

Tnnt2 Cas9-CKO Strategy

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Reviewer :

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Design Date:

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Project Overview

Project Name

Tnnt2

Project type

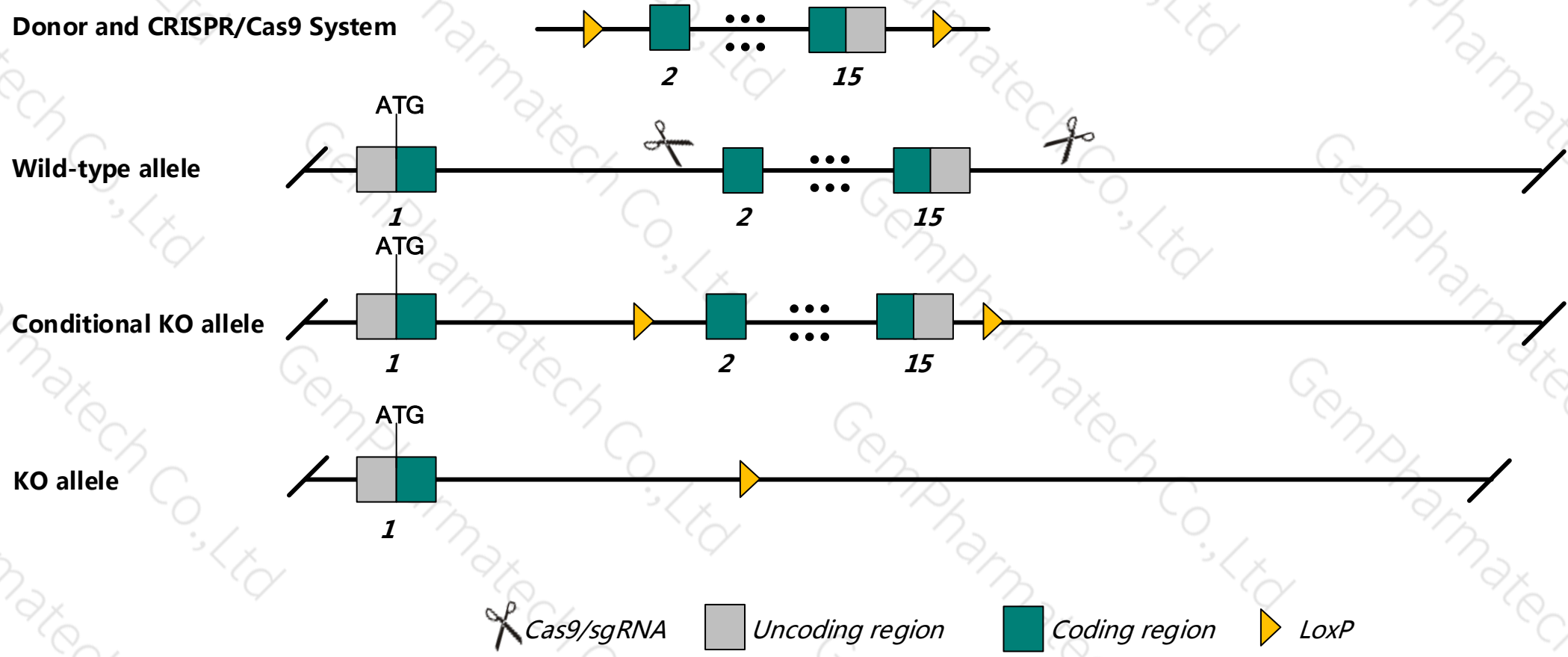
Cas9-CKO

Animal background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tnnt2* gene. The schematic diagram is as follows:



Technical routes

- The *Tnnt2* gene has 15 transcripts, According to the structure of *Tnnt2* gene, exon2-exon15 of *Tnnt2*-204 transcript is recommended as the knockout region. The region contains the most of coding sequence. Knock out the region, result in destruction of protein.
- This project uses CRISPR/Cas9 technology to modify *Tnnt2* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed, Cas9, sgRNA and donor were microinjected into fertilized eggs of C57BL/6JGpt mice and homologous recombination was carried out to obtain F0 mice. A stable and hereditary F1 generation mouse model was obtained by mating F0 generation mice with C57BL/6JGpt mice which were confirmed positive by PCR-sequencing.
- 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 and cell types.

- According to the existing MGI data , Mice homozygous for a null allele exhibit embryonic lethality during and prior to organogenesis and abnormal heart development. Mice homozygous for an allele that lacks the lysine residue at position 210 exhibit dilated cardiomyopathy.
- The *Tnnt2* gene is located in the Chr1. If the knockout mice are mixed with other mice, two target genes are avoided on the same chromosome as possible, otherwise the offspring of mice with double gene positive and homozygous gene knockout can not be obtained.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

Tnnt2 troponin T2, cardiac [*Mus musculus* (house mouse)]

Gene ID: 21956, updated on 31-Jan-2019

Summary

Official Symbol	Tnnt2 provided by MGI
Official Full Name	troponin T2, cardiac provided by MGI
Primary source	MGI:MGI:104597
See related	Ensembl:ENSMUSG00000026414
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	Tnt; cTnT
Expression	Biased expression in heart adult (RPKM 846.5) and bladder adult (RPKM 77.4) See more
Orthologs	human all

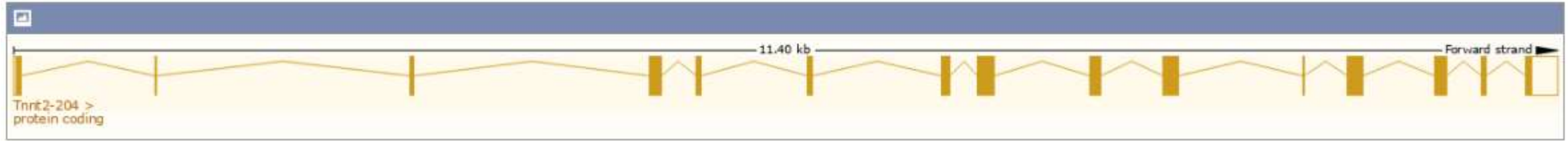
Transcript information (Ensembl)

The gene has 15 transcripts, and all transcripts are shown below :

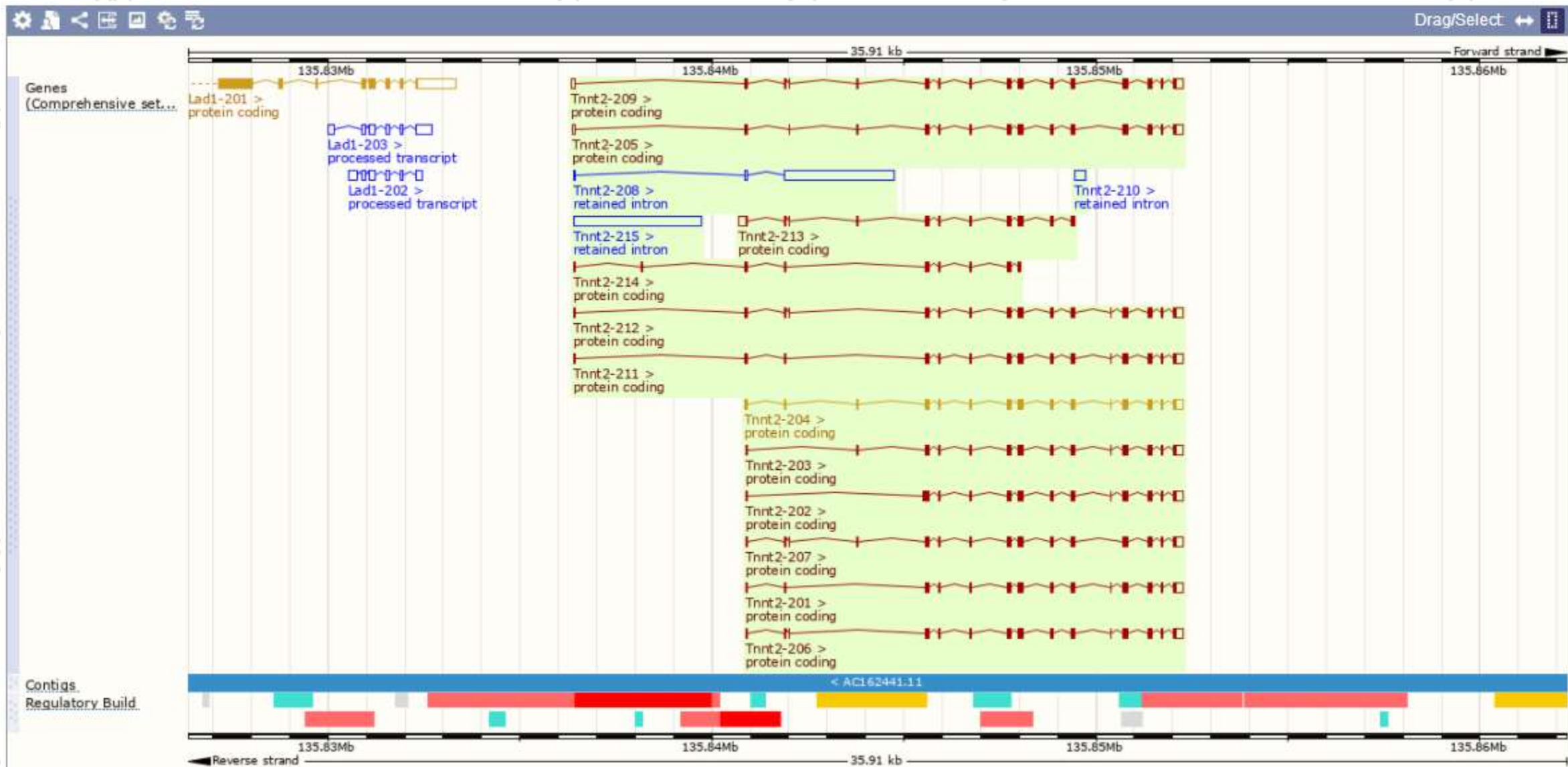
Show/hide columns (1 hidden)								Filter		
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags		
Tnnt2-209	ENSMUST00000188028.6	1200	302aa	Protein coding	CCDS48375	Q6P3Z7	NM_001130174 NM_001130175 NM_001130177 NP_001123646 NP_001123647 NP_001123649	TSL:5	GENCODE basic	APPRIS P4
Tnnt2-212	ENSMUST00000189732.6	1123	295aa	Protein coding	CCDS48376	P50752	NM_001130178 NM_001130179 NP_001123650 NP_001123651	TSL:5	GENCODE basic	APPRIS ALT2
Tnnt2-204	ENSMUST00000112087.8	1112	301aa	Protein coding	CCDS48377	P50752 Q54AB6	NM_001130176 NP_001123648	TSL:5	GENCODE basic	APPRIS ALT2
Tnnt2-211	ENSMUST00000189355.6	1112	291aa	Protein coding	CCDS48378	P50752	NM_001130180 NM_001130181 NM_011619 NP_001123652 NP_001123653 NP_035749	TSL:5	GENCODE basic	APPRIS ALT2
Tnnt2-207	ENSMUST00000179863.7	1097	302aa	Protein coding	CCDS48375	Q6P3Z7	-	TSL:5	GENCODE basic	APPRIS P4
Tnnt2-206	ENSMUST00000178854.7	1076	295aa	Protein coding	CCDS48376	P50752	-	TSL:5	GENCODE basic	APPRIS ALT2
Tnnt2-201	ENSMUST00000027671.11	1064	291aa	Protein coding	CCDS48378	P50752	-	TSL:5	GENCODE basic	APPRIS ALT2
Tnnt2-205	ENSMUST00000178204.7	1172	302aa	Protein coding	-	J3QQ13	-	TSL:5	GENCODE basic	APPRIS ALT2
Tnnt2-203	ENSMUST00000112086.2	1094	301aa	Protein coding	-	K3W4R7	-	TSL:5	GENCODE basic	APPRIS ALT2
Tnnt2-202	ENSMUST00000112085.8	1076	295aa	Protein coding	-	K3W4R6	-	TSL:5	GENCODE basic	APPRIS ALT2
Tnnt2-213	ENSMUST00000189826.6	796	206aa	Protein coding	-	A0A087WRX3	-	CDS 3' incomplete	TSL:5	
Tnnt2-214	ENSMUST00000190451.6	433	114aa	Protein coding	-	A0A087WQP1	-	CDS 3' incomplete	TSL:5	
Tnnt2-215	ENSMUST00000191055.1	3330	No protein	Retained intron	-	-	-	TSL:NA		
Tnnt2-208	ENSMUST00000186225.1	2961	No protein	Retained intron	-	-	-	TSL:2		
Tnnt2-210	ENSMUST00000188098.1	292	No protein	Retained intron	-	-	-	TSL:NA		

Transcript information (Ensembl)

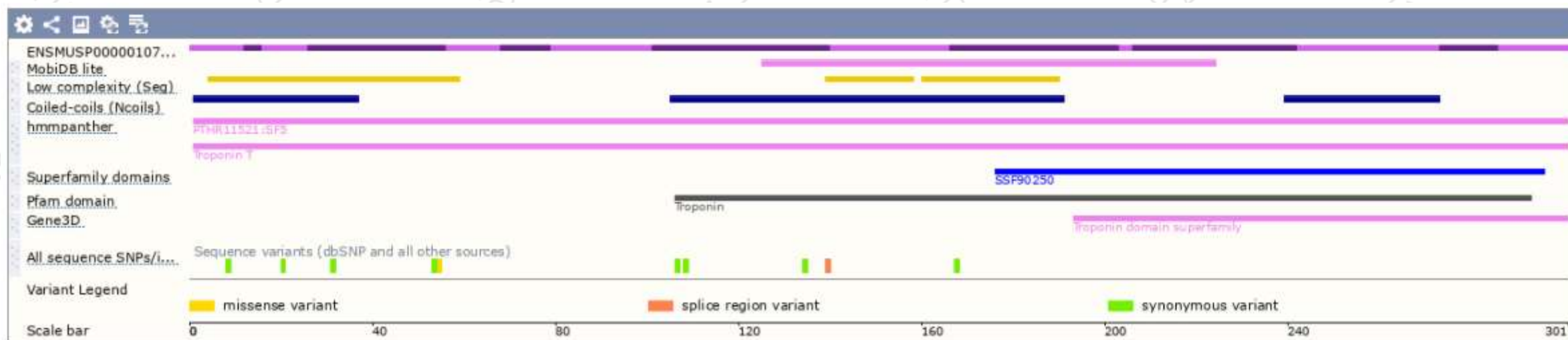
The strategy is based on the design of *Tnnt2-204* transcript, The transcription is shown below :



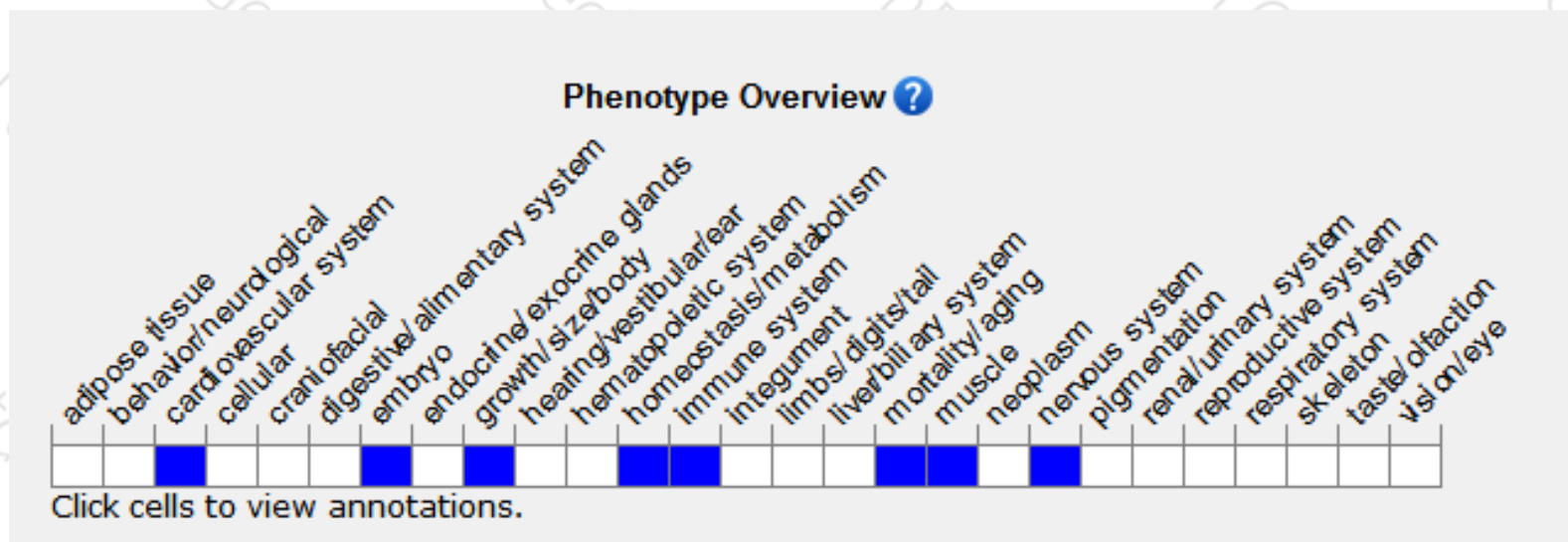
Genomic location distribution



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 null allele exhibit embryonic lethality during and prior to organogenesis and abnormal heart development. Mice homozygous for an allele that lacks the lysine residue at position 210 exhibit dilated cardiomyopathy.

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