

Actn3 Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Actn3

Project type

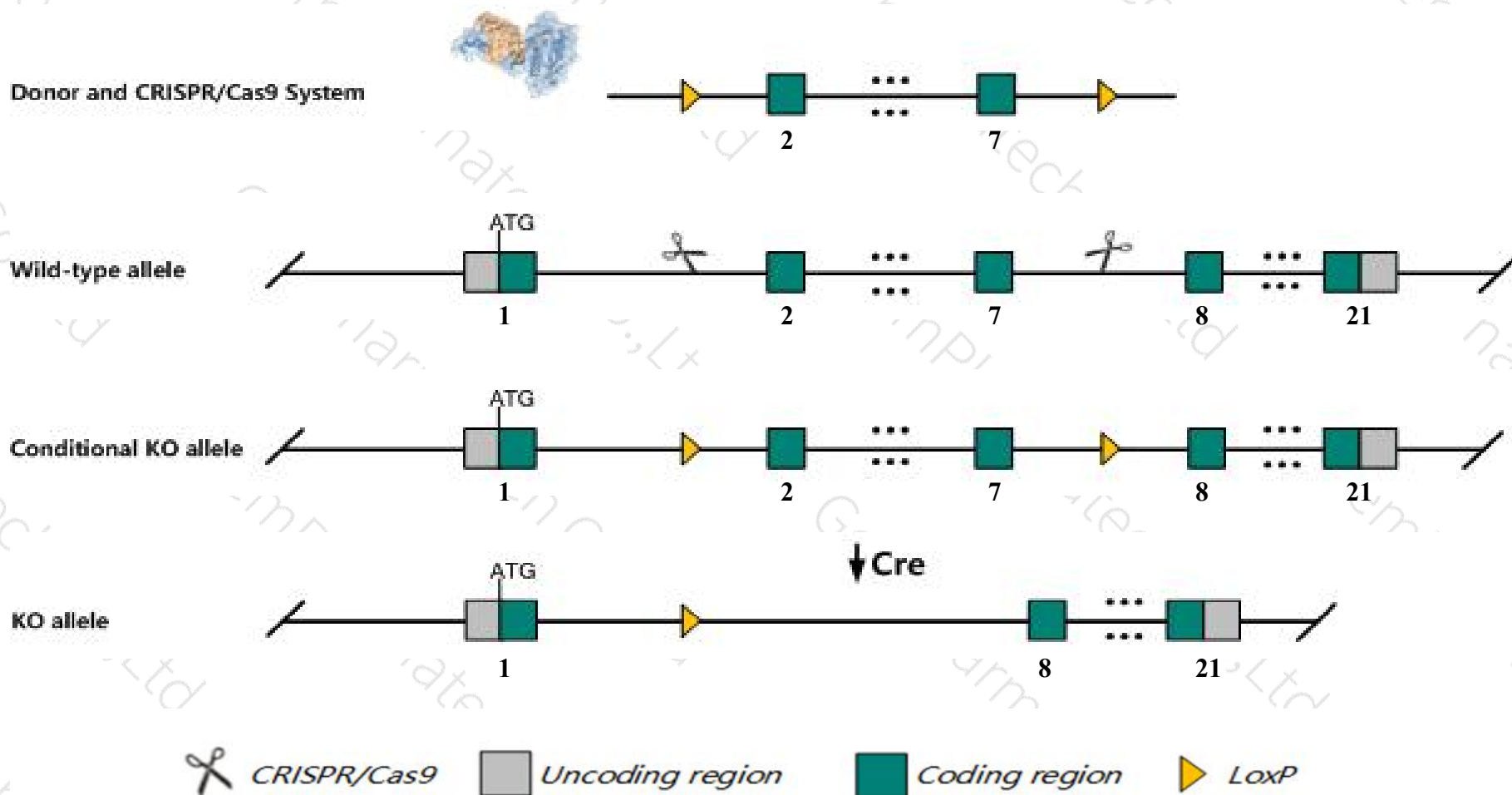
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Actn3* gene. The schematic diagram is as follows:



- The *Actn3* gene has 2 transcripts. According to the structure of *Actn3* gene, exon2-exon7 of *Actn3-201* (ENSMUST00000006626.4) transcript is recommended as the knockout region. The region contains 571bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Actn3* 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.

- According to the existing MGI data, mice homozygous for a null allele exhibit an increase mitochondria density and a shift from anaerobic to aerobic metabolism in fast muscle fiber that is associated with increased aerobic capacity.
- The *Actn3* gene is located on the Chr19. 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)

Actn3 actinin alpha 3 [*Mus musculus* (house mouse)]

Gene ID: 11474, updated on 12-Aug-2019

Summary

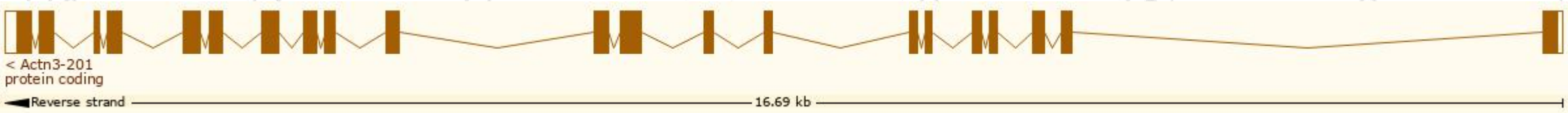
Official Symbol	Actn3 provided by MGI
Official Full Name	actinin alpha 3 provided by MGI
Primary source	MGI:MGI:99678
See related	Ensembl:ENSMUSG000000006457
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Summary	This gene encodes a member of the alpha-actin binding protein gene family. The encoded protein is primarily expressed in skeletal muscle and functions as a structural component of sarcomeric Z line. This protein is involved in crosslinking actin containing thin filaments. [provided by RefSeq, Sep 2015]
Expression	Biased expression in mammary gland adult (RPKM 125.5) and lung adult (RPKM 3.1) See more
Orthologs	human all

Transcript information (Ensembl)

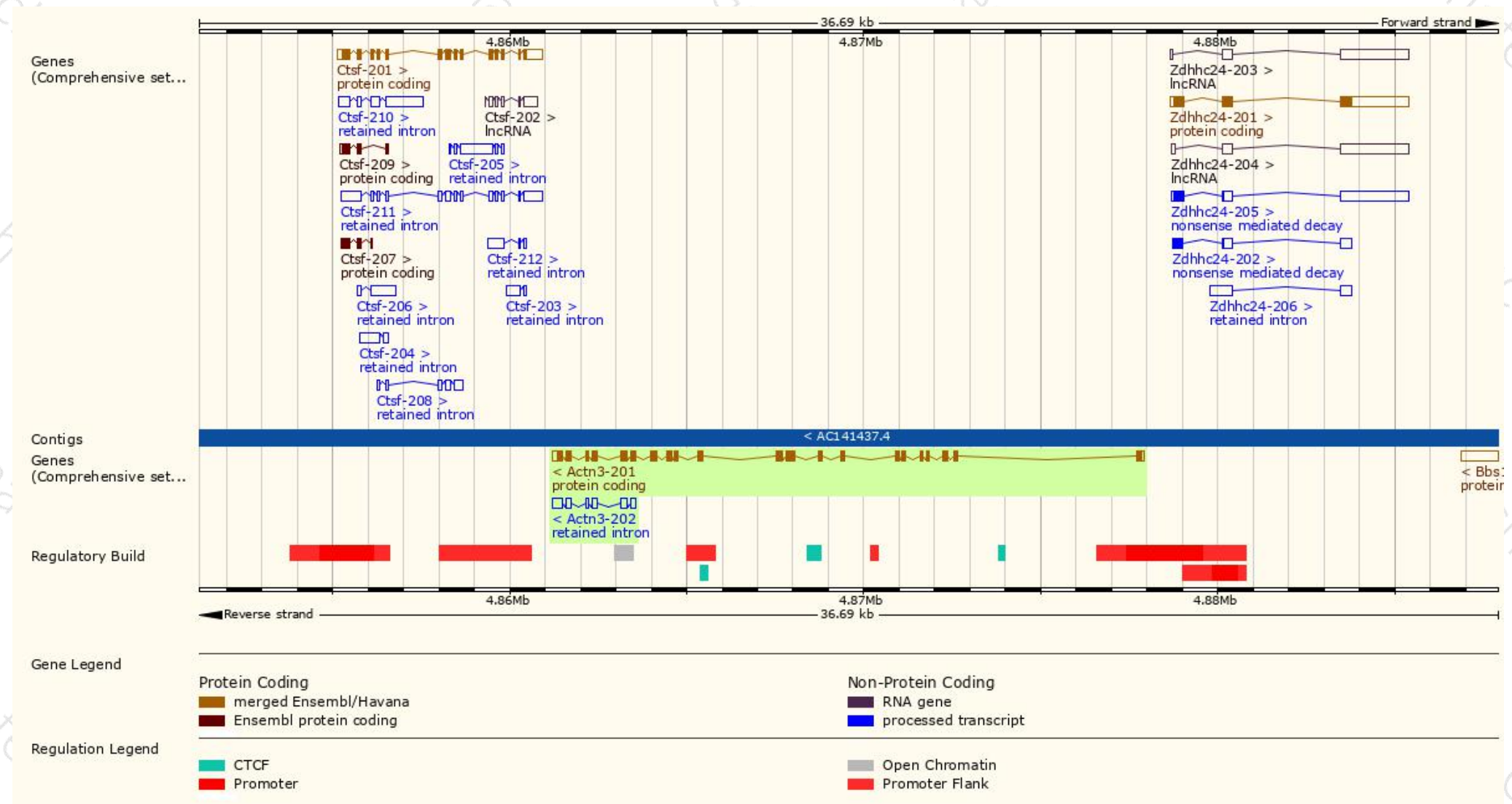
The gene has 2 transcripts,all transcripts are shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Biotype ▲	CCDS ▲	UniProt ▲	Flags ▲
Actn3-201	ENSMUST00000006626.4	2887	900aa	Protein coding	CCDS29441	O88990	TSL:1 Gencode basic APPRIS P1
Actn3-202	ENSMUST00000138811.1	982	No protein	Retained intron	-	-	TSL:2

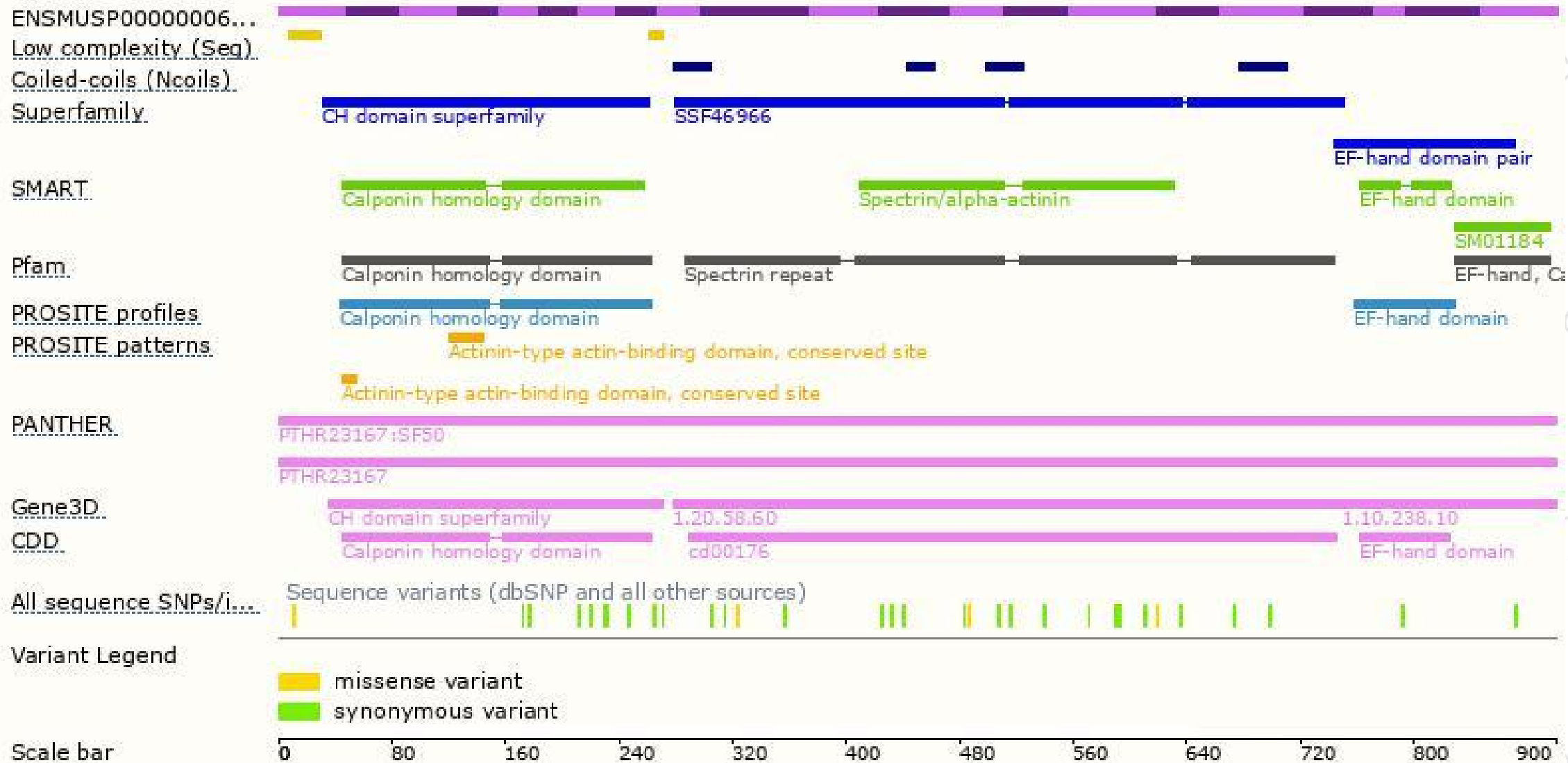
The strategy is based on the design of *Actn3-201* transcript,the transcription is shown below



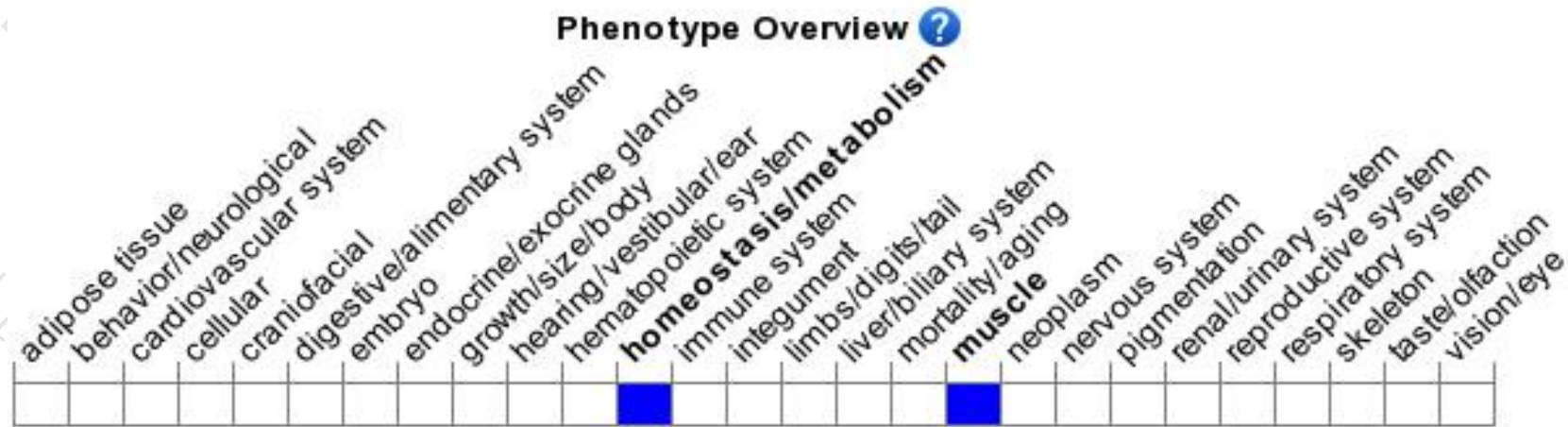
Genomic location distribution



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, Mice homozygous for a null allele exhibit an increase mitochondria density and a shift from anaerobic to aerobic metabolism in fast muscle fiber that is associated with increased aerobic capacity.

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

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