Trim68 Cas9-KO Strategy

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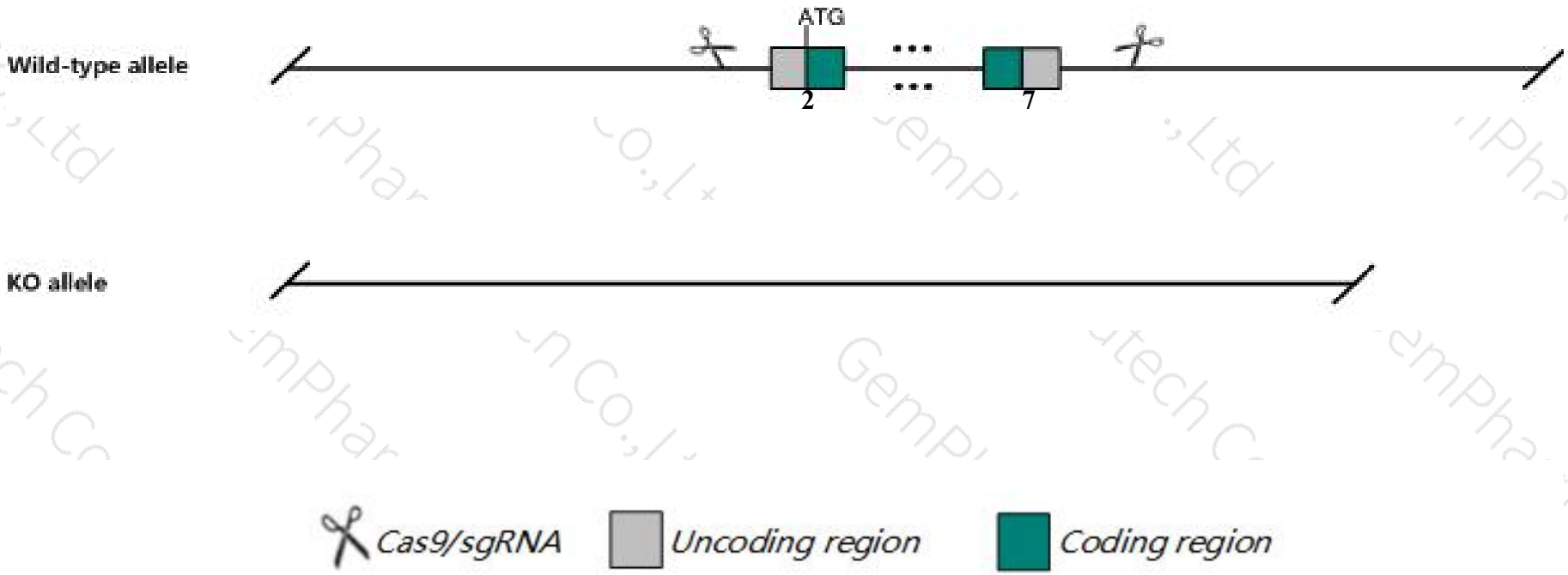
Project Overview

Project Name	***Trim68***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Trim68* gene. The schematic diagram is as follows:



Technical routes

➢ The *Trim68* gene has 5 transcripts. According to the structure of *Trim68* gene, exon2-exon7 of *Trim68-201* (ENSMUST00000082175.6) transcript is recommended as the knockout region.The region contains all 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 *Trim68* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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

➢ The knockout region is near to the C-terminal of *Gm45687* and *Gm45527* gene,this strategy may influence the regulatory function of the C-terminal of these gene.

➢ The *Trim68* 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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Trim68 tripartite motif-containing 68 [Mus musculus (house mouse)]

Gene ID: 101700, updated on 31-Jan-2019

Summary

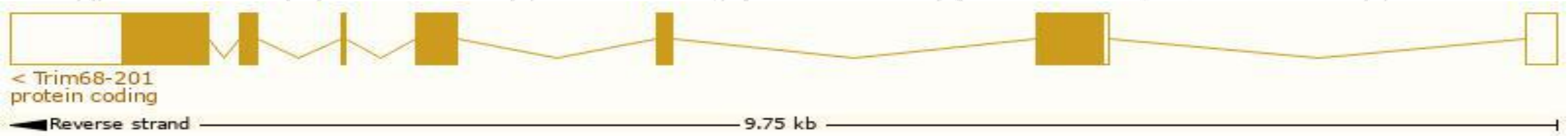
Official Symbol	Trim68 provided by MGI
Official Full Name	tripartite motif-containing 68 provided byMGI
Primary source	MGI:MGI:2142077
See related	Ensembl:ENSMUSG00000073968
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI894158, F730114J12Rik, Rnf137, SS-56
Expression	Broad expression in bladder adult (RPKM 23.0), subcutaneous fat pad adult (RPKM 9.9) and 24 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

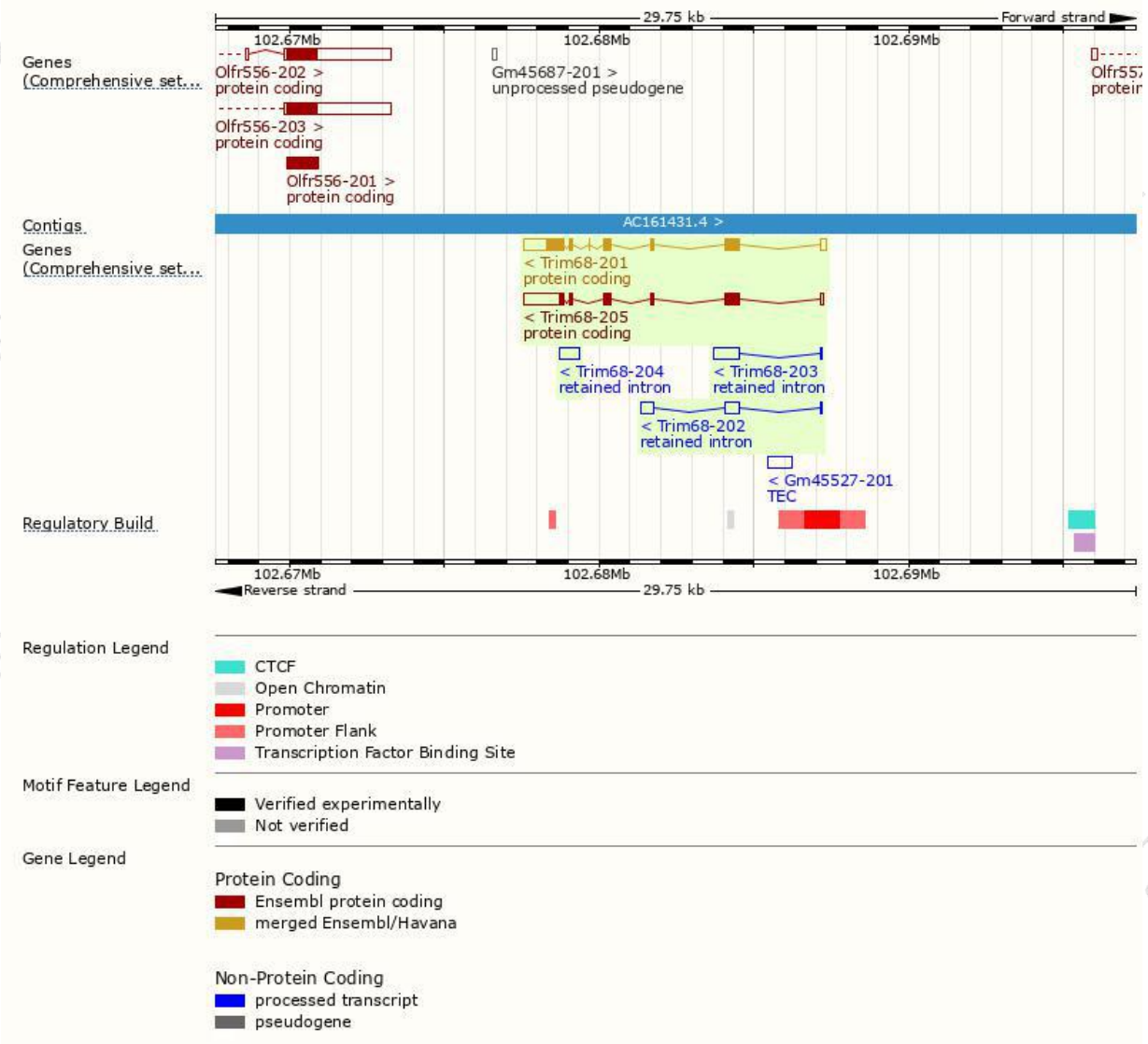
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trim68-201	ENSMUST00000082175.6	2388	485aa	Protein coding	CCDS40052	G3X9I6	TSL:1 GENCODE basic APPRIS P1
Trim68-205	ENSMUST00000210855.1	2301	327aa	Protein coding	CCDS85365	A0A1B0GSL9	TSL:1 GENCODE basic
Trim68-202	ENSMUST00000209621.1	943	No protein	Retained intron	-	-	TSL:2
Trim68-203	ENSMUST00000209654.1	890	No protein	Retained intron	-	-	TSL:2
Trim68-204	ENSMUST00000210406.1	632	No protein	Retained intron	-	-	TSL:NA

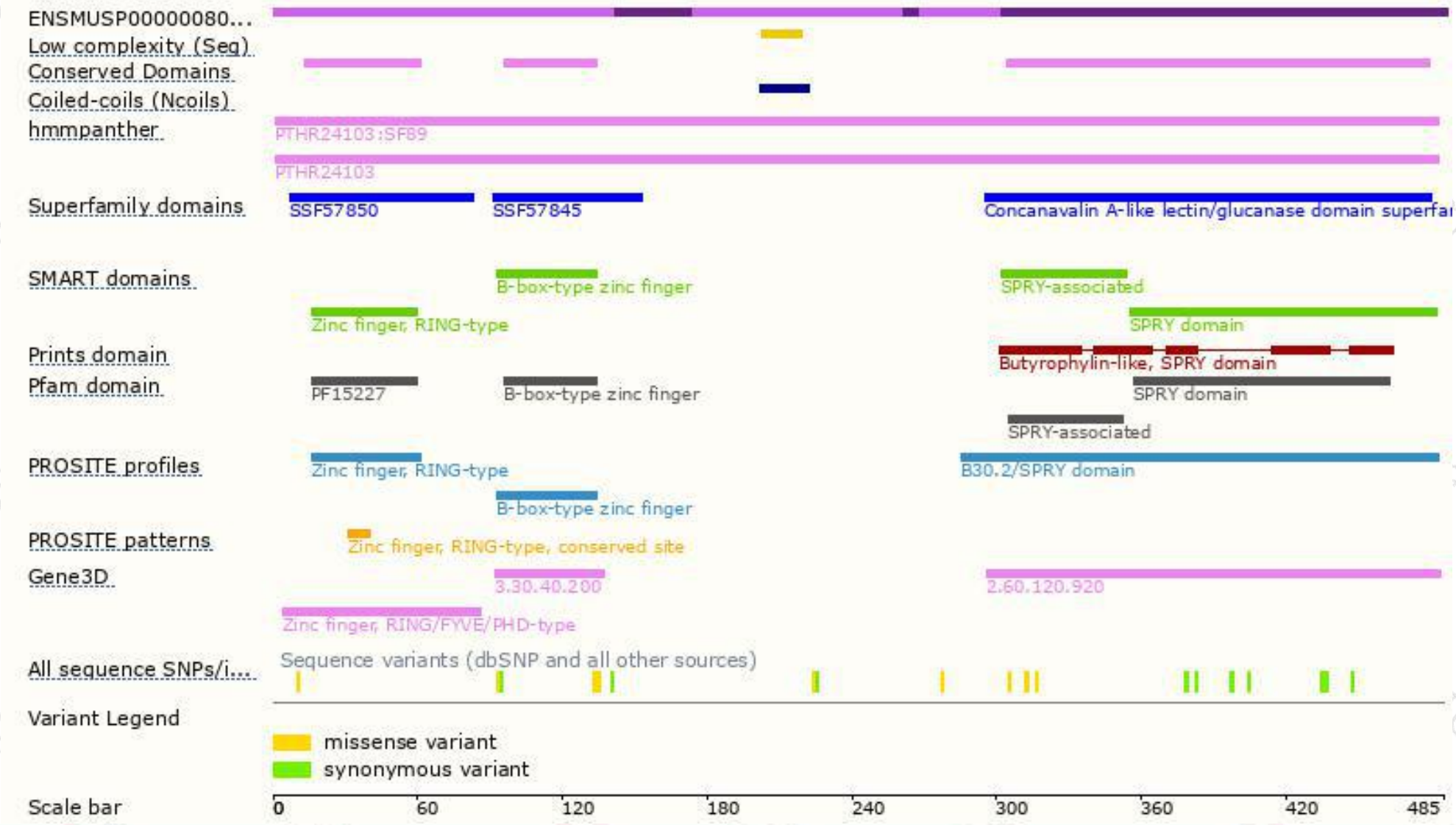
The strategy is based on the design of *Trim68-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



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

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