Tmem225 Cas9-CKO Strategy

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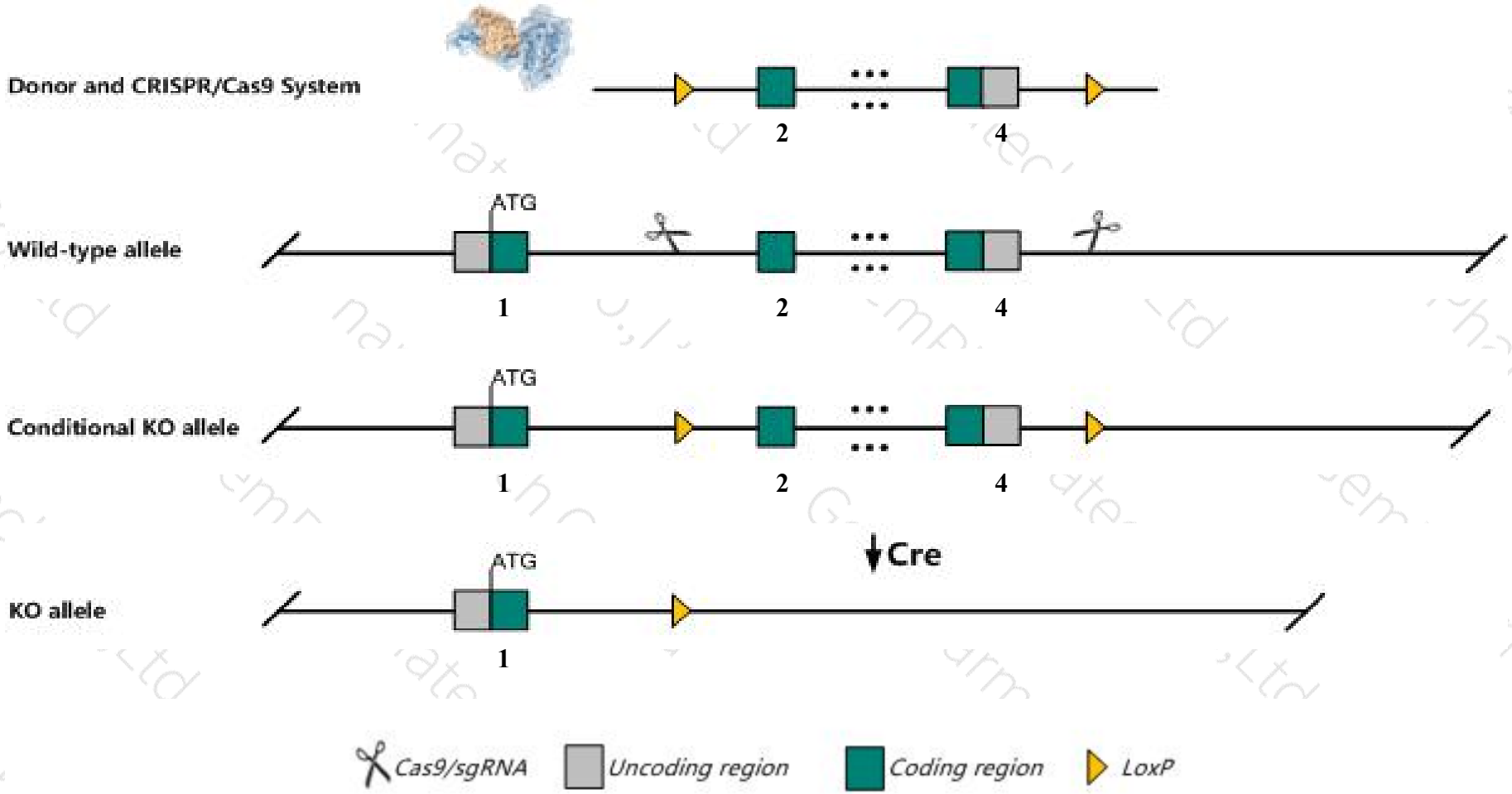
Project Overview

Project Name	***Tmem225***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tmem225* gene. The schematic diagram is as follows:



Technical routes

➢ The *Tmem225* gene has 2 transcripts. According to the structure of *Tmem225* gene, exon2-exon4 of *Tmem225-201*(ENSMUST00000046333.8) transcript is recommended as the knockout region.The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Tmem225* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector 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

➢ The *Tmem225* gene is located on the Chr9. 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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Tmem225 transmembrane protein 225 [*Mus musculus* (house mouse)]

Gene ID: 75667, updated on 25-Sep-2020

Summary

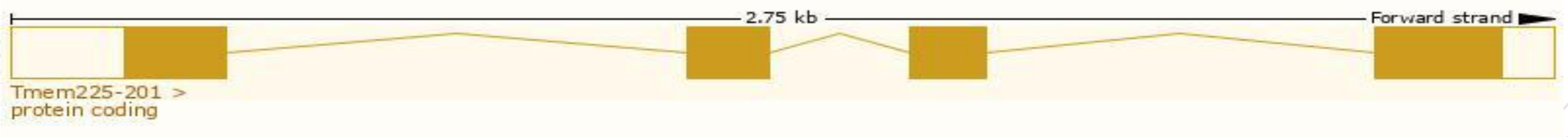
Official Symbol	Tmem225 provided by MGI
Official Full Name	transmembrane protein 225 provided by MGI
Primary source	MGI:MGI:1922917
See related	Ensembl:ENSMUSG00000040541
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Tnen225; 1700030E15Rik
Expression	Restricted expression toward testis adult (RPKM 39.4) See more
Orthologs	human all

Transcript information（Ensembl）

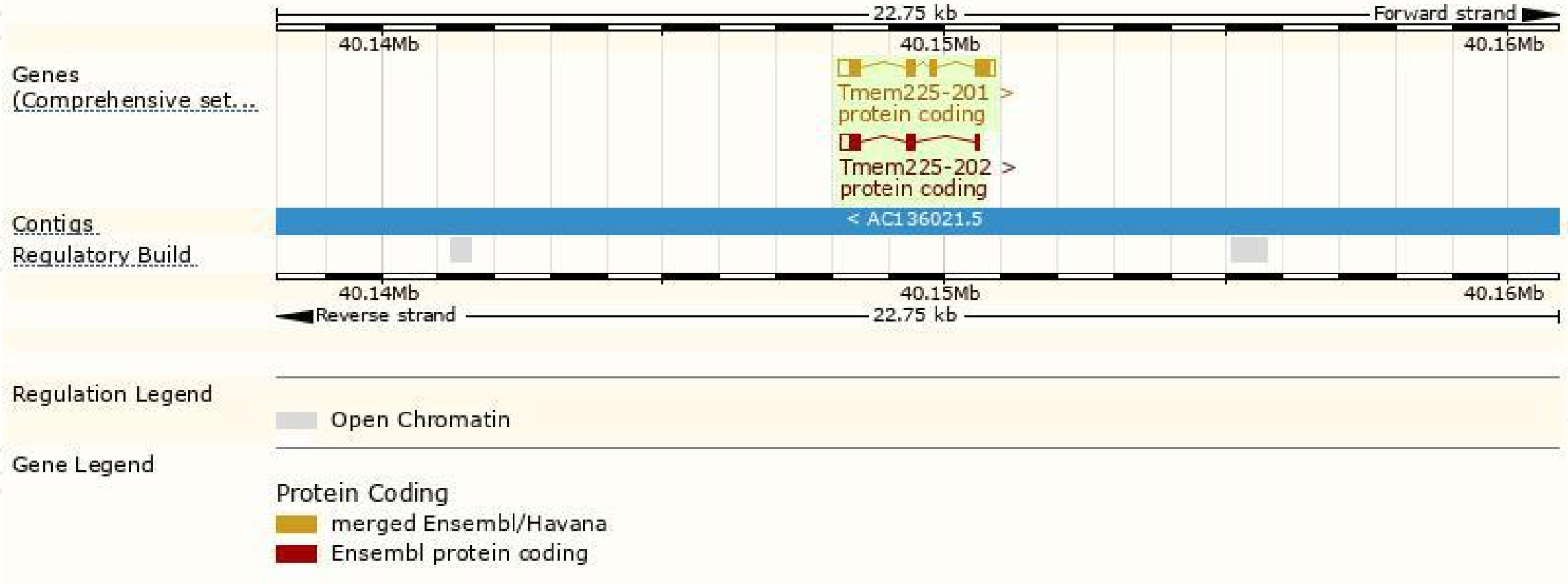
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tmem225-201	ENSMUST00000046333.8	987	230aa	Protein coding	CCDS52774	Q9D9S2	TSL:1 GENCODE basic APPRIS P1
Tmem225-202	ENSMUST00000238613.1	563	127aa	Protein coding	-	-	CDS 3' incomplete

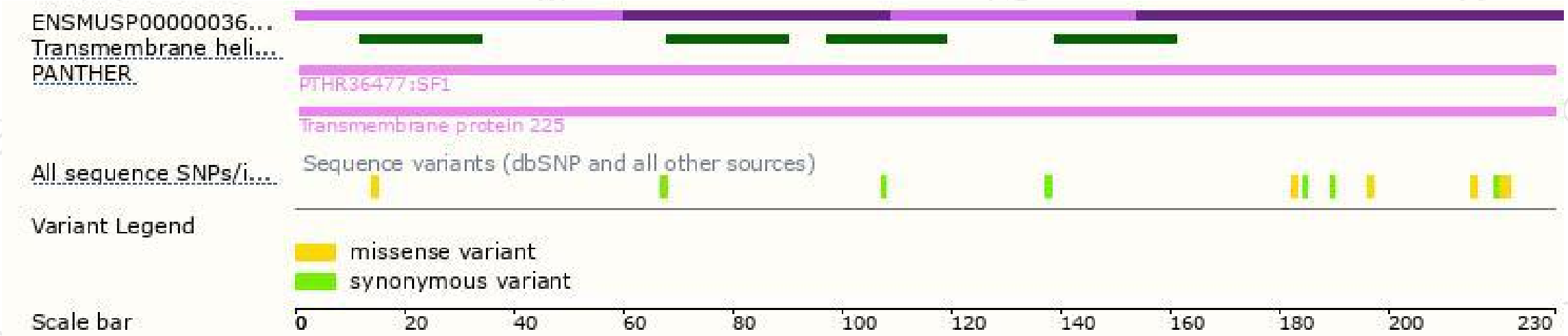
The strategy is based on the design of *Tmem225-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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