

***Mstn* Cas9-CKO Strategy**

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Project Overview

Project Name

Mstn

Project type

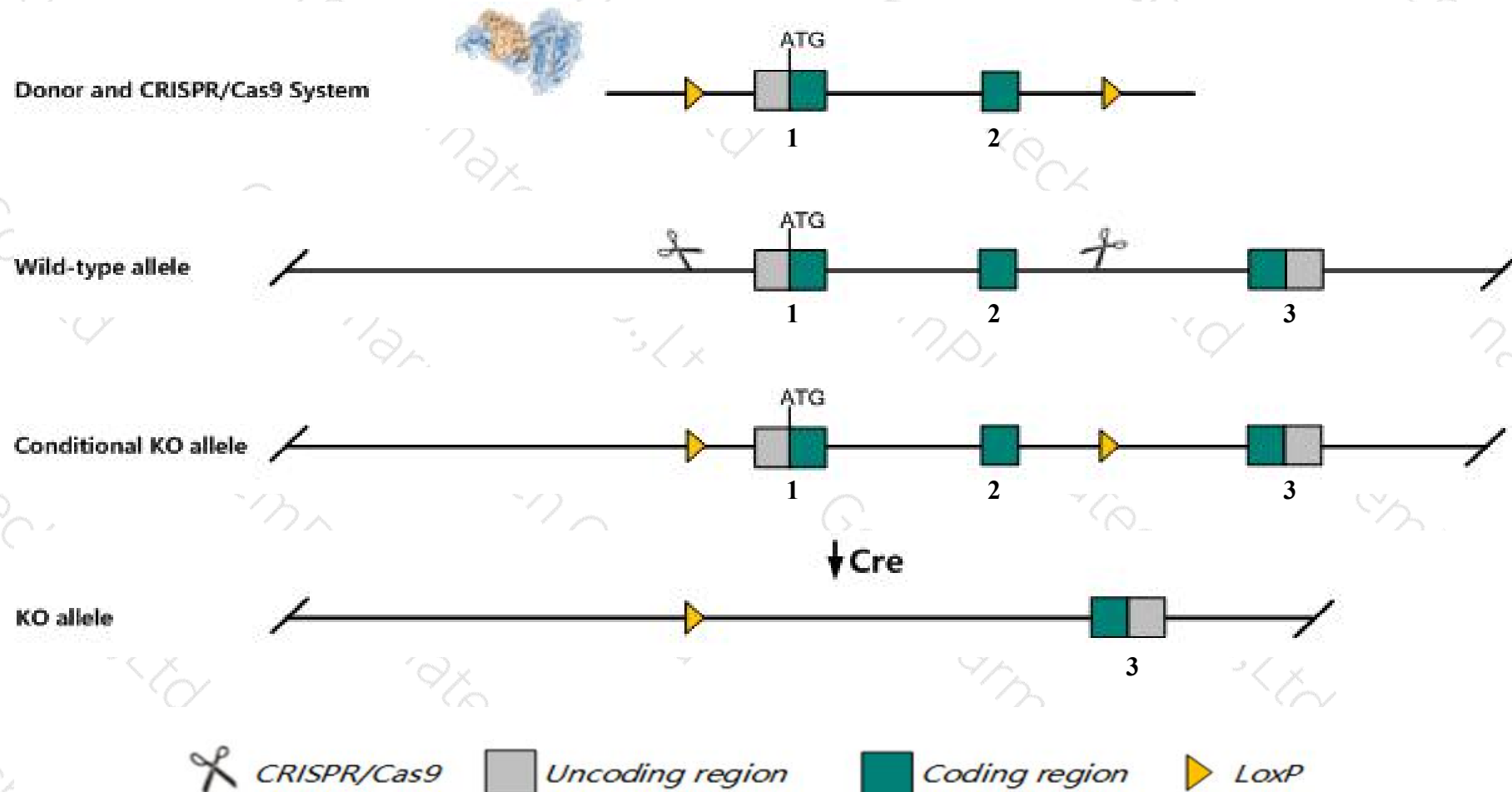
Cas9-CKO

Strain background

C57BL/6J

Conditional Knockout strategy

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



Technical routes

- The *Mstn* gene has 2 transcripts. According to the structure of *Mstn* gene, exon1-exon2 of *Mstn-201* (ENSMUST00000027269.6) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Mstn* gene. The brief process is as follows: CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6J 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/6J 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.

- According to the existing MGI data, Homozygotes for targeted and spontaneous mutations exhibit markedly increased size of striated muscle due to both hyperplasia and hypertrophy, reduced adiposity, and increased bone mineral density.
- The *Mstn* gene is located on the Chr1. 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)

Mstn myostatin [Mus musculus (house mouse)]

Gene ID: 17700, updated on 9-Apr-2019

Summary



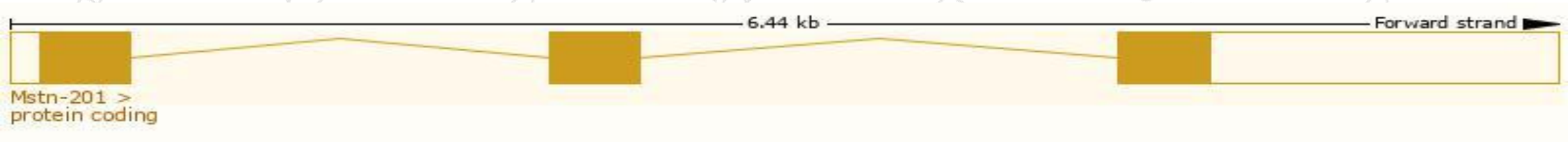
Official Symbol	Mstn provided by MGI
Official Full Name	myostatin provided by MGI
Primary source	MGI:MGI:95691
See related	Ensembl:ENSMUSG00000026100
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cmpt, Gdf8
Summary	This gene encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. The encoded preproprotein is proteolytically processed to generate each subunit of the disulfide-linked homodimer. This protein negatively regulates skeletal muscle cell proliferation and differentiation. Homozygous knockout mice for this gene exhibit increased muscle mass and bone density, and reduced adiposity. [provided by RefSeq, Jul 2016]
Expression	Biased expression in mammary gland adult (RPKM 4.7), limb E14.5 (RPKM 3.1) and 1 other tissue See more
Orthologs	human all

Transcript information (Ensembl)

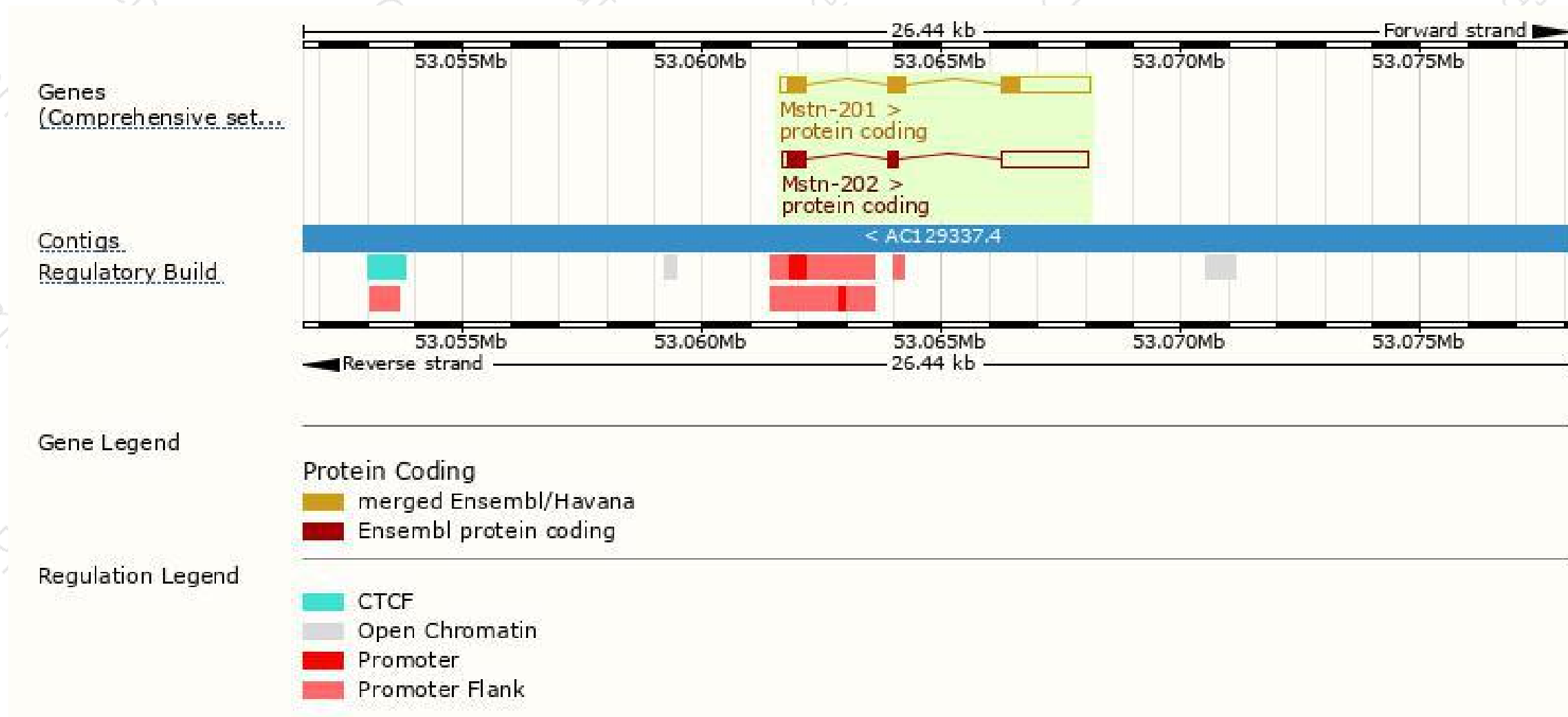
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mstn-201	ENSMUST00000027269.6	2705	376aa	Protein coding	CCDS14950	Q08689 Q540E2	TSL:1 GENCODE basic APPRIS P1
Mstn-202	ENSMUST00000191197.1	2461	189aa	Protein coding	-	A0A087WQL8	TSL:5 GENCODE basic

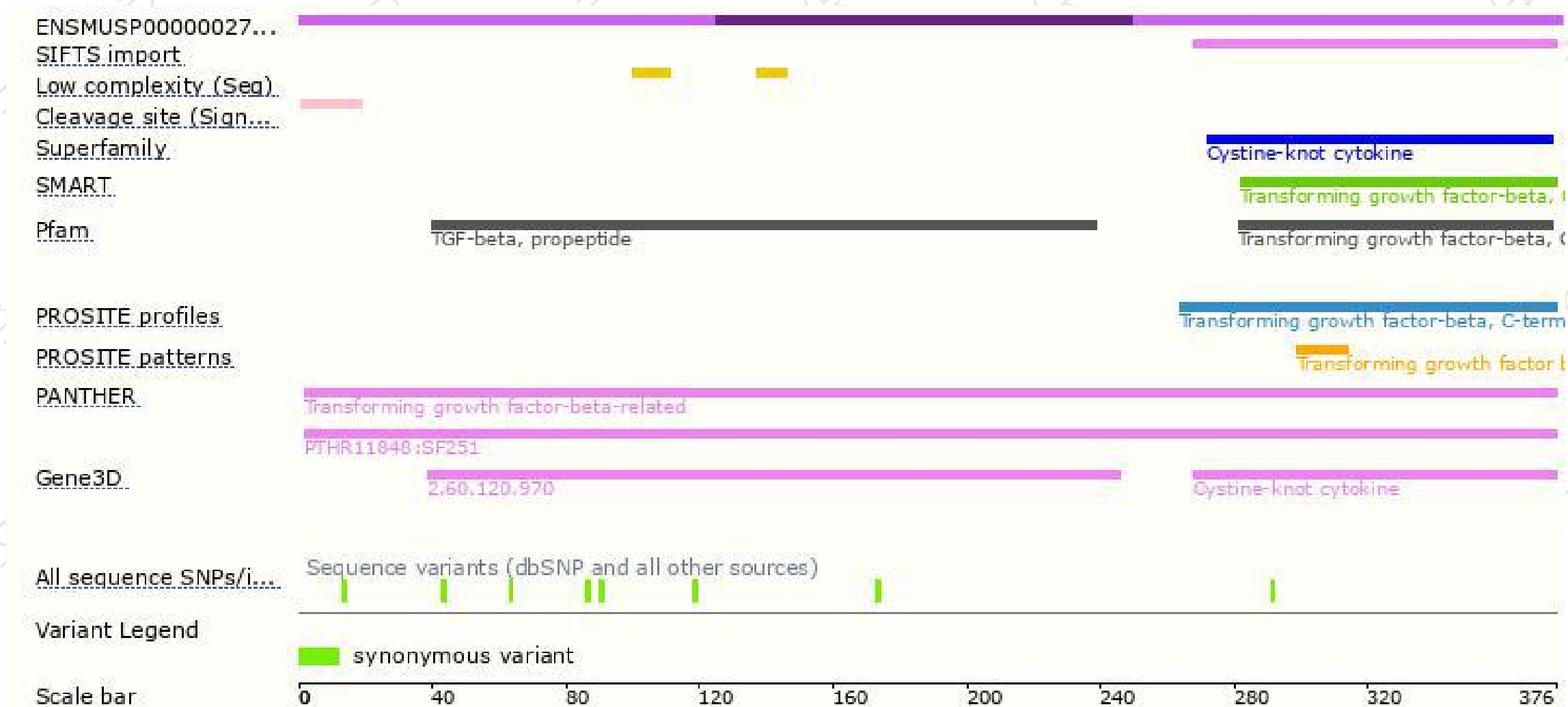
The strategy is based on the design of *Mstn-201* transcript,The transcription is shown below



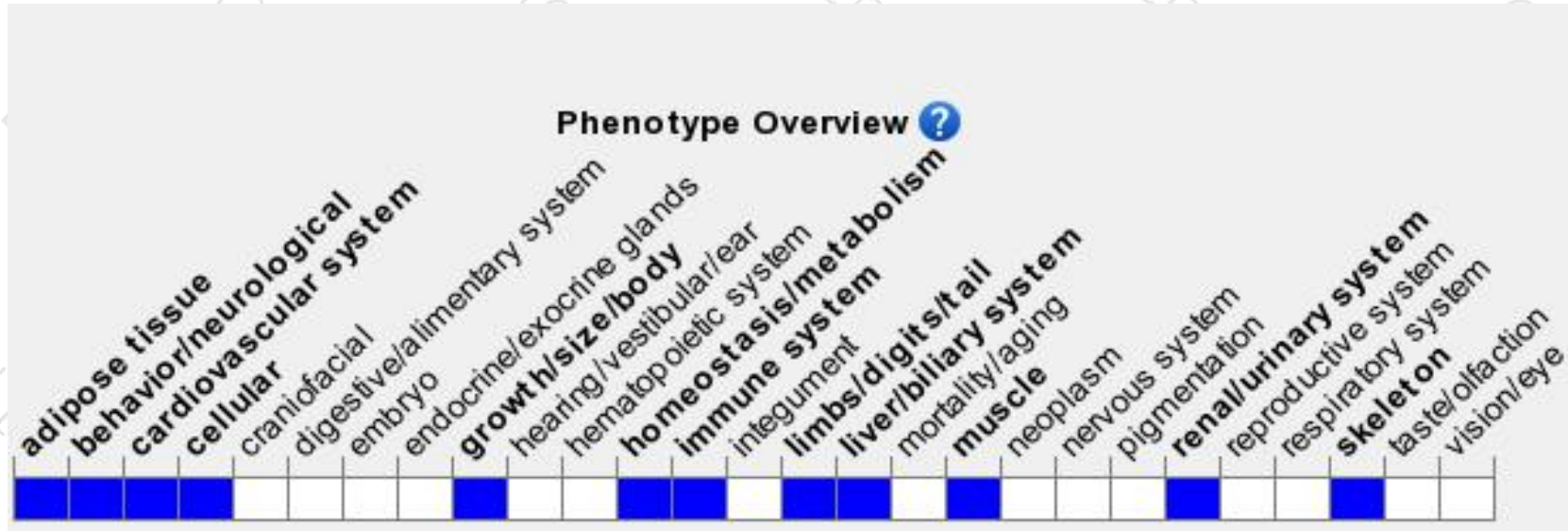
Genomic location distribution



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 targeted and spontaneous mutations exhibit markedly increased size of striated muscle due to both hyperplasia and hypertrophy, reduced adiposity, and increased bone mineral density.

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

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