Nfix Cas9-CKO Strategy

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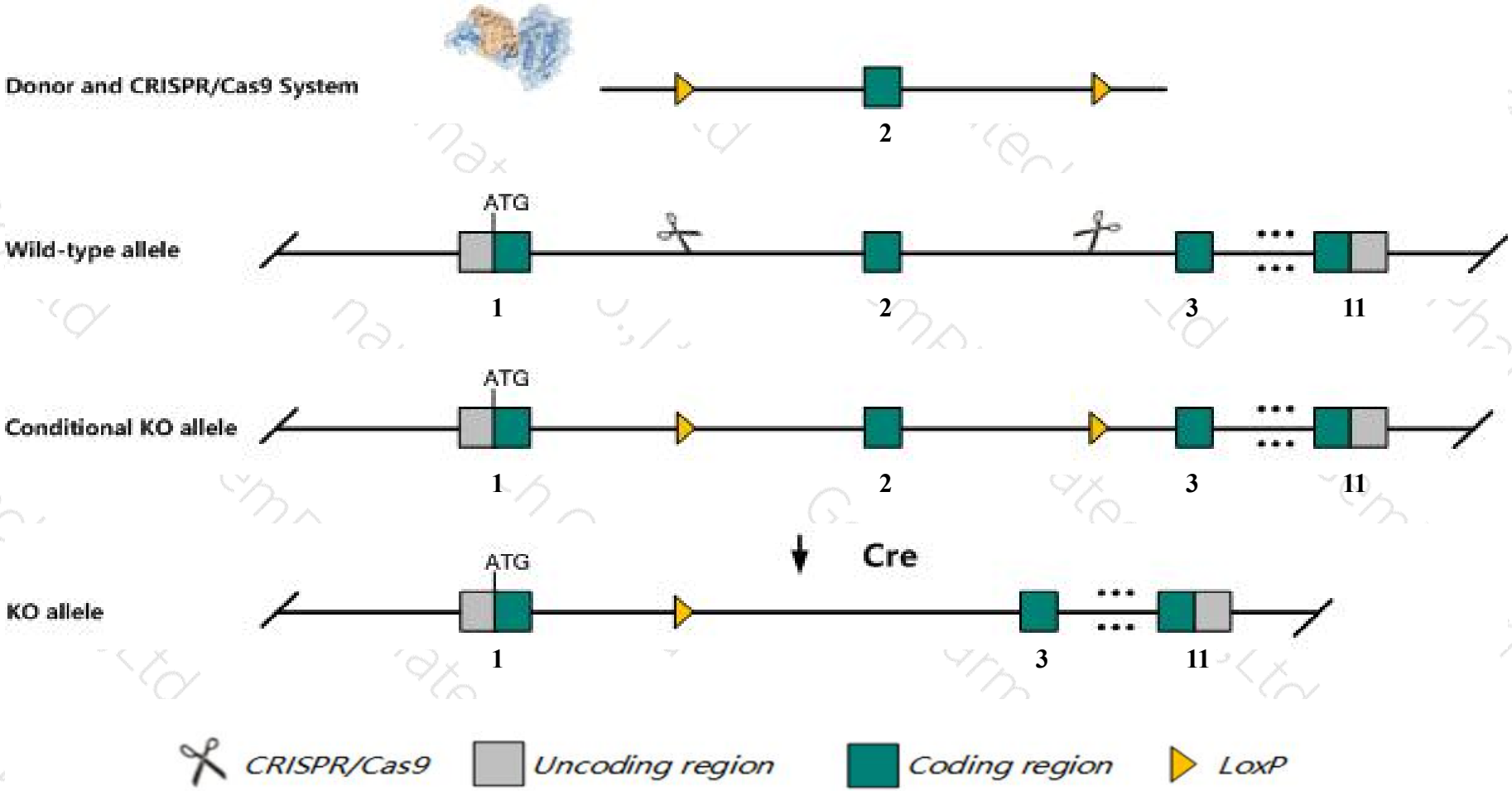
Project Overview

Project Name	***Nfix***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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



Technical routes

- The *Nfix* gene has 8 transcripts. According to the structure of *Nfix* gene, exon2 of *Nfix-204* (ENSMUST00000109764.7) transcript is recommended as the knockout region.The region contains 532bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Nfix* gene. The brief process is as follows:CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

- According to the existing MGI data,mice homozygous for a mutation in this gene display postnatal lethality, hydrocephalus, partial agenesis of the corpus callosum, deformation of the spine due to delayed vertebral body ossification, degeneration of intervertebral disks, decreased mineralization and impaired endochondral ossification.
- Transcript *Nfix*-206&207&208 may not be affected.
- The *Nfix* gene is located on the Chr8. If the knockout 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 biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Nfix nuclear factor I/X [Mus musculus (house mouse)]

Gene ID: 18032, updated on 13-Mar-2020

Summary

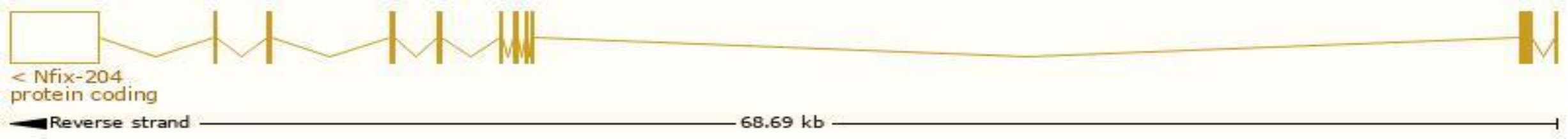
Official Symbol	Nfix provided by MGI
Official Full Name	nuclear factor I/X provided byMGI
Primary source	MGI:MGI:97311
See related	Ensembl:ENSMUSG00000001911
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CTF, NF-I/X, NF1-X, NFI-X
Expression	Ubiquitous expression in lung adult (RPKM 15.5), limb E14.5 (RPKM 14.4) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 8 transcripts,all transcripts are shown below:

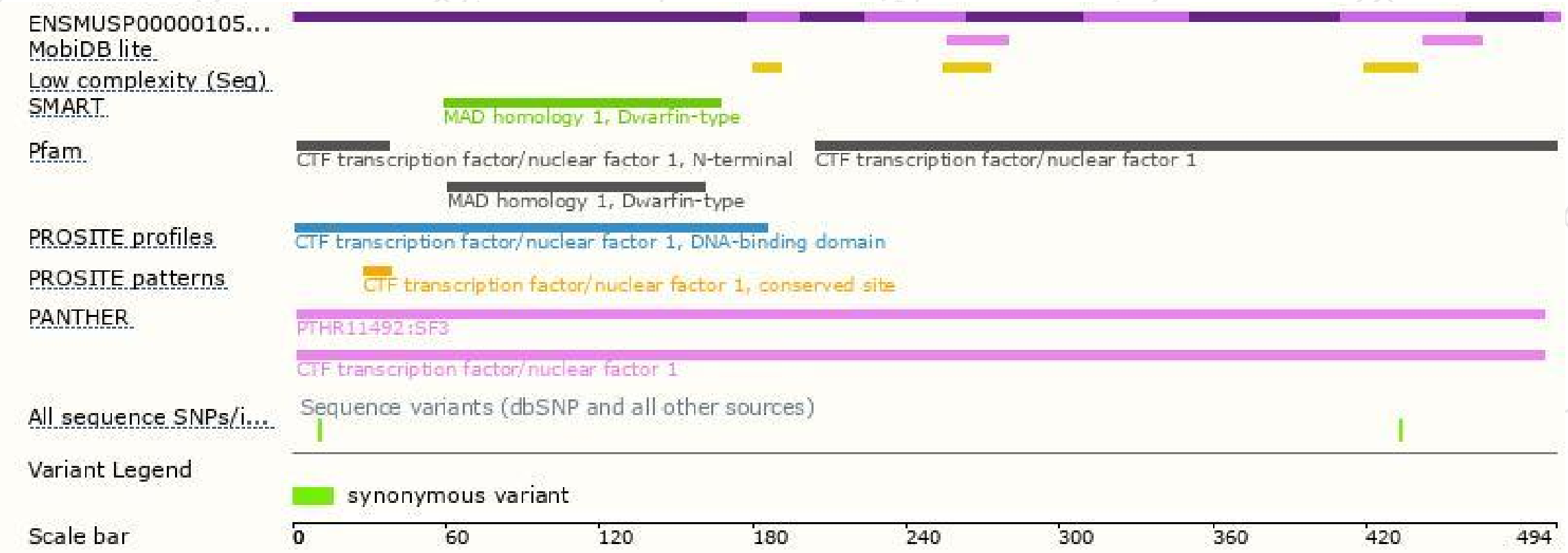
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nfix-204	ENSMUST00000109764.7	5476	494aa	Protein coding	CCDS40411	E9PUH7	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P4
Nfix-203	ENSMUST00000109762.7	2261	391aa	Protein coding	CCDS80907	Q3TYK3	TSL:1 GENCODE basic
Nfix-202	ENSMUST00000099070.9	1728	441aa	Protein coding	CCDS40413	P70257	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT1
Nfix-201	ENSMUST00000076715.12	1403	400aa	Protein coding	CCDS40412	P70257	TSL:1 GENCODE basic
Nfix-205	ENSMUST00000126806.1	1465	488aa	Protein coding	-	D3YZ00	CDS 3' incomplete TSL:5
Nfix-207	ENSMUST00000148644.1	398	No protein	Processed transcript	-	-	TSL:5
Nfix-208	ENSMUST00000148746.1	234	No protein	Processed transcript	-	-	TSL:5
Nfix-206	ENSMUST00000132236.1	347	No protein	Retained intron	-	-	TSL:2

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

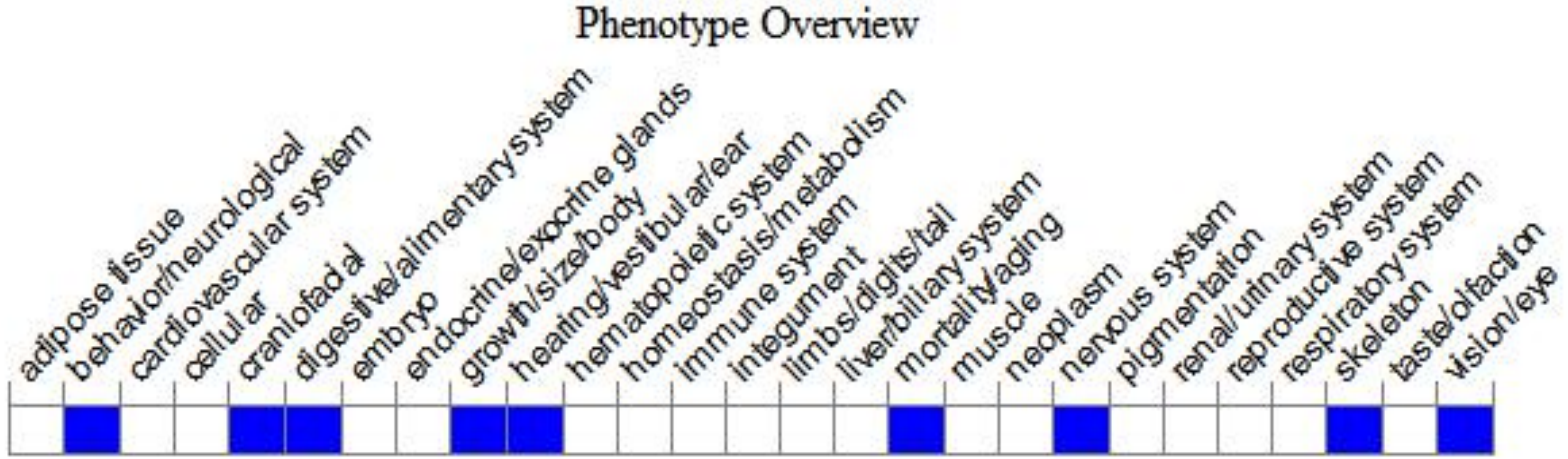


Genomic location distribution

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/).

According to the existing MGI data,mice homozygous for a mutation in this gene display postnatal lethality, hydrocephalus, partial agenesis of the corpus callosum, deformation of the spine due to delayed vertebral body ossification, degeneration of intervertebral disks, decreased mineralization and impaired endochondral ossification.

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

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