

Tmf1 Cas9-KO Strategy

Designer: JiaYu

Reviewer: Xiaojing Li

Design Date: 2020-8-1

Project Overview

Project Name

Tmf1

Project type

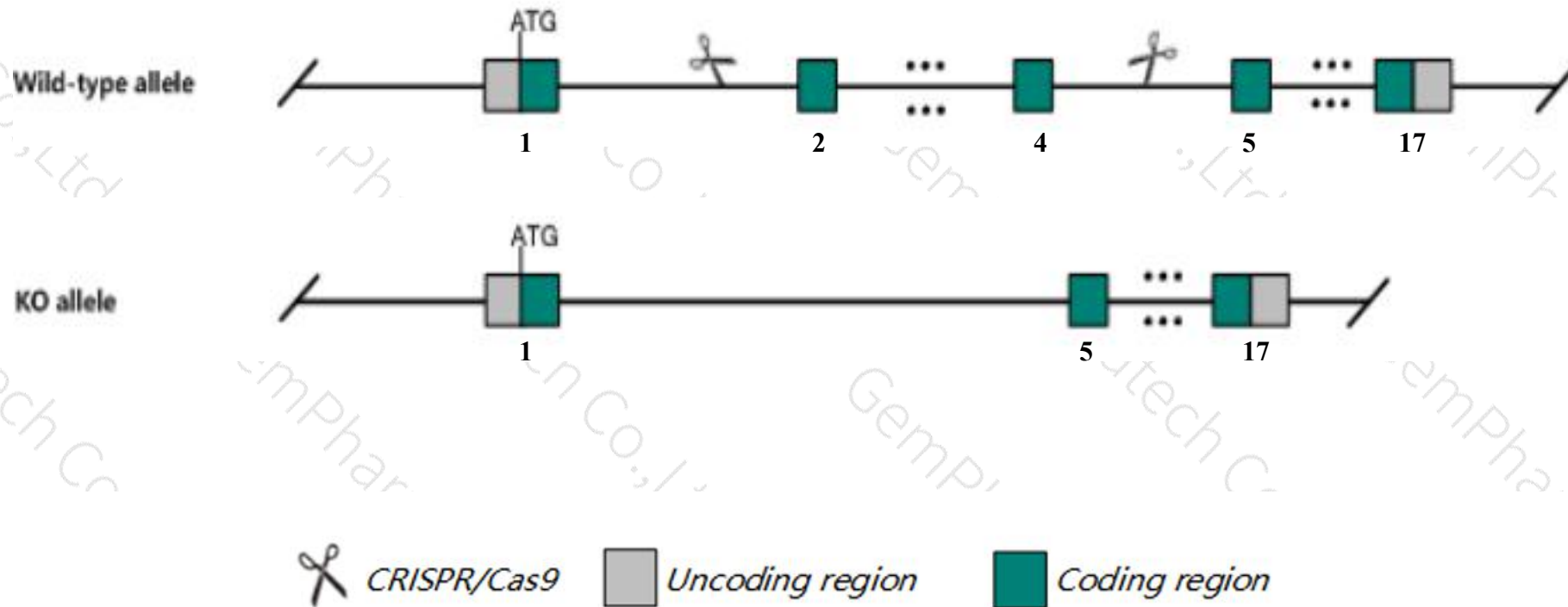
Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

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



- The *Tmfl* gene has 3 transcripts. According to the structure of *Tmfl* gene, exon2-exon4 of *Tmfl*-201(ENSMUST00000095664.5) transcript is recommended as the knockout region. The region contains 1430bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Tmfl* gene. The brief process is as follows: CRISPR/Cas9 system 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.

- According to the existing MGI data, mice homozygous for a knock-out allele exhibit male infertility associated with decreased testis weight, globozoospermia, absent acrosome, asthenozoospermia, and abnormal sperm midpiece morphology.
- The *Tmfl* gene is located on the Chr6. 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)

Tmf1 TATA element modulatory factor 1 [Mus musculus (house mouse)]

Gene ID: 232286, updated on 13-Mar-2020

Summary



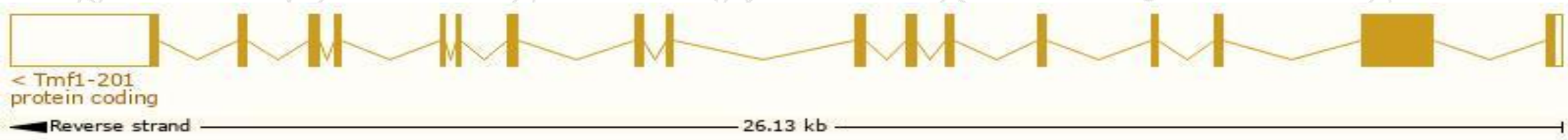
Official Symbol	Tmf1 provided by MGI
Official Full Name	TATA element modulatory factor 1 provided by MGI
Primary source	MGI:MGI:2684999
See related	Ensembl:ENSMUSG00000030059
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	7030402D04Rik, Gm153, Tmf1`
Expression	Ubiquitous expression in testis adult (RPKM 8.6), cerebellum adult (RPKM 3.1) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

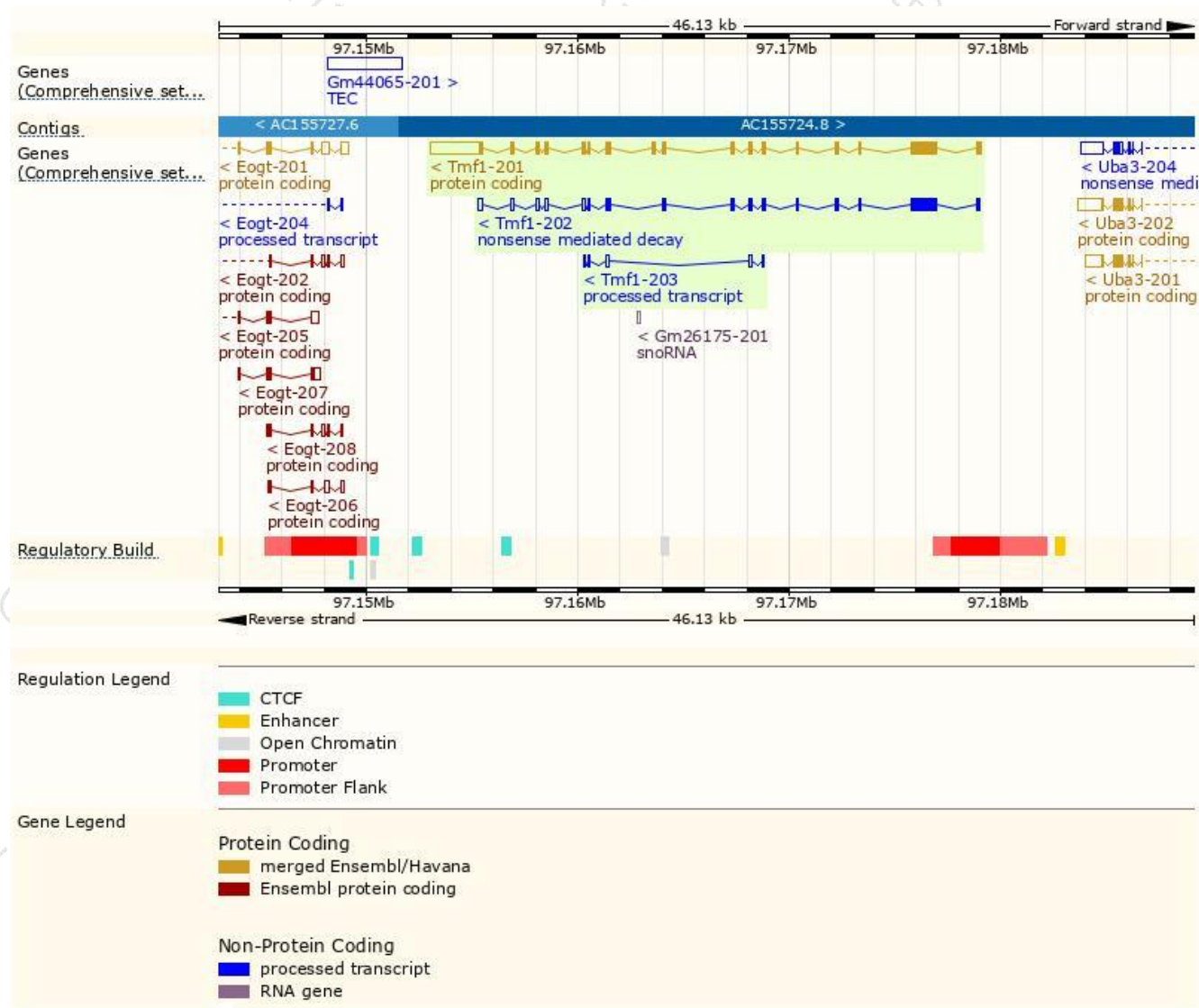
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tmf1-201	ENSMUST00000095664.5	5732	1091aa	Protein coding	CCDS39575	B9EKI3	TSL:1 GENCODE basic APPRIS P1
Tmf1-202	ENSMUST00000124173.7	3191	784aa	Nonsense mediated decay	-	D6RJK7	TSL:5
Tmf1-203	ENSMUST00000204190.1	524	No protein	Processed transcript	-	-	TSL:3

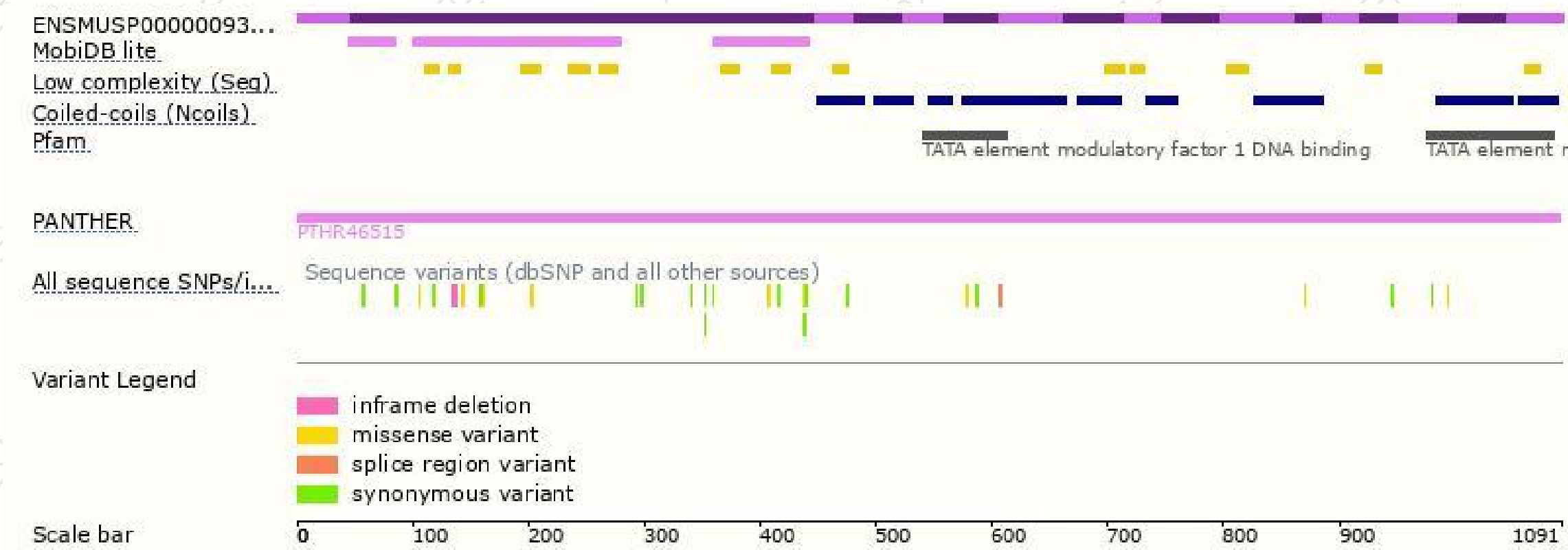
The strategy is based on the design of *Tmf1-201* transcript,the transcription is shown below:



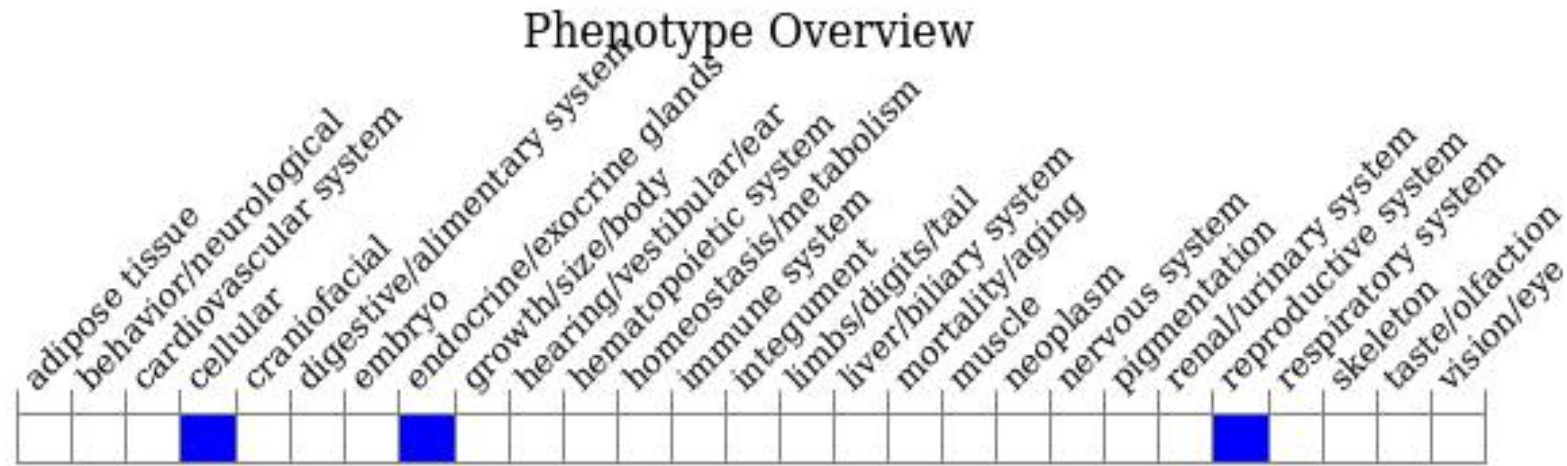
Genomic location distribution



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/>).

According to the existing MGI data, mice homozygous for a knock-out allele exhibit male infertility associated with decreased testis weight, globozoospermia, absent acrosome, asthenozoospermia, and abnormal sperm midpiece morphology.

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

Tel: 400-9660890

