Dmpk Cas9-CKO Strategy

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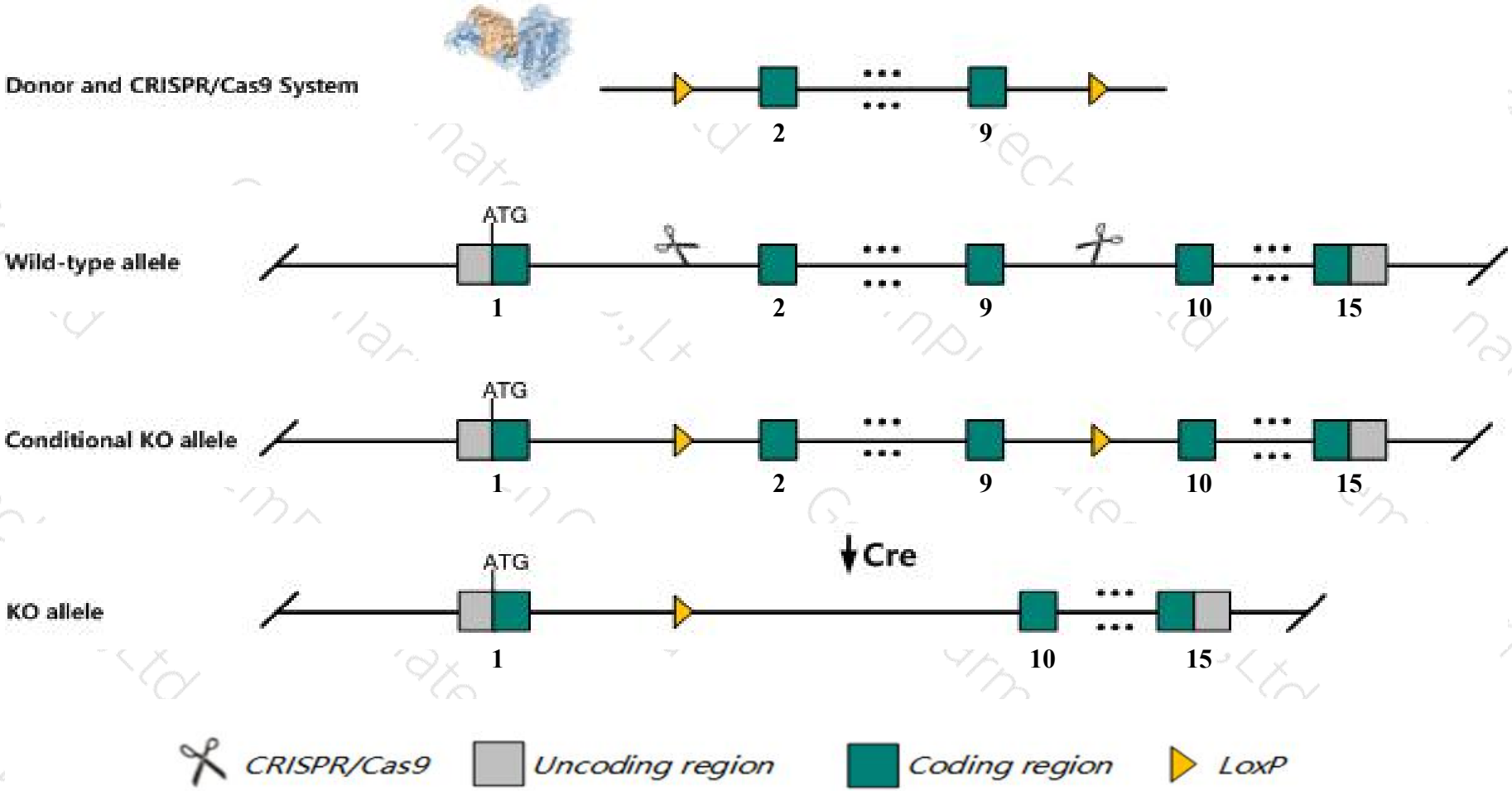
Date:2019-11-24

Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Dmpk* gene has 18 transcripts. According to the structure of *Dmpk* gene, exon2-exon9 of *Dmpk-201* (ENSMUST00000032568.13) transcript is recommended as the knockout region.The region contains 1072bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Dmpk* gene. The brief process is as follows:CRISPR/Cas9 system 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 will be 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.

Notice

➢ According to the existing MGI data,Homozygotes for a null mutation exhibit abnormal sodium channel gating in cardiac myocytes, cardiac conduction defects, and late-onset progressive skeletal myopathy. Homozygotes for a second null mutation do not develop skeletal myopathy but do have abnormal muscle intracellular calcium levels.

➢Transcript *Dmpk*-205&210&212&214 may not be affected.

➢*Mir3100* gene will be deleted together in this strategy.

➢The floxed region is near to the N-terminal of *Six5* gene,this strategy may influence the regulatory function of the N-terminal of *Six5* gene.

➢ The *Dmpk* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Dmpk dystrophia myotonica-protein kinase [*Mus musculus* (house mouse)]

Gene ID: 13400, updated on 18-Nov-2019

Summary

Official Symbol	Dmpk provided by MGI
Official Full Name	dystrophia myotonica-protein kinase provided by MGI
Primary source	MGI:MGI:94906
See related	Ensembl:ENSMUSG00000030409
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	DM; DMK; Dm15; MDPK; MT-PK
Summary	The protein encoded by this gene is a serine/threonine protein kinase that contains coiled-coil and C-terminal membrane association domains. In the embryonic mouse, it is found in cardiac and skeletal myocytes where it appears to play a role in myogenesis. In adults, the transcript is localized to several tissues including brain, heart, and skeletal and smooth muscle, and a function in cytoskeletal remodeling has been described. Transcripts with expanded CUG repeats in the 3' untranslated region mediate alternative splicing of several genes and sequester RNA binding proteins and RNA transcripts that contain CAG repeats, resulting in myotonic dystrophy, an autosomal dominant neuromuscular disorder. Alternative splicing results in multiple protein coding and non-coding transcript variants. [provided by RefSeq, Oct 2014]
Expression	Broad expression in heart adult (RPKM 189.9), bladder adult (RPKM 179.2) and 16 other tissues See more
Orthologs	human all

Genomic context

Location: 7 A3; 7 9.46 cM

See Dmpk in Genome Data Viewer

Exon count: 15

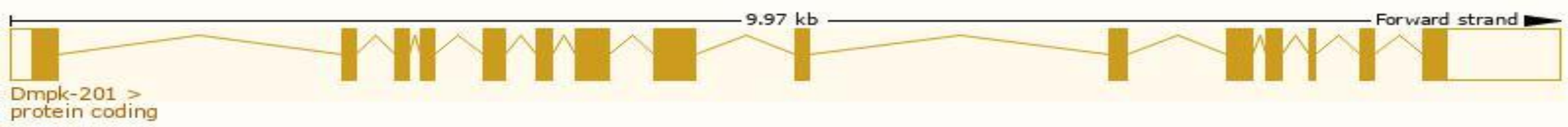
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	7	NC_000073.6 (19083646..19093821)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	7	NC_000073.5 (19669198..19679170)

Transcript information（Ensembl）

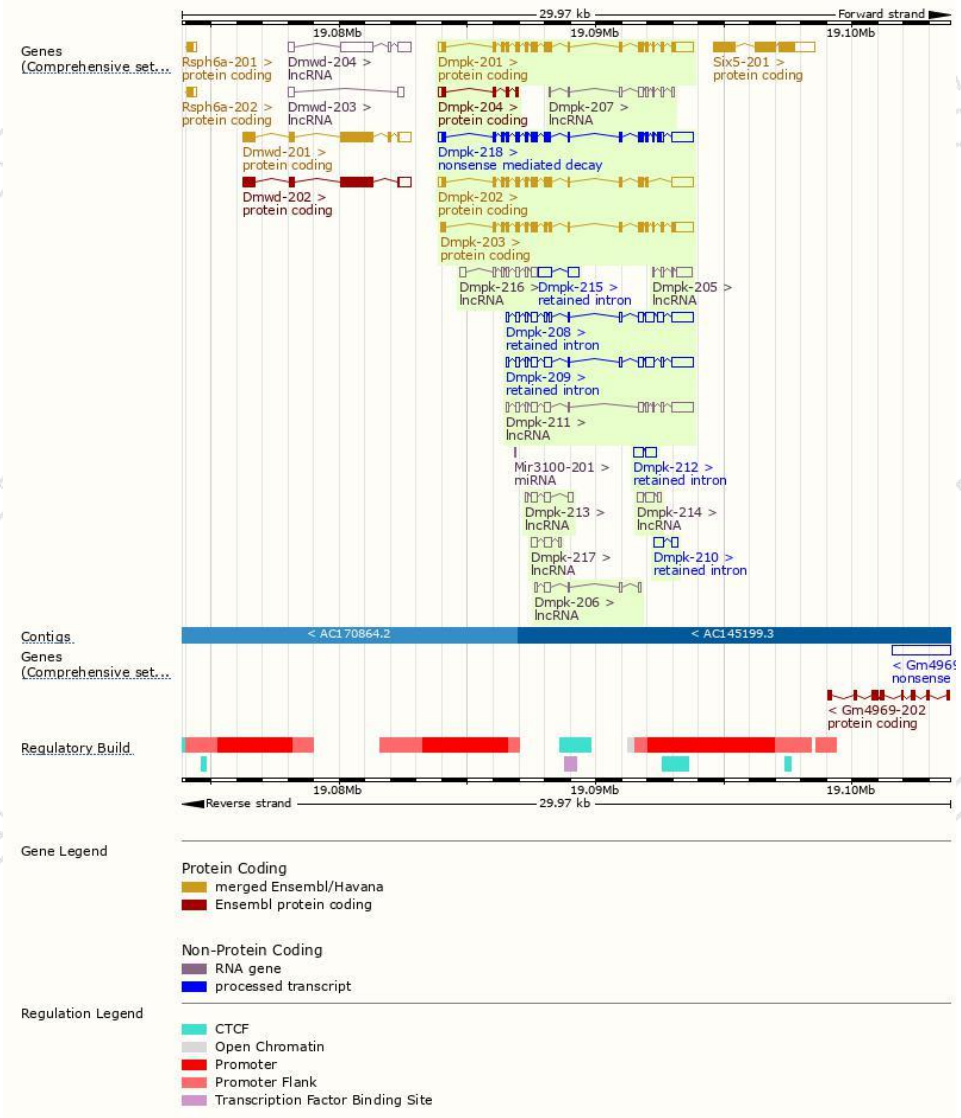
The gene has 18 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Dmpk-201	ENSMUST00000032568.13	2761	631aa	Protein coding	CCDS39794	P54265	TSL:5 GENCODE basic APPRIS P3
Dmpk-203	ENSMUST00000108474.1	2591	605aa	Protein coding	CCDS52053	E9Q6J9	TSL:1 GENCODE basic APPRIS ALT2
Dmpk-202	ENSMUST00000108473.9	2588	537aa	Protein coding	CCDS52054	P54265	TSL:1 GENCODE basic APPRIS ALT2
Dmpk-204	ENSMUST00000122999.7	544	139aa	Protein coding	-	D3YYG5	CDS 3' incomplete TSL:3
Dmpk-218	ENSMUST00000154199.7	2850	588aa	Nonsense mediated decay	-	D6RI32	TSL:1
Dmpk-208	ENSMUST00000135839.7	2475	No protein	Retained intron	-	-	TSL:1
Dmpk-209	ENSMUST00000137219.7	2433	No protein	Retained intron	-	-	TSL:1
Dmpk-215	ENSMUST00000148472.1	967	No protein	Retained intron	-	-	TSL:3
Dmpk-212	ENSMUST00000143938.1	785	No protein	Retained intron	-	-	TSL:5
Dmpk-210	ENSMUST00000140742.1	592	No protein	Retained intron	-	-	TSL:2
Dmpk-211	ENSMUST00000142725.7	2135	No protein	lncRNA	-	-	TSL:1
Dmpk-216	ENSMUST00000149188.7	923	No protein	lncRNA	-	-	TSL:5
Dmpk-205	ENSMUST00000126264.1	851	No protein	lncRNA	-	-	TSL:3
Dmpk-207	ENSMUST00000132115.7	757	No protein	lncRNA	-	-	TSL:5
Dmpk-213	ENSMUST00000147215.7	743	No protein	lncRNA	-	-	TSL:3
Dmpk-214	ENSMUST00000148380.1	663	No protein	lncRNA	-	-	TSL:3
Dmpk-206	ENSMUST00000128422.7	650	No protein	lncRNA	-	-	TSL:3
Dmpk-217	ENSMUST00000152050.1	565	No protein	lncRNA	-	-	TSL:3

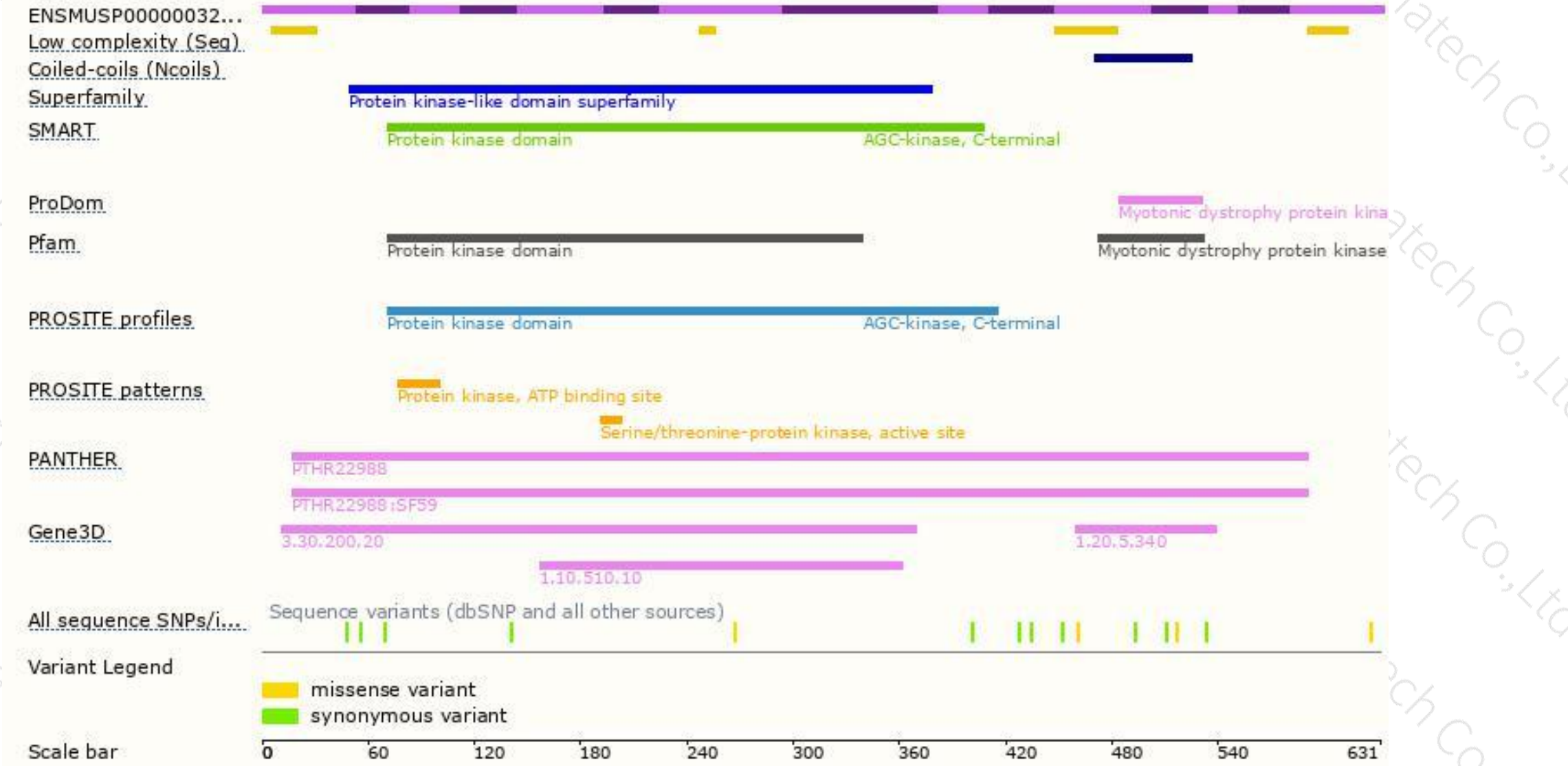
The strategy is based on the design of *Dmpk-201* transcript,The transcription is shown below



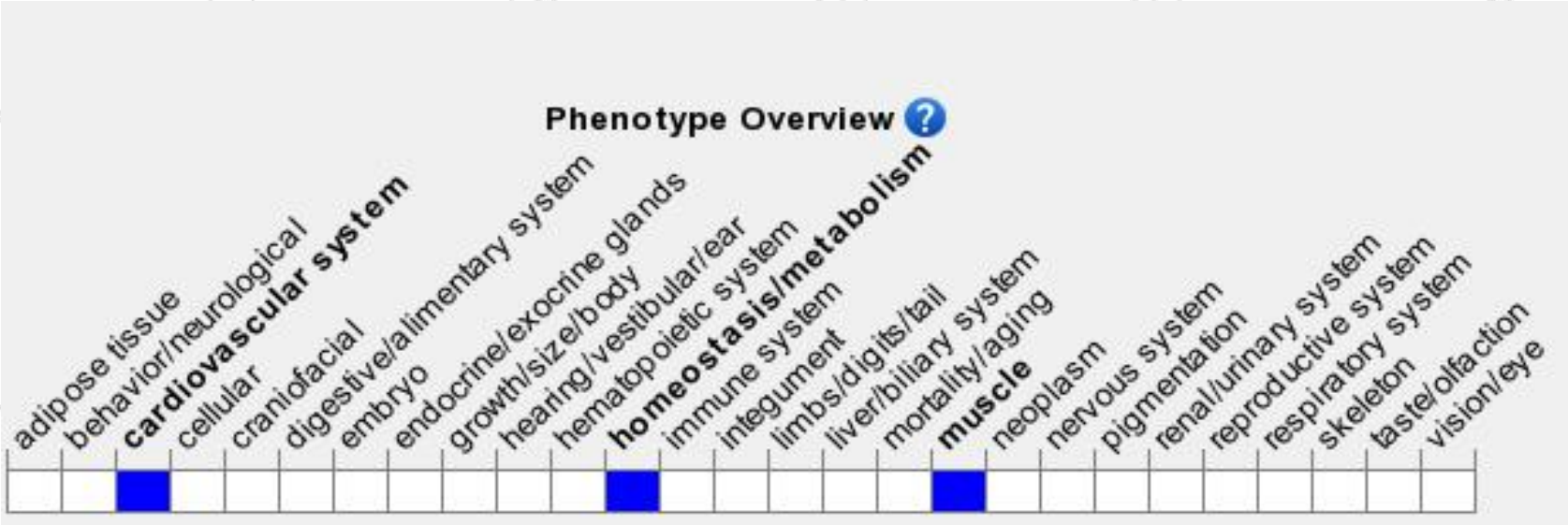
Genomic location distribution



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,Homozygotes for a null mutation exhibit abnormal sodium channel gating in cardiac myocytes, cardiac conduction defects, and late-onset progressive skeletal myopathy. Homozygotes for a second null mutation do not develop skeletal myopathy but do have abnormal muscle intracellular calcium levels.

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

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