Trpm5 Cas9-CKO Strategy

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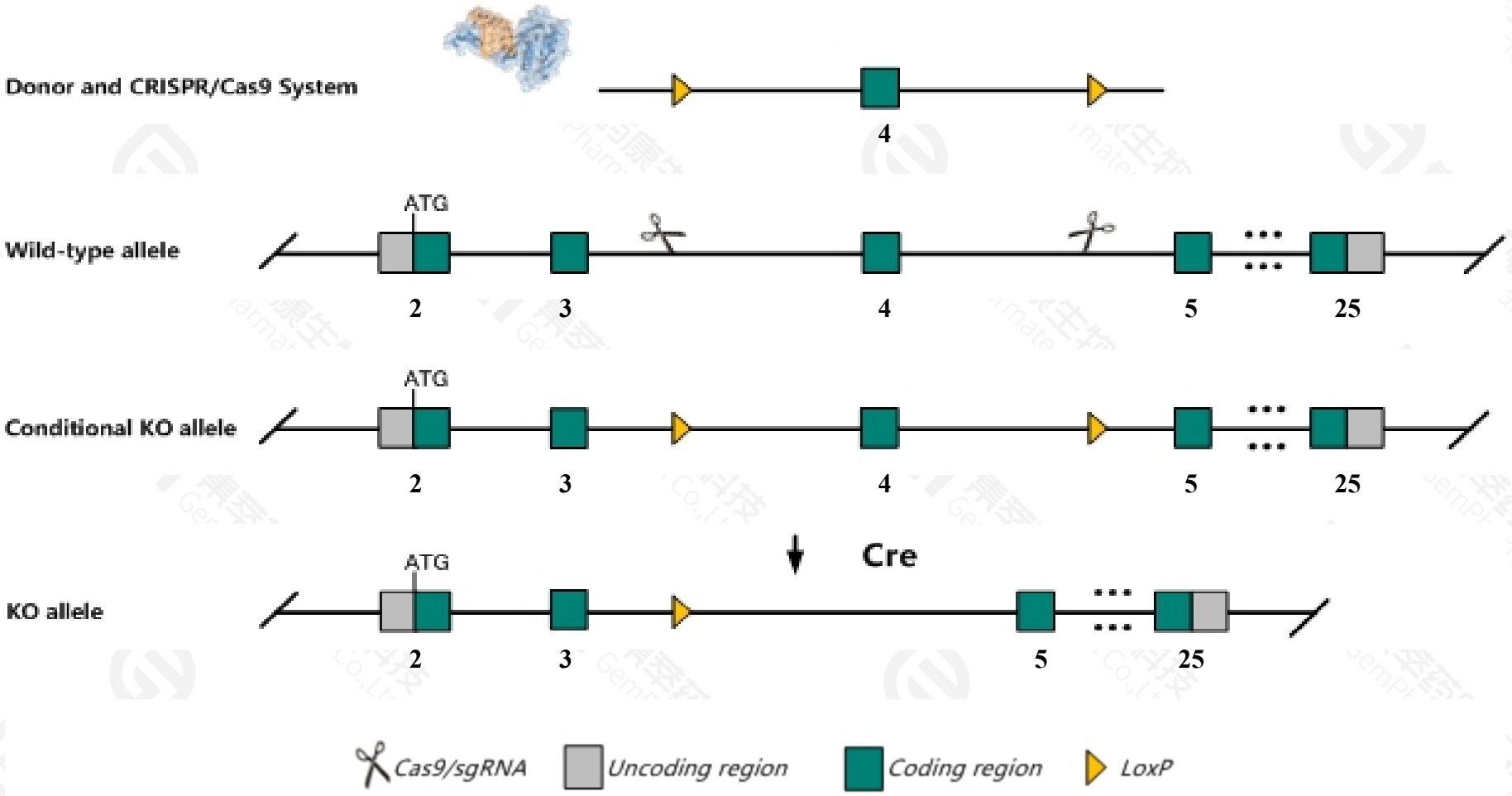
Project Overview

Project Name	***Trpm5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Trpm5* gene. The schematic diagram is as follows:



Technical routes

➢ The *Trpm5* gene has 8 transcripts. According to the structure of *Trpm5* gene, exon4 of *Trpm5-201*(ENSMUST00000009390.10) transcript is recommended as the knockout region.The region contains 173bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Trpm5* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor was constructed.Cas9, sgRNA 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 was 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,homozygous mutant mice demonstrate abnormal taste perception, responding to sour and salty stimuli but not to sweet, or bitter stimuli.

➢ The *Trpm5* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information（NCBI）

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Trpm5 transient receptor potential cation channel, subfamily M, member 5 [Mus musculus (house mouse)]

Gene ID: 56843, updated on 16-Feb-2021

Summary

Official Symbol	Trpm5 provided by MGI
Official Full Name	transient receptor potential cation channel, subfamily M, member 5 provided byMGI
Primary source	MGI:MGI:1861718
See related	Ensembl:ENSMUSG00000009246
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	9430099A16Rik, LTrpC-5, Ltr, Ltrpc5, Mtr, Mtr1
Expression	Biased expression in limb E14.5 (RPKM 15.6), small intestine adult (RPKM 3.2) and 6 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

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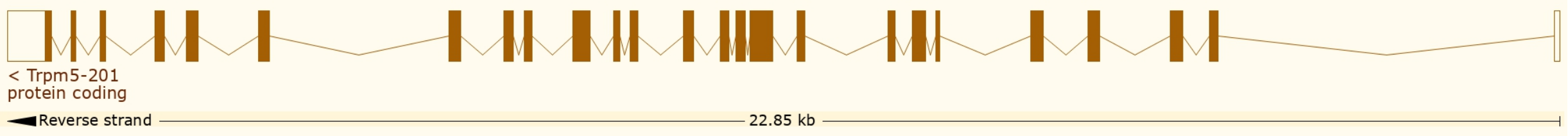
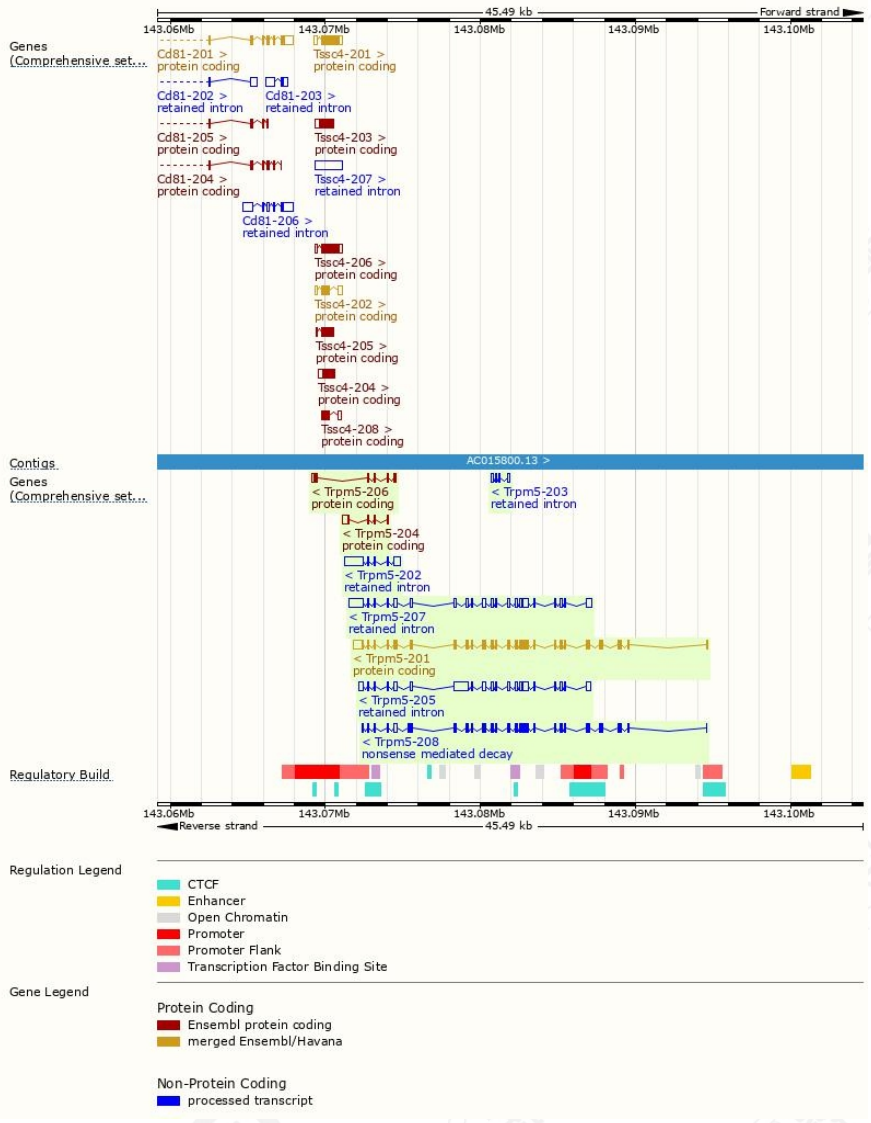
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trpm5-201	ENSMUST00000009390.10	4123	1158aa	Protein coding	CCDS52462		TSL:1 , GENCODE basic , APPRIS P1 ,
Trpm5-206	ENSMUST00000148715.8	726	190aa	Protein coding	-		CDS 5' incomplete , TSL:3 ,
Trpm5-204	ENSMUST00000136602.8	622	74aa	Protein coding	-		CDS 5' incomplete , TSL:5 ,
Trpm5-208	ENSMUST00000150867.2	3620	1000aa	Nonsense mediated decay	-		TSL:2 ,
Trpm5-207	ENSMUST00000150589.8	3997	No protein	Retained intron	-		TSL:1 ,
Trpm5-205	ENSMUST00000146075.8	3897	No protein	Retained intron	-		TSL:2 ,
Trpm5-202	ENSMUST00000126776.8	1925	No protein	Retained intron	-		TSL:2 ,
Trpm5-203	ENSMUST00000133027.2	474	No protein	Retained intron	-		TSL:5 ,

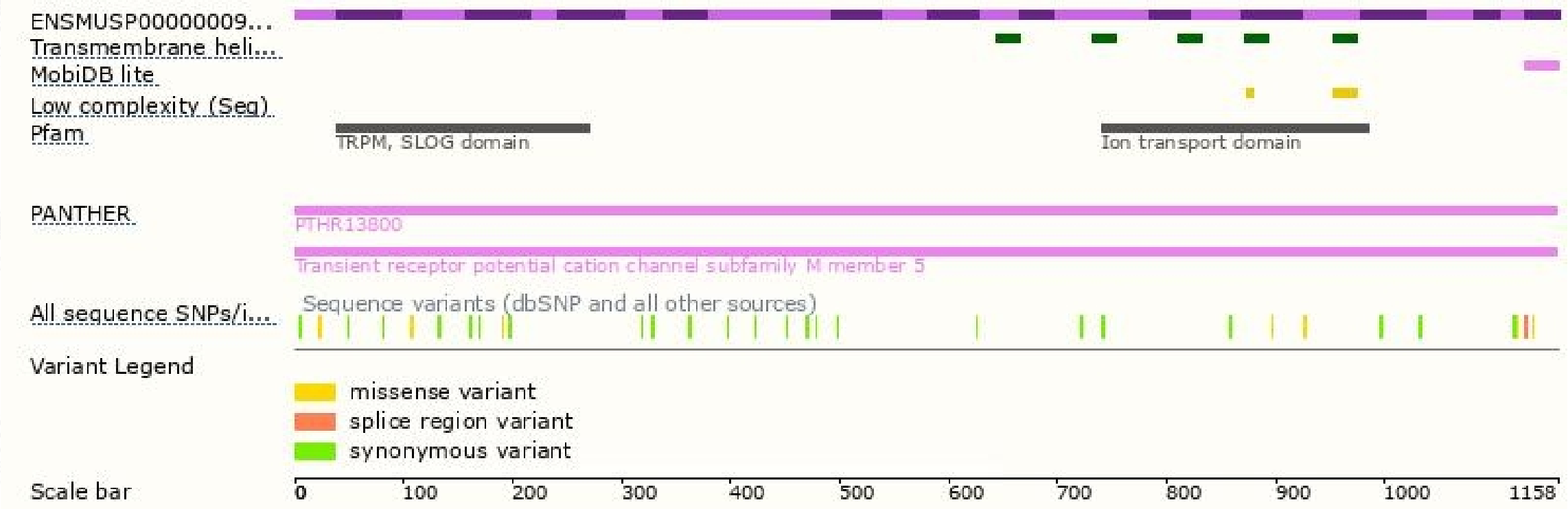
The strategy is based on the design of *Trpm5-201* transcript,the transcription is shown below:



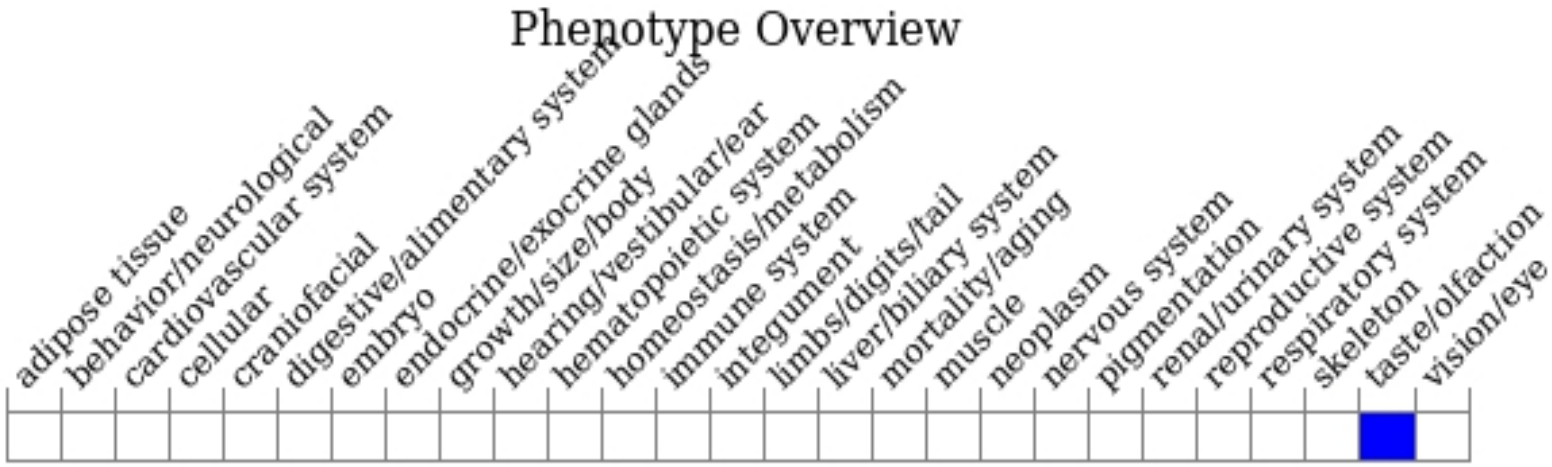
Genomic location distribution



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/).

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

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