

Cre and IL-2 PCR

Purpose: To identify the presence of Cre transgene. IL-2 is also screened as an internal control.

Cre recombinase: origin - Enterobacteria phage P1
 sequence information from genbank - NC_005856

Primers:

Cre gene (the IL2-1 and IL2-2 primer information was obtained from Jackson Lab website)

- IL2-1: 5' CTA GGC CAC AGA ATT GAA AGA TCT 3'
- IL2-2: 5' GTA GGT GGA AAT TCT AGC ATC ATC C 3'
- CreF636: 5' ACC TGA AGA TGT TCG CGA TT 3'
- CreR817: 5' CGG CAT CAA CGT TTT CTT TT 3'

PCR Reaction

1. Genomic DNA (1 µl)
2. Promega PCR Master Mix with green dye (2x; 10 µl)
 Part# M712C, Lot# 25324501, GoTaq Green Master Mix, 2X
3. Primers (10 pmol/µl), 0.28 µl/each for IL2-1, IL2-2, and 0.39 µl/each for CreF636 and CreR817
4. PCR water (7.5 µl)
 Total reaction volume is 20 µl

- Program
1. 1 cycle - 94°C for 5 min
 2. 35 cycles - 94°C for 45 sec
 57.5 °C for 1 min
 72°C for 1 min
 3. 1 cycle - 72°C 6 min
 4. Hold at 4°C.

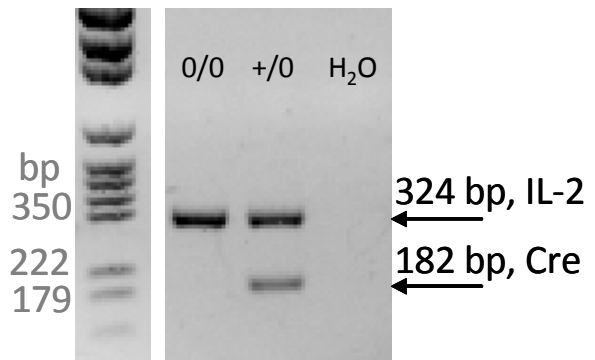
Expected Bands on TBE Agarose Gel Electrophoresis:

Resolve DNA bands on a 2% agarose gel.

	Cre0	Cre+
Cre		182 bp
IL-2	324 bp	324 bp

Notes:

Primer and DNA volumes can be slightly modified.



Prepared by Hong Lu on 7/18/2008