Tcp1 Cas9-CKO *Strategy*

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Design Date: 2019-9-24

Project Overview

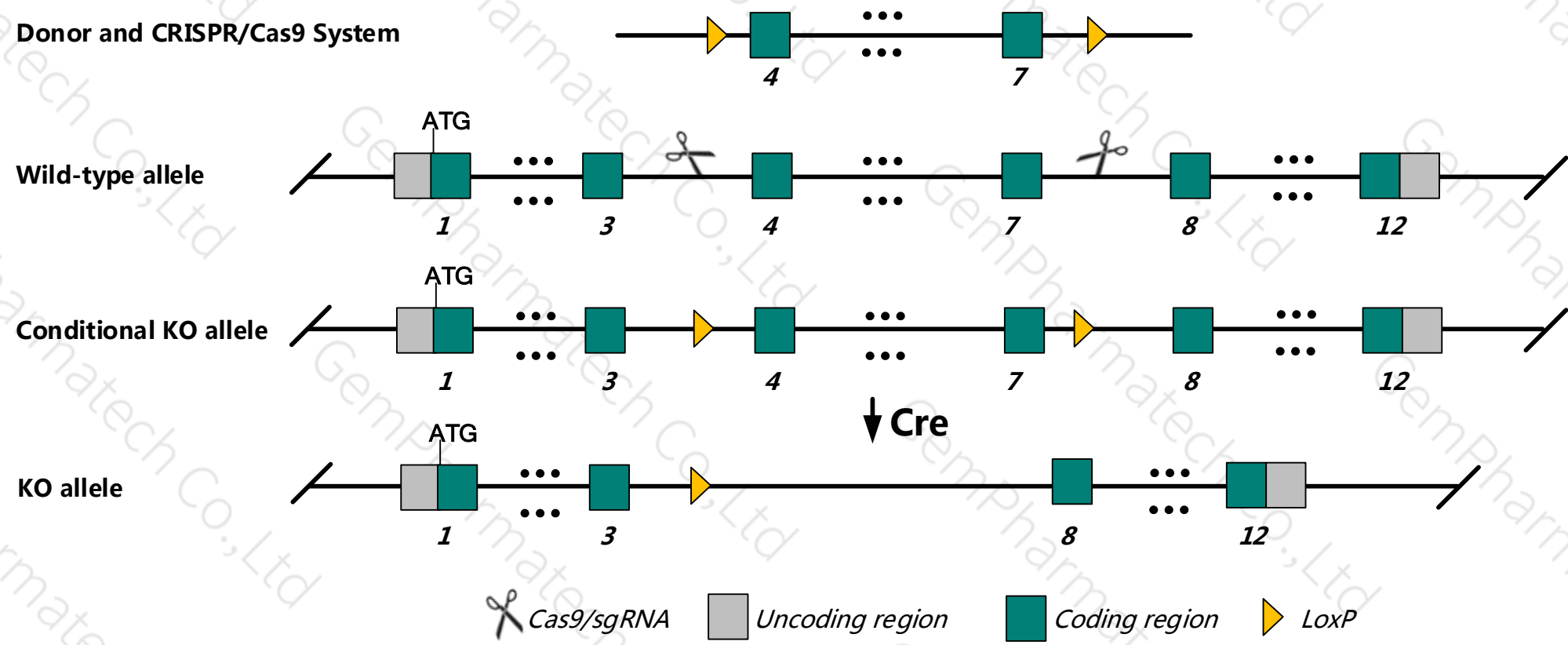|	
Project Name	*Tcp1*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tcp1* gene. The schematic diagram is as follows:

Technical routes

- The *Tcp1* gene has 12 transcript.According to the structure of *Tcp1* gene, exon4-7 of *Tcp1*-209 (ENSMUST00000151287.7) transcript is recommended as the knockout region.The region contains 518bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Tcp1* 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 or cell types.

➢ According to the existing MGI data，There are two electrophoretic alleles known; allele a occurs in all complete t haplotype chromosomes and allele b in wild-type strains. There are multiple changes between the amino acid sequences of the TCP1A and TCP1B proteins encoded by the two alleles.

➢ Transcript *Tcp1-207，211* may not be affected.

➢ The KO region contains functional region of the *GM26130* gene.Knockout the region may affect the function of *GM26130* gene.

➢ Knockout the region may affect the 5 terminal regulation function of *Mrpl18* gene.

➢ The *Tcp1* gene is located on the Chr17. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

Tcp1 t-complex protein 1 [*Mus musculus* (house mouse)]

Gene ID: 21454, updated on 12-Aug-2019

▲ Summary

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Official Symbol	Tcp1 provided by MGI
Official Full Name	t-complex protein 1 provided by MGI
Primary source	MGI:MGI:98535
See related	Ensembl:ENSMUSG00000068039
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	CCT; p63; Cct1; Ccta; TRic; Tp63; Tcp-1; c-cpn; AI528772
Expression	Ubiquitous expression in testis adult (RPKM 173.1), CNS E11.5 (RPKM 109.2) and 25 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

The gene has 12 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tcp1-209	ENSMUST00000151287.7	2446	556aa	Protein coding	CCDS28397 ☑	P11983 ☑	TSL:1 GENCODE basic APPRIS P1
Tcp1-201	ENSMUST00000089024.12	1902	507aa	Protein coding	CCDS70760 ☑	P11983 ☑	TSL:1 GENCODE basic
Tcp1-211	ENSMUST00000233286.1	678	54aa	Protein coding	-	A0A3B2WDE2 ☑	GENCODE basic
Tcp1-207	ENSMUST00000143961.1	614	103aa	Protein coding	-	F2Z483 ☑	TSL:2 GENCODE basic
Tcp1-202	ENSMUST00000129632.7	3419	No protein	Retained intron	-	-	TSL:2
Tcp1-203	ENSMUST00000133003.7	908	No protein	Retained intron	-	-	TSL:2
Tcp1-206	ENSMUST00000138709.1	768	No protein	Retained intron	-	-	TSL:2
Tcp1-212	ENSMUST00000233345.1	632	No protein	Retained intron	-	-	-
Tcp1-204	ENSMUST00000134665.1	618	No protein	Retained intron	-	-	TSL:2
Tcp1-205	ENSMUST00000137289.1	586	No protein	Retained intron	-	-	TSL:2
Tcp1-210	ENSMUST00000151715.1	578	No protein	Retained intron	-	-	TSL:2
Tcp1-208	ENSMUST00000147724.7	329	No protein	lncRNA	-	-	TSL:3

The strategy is based on the design of *Tcp1*-209 transcript,The transcription is shown below
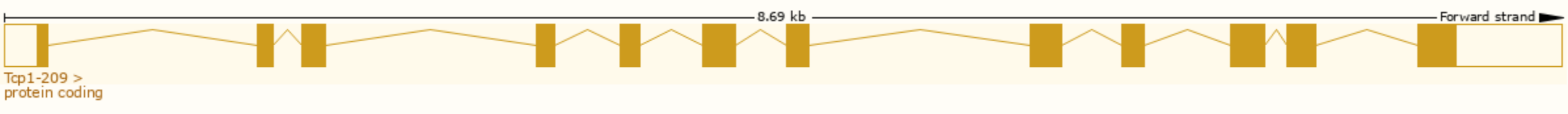
Genomic location distribution
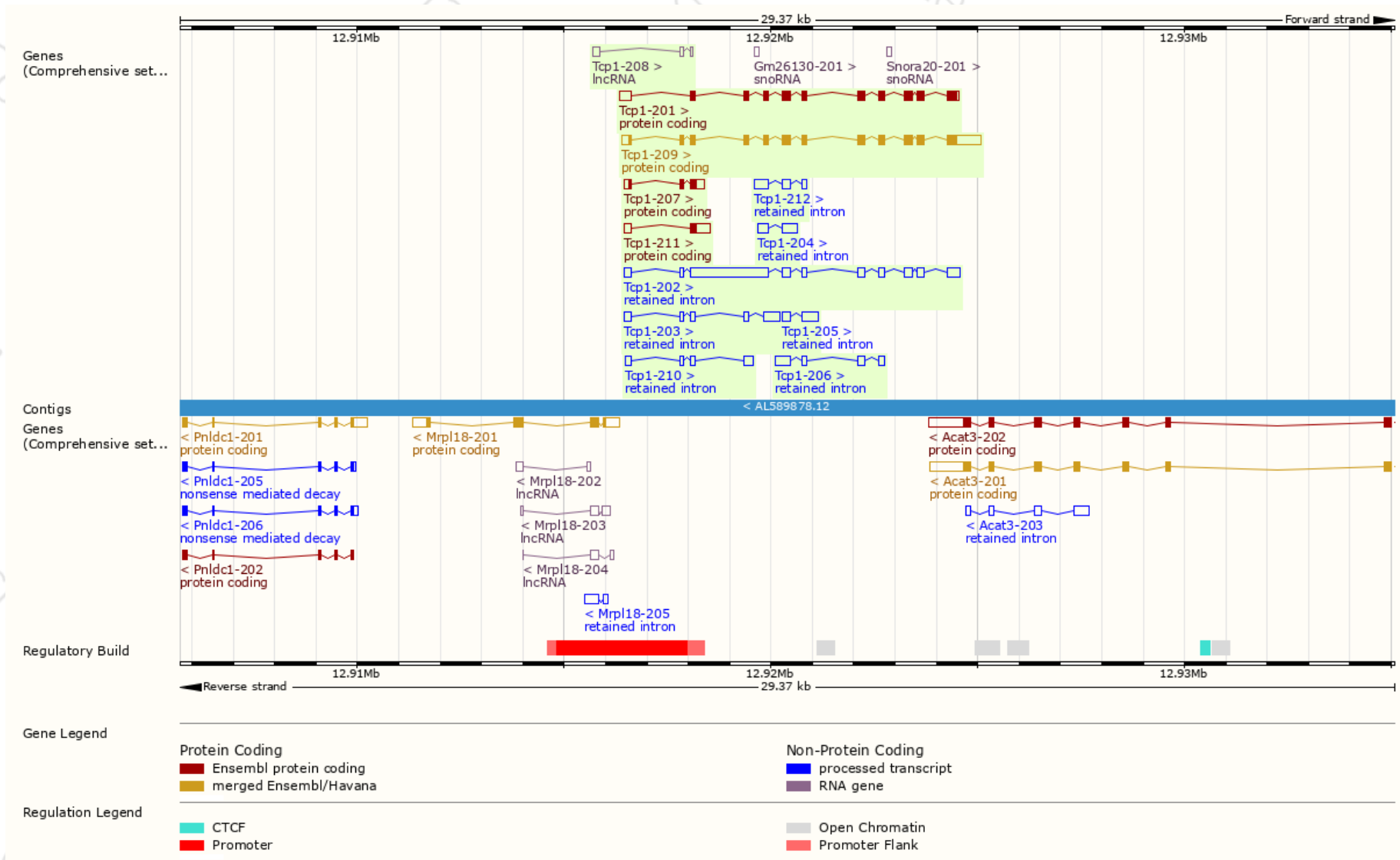
Protein domain

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, There are two electrophoretic alleles known; allele a occurs in all complete t haplotype chromosomes and allele b in wild-type strains. There are multiple changes between the amino acid sequences of the TCP1A and TCP1B proteins encoded by the two alleles.

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