Ttn Cas9-KO *Strategy*

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Reviewer: Shanhong Tao

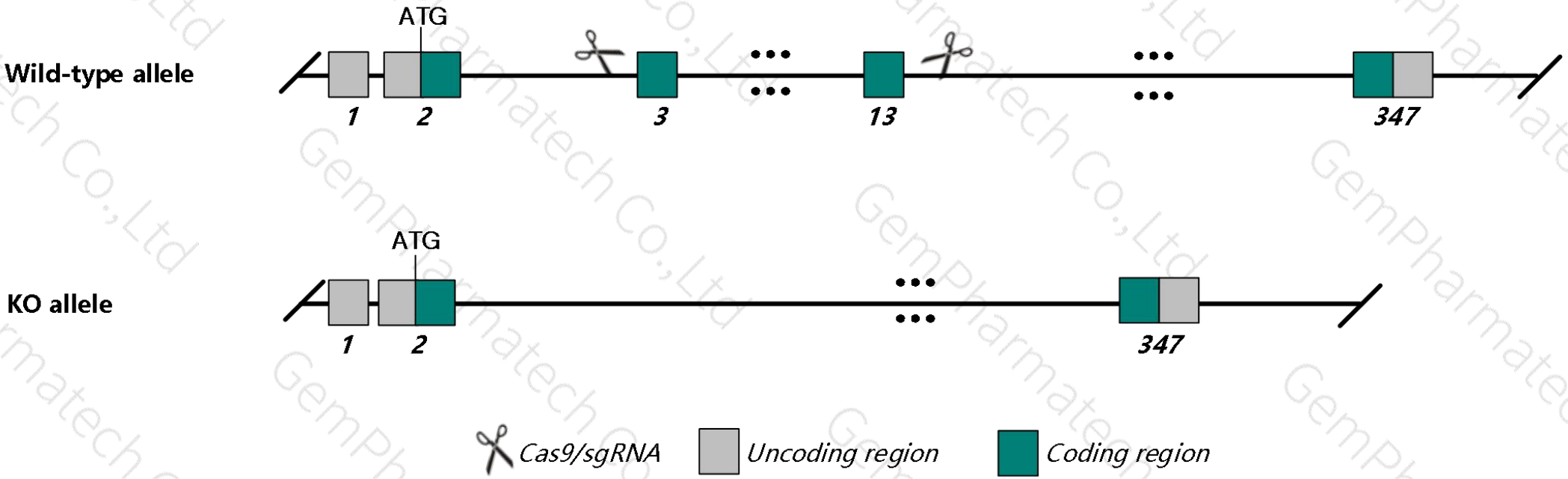
Design Date: 2021-10-25

Project Overview

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

Knockout strategy

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



Technical routes

- The *Ttn* gene has 25 transcripts.According to the structure of *Ttn* gene, exon3-13 of *Ttn*-203 (ENSMUST00000099981.10) transcript is recommended as the knockout region.The region contains 1988bp coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ttn* 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

- According to the existing MGI data，Homozygous mutant mice show embryogenesis defects in frontonasal mass, first branchial arch and somites, and vascular, cardiac and skeletal muscle defects causing growth retardation, muscle weakness, abnormal posture, and premature death ranging from embryonic day 11.5 to 8 weeks of age. A homozygous point mutation leads to reduced systolic function and mild ventricular dilation in the heart and increased cardiomyocyte size.
- Transcript *Ttn-208,209,210,211,212,213,214,216,218,219,220,221,222,223,224,225* may not be affected.
- The *Ttn* gene is located on the Chr2. 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)

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Ttn titin [*Mus musculus* (house mouse)]

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Gene ID: 22138, updated on 16-Oct-2021

Summary

Official Symbol	Ttn provided by MGI
Official Full Name	titin provided by MGI
Primary source	MGI:MGI:98864
See related	Ensembl:ENSMUSG00000051747
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	L56; mdm; shru; AF006999; AV006427; 1100001C23Rik; 2310036G12Rik; 2310057K23Rik; 2310074I15Rik; D330041I19Rik; D830007G01Rik
Expression	Biased expression in heart adult (RPKM 23.7), mammary gland adult (RPKM 4.5) and 1 other tissue See more
Orthologs	human all

NEW Try the new Gene table

Try the new Transcript table

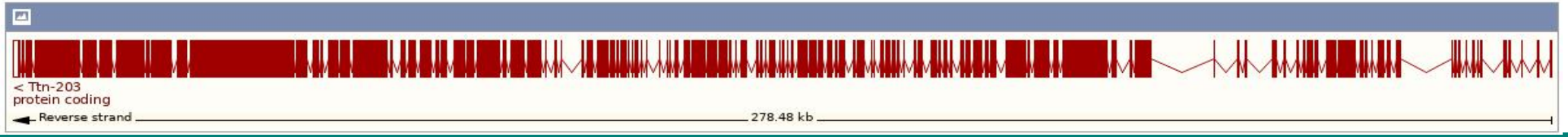
Transcript information （Ensembl）

The gene has 25 transcripts, and all transcripts are shown below:

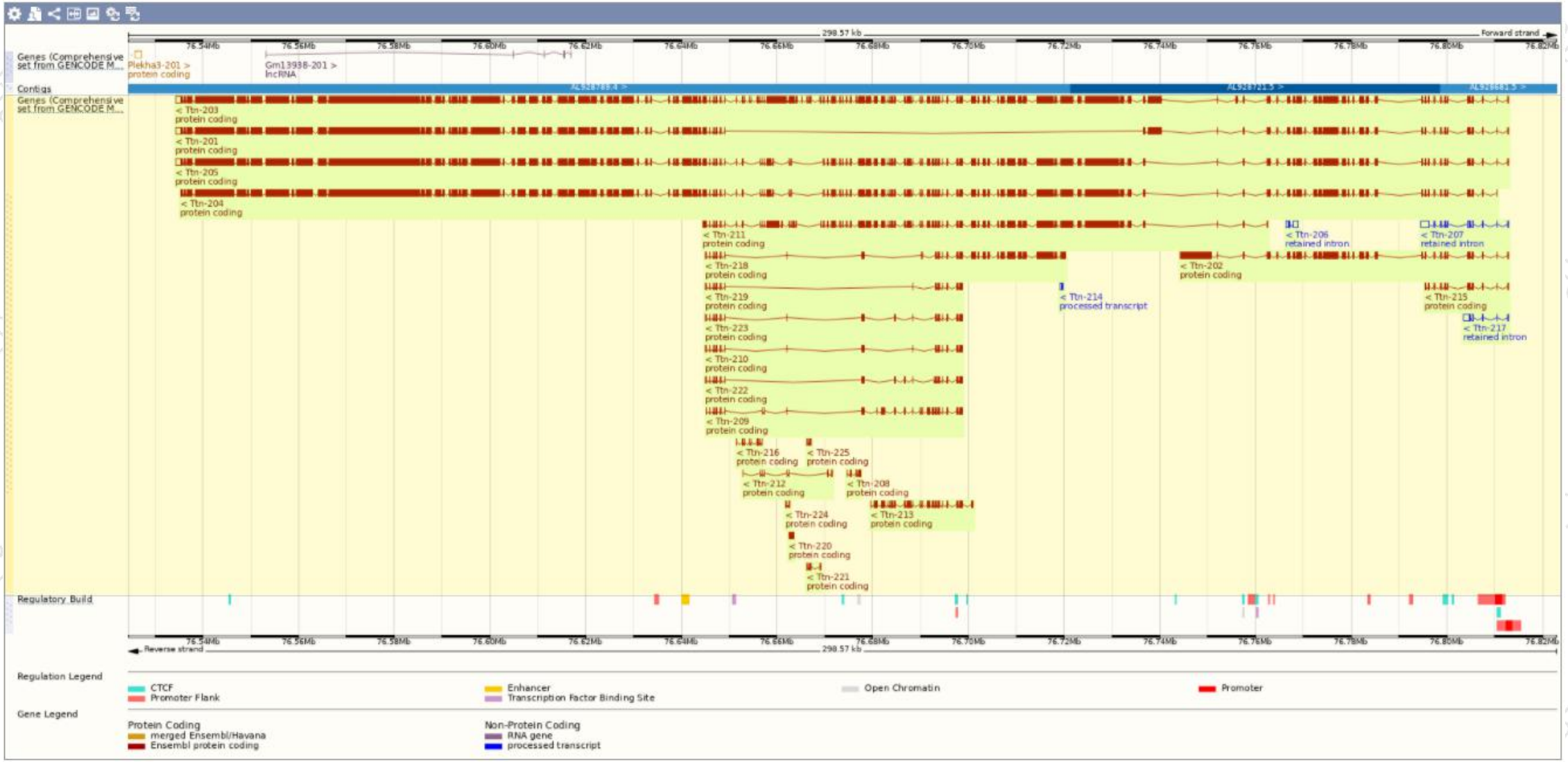
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Ttn-201	ENSMUST00000011934.13	81999	26938aa	Protein coding	-	A0A5K1VVQ1	GENCODE basic TSL:5
Ttn-202	ENSMUST00000099980.10	16891	5508aa	Protein coding	-	A2ASS6-3	GENCODE basic TSL:5
Ttn-203	ENSMUST00000099981.10	107355	35390aa	Protein coding	-	A0A5K1VVQ9	GENCODE basic APPRIS P1 TSL:5
Ttn-204	ENSMUST00000111846.9	100404	33467aa	Protein coding	CCDS38154	E9Q8N1	GENCODE basic TSL:5
Ttn-205	ENSMUST00000111882.9	101674	33467aa	Protein coding	-	E9Q8K5	GENCODE basic TSL:5
Ttn-206	ENSMUST00000123448.2	1718	No protein	Retained intron	-	-	TSL:1
Ttn-207	ENSMUST00000123804.8	3513	No protein	Retained intron	-	-	TSL:1
Ttn-208	ENSMUST00000126515.2	549	183aa	Protein coding	-	A2AT63	TSL:5 CDS 5' and 3' incomplete
Ttn-209	ENSMUST00000128071.8	3162	1054aa	Protein coding	-	A2AT68	TSL:5 CDS 5' and 3' incomplete
Ttn-210	ENSMUST00000130915.8	1887	629aa	Protein coding	-	A2AT67	TSL:5 CDS 5' and 3' incomplete
Ttn-211	ENSMUST00000134720.8	24817	8272aa	Protein coding	-	F7CR78	TSL:5 CDS 5' and 3' incomplete
Ttn-212	ENSMUST00000137854.8	609	203aa	Protein coding	-	A2AT62	TSL:5 CDS 5' and 3' incomplete
Ttn-213	ENSMUST00000138542.2	3406	1135aa	Protein coding	-	F6RSJ3	TSL:1 CDS 5' and 3' incomplete
Ttn-214	ENSMUST00000139832.2	627	No protein	Processed transcript	-	-	TSL:3
Ttn-215	ENSMUST00000140091.8	2159	645aa	Protein coding	-	Q8BUJ6	TSL:1 CDS 3' incomplete
Ttn-216	ENSMUST00000142251.8	744	248aa	Protein coding	-	F6Q6Z0	TSL:5 CDS 5' and 3' incomplete
Ttn-217	ENSMUST00000147048.2	2093	No protein	Retained intron	-	-	TSL:1
Ttn-218	ENSMUST00000148747.8	10229	3409aa	Protein coding	-	A2AT70	TSL:5 CDS 5' and 3' incomplete
Ttn-219	ENSMUST00000149616.8	1633	544aa	Protein coding	-	A2AT64	TSL:5 CDS 5' and 3' incomplete
Ttn-220	ENSMUST00000150741.2	451	150aa	Protein coding	-	F6ZRX7	TSL:5 CDS 5' and 3' incomplete
Ttn-221	ENSMUST00000151097.8	459	153aa	Protein coding	-	A2AT61	TSL:5 CDS 5' and 3' incomplete
Ttn-222	ENSMUST00000152185.8	2130	710aa	Protein coding	-	A2AT65	TSL:5 CDS 5' and 3' incomplete
Ttn-223	ENSMUST00000155365.8	2037	679aa	Protein coding	-	A2AT66	TSL:5 CDS 5' and 3' incomplete
Ttn-224	ENSMUST00000156257.2	271	91aa	Protein coding	-	F6ZZ75	TSL:3 CDS 5' and 3' incomplete
Ttn-225	ENSMUST00000190813.3	300	100aa	Protein coding	-	A0A087WRP0	GENCODE basic TSL:5

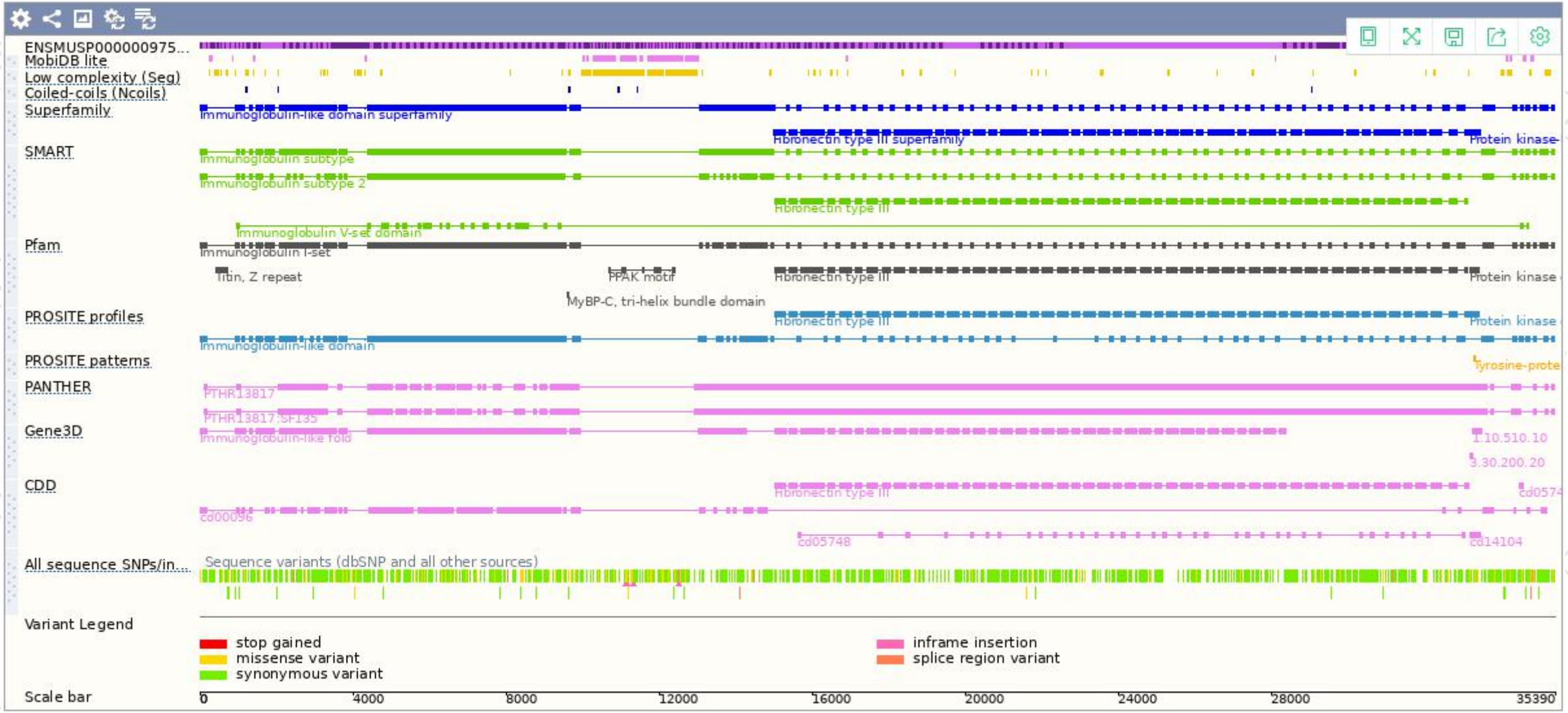
The strategy is based on the design of *Ttn*-201 transcript,The transcription is shown below



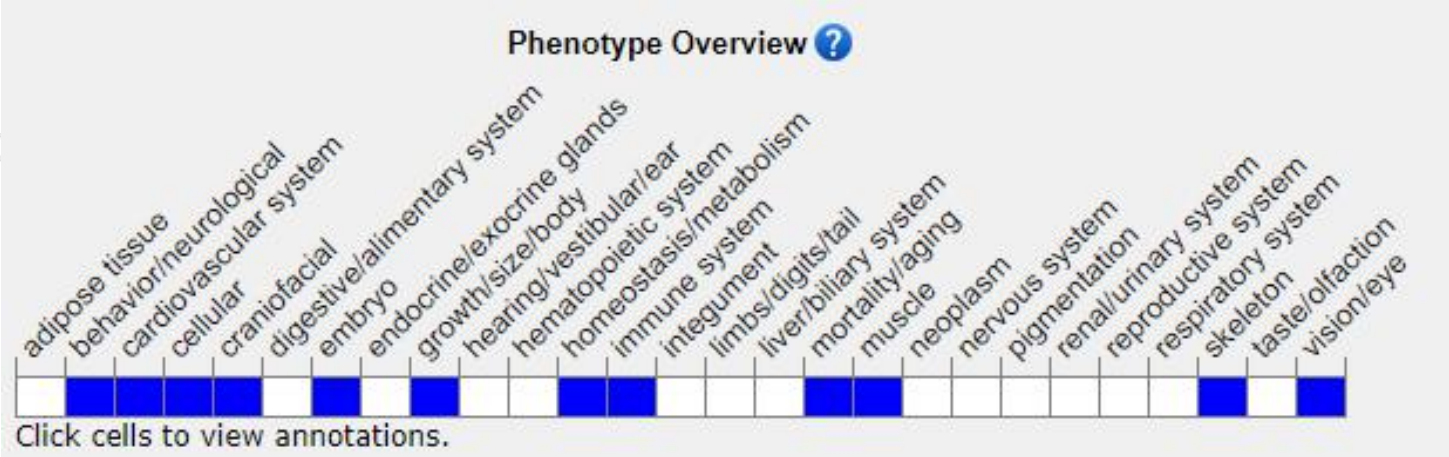
Genomic location (Ensembl)



Protein domain (Ensembl)



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/) .

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

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