Trpm8 Cas9-CKO Strategy

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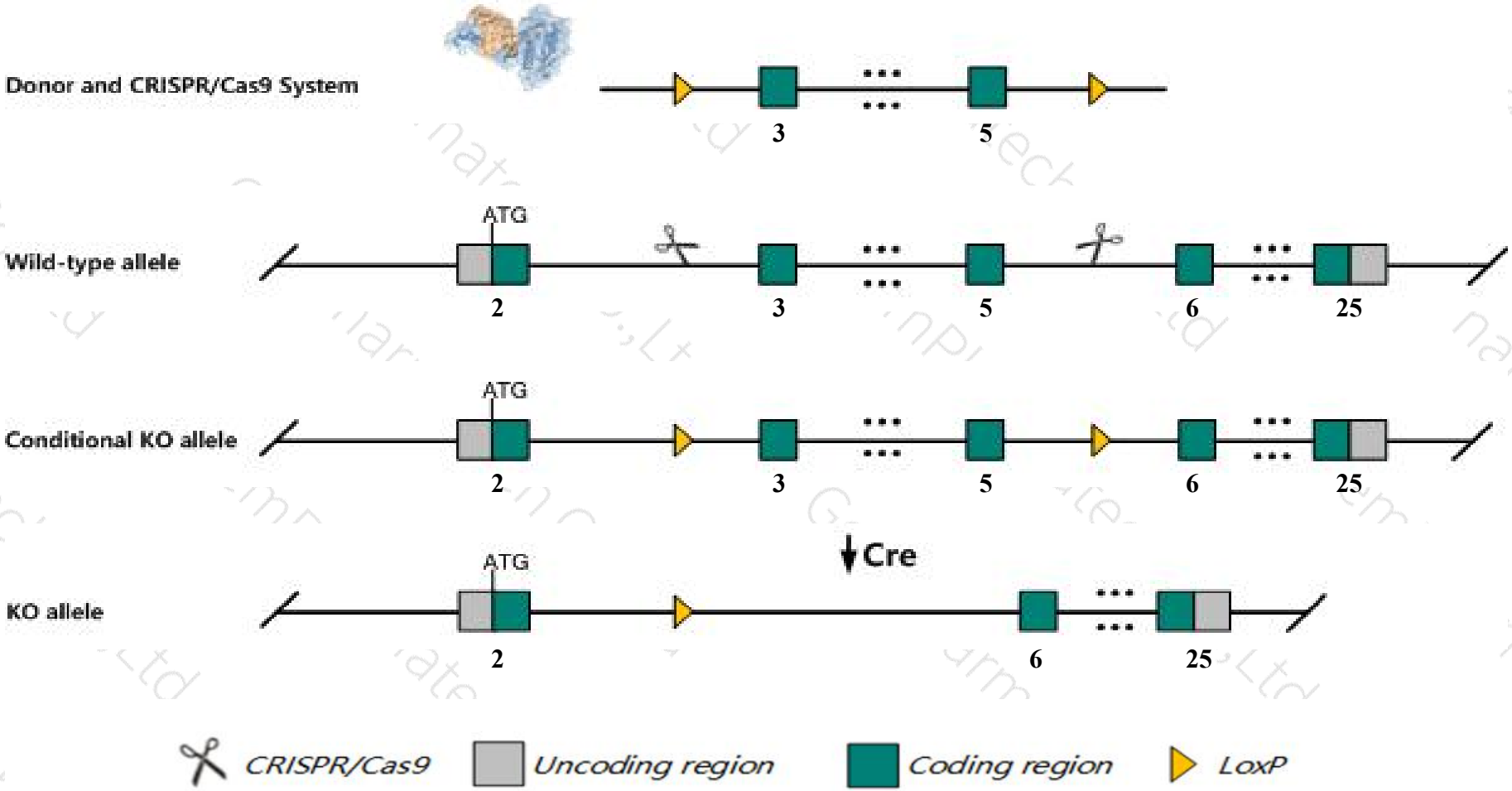
Project Overview

Project Name	***Trpm8***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Trpm8* gene. The schematic diagram is as follows:



Technical routes

- The *Trpm8* gene has 4 transcripts. According to the structure of *Trpm8* gene, exon3-exon5 of *Trpm8-201* (ENSMUST00000040210.13) transcript is recommended as the knockout region.The region contains 409bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Trpm8* 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 null allele exhibit decreased sensitivity to cold and reduced response to cold stimuli.

- The *Trpm8* gene is located on the Chr1. 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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Trpm8 transient receptor potential cation channel, subfamily M, member 8 [Mus musculus (house mouse)]

Gene ID: 171382, updated on 2-Apr-2019

Summary

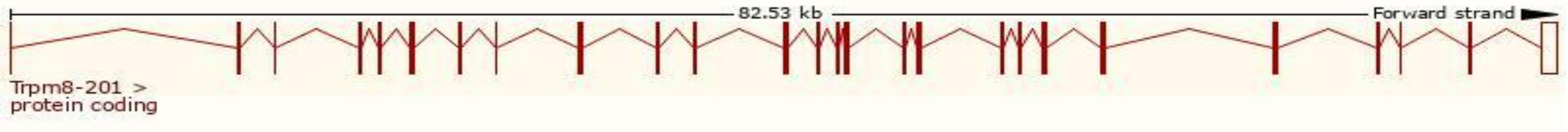
Official Symbol	Trpm8 provided by MGI
Official Full Name	transient receptor potential cation channel, subfamily M, member 8 provided by MGI
Primary source	MGI:MGI:2181435
See related	Ensembl:ENSMUSG00000036251
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CMR1, LTRPC6, LTrpC-6, TRPP8, Trp-p8
Expression	Biased expression in testis adult (RPKM 2.5), CNS E18 (RPKM 0.2) and 1 other tissue See more
Orthologs	human all

Transcript information（Ensembl）

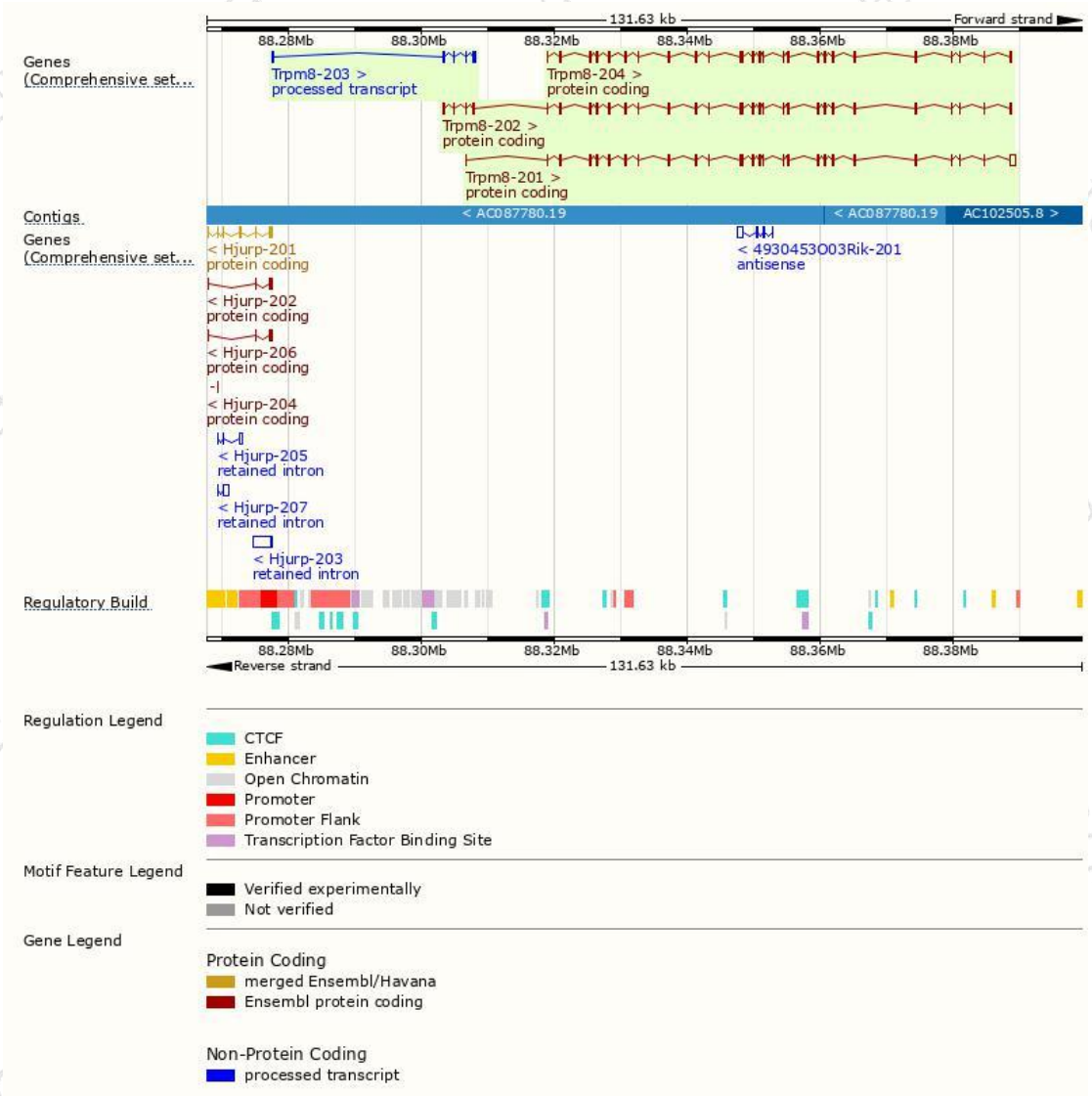
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trpm8-201	ENSMUST00000040210.13	4146	1104aa	Protein coding	CCDS48316	Q8R4D5	TSL:5 GENCODE basic APPRIS P1
Trpm8-202	ENSMUST00000113114.9	4112	1104aa	Protein coding	CCDS48316	Q8R4D5	TSL:1 GENCODE basic APPRIS P1
Trpm8-204	ENSMUST00000171176.2	3723	1104aa	Protein coding	CCDS48316	Q8R4D5	TSL:1 GENCODE basic APPRIS P1
Trpm8-203	ENSMUST00000148138.7	567	No protein	lncRNA	-	-	TSL:2

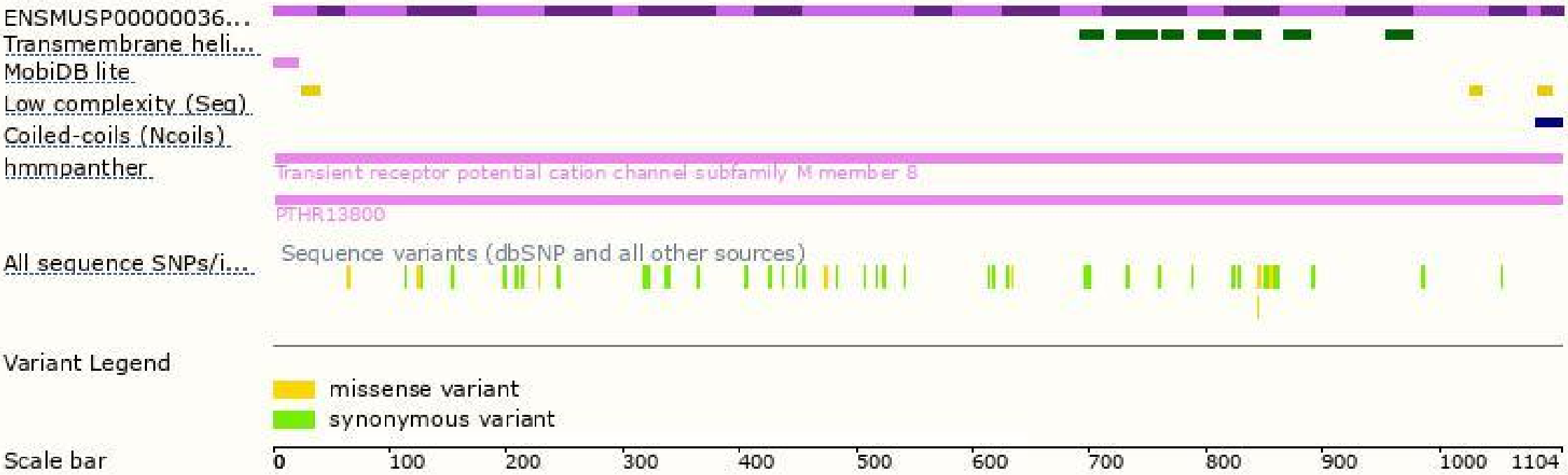
The strategy is based on the design of *Trpm8-201* transcript,The transcription is shown below



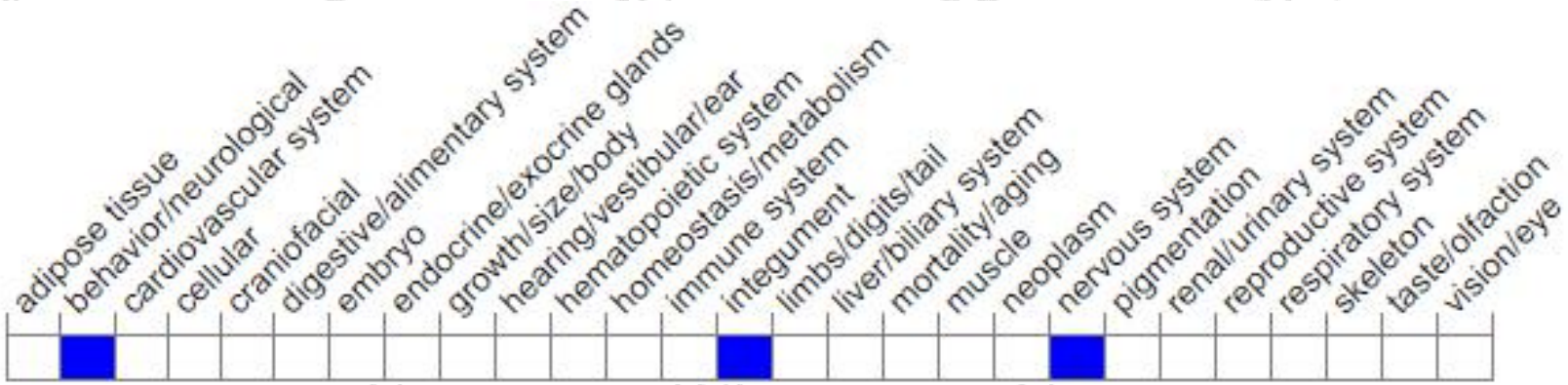
Genomic location distribution



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/).

According to the existing MGI data,Mice homozygous for a null allele exhibit decreased sensitivity to cold and reduced response to cold stimuli.

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

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