

Breyer Lab floxEP3 PCR protocol

Oligos:

- **EP3LE1.s:** tgt ttt gcc tgc atg tat gta
- **EP3m1.a:** acc caa atg aga ctt cag aaa

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

- 1.0 μ l Promega H₂O
- 5.0 μ l Sigma REDExtract-N-Amp™ PCR ReadyMix™ (catalog #R-4775)
- 1.0 μ l oligo EP3LE1.s (10.5 μ M)
- 1.0 μ l oligo EP3m1.a (10.5 μ M)

Add 8.0 μ l master mix & 2.0 μ l DNA sample to each PCR reaction tube

PCR cycle is as follows (run in a PE9600)

- Hold at 94°C for 3 minutes
- Cycle 35 times:
 - 94°C for 15 seconds
 - 58°C for 15 seconds 72°C for 30 seconds
- Hold at 72°C for 10 minutes
- Hold at 4°C ∞

Analyzing the reactions:

- Reactions are analyzed on a 1% agarose gel (1g regular agarose dissolved in 100mL 1X TAE) with 10 μ l GelRed™ Nucleic Acid Gel Stain (Biotium, catalog #41000).
- Expected sizes are **718 bp** for floxEP3 band & **641 bp** for wild type band.

