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2255 Martin Ave., Suite E. Santa Clara, CA 95050

1.2. loxP-2 PCR

Primers for loxP-2 PCR:
F2: 5'-CAATCCTATCATTTACGCCCTGC-3'
R2: 5'-GTTTCCAGTAGAAAGACAGGTGGT-3'

Expected PCR Product:
Wildtype: 227 bp
Targeted: 261 bp

Reaction Mix:

Component	x1
Mouse genomic DNA	1.5 µl
Forward primer (10 µM)	1.0 µl
Reverse primer (10 µM)	1.0 µl
Premix Taq Polymerase	12.5 µl
ddH_2O	9.0 µl
Total	25.0 µl

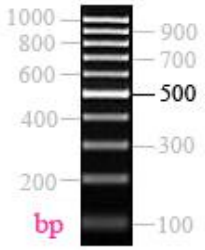
Cycling Condition:

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	
Denaturation	94 °C	30 s	
Annealing	62 °C	35 s	35 x
Extension	72 °C	35 s	
Additional extension	72 °C	5 min	

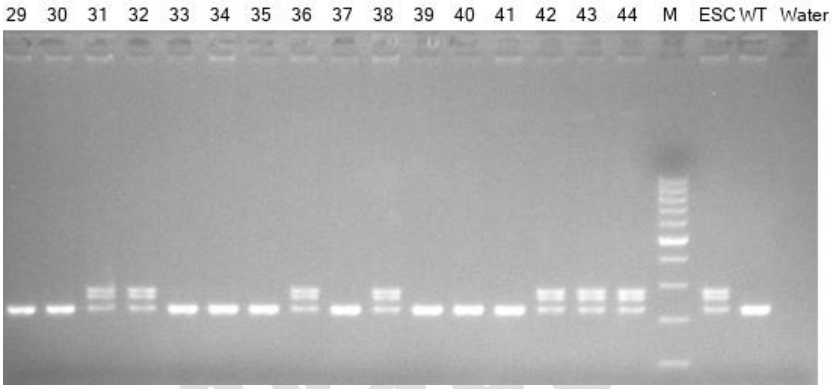
Result

Seven pups (31#, 32#, 36#, 38#, 42#, 43# and 44#) from clone 2B5 were identified positive by PCR screening for loxP-2.

Marker



loxP-2 PCR, clone: 2B5 (WT: 227 bp; MT: 261 bp)



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1.3. Neo-del PCR

Primers for Neo-del PCR:
F1: 5'-GCGCCATAACTTCGTATAGCAT-3'
R1: 5'-TTGGCTTCTTCTACTGGAGCTGTC-3'

Expected PCR Product:
Wildtype: N.A.
Targeted: 517 bp

Reaction Mix:

Component	x1
Mouse genomic DNA	1.5 µl
Forward primer (10 µM)	1.0 µl
Reverse primer (10 µM)	1.0 µl
Premix Taq Polymerase	12.5 µl
ddH_2O	9.0 µl
Total	25.0 µl

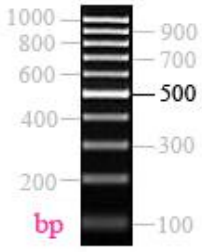
Cycling Condition:

Step	Temp.	Time	Cycles
Initial denaturation	94 °C	3 min	
Denaturation	94 °C	30 s	
Annealing	62 °C	35 s	35 x
Extension	72 °C	35 s	
Additional extension	72 °C	5 min	

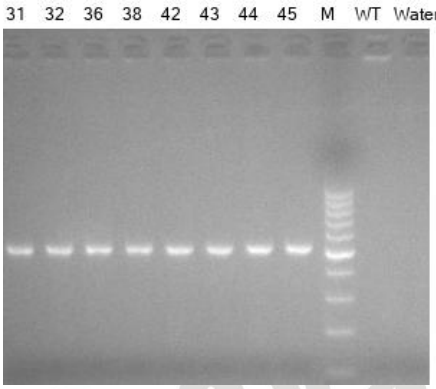
Result

Seven pups (31#, 32#, 36#, 38#, 42#, 43# and 44#) from clone 2B5 were identified positive by PCR screening for Neo-del.

Marker



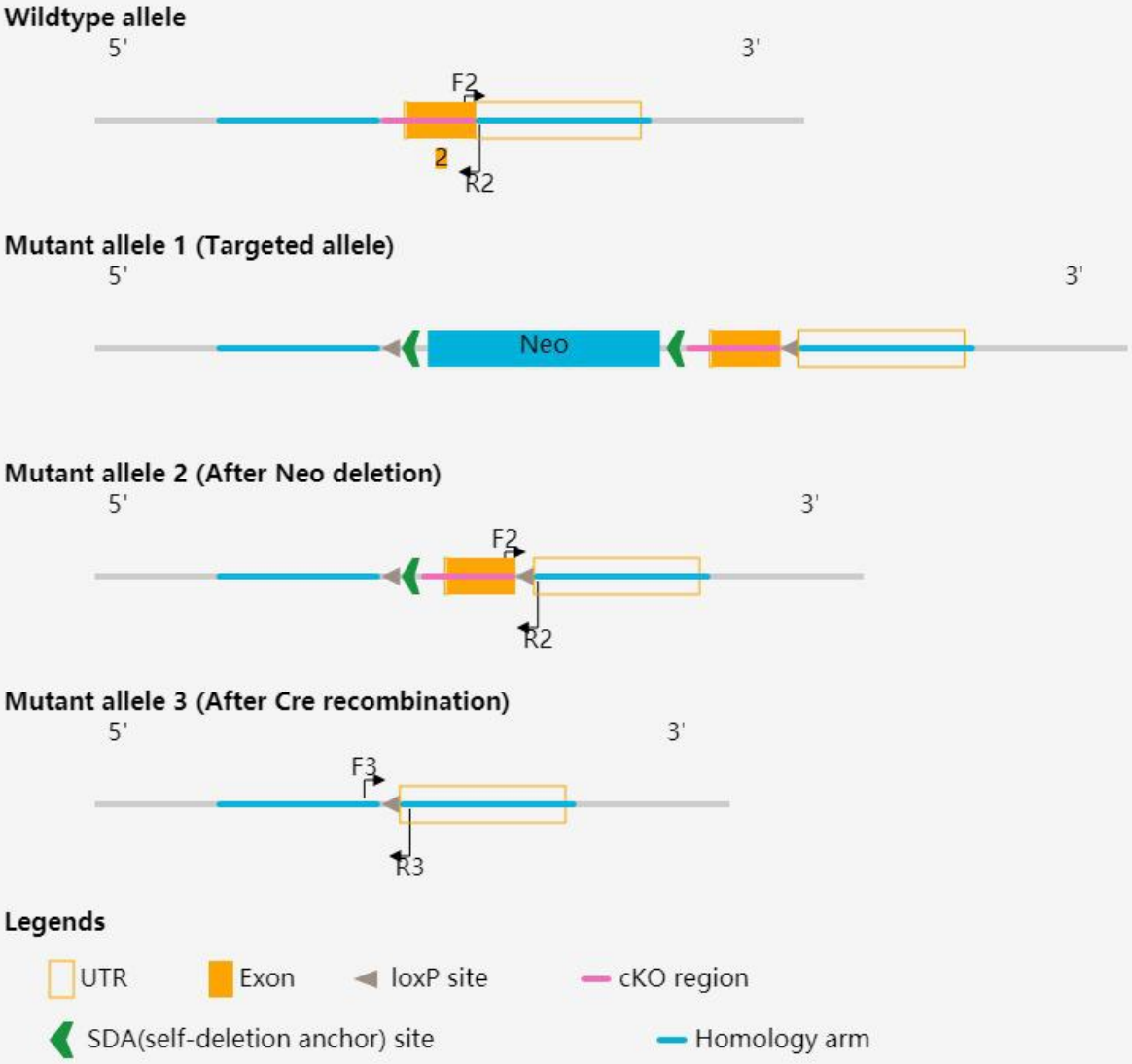
Neo-del PCR, clone: 2B5 (WT: N.A.; MT: 517 bp)



1.4. PCR Result:

Seven pups (31#, 32#, 36#, 38#, 42#, 43# and 44#) from clone 2B5 were identified positive by PCR screening for loxP-2 and Neo-del, the positive pups were reconfirmed by PCR screening for Neo-del.

1.5. Suggested Breeding and Genotyping Assay for Tissue-specific Knockout Mice Generation



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Step 1: Inter-cross heterozygous targeted mice to generate homozygous targeted mice

Primers for targeted allele:
F2: 5'-CAATCCTATCATTTACGCCCTGC-3'
R2: 5'-GTTTCCAGTAGAAAGACAGGTGGT-3'

Wildtype: 227 bp
Homozygotes: 261 bp
Heterozygotes: 261 bp/227 bp

Step 2: Breed a homozygous targeted mouse with a tissue-specific Cre delete mouse to generate mice that are heterozygous for a targeted allele and a hemizygous/heterozygous for the Cre transgene

Primers for targeted allele:
F2: 5'-CAATCCTATCATTTACGCCCTGC-3'
R2: 5'-GTTTCCAGTAGAAAGACAGGTGGT-3'

Heterozygotes: 261 bp/227 bp

Primers for Cre transgene:
Forward1: 5'-CATATTGGCAGAACGAAAACGC-3'
Reverse1: 5'-CCTGTTTCACTATCCAGGTTACGG-3'

Cre amplicon: 413 bp

Step 3: Breed heterozygous, Cre+ mice with homozygous mice. Approximately 25% of the progeny from this mating will be homozygous for the targeted allele and hemizygous/heterozygous for the Cre transgene. The pups can be screened by the same assay as described above. The tissue-specific gene deletion can be confirmed by the following primers:

Primers for targeted allele:
F2: 5'-CAATCCTATCATTTACGCCCTGC-3'
R2: 5'-GTTTCCAGTAGAAAGACAGGTGGT-3'

Conditional KO allele: 261 bp
Wildtype allele: 227 bp

F3: 5'-TGCTTGGCAGCAAACTATAAATGG-3'
R3: 5'-TTCCATTACCCTAGAACTGGCTTC-3'

Constitutive KO allele: 456 bp