Breyer Lab EP2 PCR protocol

**EP2 oligos:**

- **EP2.806a** mer: 21  
  Sequence: 5’ gtg cat gcg aat gag gtt gag  3’

- **EP2.249s2** mer: 18  
  Sequence: 5’ ccg ggg ttc tgg gga atc  3’

- **pPNT.1803s** mer: 24  
  Sequence: 5’ ttg cca agt tct aat tcc atc aga  3’

**For wild type master mix (per DNA sample to be run):**

- 23.2 µl Invitrogen Platinum PCR mix (catalog #11306-016)
- 0.5 µl oligo 806 at 20 µM concentration
- 0.5 µl oligo 249 at 20 µM conc.

  Add 24.2 µl of master mix & 0.8 µl DNA sample to each PCR reaction tube

**For knock out master mix (per DNA sample to be run):**

- 23.2 µl Invitrogen Platinum PCR mix
- 0.5 µl oligo 806 at 20 µM conc.
- 0.5 µl oligo 1803 at 20 µM conc.

  Add 24.2 µl of master mix & 0.8 µl DNA sample to each PCR reaction tube

**PCR cycle is as follows: (run in a PE9600)**

- Hold at 94°C for 3 minutes
- Cycle through 35 times:  
  94°C for 30 seconds  
  56°C for 30 seconds  
  72°C for 1 minute
- Hold at 72°C for 10 minutes
- Hold at 4°C ∞

✔ Reactions are analyzed on a 1.5% 3:1::low melt: regular agarose gel.  
✔ Expected sizes are 578 bp for wild type & 298 bp for knock-out.  
✔ We do not multiplex the two reactions because this increases false negatives.
Sample EP2 gel:

Wild type = 578 bp

Knock out = 298 bp