Tmc1 Cas9-CKO Strategy

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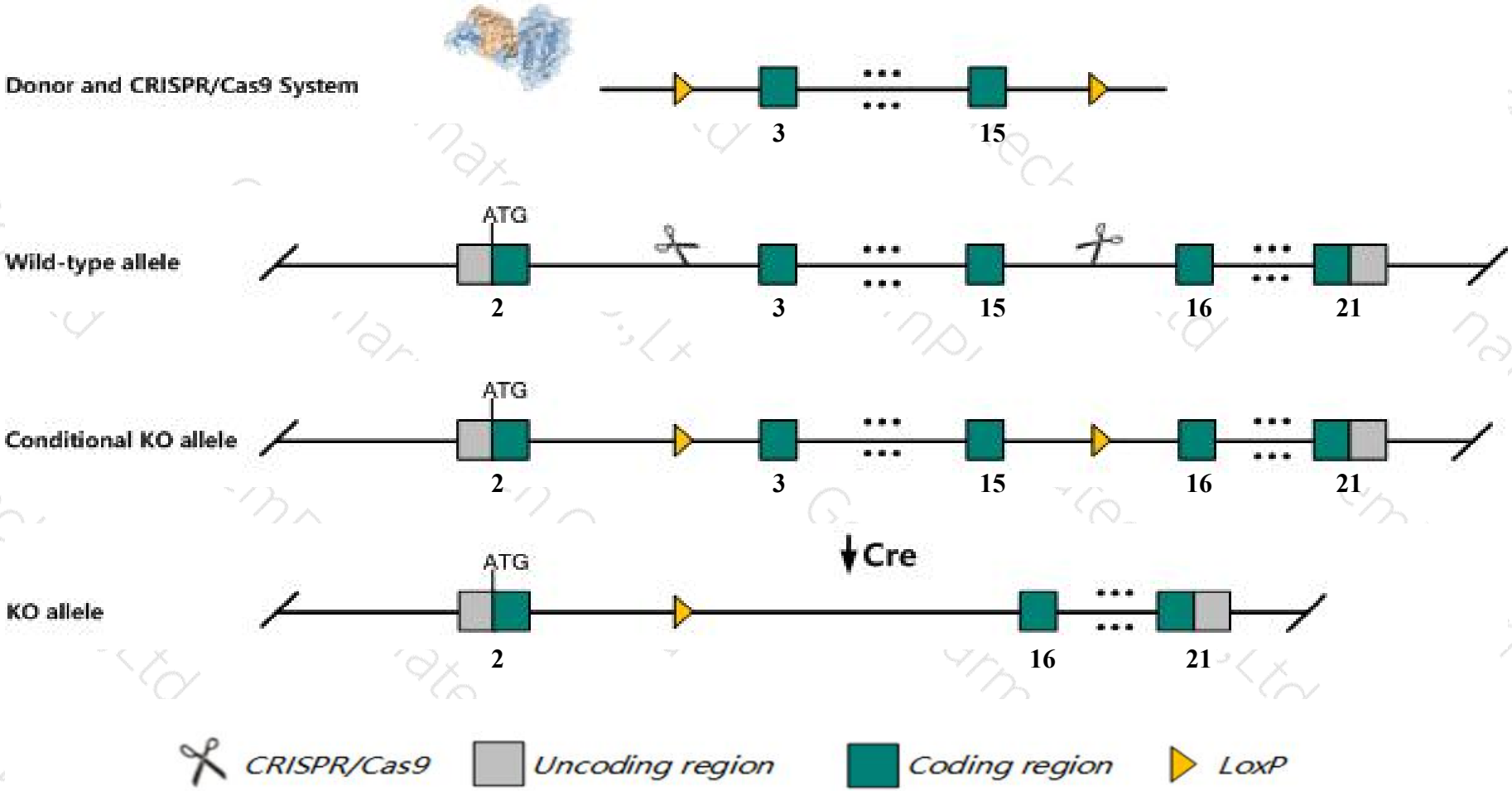
Design Date: 2019-12-16

Project Overview

Project Name	***Tmc1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tmc1* gene. The schematic diagram is as follows:



Technical routes

- The *Tmc1* gene has 4 transcripts. According to the structure of *Tmc1* gene, exon3-exon15 of *Tmc1-201* (ENSMUST00000039500.3) 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 *Tmc1* 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,Mutant mice are characterized by progressive degeneration of the cochlear inner hair cells and concomitant deafness. Different alleles causing progressive deafness or profound congenital deafness.

- The *Tmc1* gene is located on the Chr19. 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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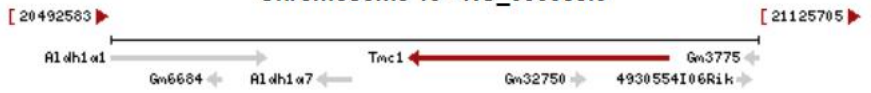
Tmc1 transmembrane channel-like gene family 1 [*Mus musculus* (house mouse)]

Gene ID: 13409, updated on 5-Nov-2019

Summary

Official Symbol	Tmc1 provided by MGI
Official Full Name	transmembrane channel-like gene family 1 provided by MGI
Primary source	MGI:MGI:2151016
See related	Ensembl:ENSMUSG00000024749
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	dn; Bth; CWEA1; Beethoven; 4933416G09Rik
Expression	Biased expression in testis adult (RPKM 1.8), colon adult (RPKM 0.1) and 1 other tissue See more
Orthologs	human all

Chromosome 19 - NC_000085.6

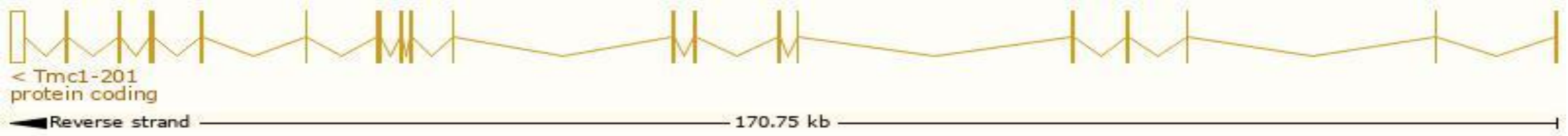


Transcript information（Ensembl）

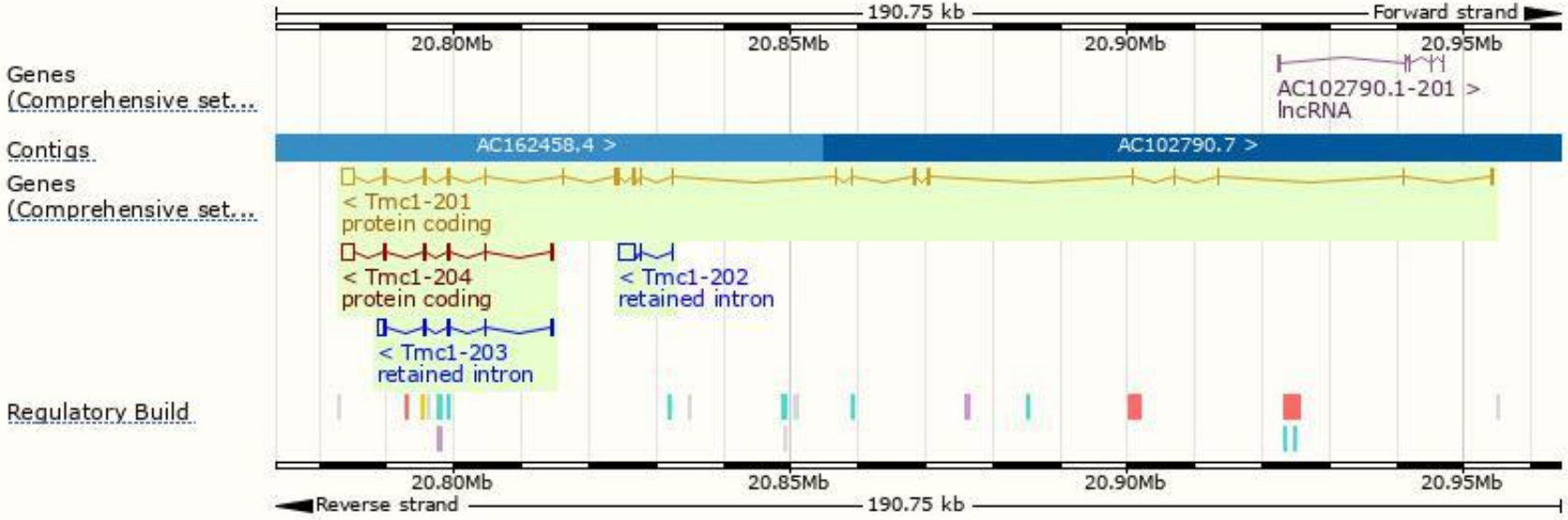
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Tmc1-201	ENSMUST00000039500.3	4073	757aa	ENSMUSP00000040859.3	Protein coding	CCDS50403	Q8R4P5	TSL:1 GENCODE basic APPRIS P1
Tmc1-204	ENSMUST00000236437.1	2432	212aa	ENSMUSP00000158304.1	Protein coding	-	A0A494BB39	GENCODE basic
Tmc1-202	ENSMUST00000235546.1	2393	No protein	-	Retained intron	-	-	-
Tmc1-203	ENSMUST00000235605.1	1645	No protein	-	Retained intron	-	-	-

The strategy is based on the design of *Tmc1-201* transcript,The transcription is shown below



Genomic location distribution



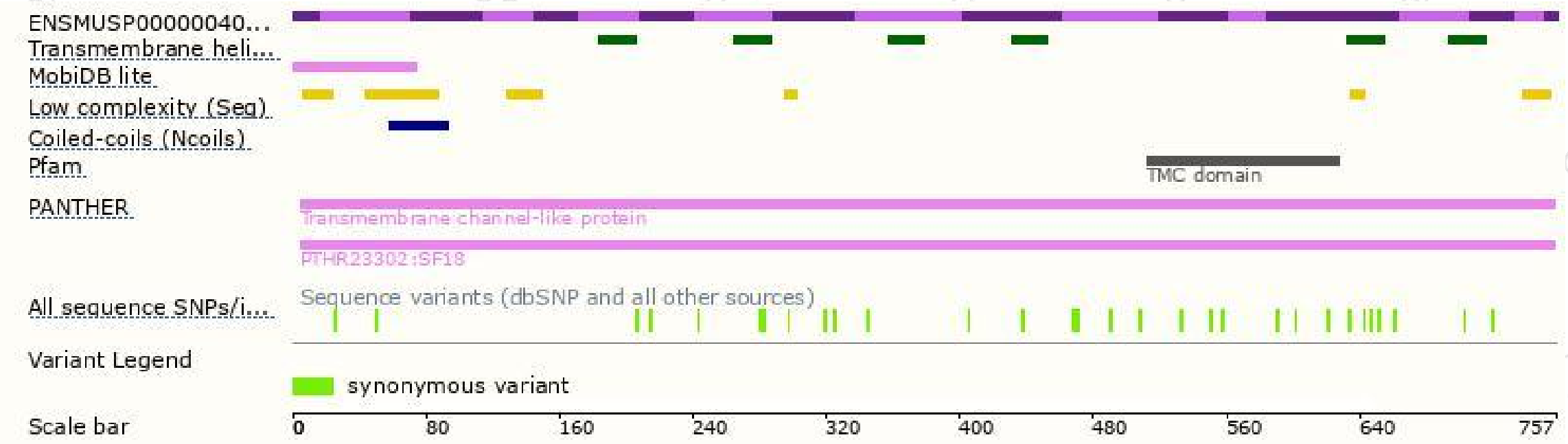
Gene Legend

Protein Coding
- merged Ensembl/Havana
- Ensembl protein coding

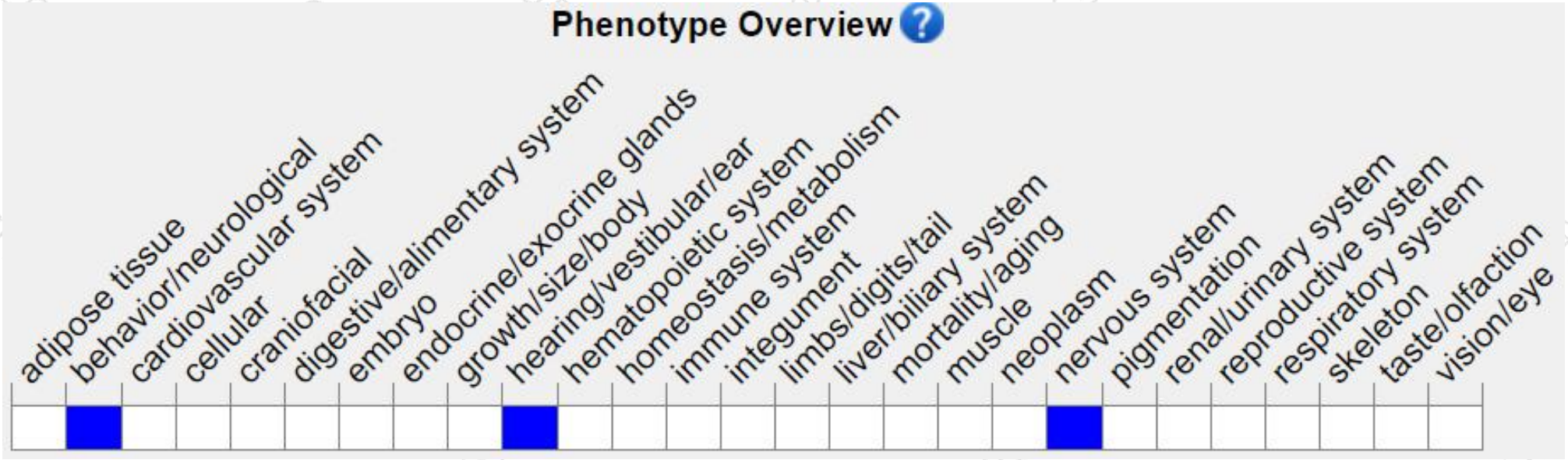
Non-Protein Coding
- processed transcript
- RNA gene

Regulation Legend
- CTCF
- Enhancer
- Open Chromatin
- Promoter Flank
- Transcription Factor Binding Site

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,Mutant mice are characterized by progressive degeneration of the cochlear inner hair cells and concomitant deafness. Different alleles causing progressive deafness or profound congenital deafness.

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

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