Nfatc1-P2A-iCre Cas9-KI Strategy

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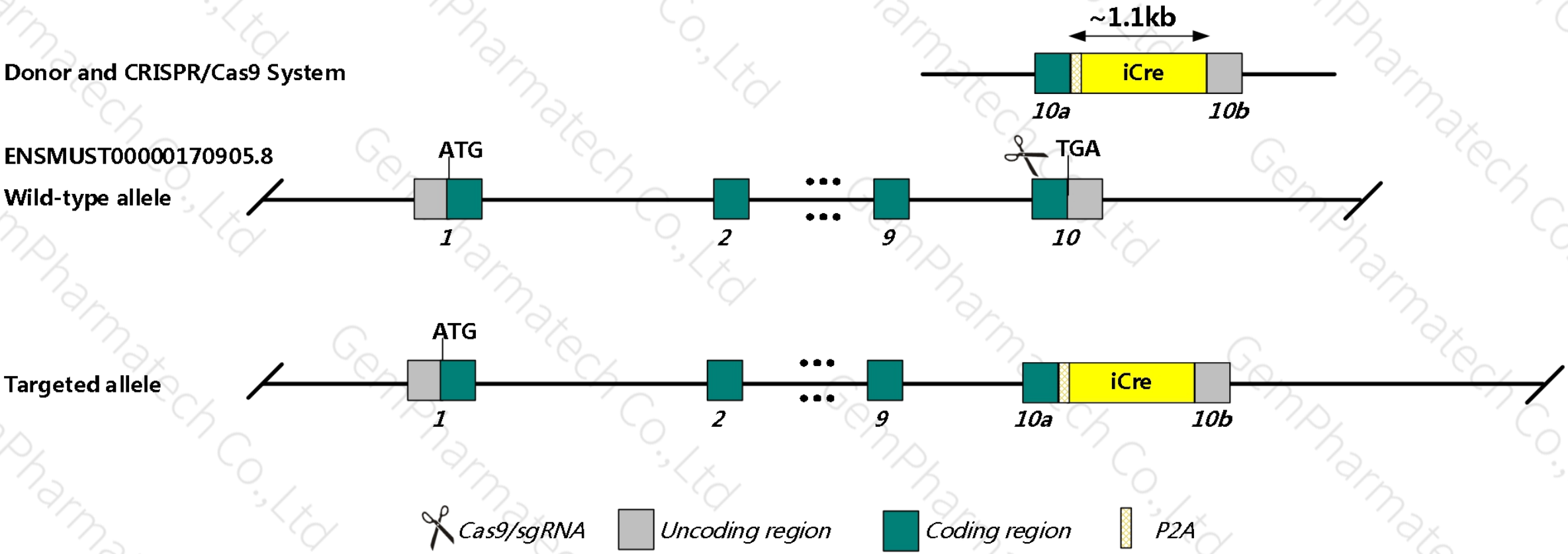
Design Date:	**2019-8-14**
Reviewer	**Xueting Zhang**

Project Overview

Project Name	***Nfatc1-P2A-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Nfatc1* gene. The schematic diagram is as follows:



Technical routes

- The *Nfatc1* gene has 7 transcripts.According to the structure of *Nfatc1* gene, *Nfatc1-204*(ENSMUST00000170905.8) is selected for presentation of the recommended strategy.

- *Nfatc1-204* gene has 10 exons, with the ATG start codon in exon1 and TGA stop codon in exon10.

- We make *Nfatc1-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 coding(TGA) of Nfatc1 gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-iCre after stop coding(TGA) of Nfatc1 gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, Homozygous mutation of this gene results in lethality throughout fetal growth and development due to cardiac failure. Mutants exhibit blood circulation, cardiac valve and ventricular septal abnormalities, edema, abdominal hemorrhage, and semilunar valveregurgitation.
- According to the existing articles, the site of expression is in the endocardium of the heart (expressed during embryonic stage).
- 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 *Nfatc1* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon10 of *Nfatc1* gene in this strategy.
- The *Nfatc1* 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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Nfatc1 nuclear factor of activated T cells, cytoplasmic, calcineurin dependent 1 [*Mus musculus* (house mouse)]

Gene ID: 18018, updated on 13-Aug-2019

Summary

Official Symbol	Nfatc1 provided by MGI
Official Full Name	nuclear factor of activated T cells, cytoplasmic, calcineurin dependent 1 provided by MGI
Primary source	MGI:MGI:102469
See related	Ensembl:ENSMUSG00000033016
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	NFAT2; NFATc; NF-ATc; Nfatcb; AI449492; AV076380; 2210017P03Rik
Expression	Broad expression in spleen adult (RPKM 23.0), thymus adult (RPKM 14.6) and 15 other tissues See more
Orthologs	human all

Genomic context

Location: 18 E3; 18 53.66 cM

See Nfatc1 in Genome Data Viewer

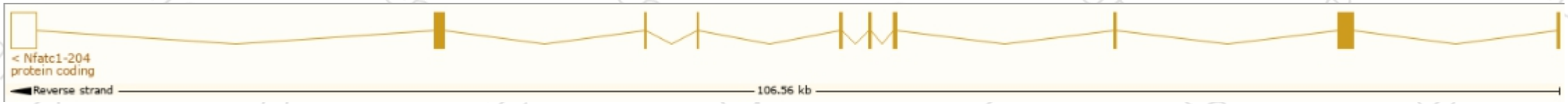
Exon count: 11

Transcript information（Ensembl）

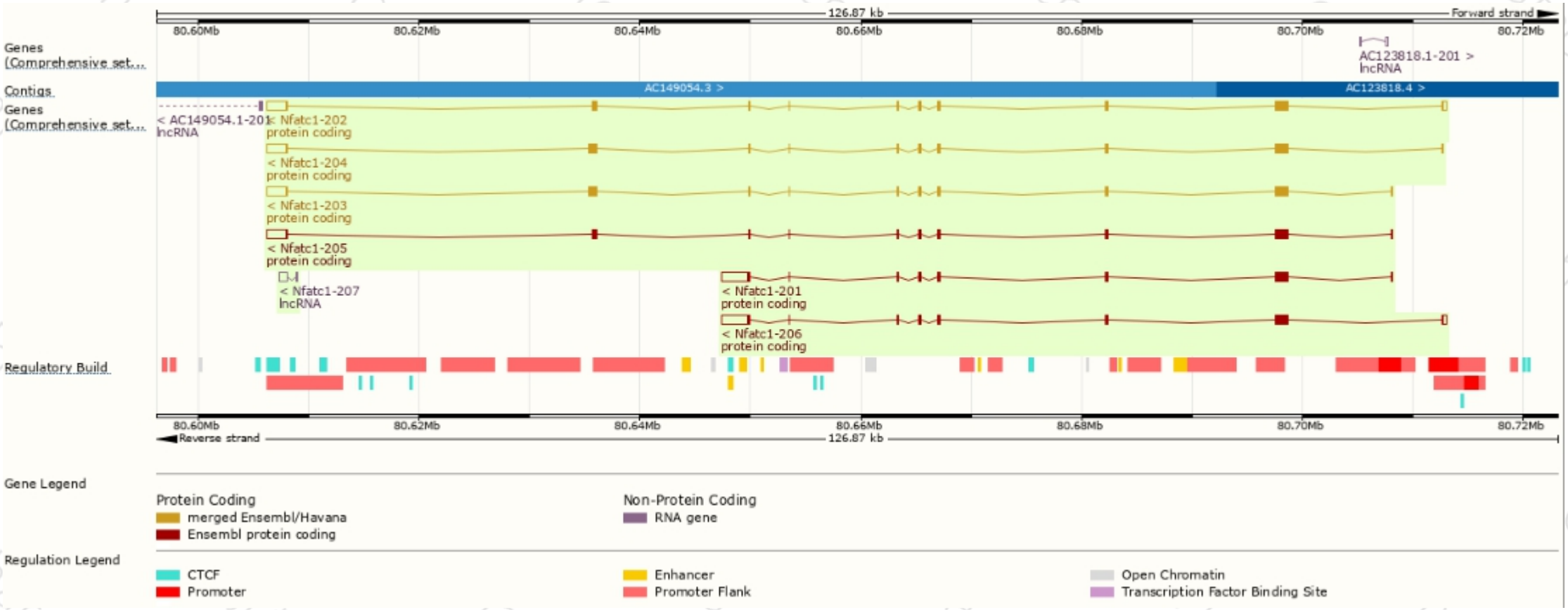
The gene has 7 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nfatc1-203	ENSMUST00000167977.7	4604	925aa	Protein coding	CCDS50335	B5B2N7	TSL:1 GENCODE basic APPRIS ALT2
Nfatc1-202	ENSMUST00000078049.11	4594	827aa	Protein coding	CCDS37873	Q6P7T9	TSL:1 GENCODE basic APPRIS ALT2
Nfatc1-204	ENSMUST00000170905.8	4591	939aa	Protein coding	CCDS50336	B5B2N2	TSL:1 GENCODE basic APPRIS ALT2
Nfatc1-201	ENSMUST00000035800.7	4505	703aa	Protein coding	CCDS29369	Q9DBQ6	TSL:1 GENCODE basic APPRIS P3
Nfatc1-206	ENSMUST00000236711.1	4797	717aa	Protein coding	-	B5B2N5	GENCODE basic APPRIS ALT2
Nfatc1-205	ENSMUST00000236310.1	4315	813aa	Protein coding	-	B5B2N4	GENCODE basic APPRIS ALT2
Nfatc1-207	ENSMUST00000237776.1	672	No protein	lncRNA	-	-	-

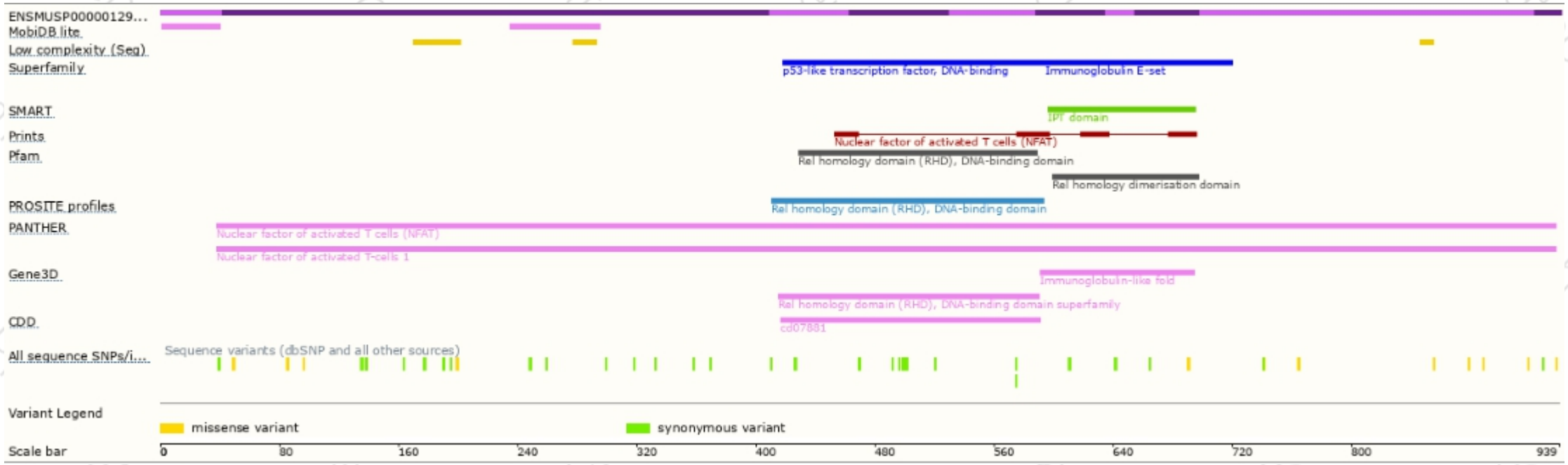
The strategy is based on the design of *Nfatc1-204* transcript,The transcription is shown below



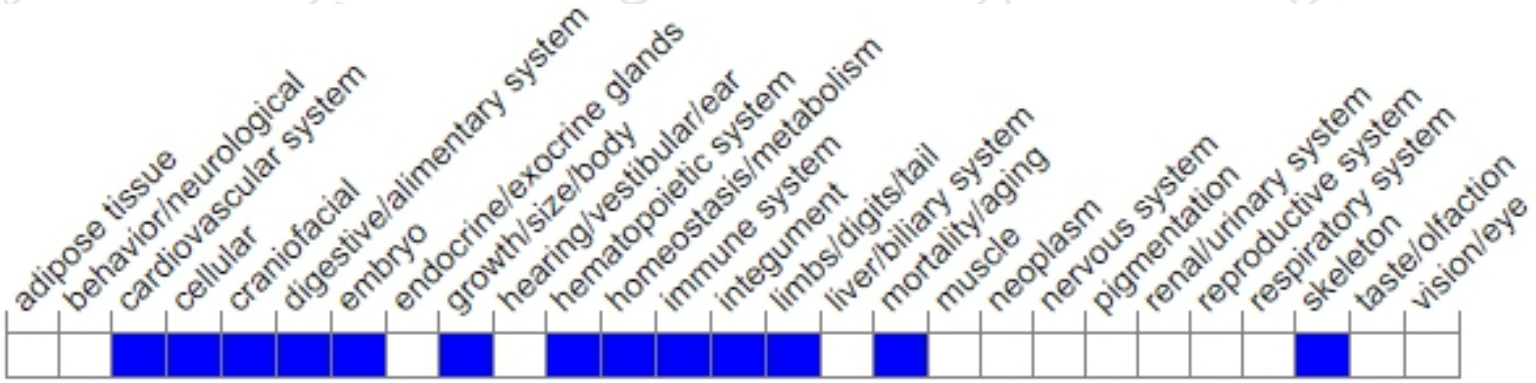
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)

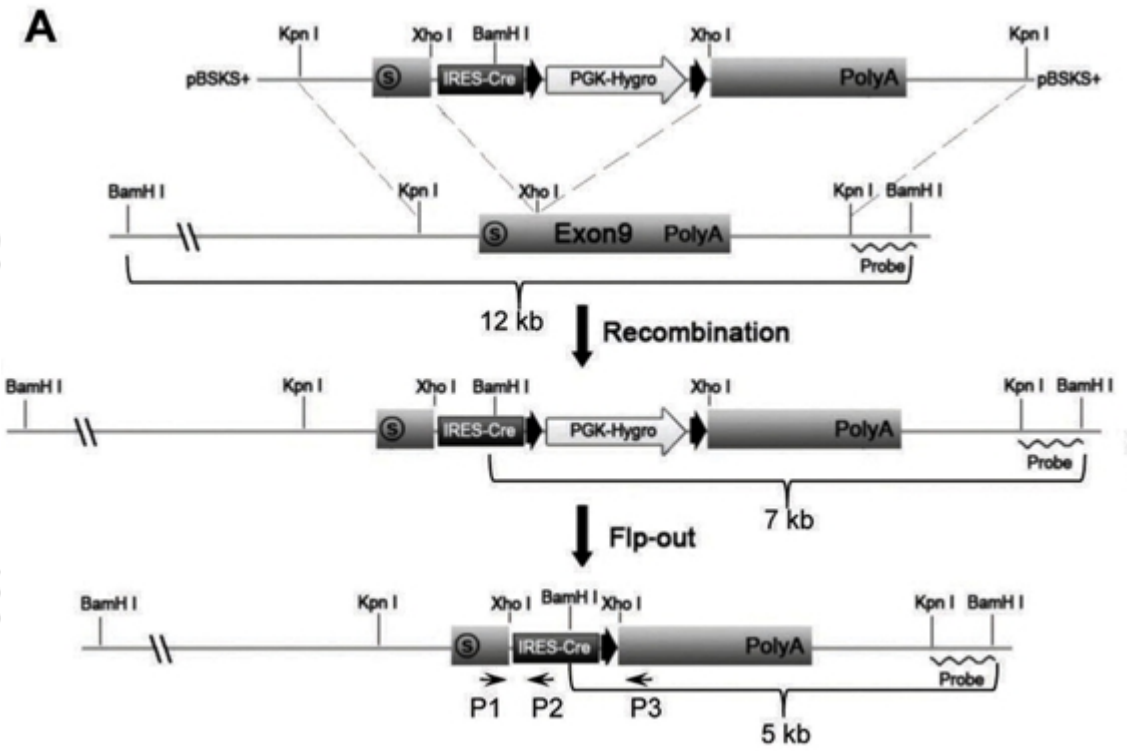


Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/marker/MGI:102469) .

Homozygous mutation of this gene results in lethality throughout fetal growth and development due to cardiac failure. Mutants exhibit blood circulation, cardiac valve and ventricular septal abnormalities, edema, abdominal hemorrhage, and semilunar valveregurgitation.

References

Wu B, et al., Endocardial cells form the coronary arteries by angiogenesis through myocardial-endocardial VEGF signaling. Cell. 2012 Nov 21;151(5):1083-96



Nfatc1^{+} Endocardial Precursors Generate Coronary Plexuses

To study the developmental fate and function of endocardial cells, we generated a *Cre* knockin mouse strain, *Nfatc1*^{Cre}, in which *Cre* cDNA with an internal ribosomal entry site was inserted downstream of the stop codon of the mouse *Nfatc1* (Zhou et al., 2002) (Figure S2A). *Nfatc1*^{Cre} mice developed normally and bred to the *RCE*^{fsEGFP} (Miyoshi et al., 2010; Sousa et al., 2009) or *R26*^{fslz} mice (Soriano, 1999). *Cre* expression was restricted to the endocardium of *Nfatc1*^{Cre} embryos; no expression was seen in the sinus venosus, liver, pharyngeal arch, proepicardium/epicardium, or myocardium at E9.5–E10.5 (Figure S2B), or developing coronary vessels (data not shown).

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

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