

Breyer Lab floxed EP₄ PCR protocol

EP₄ oligos:

- **EP₄flox.a** mer: 23
sequence: TCT GTG AAG CGA GTC CTT AGG CT
- **EP₄flox.s** mer: 24
sequence: GTT AGA TGG GGG GAG GGG ACA ACT

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

- 23.2 µl Invitrogen Platinum PCR mix (catalog #11306-016)
- 0.5 µl oligo EP₄flox.a (at 20 µM concentration)
- 0.5 µl oligo EP₄flox.s (at 20 µM concentration)

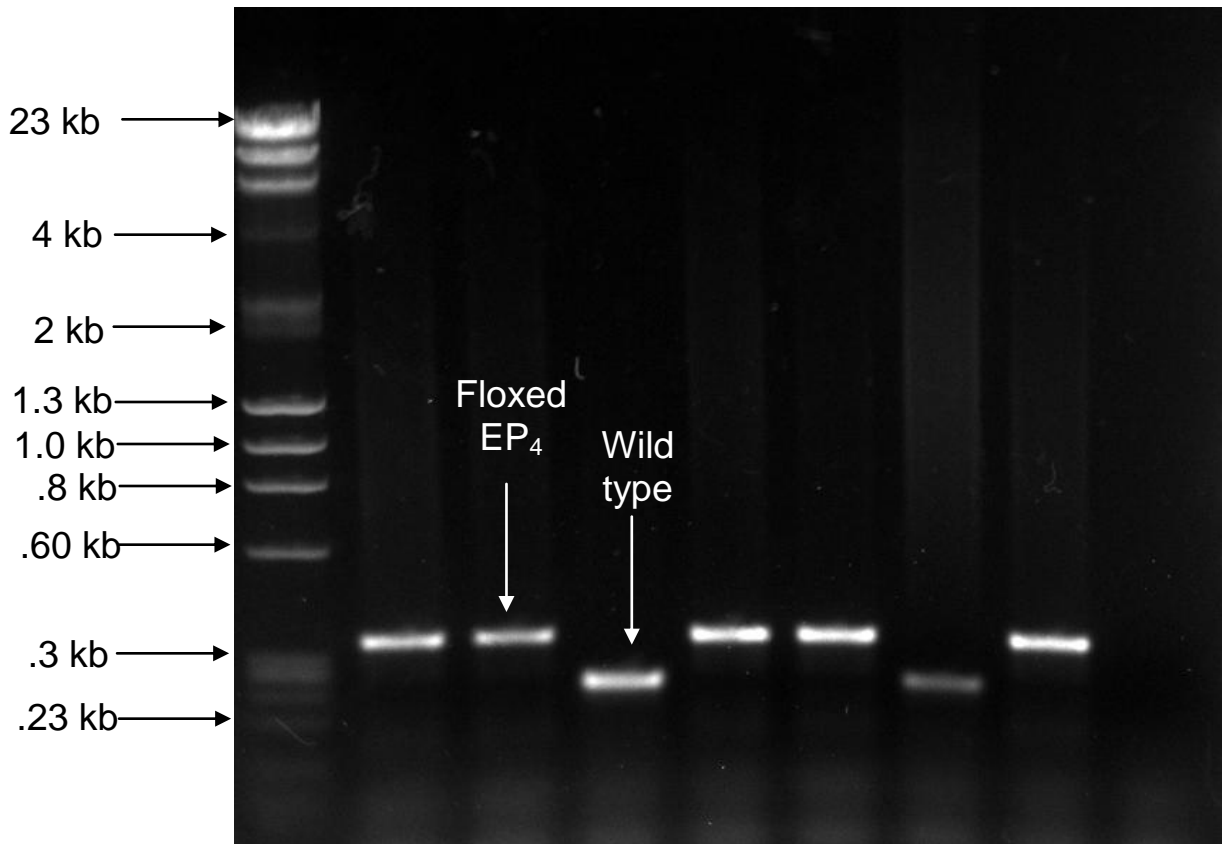
Add 24.2 µl master mix to each PCR reaction tube. Then add 0.8 µl of each DNA sample to PCR reaction tubes.

PCR cycle is as follows (run in a Perkin Elmer 9600):

- Hold at 94°C for 3 minutes
- Cycle 35 times:
 - 94°C for 15 seconds
 - 57.4°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 10µl GelRed™ Nucleic Acid Gel Stain (Biotium, catalog #41000).
- Expected sizes are **243 bp for wild type & 344 bp for floxEP₄**.
- See sample gel on following page:



Expected sizes:
Floxed EP4= **344 bp**
Wild type= **243 bp**