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.1803**s 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:

