Kirrel Cas9-CKO Strategy

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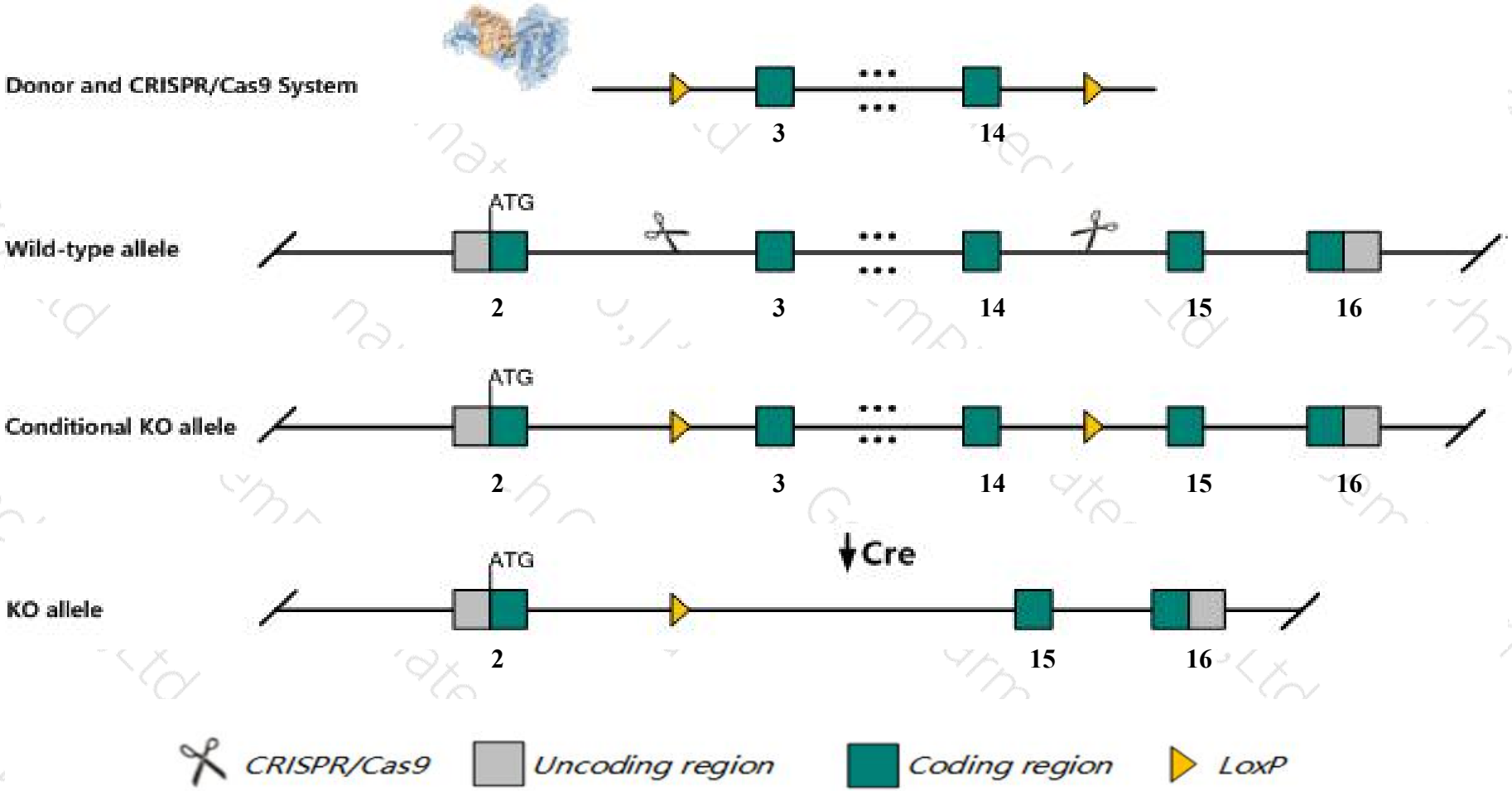
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Kirrel* gene has 3 transcripts. According to the structure of *Kirrel* gene, exon3-exon14 of *Kirrel-202*(ENSMUST00000107618.8) transcript is recommended as the knockout region.The region contains 1682bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Kirrel* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 gene trap insertion exhibit postnatal lethality and are small and sickly. Glomerular and tubular defects in the kidney result in severe proteinuria.

➢ The *Kirrel* gene is located on the Chr3. 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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Kirrel kirre like nephrin family adhesion molecule 1 [*Mus musculus* (house mouse)]

Gene ID: 170643, updated on 26-Jun-2020

Summary

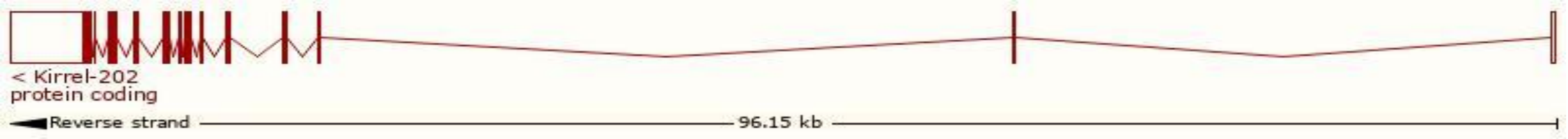
Official Symbol	Kirrel provided by MGI
Official Full Name	kirre like nephrin family adhesion molecule 1 provided by MGI
Primary source	MGI:MGI:1891396
See related	Ensembl:ENSMUSG00000041734
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Neph1; Kirrel1; 6720469N11Rik
Expression	Broad expression in limb E14.5 (RPKM 16.4), subcutaneous fat pad adult (RPKM 10.9) and 21 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

The gene has 3 transcripts,all transcripts are shown below:

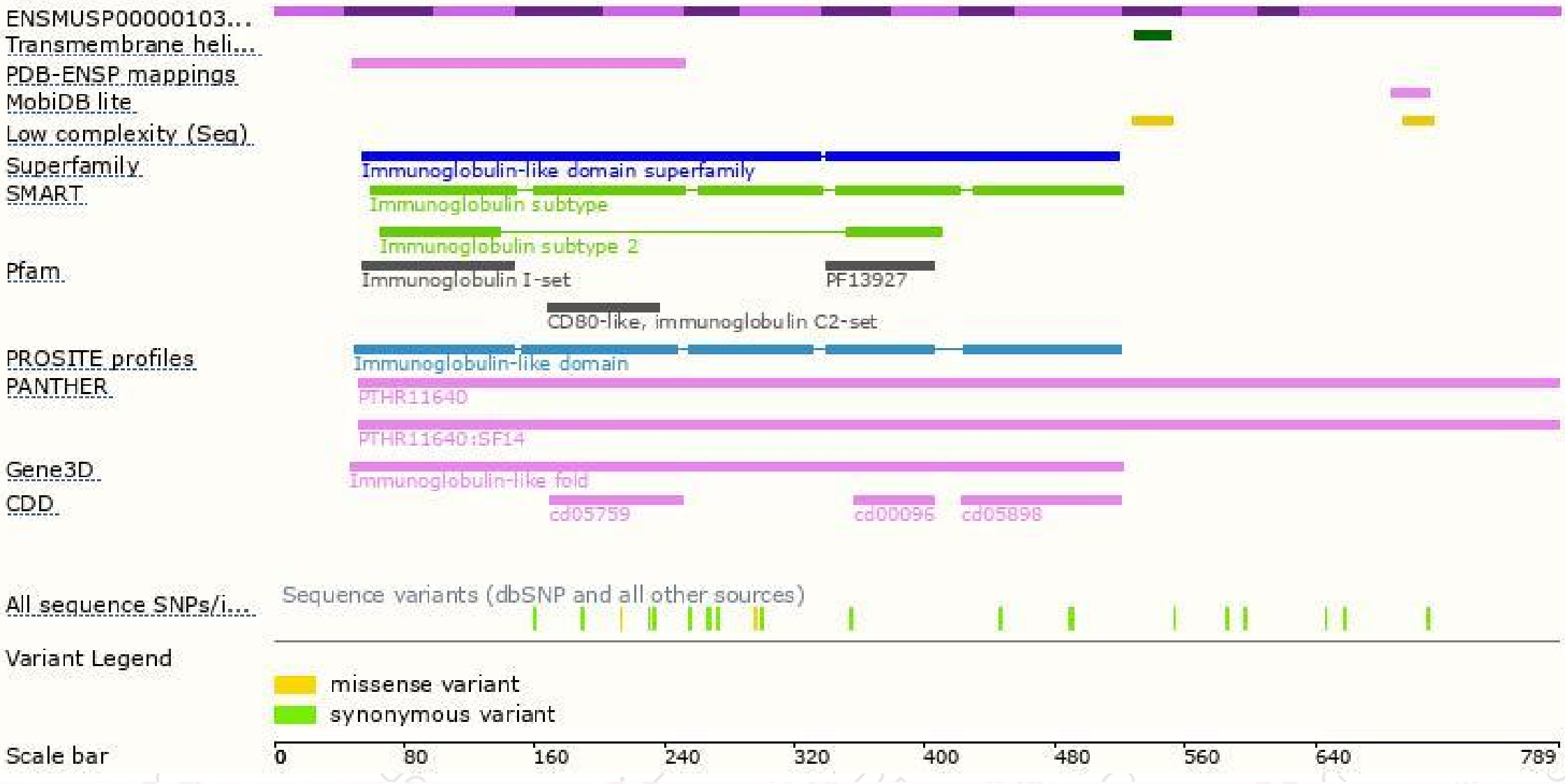
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kirrel-202	ENSMUST00000107618.8	7278	789aa	Protein coding	CCDS17450	Q80W68	TSL:5 GENCODE basic APPRIS P2
Kirrel-203	ENSMUST00000159976.7	7236	789aa	Protein coding	CCDS17450	Q80W68	TSL:1 GENCODE basic APPRIS P2
Kirrel-201	ENSMUST00000041732.8	2160	634aa	Protein coding	-	Q80W68	TSL:1 GENCODE basic APPRIS ALT2

The strategy is based on the design of *Kirrel-202* transcript,the transcription is shown below:

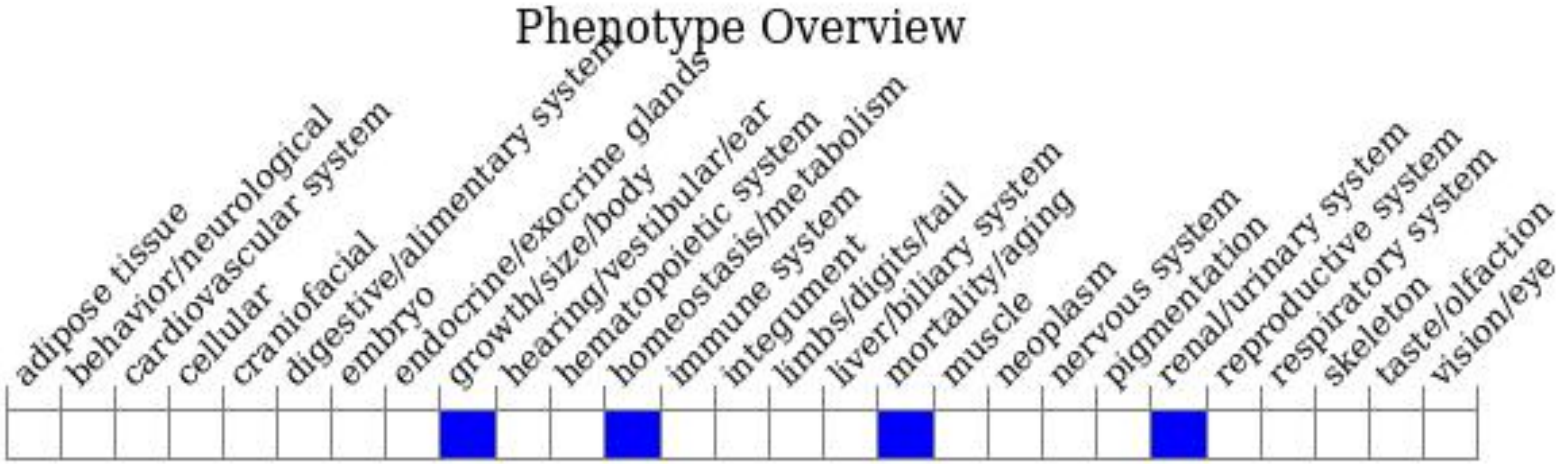


Genomic location distribution

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/).

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

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