PCR of mouse LOXP (Cleavage Template)

Jing-Yi Chung

Abstract

Citation: Jing-Yi Chung PCR of mouse LOXP (Cleavage Template). **protocols.io**
dx.doi.org/10.17504/protocols.io.hedb3a6
Published: 14 Apr 2017

Protocol

PCR Mix

Step 1.

Prepare PCR mix by adding 50µl of H2O, 16µl of GC Buffer, 1.6µl of dNTPs, 4µl of 10µM LOX fwd (57.3℃) primer, 4µl of 10µM LOX rev (55.9℃) primer, and 200ng of LOXP DNA (target DNA).

Thermocycler

Step 2.

Prior to adding Phusion, set up thermocycler protocol: 95℃ 5mins, [95℃ 30sec (Denaturation), 56℃ 30sec (Annealing), 72℃ 30sec (Elongation)] x 30, 72℃ 5mins, 4℃ on hold.

Add Phusion 0.8µl into PCR mix and mix thoroughly (pipette up and down), and divide the mix into 20µl or 40µl per tube.

Place tubes in the thermocycler and run thermocycler protocol.

Gel Preparation

Step 3.

Prepare 15% gel by adding 600mg of agorose into 40ml 1xTB Buffer. Heat mixture in microwave for 1.5 min and add 4µl cybersafe. Insert two small combs and pour gel mixture. Let gel cool and solidify.

Load Sample into Gel

Step 4.

Load 2µl 1kb ladder with 2µl 6xLD in first well. Load 2µl of the sample from each tube with 2µl of 6xLD. Load total 4µl into well. Run gel on 80V for 40 mins.

See whether the PCR is successful.