www.rrc.uic.edu/SERVICES/DNAS/dna_sequencing_policies_and_procedures.html).

Sequencing primers:

Sequence 3' end with primer Sp_{ep}1 (GAC ACT CAG AAT ACT ATT C)

Sequence 5' end with primer Sp₁ (ACA CAA CCT TTC CTC TCA ACA A)