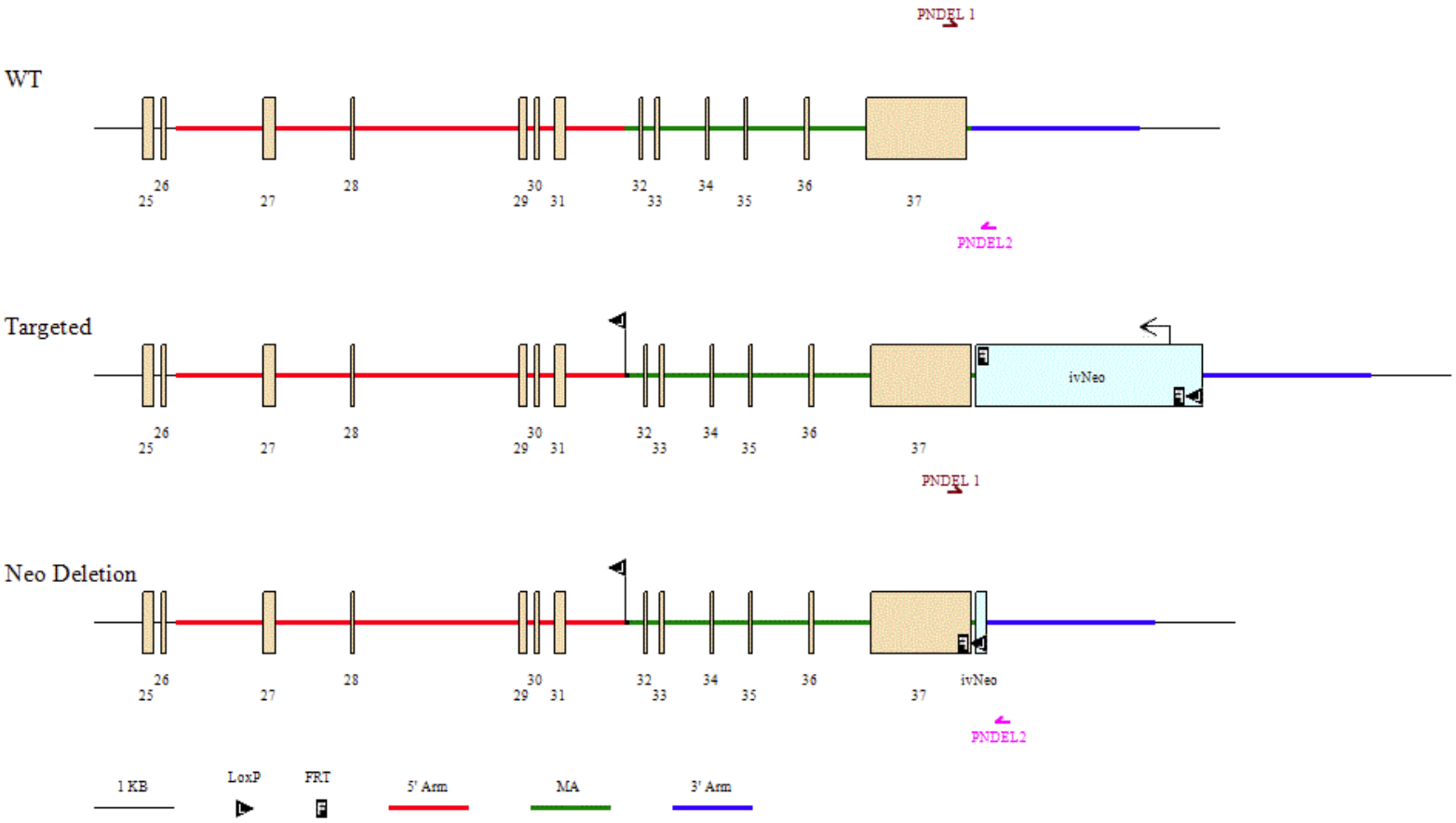
Conditional KO: PCR Screening of Mice

Sections:

I. Identification of Germline Neo Deleted Mice.

1. Schematic and Information

Tail DNA was extracted and analyzed as described below.



Primers for PCR Screening:

Forward Oligos

PNDEL1: 5′- PROJECT SPECIFIC -3′

Reverse Oligos

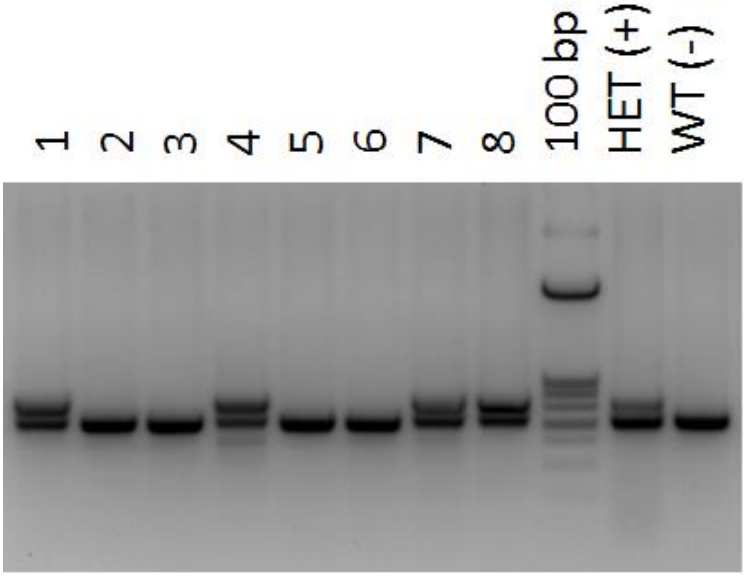
PNDEL2: 5′- PROJECT SPECIFIC -3′

newFLP1: 5′- PROJECT SPECIFIC -3′
newFLP2: 5′- PROJECT SPECIFIC -3'

*The FLP primers cannot be seen in the schematic above.

2. Screening for Neo Deletion

Primer set PNDEL1 and PNDEL2 was used to screen mice for the deletion of the Neo cassette. The PCR product for the wild-type is 487 bp. After Neo deletion, one set of LoxP-FRT sites remain (123 bp). A second band with a size of 610 bp indicates Neo deletion.

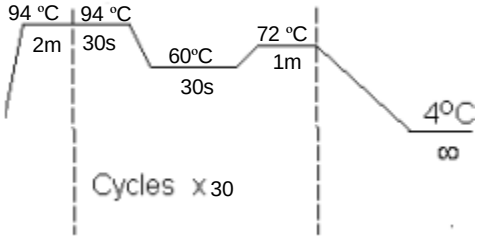


PNDEL1 / PNDEL2 (487 bp / 610 bp)

PCR Parameters for PNDEL1 / PNDEL2:

EconoTaq Plus Green 2x Master Mix (Lucigen catalog# 30033-1)

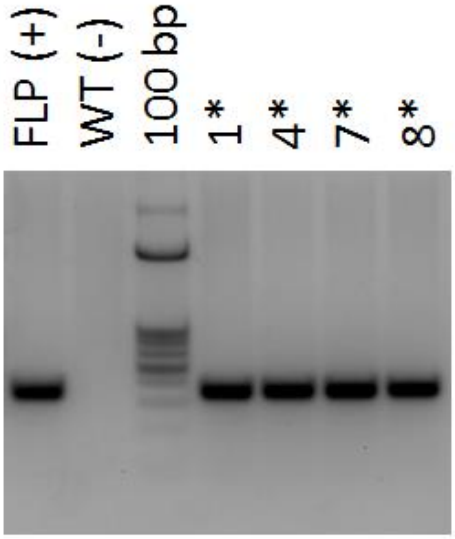
11.00 μL ddH_20
12.50 μL EconoTaq Plus Green 2x Master Mix
0.25μL 100 μM Primer
1.00 μL DNA



After a 2 minute hot start at 94°C the samples were run using the above conditions. The PCR product was run on a 2% gel with a 100 bp ladder as reference. The top band representing Neo deletion was excised and sequenced in positive samples. The expanded ES clone, which was used as a positive control, is denoted by a (+) in the gel photograph above.

3. Screening for FLP Transgene

Primer set newFLP1 and newFLP2 was used to screen mice for the FLP transgene. The amplified product for primer set newFLP1 and newFLP2 is 330bp.

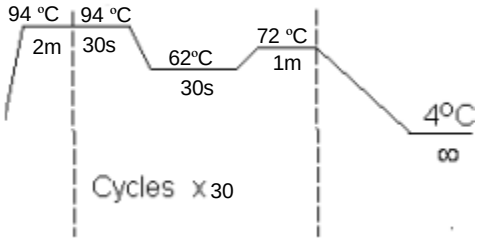


newFLP1 / newFLP2 (330 bp if FLP transgene present)
(*Asterisked mice are FLP present)

PCR Parameters for newFLP1 / newFLP2:

EconoTaq Plus Green 2x Master Mix (Lucigen catalog# 30033-1)

11 μL ddH_20
12.5 μL EconoTaq Plus Green 2x Master Mix
.25μL 100 μM Primer
1.0 μL DNA

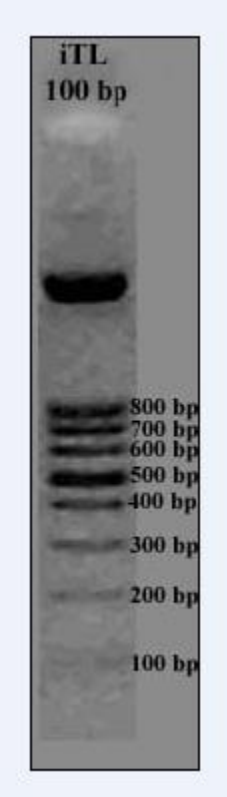


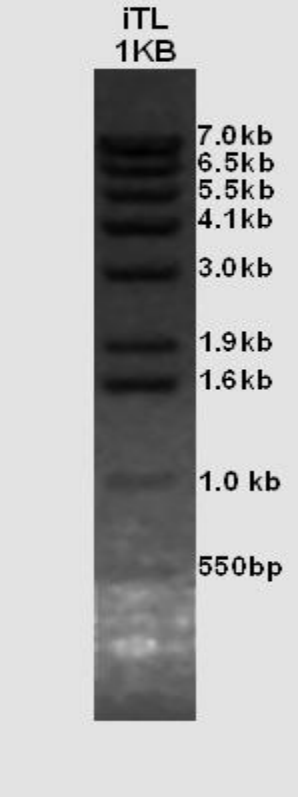
After a 2 minute hot start at 94°C the samples were run using the above conditions. The PCR product was run on a 2% gel with a 100 bp ladder as reference. Tail DNA sample from a FLP mouse was used as a positive control and is denoted by a (+) in the gel photographs.

4. Mouse Genotype Information

Mouse #	Genotype
1	Heterozygous, FLP+
2	Wild Type
3	Wild Type
4	Heterozygous, FLP+
5	Wild Type
6	Wild Type
7	Heterozygous, FLP+
8	Heterozygous, FLP+

5. Reference


iTL
100 bp
800 bp
700 bp
600 bp
500 bp
400 bp
300 bp
200 bp
100 bp


iTL
1KB
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5.5kb
4.1kb
3.0kb
1.9kb
1.6kb
1.0 kb
550bp