Cspg4-CreERT2-P2A Cas9-KI Strategy

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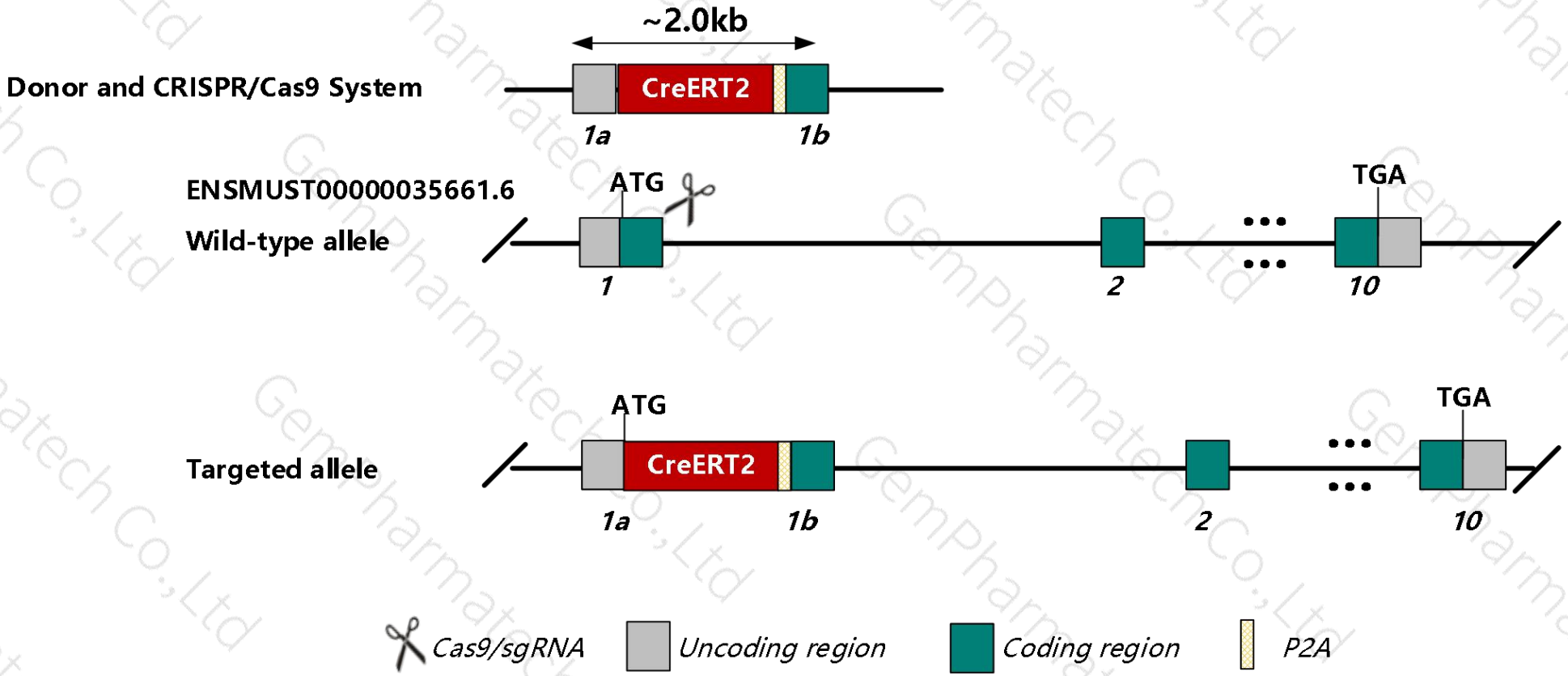
Design Date: 2019/9/26

Project Overview

Project Name	***Cspg4-CreERT2-P2A***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Cspg4* gene. The schematic diagram is as follows:



Technical routes

- The *Cspg4* gene has 4 transcripts. According to the structure of *Cspg4* gene, *Cspg4-201*(ENSMUST00000035661.6) is selected for presentation of the recommended strategy.

- *Cspg4-201* gene has 10 exons, with the ATG start codon in exon1 and TGA stop codon in exon10.

- We make *Cspg4-CreERT2-P2A* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. gRNA direct Cas9 endonuclease cleavage near translational start coding of *Cspg4* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in the insertion of *CreERT2-P2A* after translational start coding of *Cspg4* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, Mice homozygous for a null mutation display abnormal dentate gyrus morphology and abnormal smooth muscle cell physiology.
- The expression of two genes linked with P2A peptide is drived by the same promoter, and the fused protein will be cleaved into two proteins folding independently, while the former will carry the P2A-translated polypeptide.
- The insertion of *CreERT2* may affect the 5-terminal regulation of the *Cspg4* gene.
- The *Cspg4* gene is located on the Chr9. If the knock in mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

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Cspg4 chondroitin sulfate proteoglycan 4 [*Mus musculus* (house mouse)]

Gene ID: 121021, updated on 6-Aug-2019

Summary

Official Symbol	Cspg4 provided by MGI
Official Full Name	chondroitin sulfate proteoglycan 4 provided by MGI
Primary source	MGI:MGI:2153093
See related	Ensembl:ENSMUSG00000032911
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	AN2; NG2; 4732461B14Rik
Expression	Broad expression in limb E14.5 (RPKM 17.6), mammary gland adult (RPKM 12.2) and 20 other tissues See more
Orthologs	human all

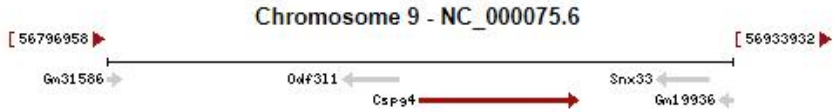
Genomic context

Location: 9; 9 B

See Cspg4 in Genome Data Viewer

Exon count: 10

Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	9	NC_000075.6 (56865104..56899870)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	9	NC_000075.5 (56712911..56747677)

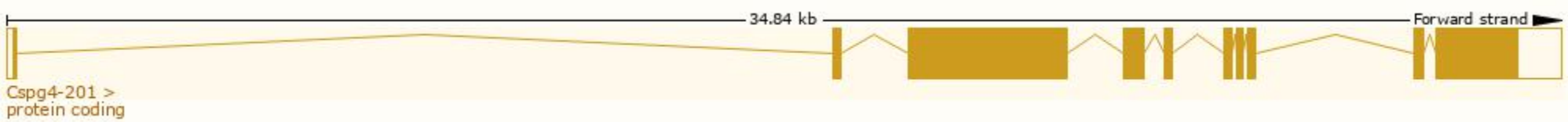


Transcript information （Ensembl）

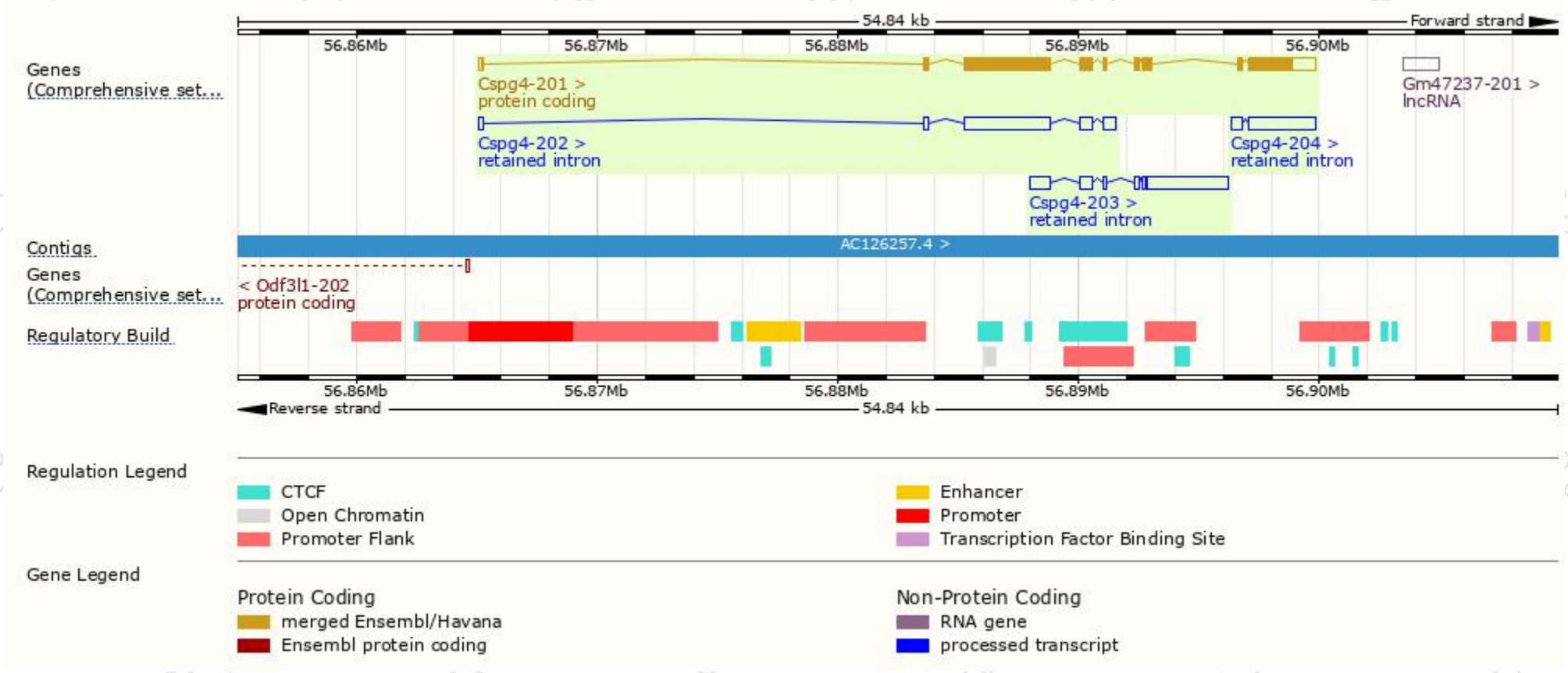
The gene has 4 transcripts, and the transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cspg4-201	ENSMUST00000035661.6	8121	2327aa	Protein coding	CCDS23211	Q8VHY0	TSL:1 GENCODE basic APPRIS P1
Cspg4-203	ENSMUST00000215666.1	5158	No protein	Retained intron	-	-	TSL:2
Cspg4-202	ENSMUST00000214057.1	4976	No protein	Retained intron	-	-	TSL:1
Cspg4-204	ENSMUST00000217052.1	3267	No protein	Retained intron	-	-	TSL:1

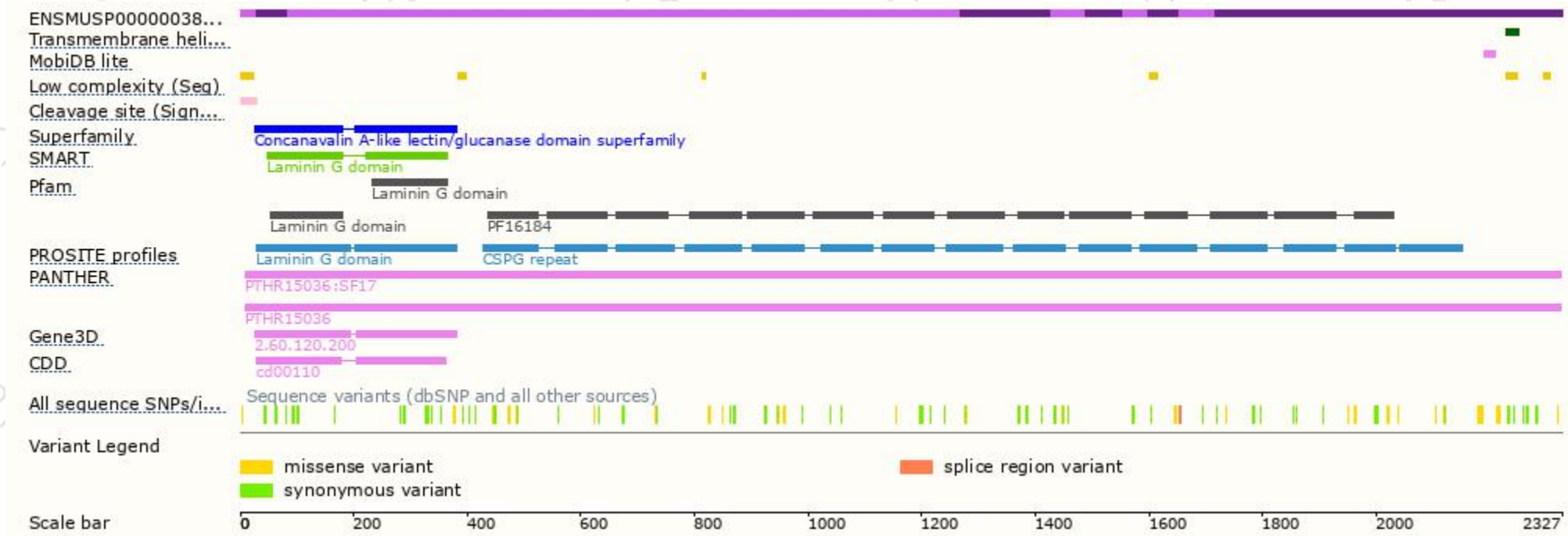
The strategy is based on the design of *Cspg4-201* transcript,The transcription is shown below



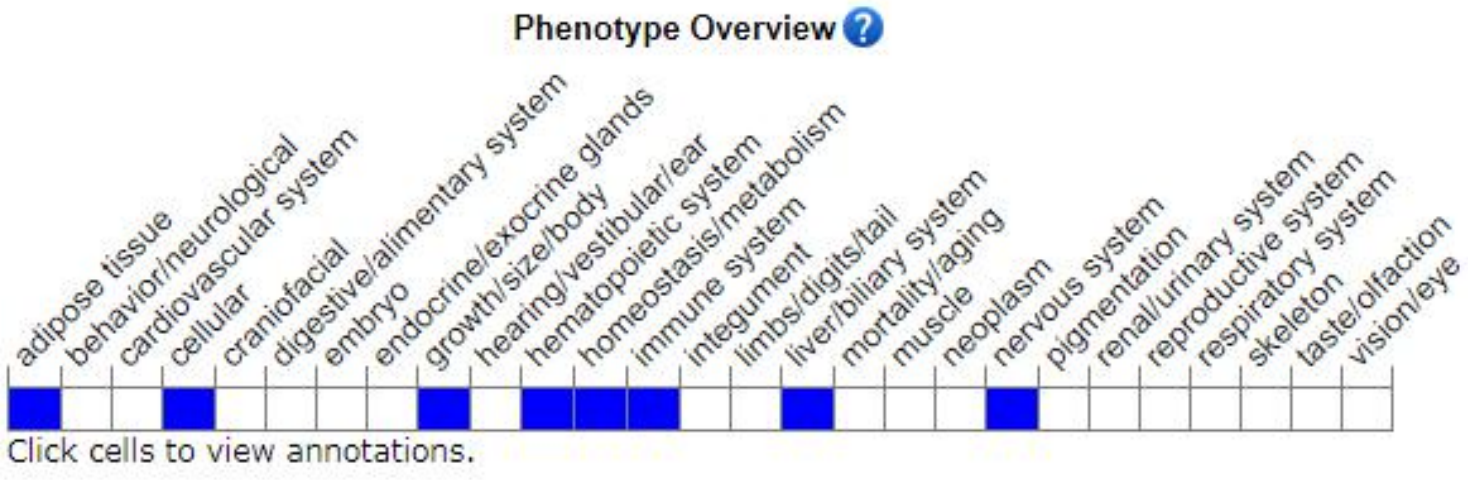
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/marker/MGI:2153093) .

Mice homozygous for a null mutation display abnormal dentate gyrus morphology and abnormal smooth muscle cell physiology.

Knockin mouse model

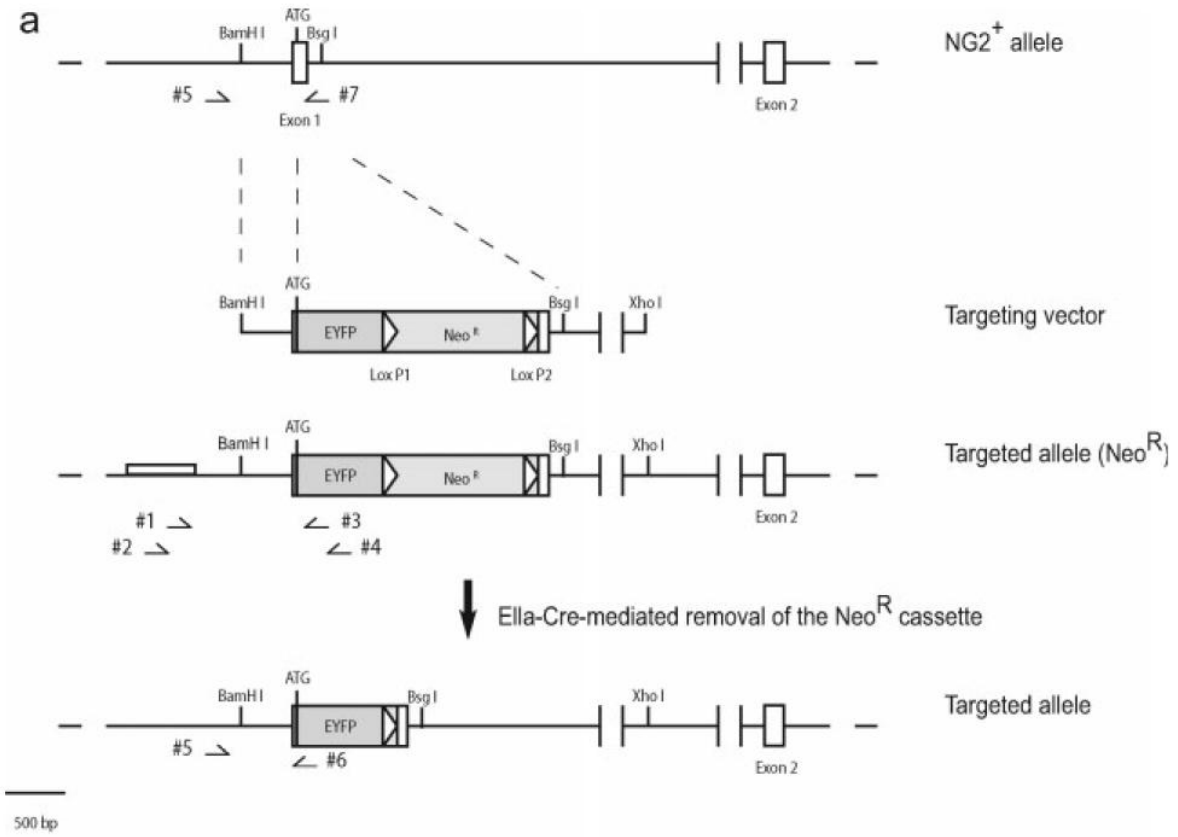


FIG. 1. Homologous recombination of the murine *NG2* gene. (a) Exons 1 and 2 of the wild-type NG2 allele (NG2+ allele, top). The NG2 locus comprises of 8 exons (white boxes) with the coding region depicted in grey. The targeting vector harbored an EYFP gene fused to the start codon in exon 1 of the NG2 gene followed by a lox P flanked neo resistance cassette (middle). Modified targeted allele (targeted allele

References

[1]. Huang W, Zhao N, Bai X, et al. Novel NG2-iCre knock-in mice demonstrate heterogeneous differentiation potential of NG2 glia during development.[J]. Glia, 2014, 62(6):896-913.

[2]. Karram K, Goebbels S, Schwab M, et al. NG2-expressing cells in the nervous system revealed by the NG2-EYFP-knockin mouse[J]. Genesis, 2010, 46(12):743-757.

If you have any questions, you are welcome to inquire.

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