Csf1r-P2A-CreERT2 Cas9-KI Strategy

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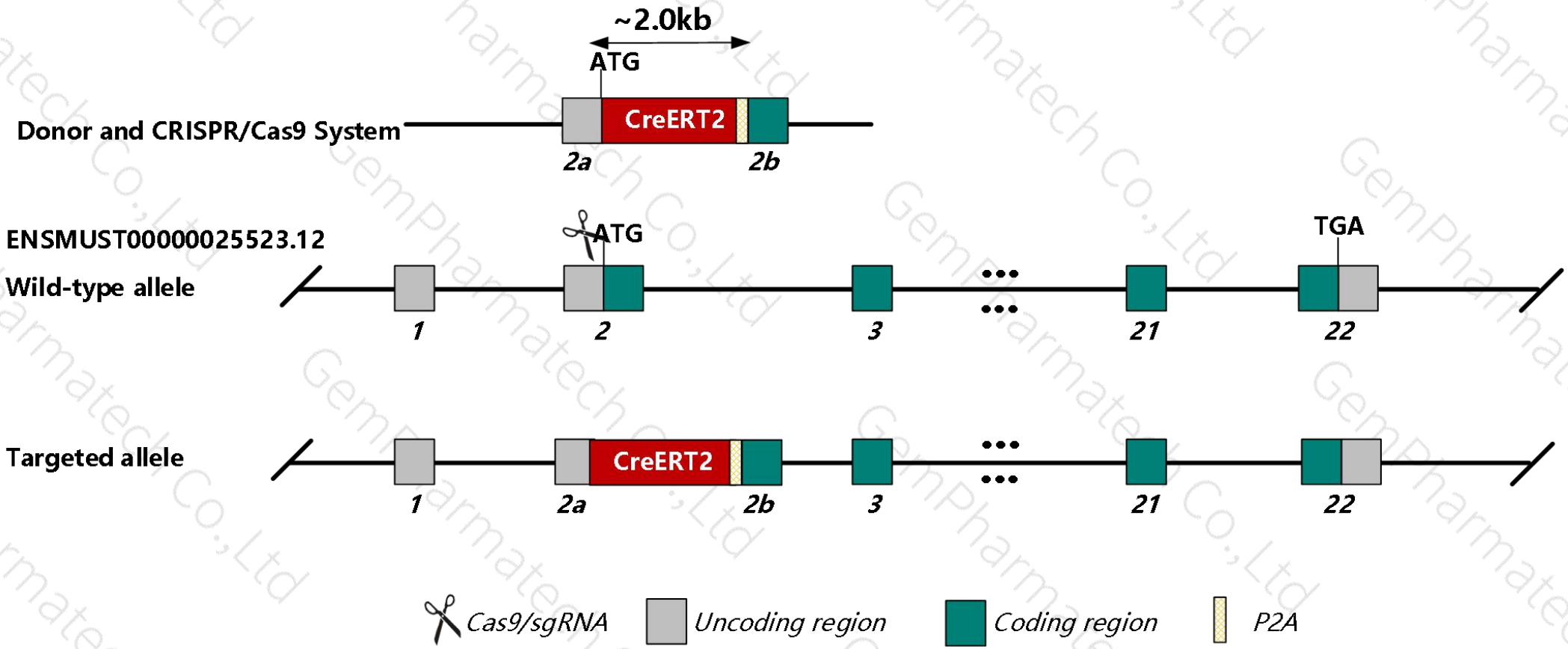
Project Overview

Project Name	***Csf1r-P2A-CreERT2***
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 exon2 and TGA stop codon in exon22.

- We make *Csf1r-P2A-CreERT2* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near start coding(ATG) of *Csf1r* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in *CreERT2-P2A* after start coding(ATG) 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.
- According to the existing JAX data, tamoxifen-inducible cre activity is detected in bone-marrow-derived macrophages and yolk sac macrophages.
- Insertion of *CreERT2* may affect the regulation of the 5' end of the *Csf1r* gene.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- 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 17-Sep-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

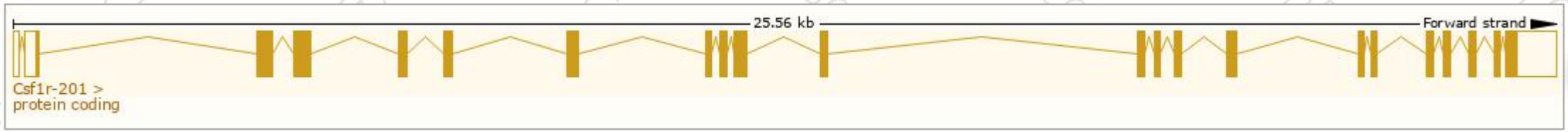
Exon count: 22

Transcript information （Ensembl）

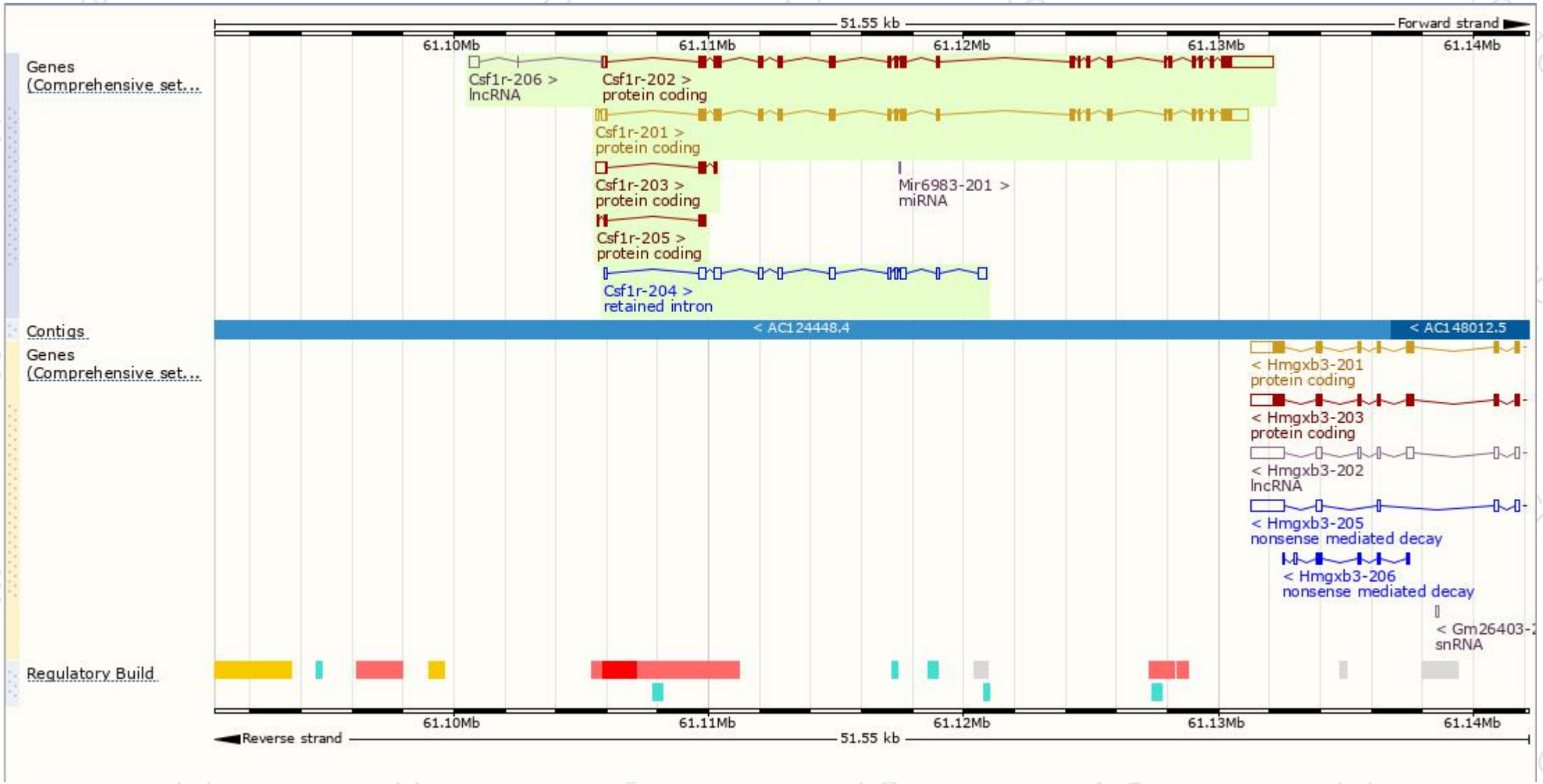
The gene has 6 transcripts, and the transcript is shown below：

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	-	-	-

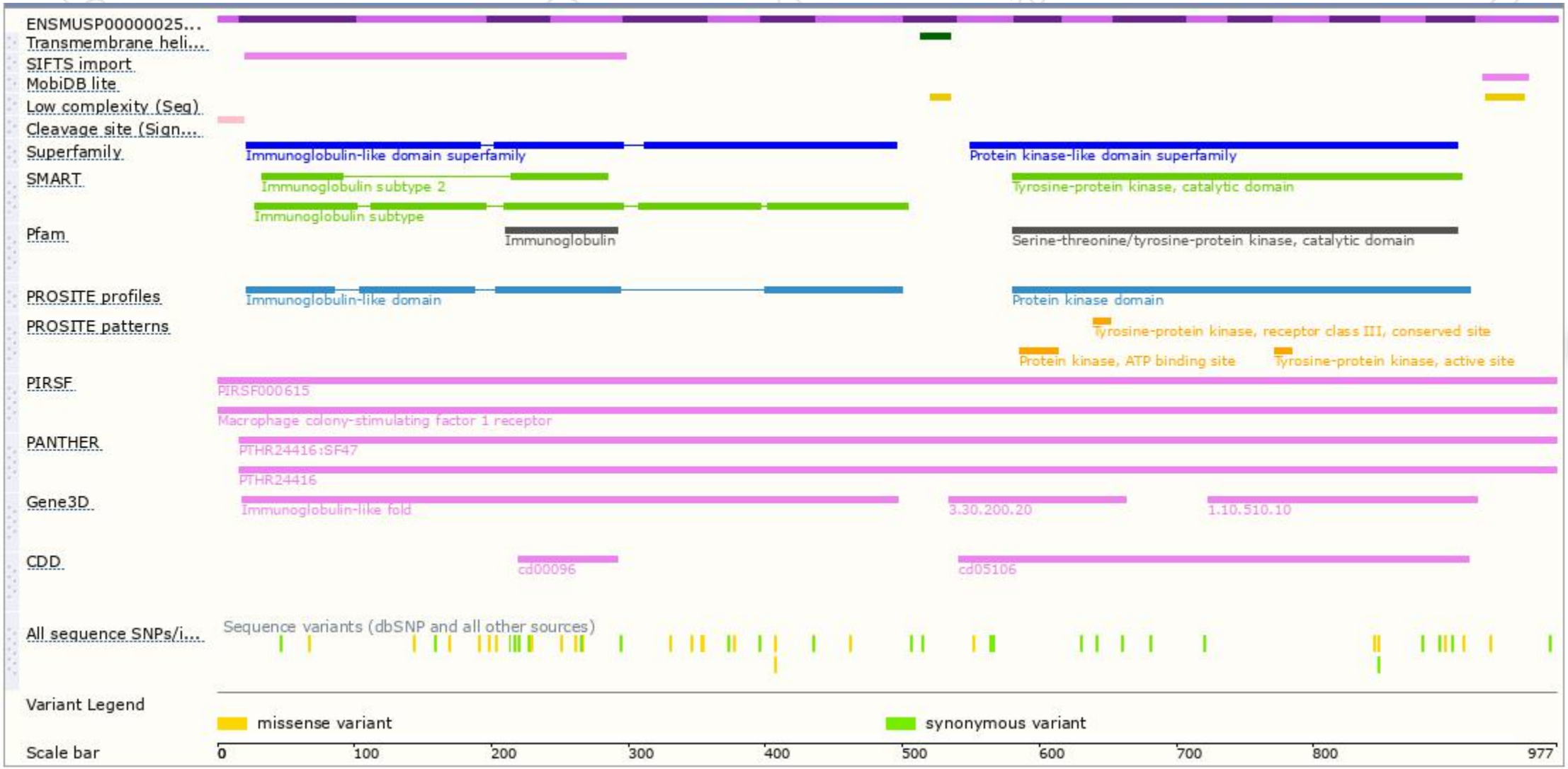
The strategy is based on the design of *Csf1r-201* transcript.The transcription is shown below



Genomic location distribution



Protein domain



Targeted Progress (from JAX)

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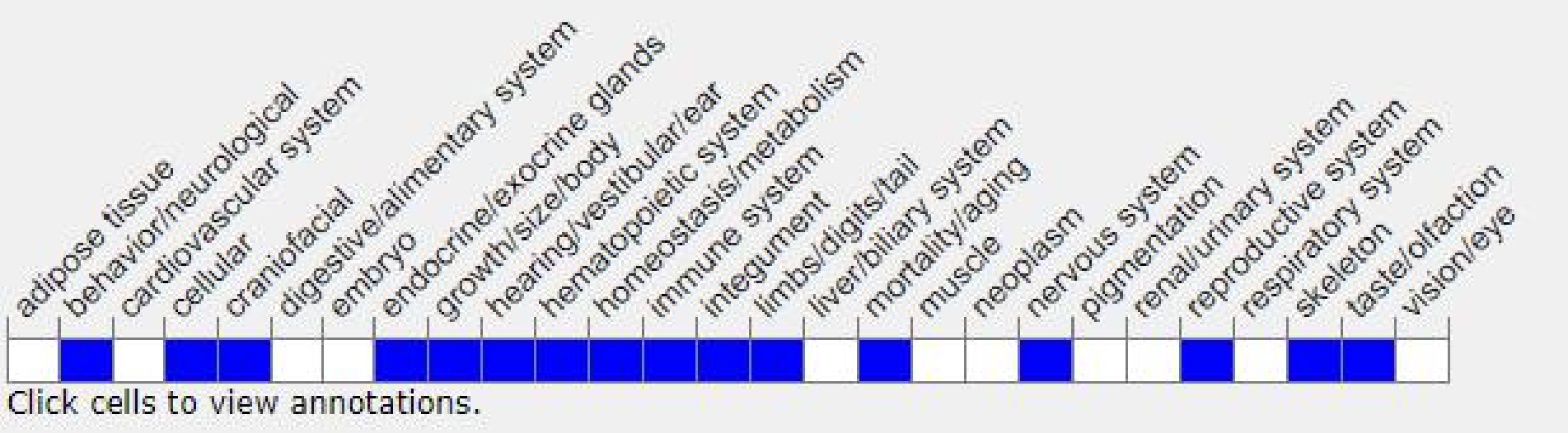
Tg(Csf1r-cre/Esr1*)1Jwp

Allele Symbol: Tg(Csf1r-cre/Esr1*)1Jwp MGI

Allele Name	transgene insertion 1, Jeffrey W Pollard
Allele Type	Transgenic (Recombinase-expressing, Inducible)
Allele Synonym(s)	Tg(Csf1r-cre/Esr1*)1Jwp; transgene insertion 1, Jeffrey W Pollard
Gene Symbol and Name	Tg(Csf1r-cre/Esr1*)1Jwp MGI , transgene insertion 1, Jeffrey W Pollard
Gene Synonym(s)	
Promoter	*Csf1r*, colony stimulating factor 1 receptor, mouse, laboratory
Expressed Gene	*cre/Esr1*, Cre recombinase and estrogen receptor 1 fusion gene,
Site of Expression	Tamoxifen-inducible cre activity is detected in bone-marrow-derived macrophages and yolk sac macrophages.
Strain of Origin	FVB/N
Chromosome	UN
Molecular Note	A transgene construct was generated in which the Csf1r promoter was used to drive expression of icre fused to 2 tamoxifen-inducible mutated estrogen receptors (Mer-icre-Mer or icre/Esr1*). Line 1 was used in the primary reference.

https://www.jax.org/strain/019098

Mouse phenotype description(MGI)



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

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

Coding Sequence of *CreERT2* (1983 bp)

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ATGTCCAATTTACTGACCGTACACCAAAATTTGCCTGCATTACCGGTCGATGCAACGAGTGATGAGGTTCGCAAGAACCTGATGG
ACATGTTCAGGGATCGCCAGGCGTTTTCTGAGCATACCTGGAAAATGCTTCTGTCCGTTTGCCGGTCGTGGGCGGCATGGTGCAA
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CAGCTATCAACTCGCGCCCTGGAAGGGATTTTTGAAGCAACTCATCGATTGATTTACGGCGCTAAGGATGACTCTGGTCAGAGAT
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AAGCTGGTGGCTGGACCAATGTAAATATTGTCATGAACTATATCCGTAACCTGGATAGTGAAACAGGGGCAATGGTGCGCCTGCT
GGAAGATGGCGATCTCGAGCCATCTGCTGGAGACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTCTAA
GAAGAACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGATGCTGAGCCCCCCATACTCTATTCCGA
GTATGATCCTACCAGACCCTTCAGTGAAGCTTCGATGATGGGCTTACTGACCAACCTGGCAGACAGGGAGCTGGTTCACATGATC
AACTGGGCGAAGAGGGTGCCAGGCTTTGTGGATTTGACCCTCCATGATCAGGTCCACCTTCTAGAATGTGCCTGGCTAGAGATC
CTGATGATTGGTCTCGTCTGGCGCTCCATGGAGCACCCAGTGAAGCTACTGTTTGCTCCTAACTTGCTCTTGGACAGGAACCAGG
GAAAATGTGTAGAGGGCATGGTGGAGATCTTCGACATGCTGCTGGCTACATCATCTCGGTTCCGCATGATGAATCTGCAGGGAGA
GGAGTTTGTGTGCCTCAAATCTATTATTTTGCTTAATTCTGGAGTGTACACATTTCTGTCCAGCACCCTGAAGTCTCTGGAAGAGA
AGGACCATATCCACCGAGTCCTGGACAAGATCACAGACACTTTGATCCACCTGATGGCCAAGGCAGGCCTGACCCTGCAGCAGC
AGCACCAGCGGCTGGCCCAGCTCCTCCTCATCCTCTCCCACATCAGGCACATGAGTAACAAAGGCATGGAGCATCTGTACAGCA
TGAAGTGCAAGAACGTGGTGCCCCTCTATGACCTGCTGCTGGAGGCGGCGGACGCCCACCGCCTACATGCGCCCACTAGCCGTG
GAGGGGCATCCGTGGAGGAGACGGACCAAAGCCACTTGGCCACTGCGGGCTCTACTTCATCGCATTCCTTGCAAAAGTATTACA
TCACGGGGGAGGCAGAGGGTTTCCCTGCCACAGCTTAA

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

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