Pf4-P2A-iCre Cas9-KI Strategy

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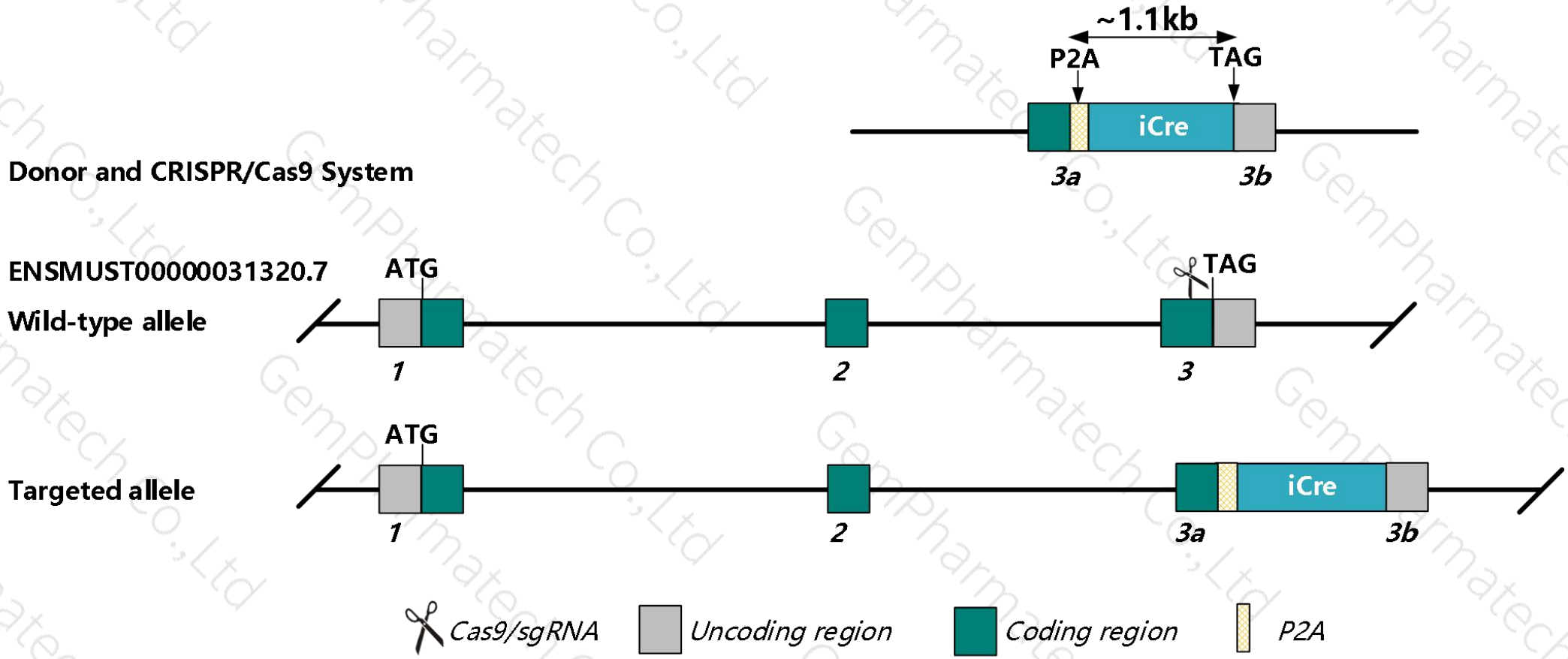
Project Overview

Project Name	***Pf4-P2A-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Pf4* gene. The schematic diagram is as follows:



Technical routes

- The *Pf4* gene has 3 transcripts. According to the structure of *Pf4* gene, *Pf4-201*(ENSMUST00000031320.7) is selected for presentation of the recommended strategy.

- *Pf4-201* gene has 3 exons, with the ATG start codon at exon1 and TAG stop codon at exon3.

- We make *Pf4-P2A-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 codon(TAG) of *Pf4* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A before stop codon(TAG) of *Pf4* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, homozygous and heterozygous null mice display increased platelet counts and reduced thrombus formation following vascular injury.
- According to the existing references, Cre-mediated recombination is expressed in the majority of megakaryocytes.
- 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.
- Insertion of iCre may affect the regulation of the 3' end of the *Pf4* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon3 of *Pf4* gene in this strategy.
- The *Pf4* gene is located on the Chr5. 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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Pf4 platelet factor 4 [*Mus musculus* (house mouse)]

Gene ID: 56744, updated on 12-Aug-2019

Summary

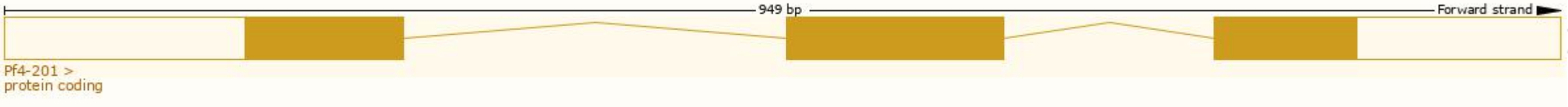
Official Symbol	Pf4 provided by MGI
Official Full Name	platelet factor 4 provided by MGI
Primary source	MGI:MGI:1888711
See related	Ensembl:ENSMUSG00000029373
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cxcl4; Scyb4
Expression	Biased expression in liver E14 (RPKM 119.8), liver E14.5 (RPKM 114.1) and 13 other tissues See more
Orthologs	human all

Transcript information （Ensembl）

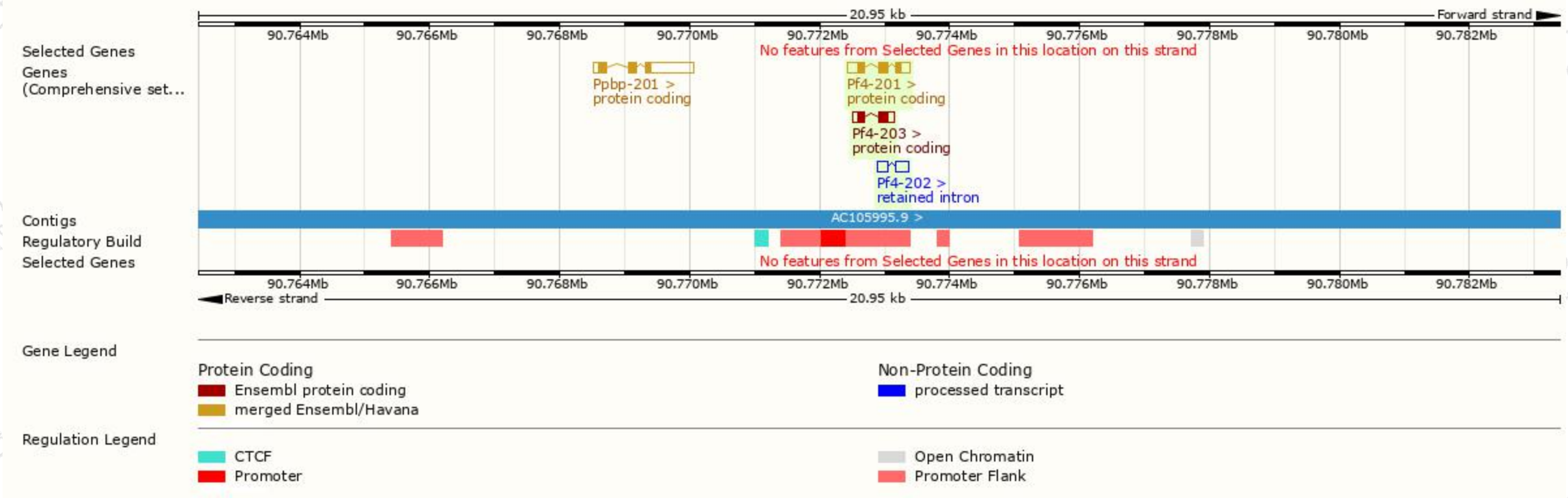
The gene has 3 transcripts, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pf4-201	ENSMUST00000031320.7	588	105aa	Protein coding	CCDS19416	Q3TVN6 Q9Z126	TSL:1 GENCODE basic APPRIS P1
Pf4-203	ENSMUST00000202625.1	411	77aa	Protein coding	-	A0A0J9YTR7	TSL:2 GENCODE basic
Pf4-202	ENSMUST00000201990.1	360	No protein	Retained intron	-	-	TSL:2

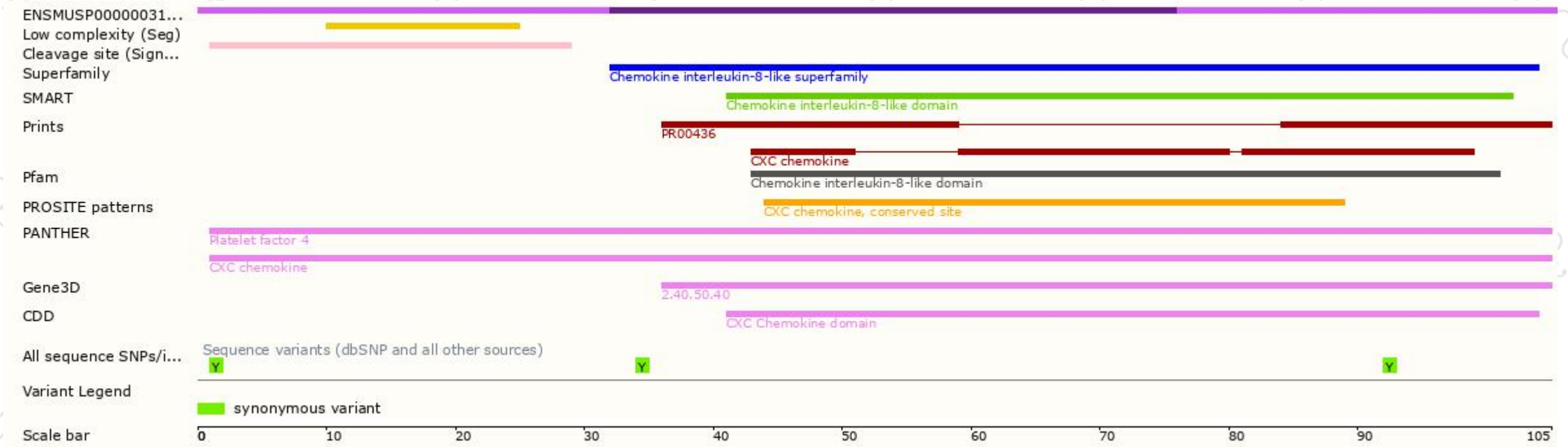
The strategy is based on the design of *Pf4-201* transcript,The transcription is shown below:



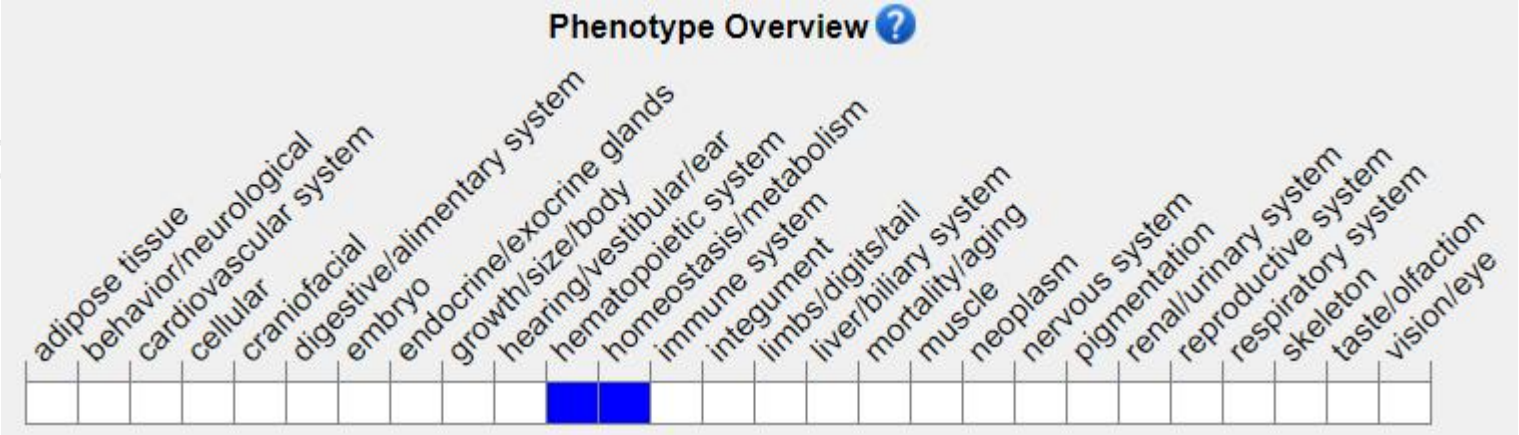
Genomic location distribution



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:1888711) .

Homozygous and heterozygous null mice display increased platelet counts and reduced thrombus formation following vascular injury.

Targeted Progress (from JAX)

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The Jackson Laboratory

MOUSE STRAIN DATASHEET - 008535

C57BL/6-Tg(Pf4-icre)Q3Rsko/J

Details

Detailed Description

Development

A 100kb BAC containing the mouse *Pf4* (platelet factor 4), or *Cxcl4*, gene with exon 1 replaced by a construct containing the mouse *Pf4* (platelet factor 4), or *Cxcl4*, promoter, a codon-improved Cre recombinase (iCre) sequence, bovine growth hormone polyadenylation signal, nuclear localization signal and a myc tag was injected into fertilized C57BL/6 fertilized oocytes. Founder line Q3 was established, carrying 1 copy of the transgene. Founder animals were maintained as hemizygotes on the C57BL/6 background.

A 32 SNP (single nucleotide polymorphism) panel analysis, with 27 markers covering all 19 chromosomes and the X chromosome, as well as 5 markers that distinguish between the C57BL/6J and C57BL/6N substrains, was performed on the rederived living colony at The Jackson Laboratory Repository. While the 27 markers throughout the genome suggested a C57BL/6 genetic background, 2 of 5 markers that determine C57BL/6J from C57BL/6N were found to be segregating. These data suggest the mice sent to The Jackson Laboratory Repository were on a C57BL/6N genetic background.

https://www.jax.org/strain/008535

References

1. ShimshekDR, Kim J, HübnerMR, SpergelDJ. Codon-improved Cre recombinase (iCre) expression in the mouse. Genesis.2002 Jan.32(1):19-26.

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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