Tnnt1 Cas9-CKO Strategy

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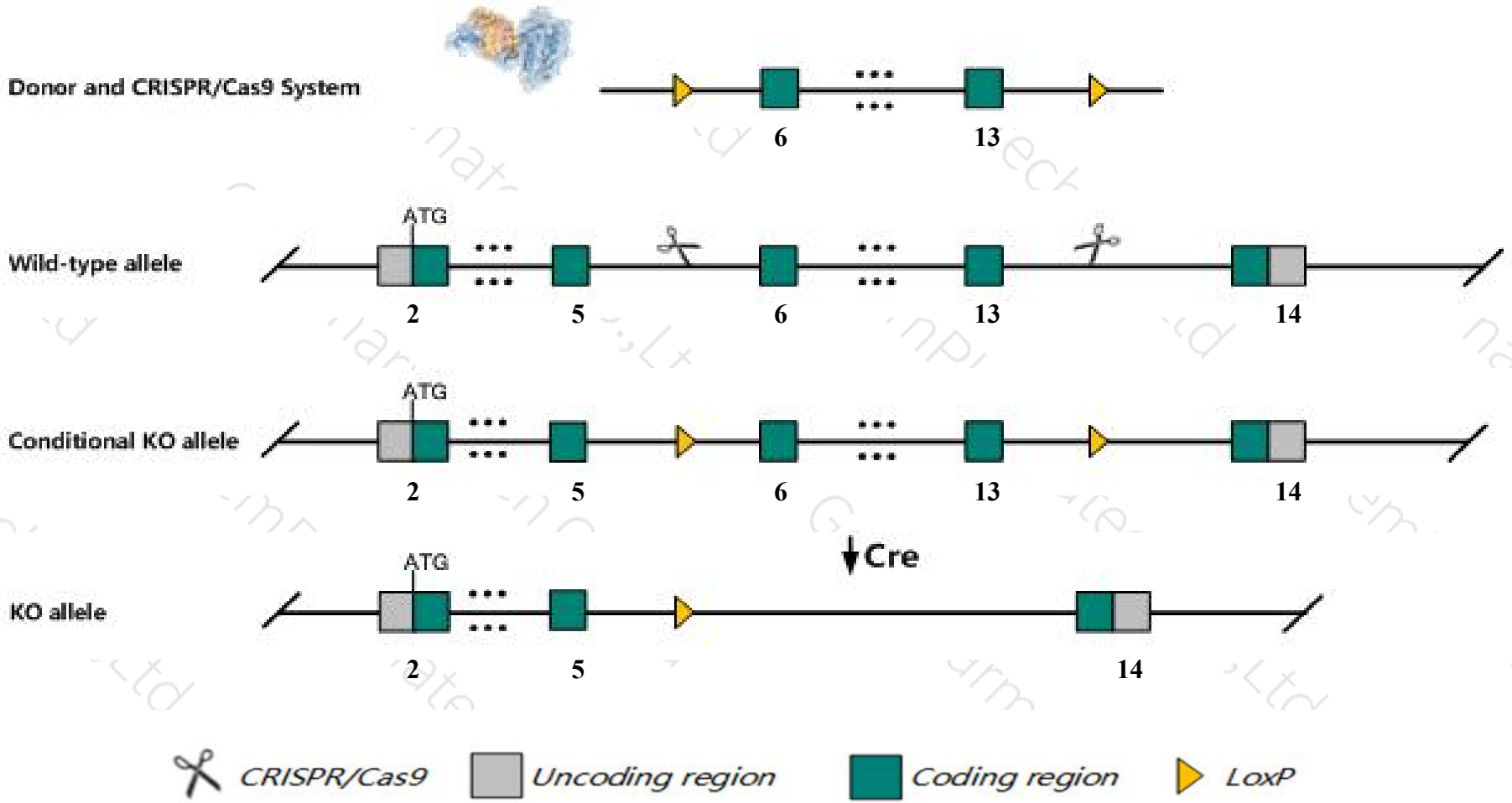
Project Overview

Project Name	***Tnnt1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tnnt1* gene. The schematic diagram is as follows:



Technical routes

- The *Tnnt1* gene has 13 transcripts. According to the structure of *Tnnt1* gene, exon6-exon13 of *Tnnt1-203* (ENSMUST00000108587.8) transcript is recommended as the knockout region.The region contains 637bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Tnnt1* 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,Mice homozygous for a null or hypomorphic allele show small and loss of type I slow skeletal muscle fibers with compensatory hypertrophy of type II fast fibers and reduced contractile force and tolerance of skeletal muscle fibers.

➢ The *Tnnt1* 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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Tnnt1 troponin T1, skeletal, slow [Mus musculus (house mouse)]

Gene ID: 21955, updated on 19-Mar-2019

Summary

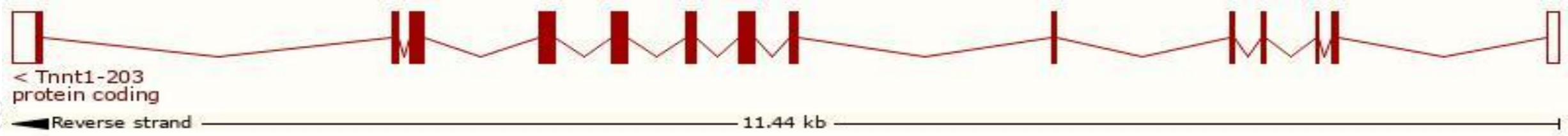
Official Symbol	Tnnt1 provided by MGI
Official Full Name	troponin T1, skeletal, slow provided by MGI
Primary source	MGI:MGI:1333868
See related	Ensembl:ENSMUSG00000064179
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW146156, Tnt, sTnT, ssTnT
Summary	This gene encodes the slow skeletal tropomyosin-binding subunit of the troponin complex and plays an essential role in the regulation of striated muscle contraction. In humans, mutations in this gene are associated with nemaline myopathy type 5. Alternative splicing of this gene results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Apr 2013]
Expression	Biased expression in limb E14.5 (RPKM 26.9), CNS E11.5 (RPKM 6.4) and 5 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

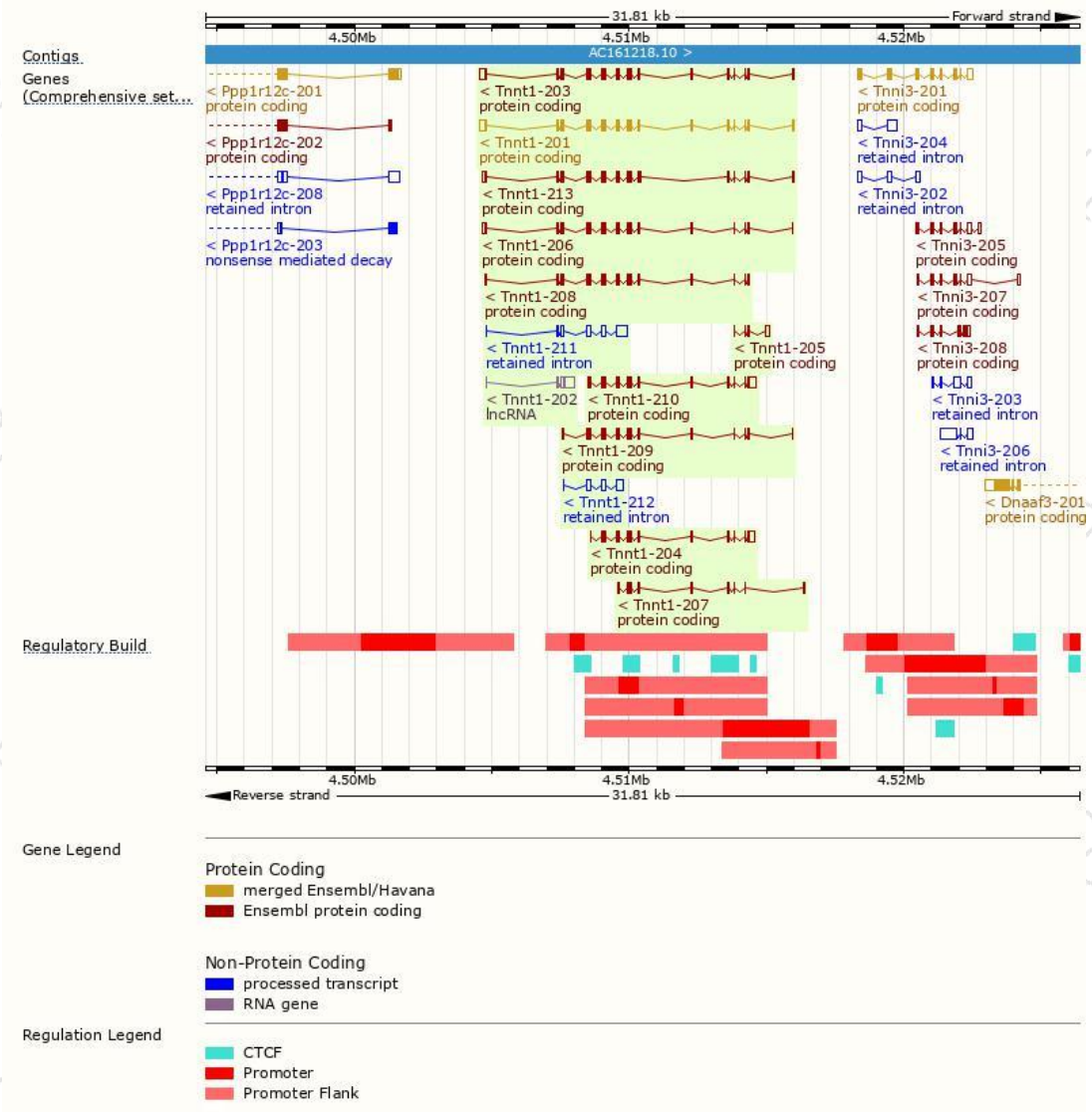
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tnnt1-203	ENSMUST00000108587.8	1059	262aa	Protein coding	CCDS71880	O88346	TSL:1 GENCODE basic APPRIS ALT2
Tnnt1-201	ENSMUST00000071798.12	1027	261aa	Protein coding	CCDS51973	O88346 Q3TVB8	TSL:1 GENCODE basic APPRIS P3
Tnnt1-206	ENSMUST00000163710.7	885	250aa	Protein coding	CCDS71879	O88346	TSL:1 GENCODE basic APPRIS ALT2
Tnnt1-213	ENSMUST00000178163.7	934	261aa	Protein coding	-	J3QPC8	TSL:5 GENCODE basic APPRIS ALT2
Tnnt1-210	ENSMUST00000166959.7	822	191aa	Protein coding	-	E9Q0G9	CDS 3' incomplete TSL:5
Tnnt1-208	ENSMUST00000166161.7	750	249aa	Protein coding	-	E9Q688	TSL:5 GENCODE basic APPRIS ALT2
Tnnt1-204	ENSMUST00000163538.7	710	168aa	Protein coding	-	E9PZB4	CDS 3' incomplete TSL:5
Tnnt1-209	ENSMUST00000166268.7	665	207aa	Protein coding	-	E9QA86	CDS 3' incomplete TSL:5
Tnnt1-207	ENSMUST00000163722.7	358	118aa	Protein coding	-	E9PVN9	CDS 3' incomplete TSL:2
Tnnt1-205	ENSMUST00000163560.1	218	18aa	Protein coding	-	E9Q334	CDS 3' incomplete TSL:5
Tnnt1-211	ENSMUST00000168111.7	796	No protein	Retained intron	-	-	TSL:5
Tnnt1-212	ENSMUST00000169571.1	478	No protein	Retained intron	-	-	TSL:5
Tnnt1-202	ENSMUST00000086502.4	485	No protein	lncRNA	-	-	TSL:5

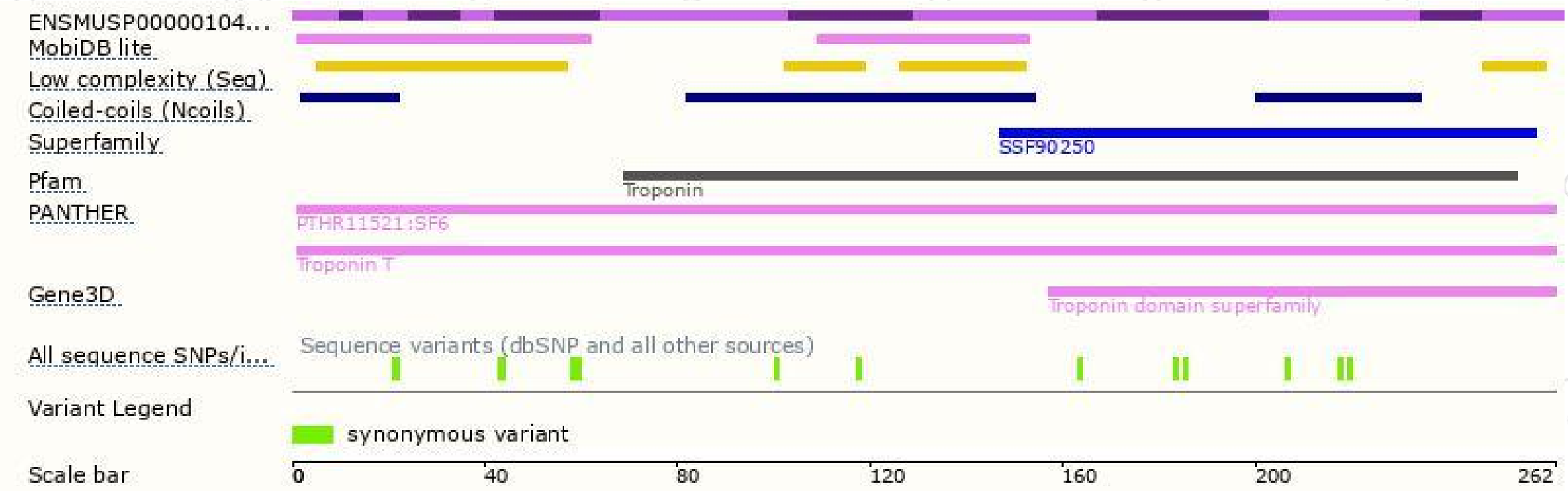
The strategy is based on the design of *Tnnt1-203* transcript,The transcription is shown below



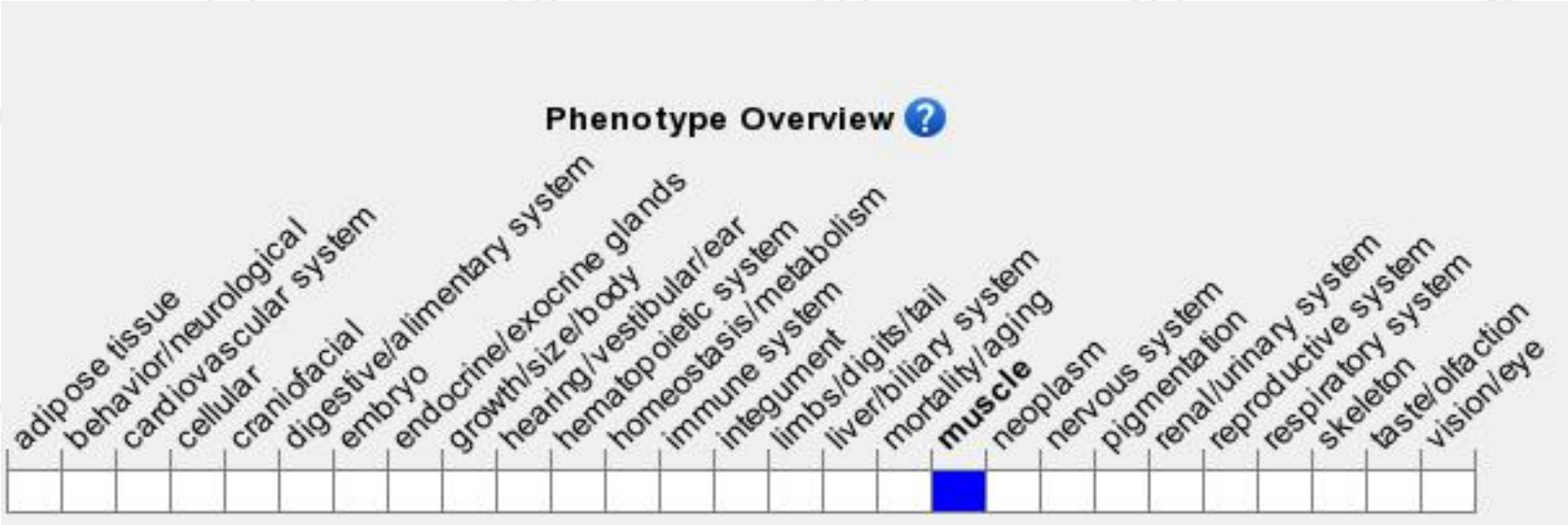
Genomic location distribution



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/).

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

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