Csf1r-IRES-iCre Cas9-KI Strategy

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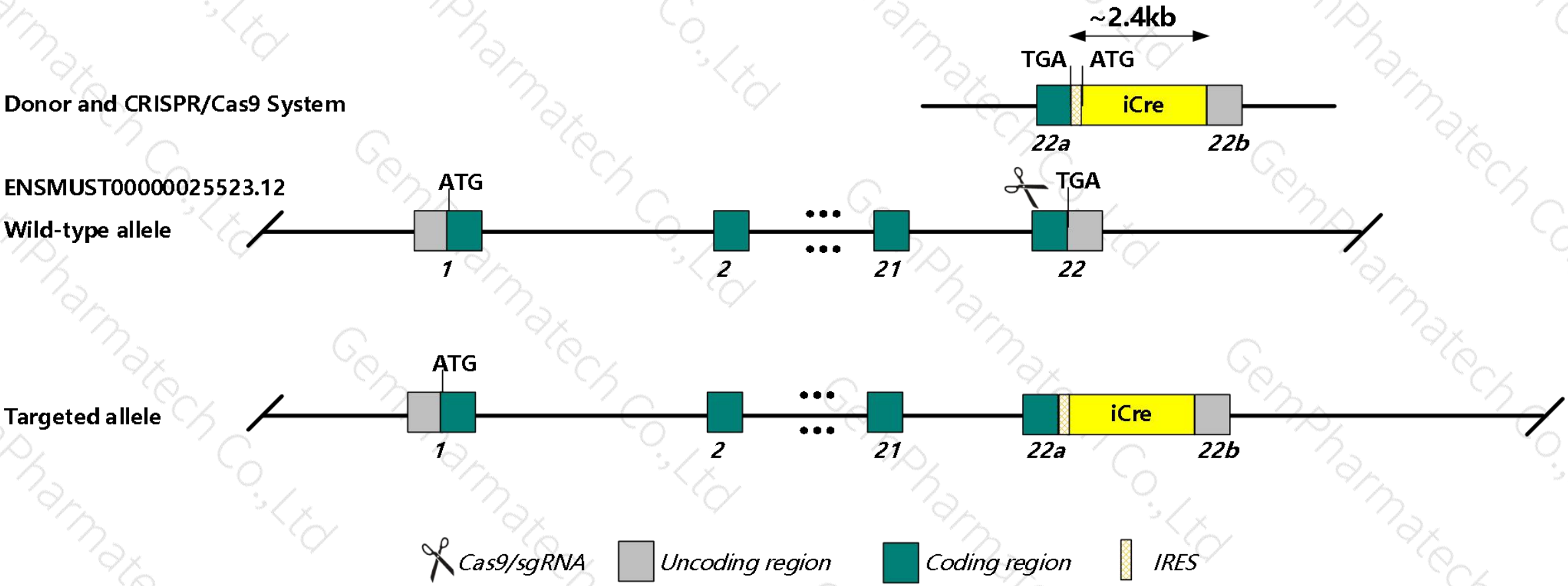
Design Date:	**2019-8-14**
Reviewer	**JiaYu**

Project Overview

Project Name	***Csf1r-IRES-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Csf1r* gene. The schematic diagram is as follows:



Technical routes

- The *Csf1r* gene has 6 transcripts.According to the structure of *Csf1r* gene, *Csf1r-201*(ENSMUST00000025523.12) is selected for presentation of the recommended strategy.

- *Csf1r-201* gene has 22 exons, with the ATG start codon in exon1 and TGA stop codon in exon22.

- We make *Csf1r-IRES-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop coding(TGA) of Csf1r gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in IRES-iCre near stop coding(TGA) of Csf1r gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, Homozygotes for a targeted null mutation exhibit skeletal, sensory, and reproductive abnormalities associated with severe deficiencies in osteoclasts, macrophages, and brain microglia.
- The IRES-linked *Csf1r* gene and the iCre gene are expressed by the same promoter driver. The transcription levels are consistent but the translation is independent. Often the latter gene translates at a lower level than the former.
- Insertion of iCre may affect the regulation of the 3' end of the *Csf1r* gene.
- There will be 2 to 4 base mutations in exon22 of *Csf1r* gene in this strategy.
- The distance between the 3'regulation of *Hmgxb3* gene is about 0.9kb, insertion of IRES-iCre may influence the 3'regulation of this gene.
- The *Csf1r* gene is located on the Chr18. If the knockin 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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Csf1r colony stimulating factor 1 receptor [*Mus musculus* (house mouse)]

Gene ID: 12978, updated on 12-Aug-2019

Summary

Official Symbol	Csf1r provided by MGI
Official Full Name	colony stimulating factor 1 receptor provided by MGI
Primary source	MGI:MGI:1339758
See related	Ensembl:ENSMUSG00000024621
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	Fms; Fim2; CD115; Csfmr; Fim-2; CSF-1R; M-CSFR; M-CSF-R; AI323359
Expression	Broad expression in spleen adult (RPKM 69.7), placenta adult (RPKM 34.8) and 23 other tissues See more
Orthologs	human all

Genomic context

Location: 18 E1; 18 34.41 cM

See Csf1r in Genome Data Viewer

Exon count: 22

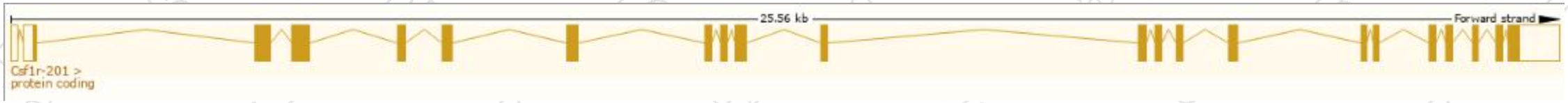
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	18	NC_000084.6 (61105572..61131139)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	18	NC_000084.5 (61265226..61290793)

Transcript information （Ensembl）

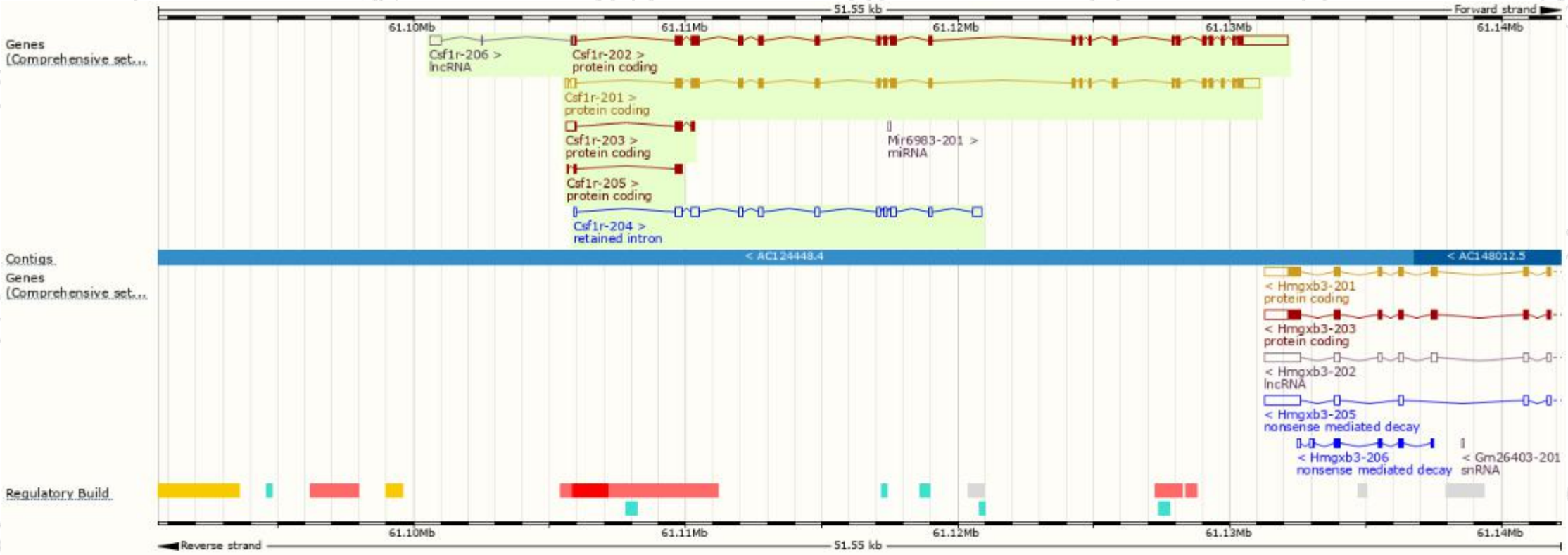
The gene has 6 transcripts, and all transcripts are shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Csf1r-202	ENSMUST00000115268.3	4701	977aa	Protein coding	CCDS29280	P09581 Q0P635	TSL:1 GENCODE basic APPRIS P1
Csf1r-201	ENSMUST00000025523.12	3870	977aa	Protein coding	CCDS29280	P09581 Q0P635	TSL:1 GENCODE basic APPRIS P1
Csf1r-203	ENSMUST00000235447.1	776	139aa	Protein coding	-	-	CDS 3' incomplete
Csf1r-205	ENSMUST00000237706.1	416	103aa	Protein coding	-	-	CDS 3' incomplete
Csf1r-204	ENSMUST00000237485.1	2035	No protein	Retained intron	-	-	-
Csf1r-206	ENSMUST00000237873.1	465	No protein	lncRNA	-	-	-

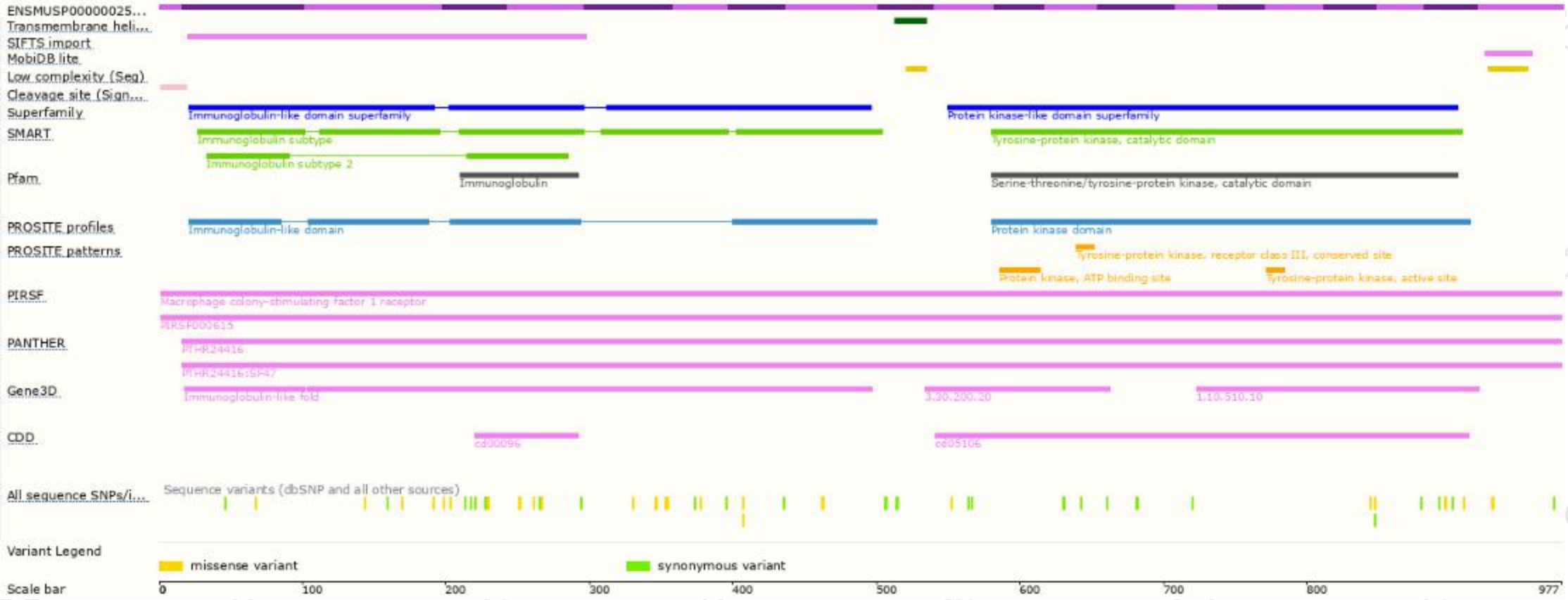
The strategy is based on the design of *Csf1r-201* transcript,The transcription is shown below



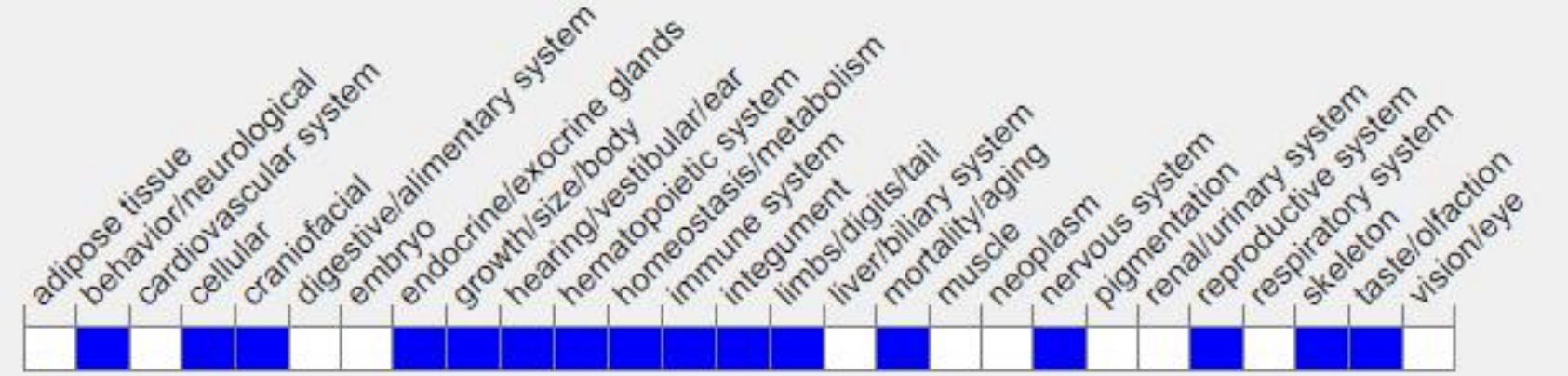
Genomic location distribution



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:1339758) .

Homozygotes for a targeted null mutation exhibit skeletal, sensory, and reproductive abnormalities associated with severe deficiencies in osteoclasts, macrophages, and brain microglia

Targeted Progress (from Jax)

The Jackson Laboratory

MOUSE STRAIN DATASHEET - 029206

C57BL/6-Tg(Csf1r-cre)1Mnz/J

Detailed Description

These *Csfr1*^{Cre} BAC transgenic mice express cre recombinase from the mouse *Csfr1* (colony stimulating factor 1 receptor) promoter.

Crosses with fluorescent floxed stop reporter mice demonstrate expression in pre-dendritic cells and conventional dendritic cells (cDCs), plasmacytoid dendritic cells (pDCs), monocytes and macrophages. The percentage of labeled pDCs is lower than in cDCs, macrophages and monocytes. Although cre expression is not specific for cDCs, cDCs are efficiently targeted in these mice (near 100% recombination efficiency), therefore they are considered suitable for cDC targeting.

An inducible *in vivo* system for cDC depletion can be generated by crossing these mice with zDC-loxStoplox-DTR (see Stock No. 028539; also called zDC$^{[lslDTR]}$) animals. zDC$^{[lslDTR]}$ mice express human diptheria toxin receptor (DTR) under the control of the *Zbtb46* promoter after the excision of a floxed Stop signal and the administration of diptheria toxin (DT). Sixteen hours after DT administration, cDCs (but not monocytes, macrophages, or CD8^{+} T or B cells) are efficiently depleted in the compound mutant mice.

Development

IRES-cre was introduced to the 3' UTR of the mouse *Csf1r* gene encoded by BAC RP23-174D18, right after the endogenous Stop codon in exon 21. The BAC was microinjected into C57BL/6 mouse oocytes and founders were bred to C57BL/6J by the donating laboratory.

https://www.jax.org/strain/029206

References

1. Loschko J; Rieke GJ; Schreiber HA; Meredith MM; Yao KH; Guermonprez P; Nussenzweig MC. 2016. Inducible targeting of cDCs and their subsets in vivo. J Immunol Methods 434:32-8.
2. Tiedt R; Schomber T; Hao-Shen H; Skoda RC. 2007. Pf4-Cre transgenic mice allow the generation of lineage-restricted gene knockouts for studying megakaryocyte and platelet function in vivo. Blood 109(4):1503-6.

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

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