Trpm4 Cas9-KO Strategy

Designer: Huan Wang

Reviewer: Huan Fan

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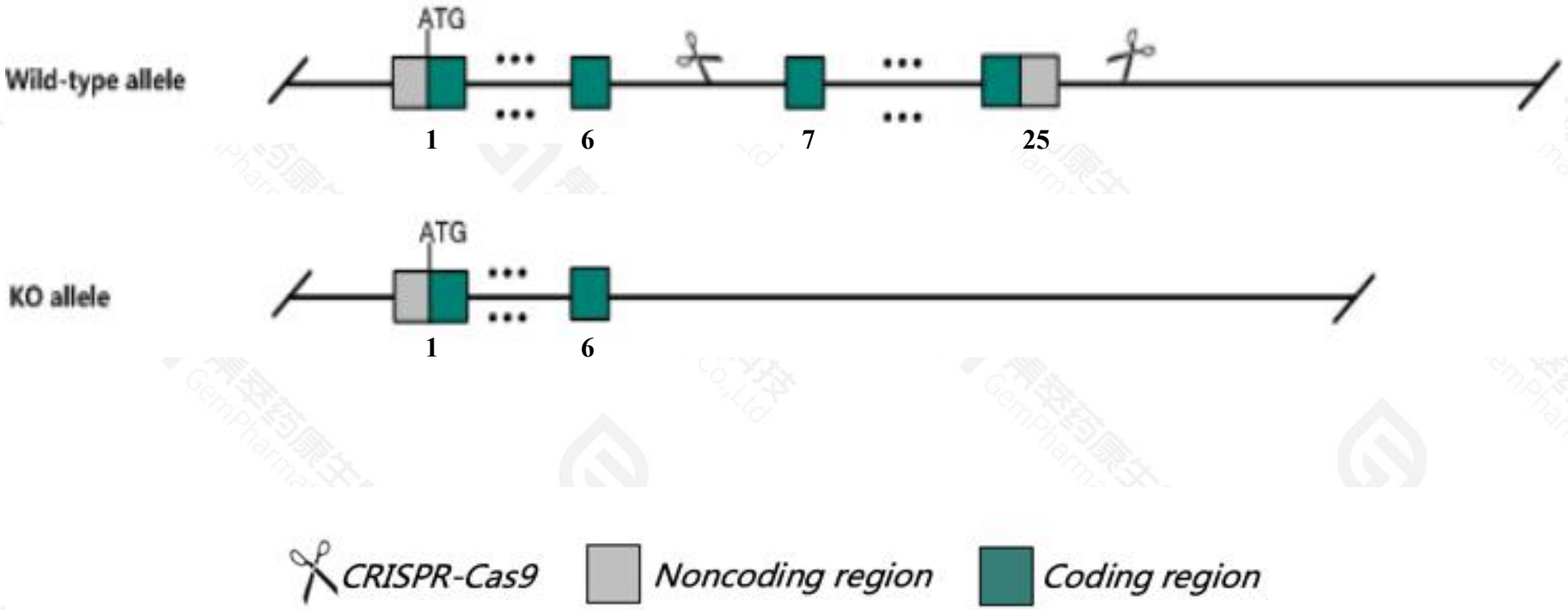
Project Overview

Project Name	***Trpm4***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR-Cas9 technology to edit the *Trpm4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Trpm4* gene has 9 transcripts. According to the structure of *Trpm4* gene, exon7-exon25 of *Trpm4-201*(ENSMUST00000042194.9) transcript is recommended as the knockout region. The region contains most of coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR-Cas9 technology to modify *Trpm4* gene. The brief process is as follows: CRISPR-Cas9 system 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.

Notice

➢ According to the existing MGI data,mice homozygous for a knock-out allele display increased Ca2+ influx and IgE-dependent mast cell activation, increased vascular permeability, and enhanced acute anaphylactic responses. Mice homozygous for a different knock-out allele show Ca2+ overload and impaired dendritic cell migration.

➢ The *Trpm4* gene is located on the Chr7. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Trpm4 transient receptor potential cation channel, subfamily M, member 4 [Mus musculus (house mouse)]

Gene ID: 68667, updated on 31-Jan-2019

Summary

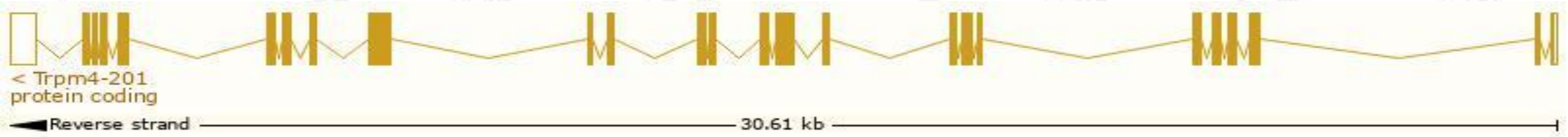
Official Symbol	Trpm4 provided by MGI
Official Full Name	transient receptor potential cation channel, subfamily M, member 4 provided by MGI
Primary source	MGI:MGI:1915917
See related	Ensembl:ENSMUSG00000038260
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1110030C19Rik, AW047689, LTRPC4, LTrpC-4, TRPM4B
Expression	Biased expression in colon adult (RPKM 55.6), duodenum adult (RPKM 35.6) and 14 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

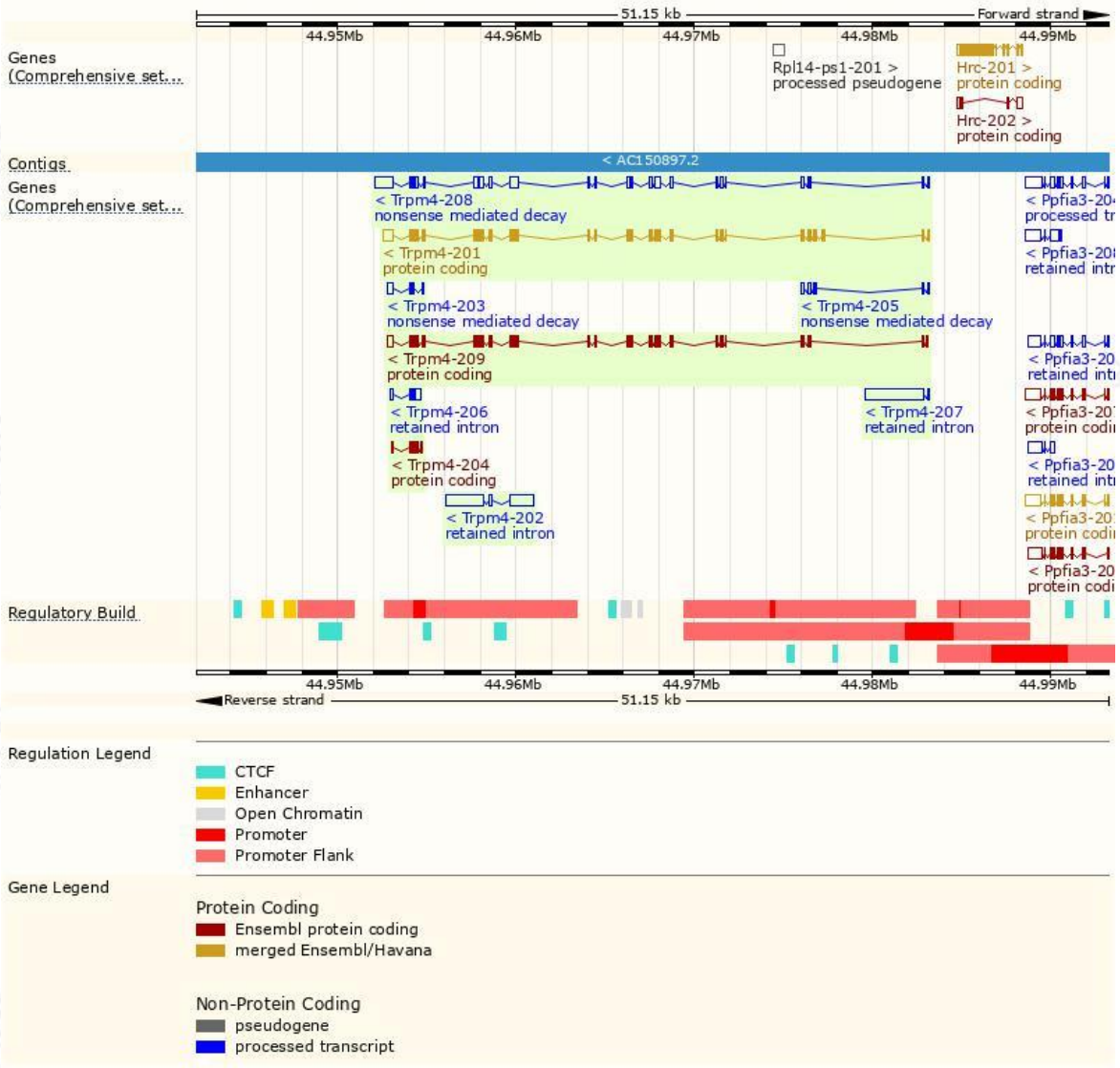
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trpm4-201	ENSMUST00000042194.9	4234	1213aa	Protein coding	CCDS52245	Q7TN37	TSL:1 GENCODE basic APPRIS P2
Trpm4-209	ENSMUST00000211743.1	3673	1066aa	Protein coding	-	A0A1B0GS49	TSL:1 GENCODE basic APPRIS ALT2
Trpm4-204	ENSMUST00000210311.1	498	128aa	Protein coding	-	A0A1B0GT86	CDS 5' incomplete TSL:3
Trpm4-208	ENSMUST00000211431.1	4315	97aa	Nonsense mediated decay	-	A0A1B0GRQ4	TSL:2
Trpm4-205	ENSMUST00000210541.1	685	85aa	Nonsense mediated decay	-	A0A1B0GRR7	TSL:3
Trpm4-203	ENSMUST00000209506.1	609	65aa	Nonsense mediated decay	-	A0A1B0GSM7	TSL:1
Trpm4-202	ENSMUST00000209239.1	3521	No protein	Retained intron	-	-	TSL:1
Trpm4-207	ENSMUST00000211411.1	3350	No protein	Retained intron	-	-	TSL:1
Trpm4-206	ENSMUST00000210639.1	625	No protein	Retained intron	-	-	TSL:2

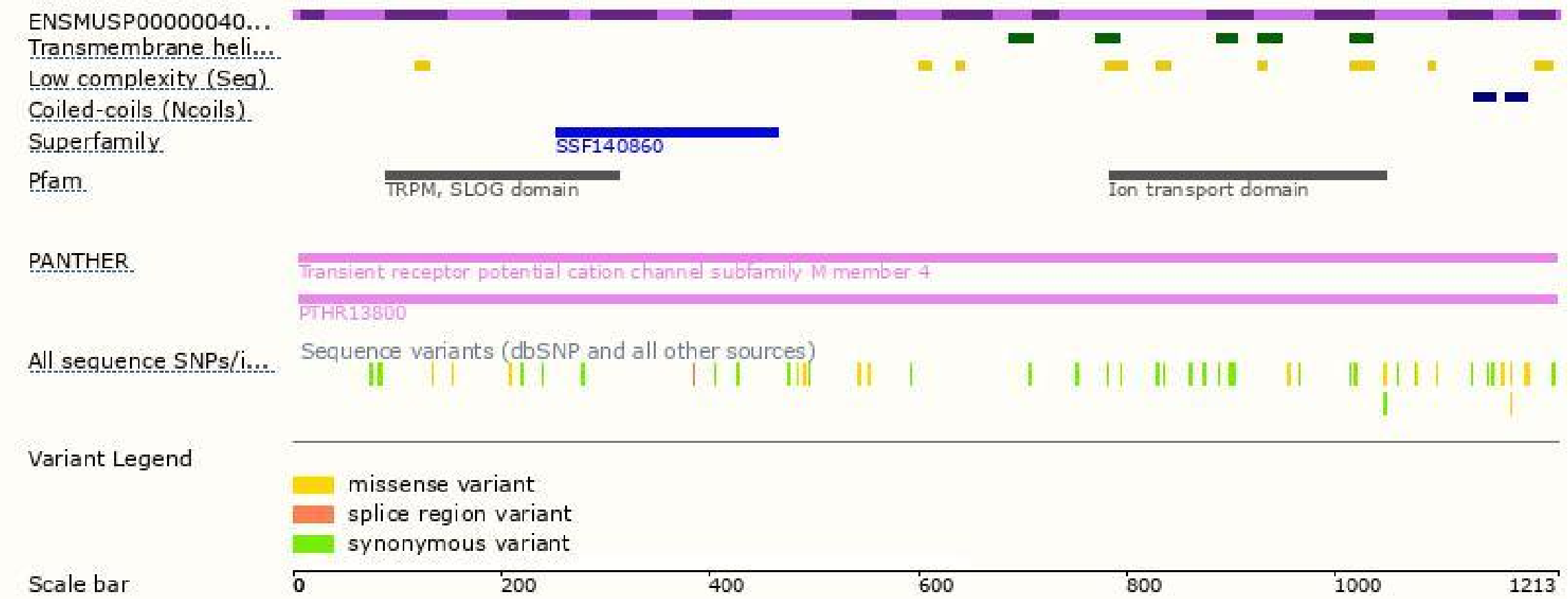
The strategy is based on the design of *Trpm4-201* transcript,the transcription is shown below:



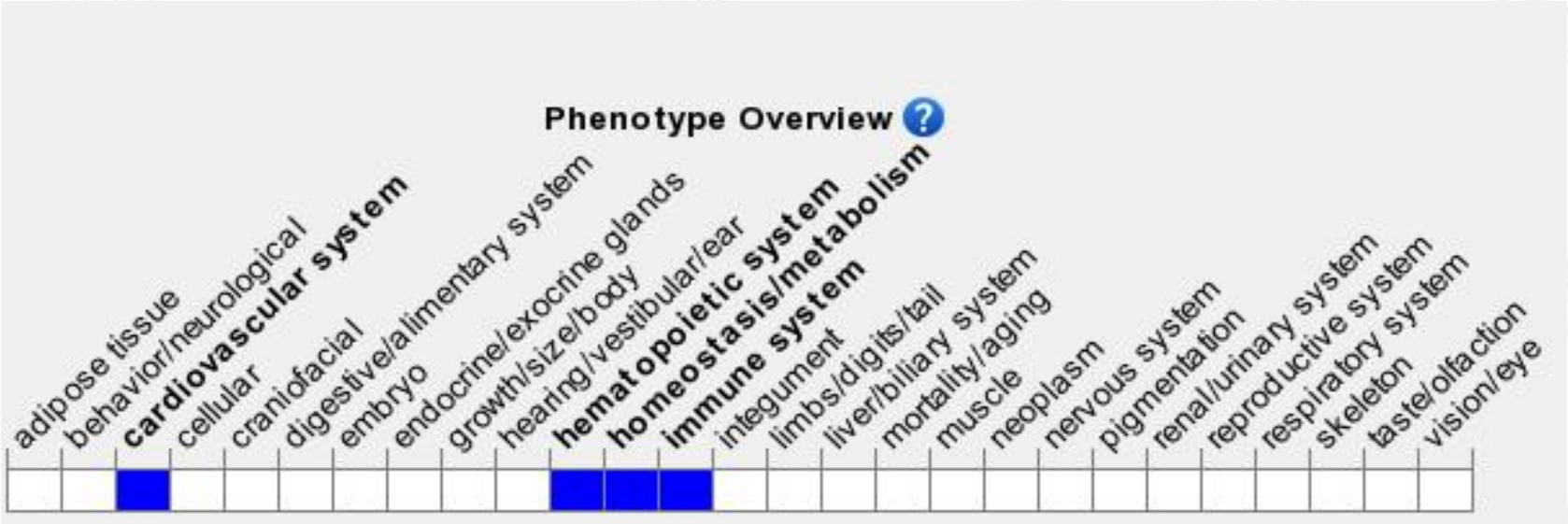
Genomic location distribution



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 knock-out allele display increased Ca2+ influx and IgE-dependent mast cell activation, increased vascular permeability, and enhanced acute anaphylactic responses. Mice homozygous for a different knock-out allele show Ca2+ overload and impaired dendritic cell migration.

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If you have any questions, you are welcome to inquire.
Tel: 400-9660890

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