

Mouse Cnr2 Conditional Knockout Project

Objective:

To create a mouse Cnr2 conditional knockout model in C57BL/6N mice.

Strategy summary:

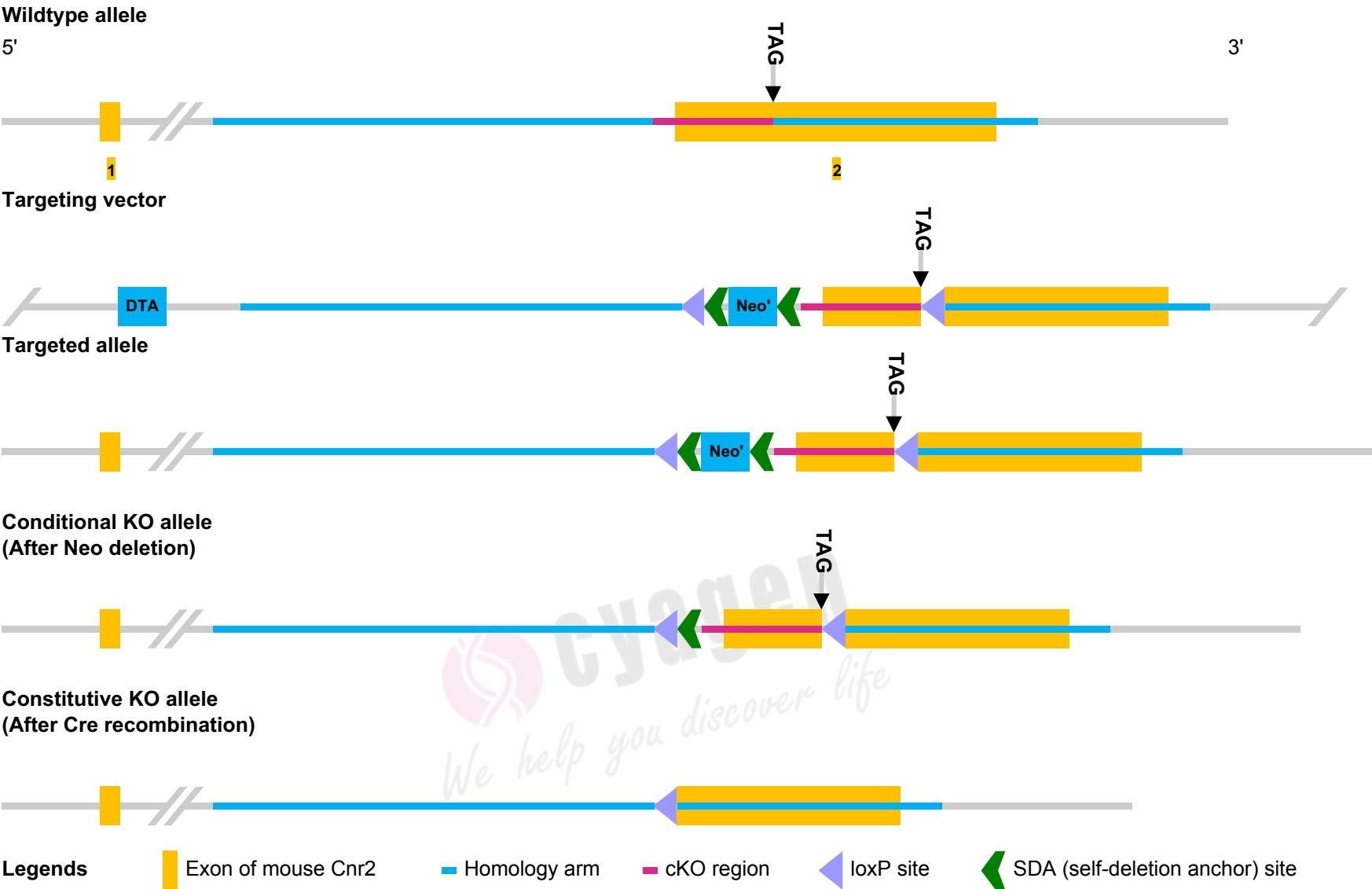
- The Cnr2 gene (NCBI Reference Sequence: NM_009924; Ensembl: ENSMUSG00000062585) is located on mouse chromosome 4.
- 2 exons are identified, with the ATG start codon in exon 2 and the TAG stop codon in exon 2 (Transcript: ENSMUST00000097843).
- Exon 2 will be selected as conditional knockout region (cKO region). Deletion of this region should result in the loss of function of the mouse Cnr2 gene.
- To engineer the targeting vector, homologous arms and cKO region will be generated by PCR using BAC clone [RP23-46F2](#) as template.
- In the targeting vector, the Neo cassette will be flanked by SDA (self-deletion anchor) sites. DTA will be used for negative selection.
- C57BL/6N ES cells will be used for gene targeting.
- The KO allele will be obtained after certain Cre-mediated recombination.

Note: Macrophages from homozygous mutant animals are resistant to the inhibitory effects of delta9-Tetrahydrocannabinol. Alopecia is seen in some but not all homozygotes.

- Exon 2 covers 100.0% of the coding region. Start codon is in exon 2, and stop codon is in exon 2.
- The size of effective cKO region: ~1360 bp.
- The cKO region does not have any other known gene.

Template Needed	Cost of Purchase
BAC: RP23-46F2	TBD

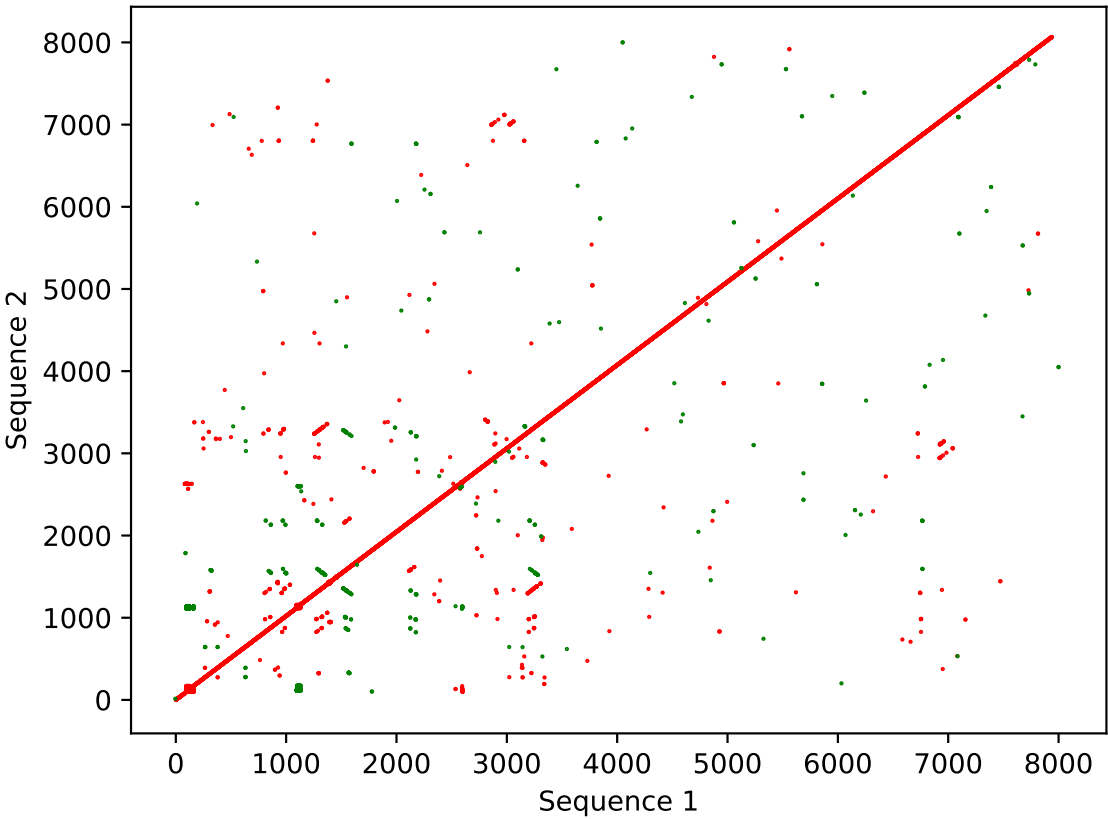
Overview of the Targeting Strategy



Overview of the Dot Plot

Window size: 10 bp

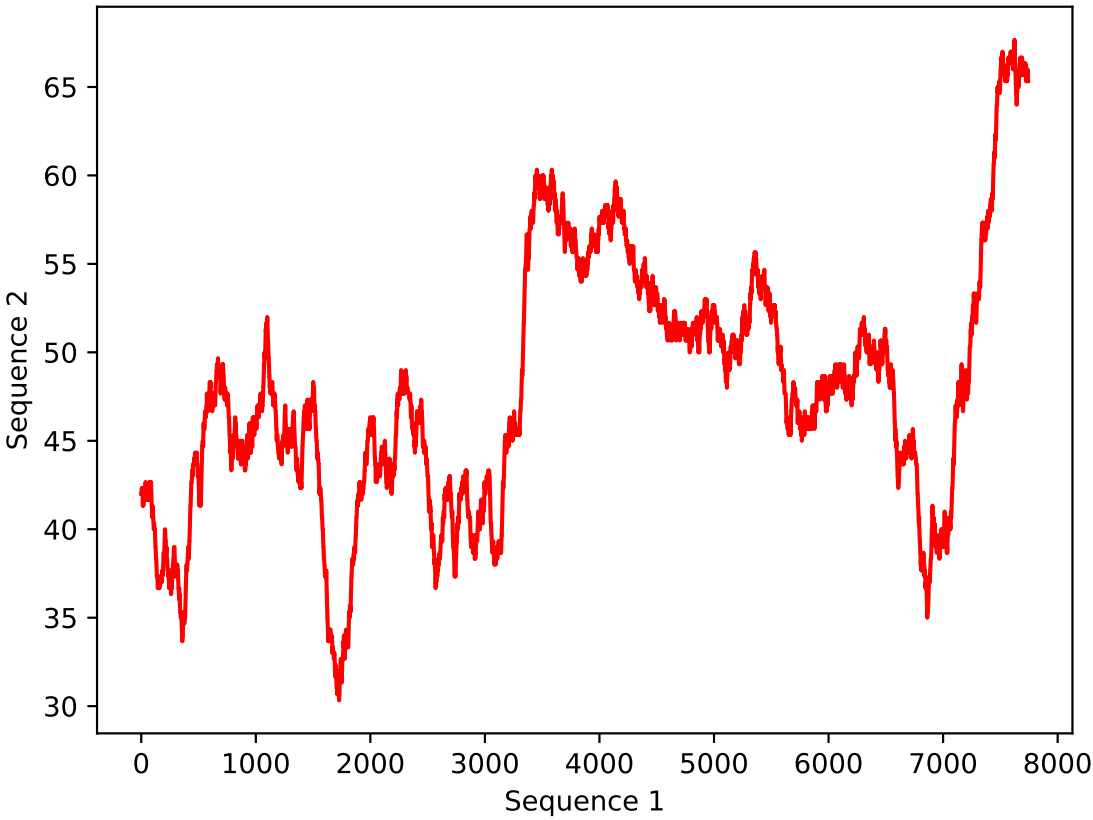
● Forward ● Reverse Complement



Note: The sequence of homologous arms and cKO region is aligned with itself to determine if there are tandem repeats. Tandem repeats are found in the dot plot matrix. It may be difficult to construct this targeting vector.

Overview of the GC Content Distribution

Window size: 300 bp



Summary: Full Length(8041bp) | A(25.68% 2065) | C(24.28% 1952) | T(26.14% 2102) | G(23.9% 1922)

Note: The sequence of homologous arms and cKO region is analyzed to determine the GC content. Significant high GC-content regions are found. It may be difficult to construct this targeting vector.

BLAT Search Results (up)

QUERY	SCORE	START	END	QSIZE	IDENTITY	CHROM	STRAND	START	END	SPAN	
browser details	YourSeq	3500	1	3500	3500	100.0%	chr4	+	135913114	135916613	3500
browser details	YourSeq	299	2899	3333	3500	89.3%	chr18	+	53101114	53101637	524
browser details	YourSeq	293	2874	3315	3500	89.9%	chr18	-	34227620	34228149	530
browser details	YourSeq	293	2878	3303	3500	91.1%	chr2	+	165893967	165894583	617
browser details	YourSeq	291	2878	3332	3500	91.2%	chr2	-	120883603	120884123	521
browser details	YourSeq	282	2868	3287	3500	88.9%	chr5	-	92852605	92853019	415
browser details	YourSeq	275	2899	3303	3500	87.6%	chr14	+	27246990	27247377	388
browser details	YourSeq	274	2878	3321	3500	92.1%	chr4	-	116196351	116511297	314947
browser details	YourSeq	273	2878	3329	3500	91.7%	chr11	-	114528235	114528696	462
browser details	YourSeq	265	2882	3301	3500	92.1%	chr7	-	29545976	29546400	425
browser details	YourSeq	261	2874	3303	3500	90.8%	chr10	-	82163592	82164067	476
browser details	YourSeq	247	2878	3332	3500	89.7%	chr11	+	61386456	61387320	865
browser details	YourSeq	239	2867	3264	3500	85.2%	chr17	-	28283912	28284290	379
browser details	YourSeq	235	2869	3166	3500	91.1%	chrX	+	151546165	151546470	306
browser details	YourSeq	226	2878	3166	3500	92.9%	chr6	+	39369025	39369326	302
browser details	YourSeq	224	2878	3166	3500	91.9%	chr19	-	37614718	37615021	304
browser details	YourSeq	223	2899	3297	3500	92.2%	chr10	-	128901473	128902124	652
browser details	YourSeq	223	2899	3332	3500	86.5%	chr9	+	88555393	88555705	313
browser details	YourSeq	222	2879	3166	3500	90.5%	chr6	+	100762706	100762989	284
browser details	YourSeq	221	2874	3166	3500	93.0%	chr13	-	43593883	43594220	338

Note: The 3500 bp section upstream of Exon 2 is BLAT searched against the genome. No significant similarity is found.

BLAT Search Results (down)

QUERY	SCORE	START	END	QSIZE	IDENTITY	CHROM	STRAND	START	END	SPAN	
browser details	YourSeq	3500	1	3500	3500	100.0%	chr4	+	135917655	135921154	3500
browser details	YourSeq	172	2358	2564	3500	90.5%	chr11	-	63516568	63516769	202
browser details	YourSeq	166	2369	2562	3500	91.1%	chr13	-	118841370	118841558	189
browser details	YourSeq	165	2342	2562	3500	91.0%	chr11	+	62682702	62683002	301
browser details	YourSeq	164	2373	2563	3500	93.2%	chr2	-	148591883	148592076	194
browser details	YourSeq	164	2357	2561	3500	90.0%	chr12	-	75629280	75629478	199
browser details	YourSeq	163	2350	2554	3500	91.4%	chr5	-	77744427	77744632	206
browser details	YourSeq	163	2359	2561	3500	88.2%	chr1	-	155506263	155506457	195
browser details	YourSeq	162	2370	2568	3500	91.8%	chr5	-	129871133	129871332	200
browser details	YourSeq	161	2374	2561	3500	90.8%	chr16	-	7811751	7811933	183
browser details	YourSeq	161	2372	2562	3500	91.1%	chr13	-	58650733	58650922	190
browser details	YourSeq	161	2369	2560	3500	90.6%	chr3	+	108548613	108548802	190
browser details	YourSeq	158	2354	2562	3500	90.3%	chr2	-	156986190	156986400	211
browser details	YourSeq	158	2370	2562	3500	88.3%	chr1	-	58608306	58608492	187
browser details	YourSeq	158	2373	2567	3500	95.4%	chr11	+	98093231	98117569	24339
browser details	YourSeq	157	2370	2557	3500	92.5%	chr2	+	132723123	132723311	189
browser details	YourSeq	156	2223	2560	3500	92.9%	chr17	-	87631090	87631468	379
browser details	YourSeq	156	2372	2561	3500	92.5%	chr10	-	117198370	117206833	8464
browser details	YourSeq	156	2368	2562	3500	90.3%	chr6	+	8241801	8241996	196
browser details	YourSeq	155	2369	2561	3500	88.9%	chr11	-	105189257	105189446	190

Note: The 3500 bp section downstream of Exon 2 is BLAT searched against the genome. No significant similarity is found.

Gene and protein information:

Cnr2 cannabinoid receptor 2 (macrophage) [*Mus musculus* (house mouse)]

Gene ID: 12802, updated on 25-Jun-2017

Gene summary

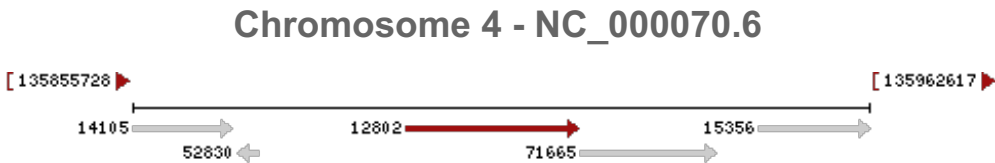
Official Symbol	Cnr2 provided by MGI
Official Full Name	cannabinoid receptor 2 (macrophage) provided by MGI
Primary source	MGI:MGI:104650
See related	Ensembl:ENSMUSG000000062585 Vega:OTTMUSG000000009846
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CB2; CB-2; CB2-R
Orthologs	human all

Genomic context

Location: 4; 4 D3 See Cnr2 in [Genome Data Viewer](#) [Map Viewer](#)

Exon count: 3

Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	4	NC_000070.6 (135895189..135920219)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	4	NC_000070.5 (135451319..135476130)



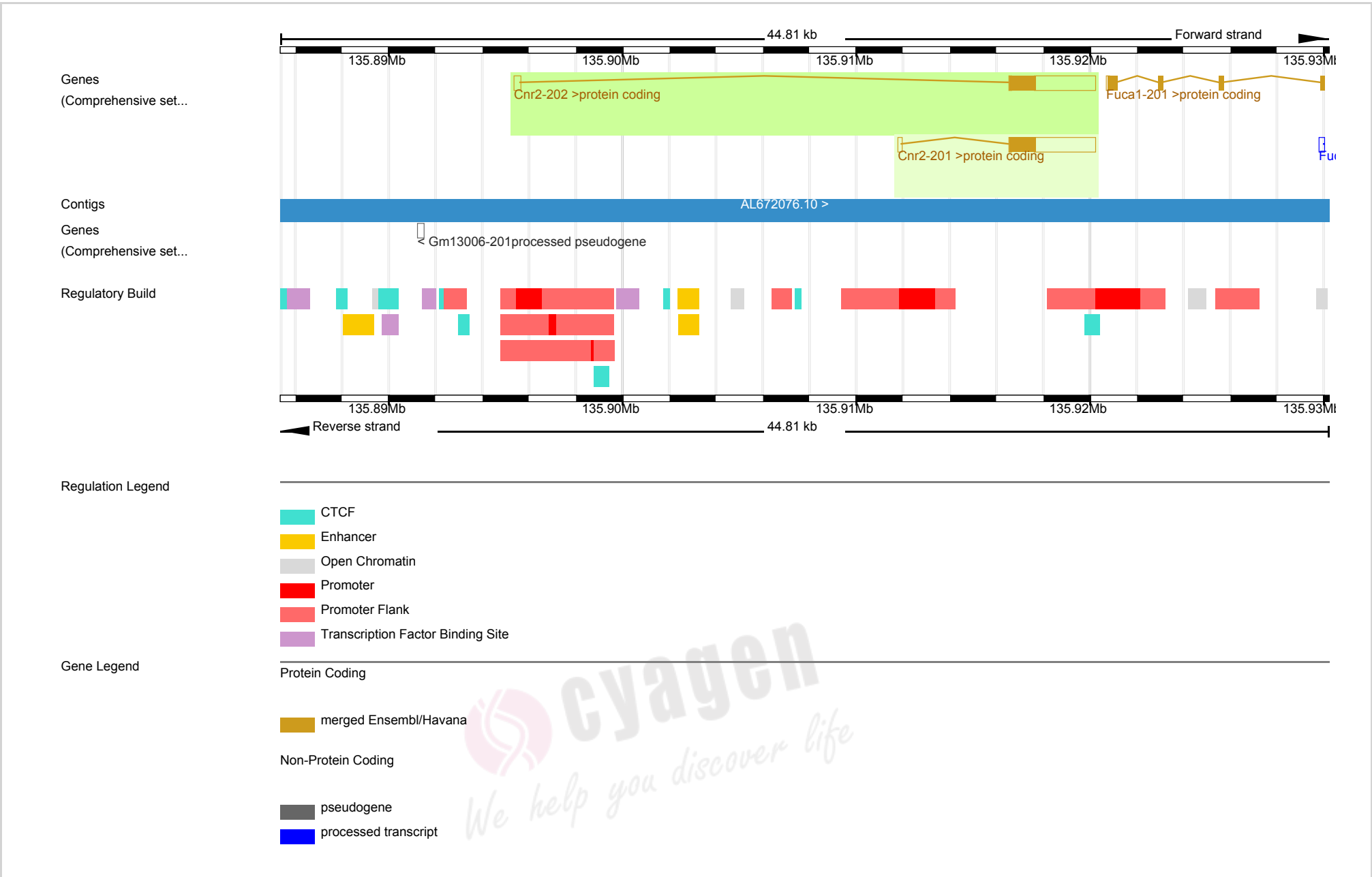
Transcript information: This gene has 2 transcripts

Gene: Cnr2 ENSMUSG00000062585

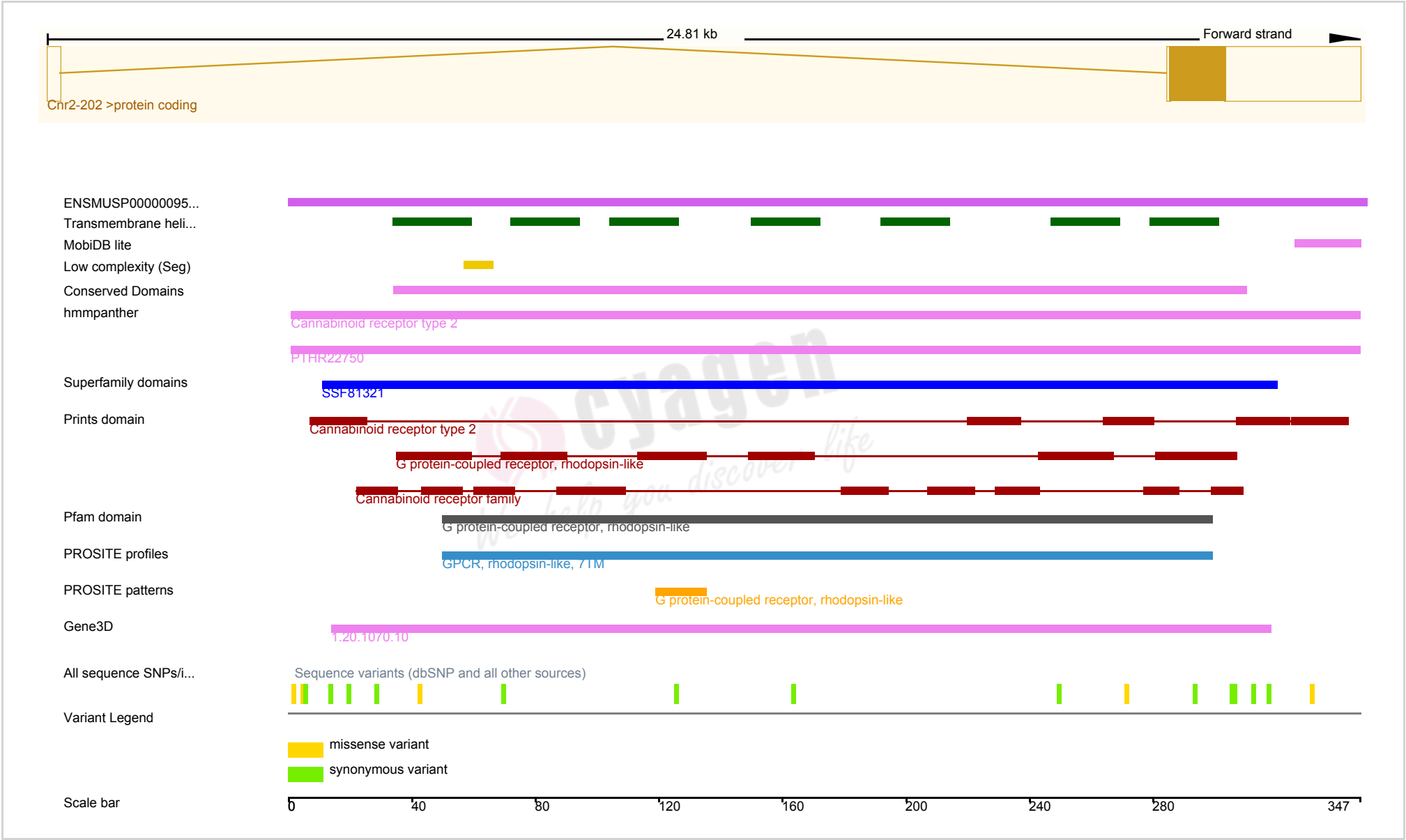
Description cannabinoid receptor 2 (macrophage) [Source:MGI Symbol;Acc:[MGI:104650](#)]
Gene Synonyms CB2, CB2-R, cannabinoid receptor 2 (spleen)
Location [Chromosome 4: 135,895,394-135,920,207](#) forward strand.
GRCm38:CM000997.2
About this gene This gene has 2 transcripts ([splice variants](#)), [118 orthologues](#), [18 paralogues](#), is a member of [1 Ensembl protein family](#) and is associated with [2 phenotypes](#).

Transcripts

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	RefSeq	Flags
Cnr2-202	ENSMUST00000097843.8	3867	347aa	ENSMUSP00000095454	Protein coding	CCDS18793	P47936	NM_009924 NP_034054	TSL:1 GENCODE basic APPRIS P1
Cnr2-201	ENSMUST00000068830.3	3756	347aa	ENSMUSP00000069957	Protein coding	CCDS18793	P47936	NM_001305278 NP_001292207	TSL:1 GENCODE basic APPRIS P1



Transcript: ENSMUST00000097843



We wish to acknowledge the following valuable scientific information resources: Ensembl, MGI, NCBI, UCSC.



Animal Report

Quote: TKC-191005-ADJ-01-tac
Project: Mouse Cnr2 Conditional Knockout

-Confidential-

1. Animal Generation

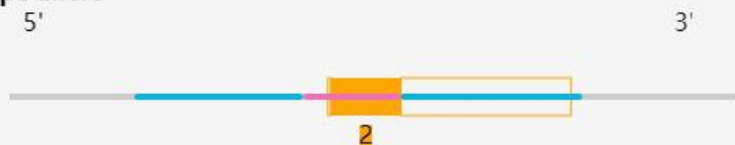
Targeted ES cell clone 2B5 was injected into C57BL/6 albino embryos, which were then re-implanted into CD-1 pseudo-pregnant females. Founder animals were identified by their coat color, their germline transmission was confirmed by breeding with C57BL/6 females and subsequent genotyping of the offspring. Three male and four female heterozygous targeted mice were generated from clone 2B5 as final deliverables for this project.

2B5 ESC, F1 mice			
3 Males ♂		4 Females ♀	
D.O.B	06-04-2020	D.O.B	06-04-2020
Mouse ID	31, 32, 36	Mouse ID	38, 42, 43, 44



1.1. Genotyping Strategy

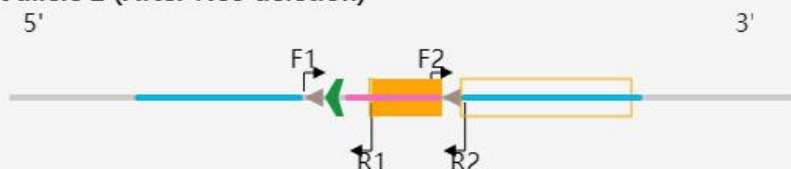
Wildtype allele



Mutant allele 1 (Targeted allele)



Mutant allele 2 (After Neo deletion)



Mutant allele 3 (After Cre recombination)



Legends



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:

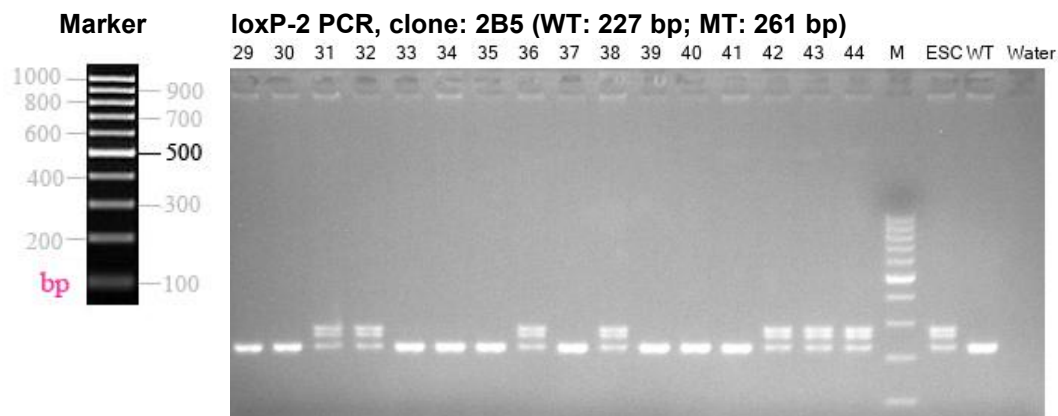
Component	x1
Mouse genomic DNA	1.5 µl
Forward primer (10 µM)	1.0 µl
Reverse primer (10 µM)	1.0 µl
Premix Taq Polymerase	12.5 µl
ddH ₂ O	9.0 µl
Total	25.0 µl

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.



cyagen
We help you discover life

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:

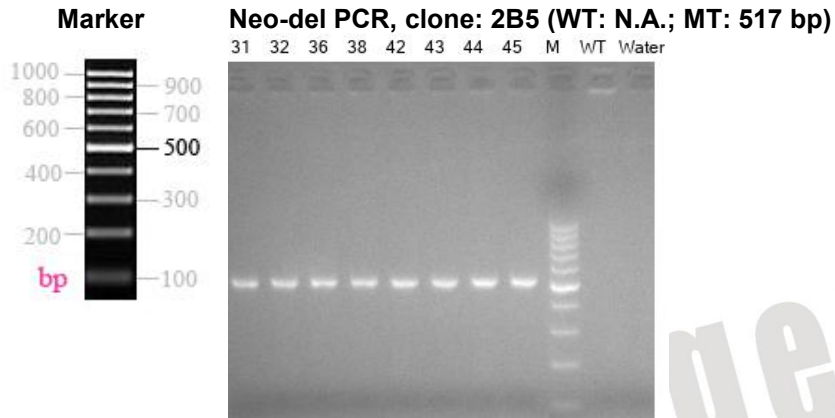
Component	x1
Mouse genomic DNA	1.5 µl
Forward primer (10 µM)	1.0 µl
Reverse primer (10 µM)	1.0 µl
Premix Taq Polymerase	12.5 µl
ddH ₂ O	9.0 µl
Total	25.0 µl

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.



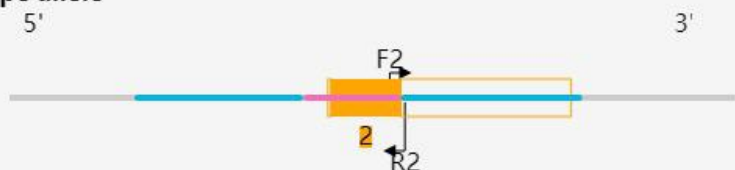
1.4. PCR Result:

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

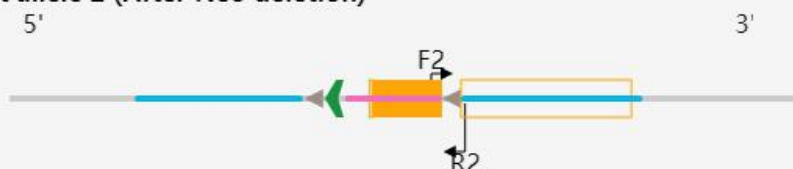
Wildtype allele



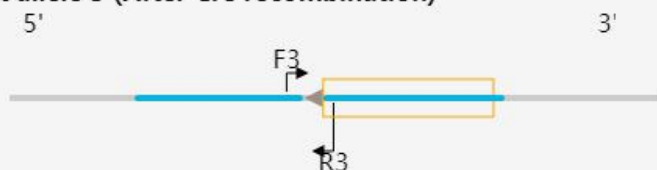
Mutant allele 1 (Targeted allele)



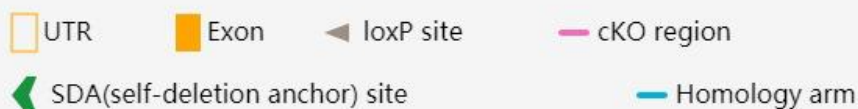
Mutant allele 2 (After Neo deletion)



Mutant allele 3 (After Cre recombination)



Legends



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'-CCTGTTTCACTATCCAGGTACGG-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'-TGCTTGGCAGCAAACCTATAAATGG-3'

R3: 5'-TTCCATTACCCTAGAACTGGCTTC-3'

Constitutive KO allele: 456 bp