Pygm Cas9-KO Strategy

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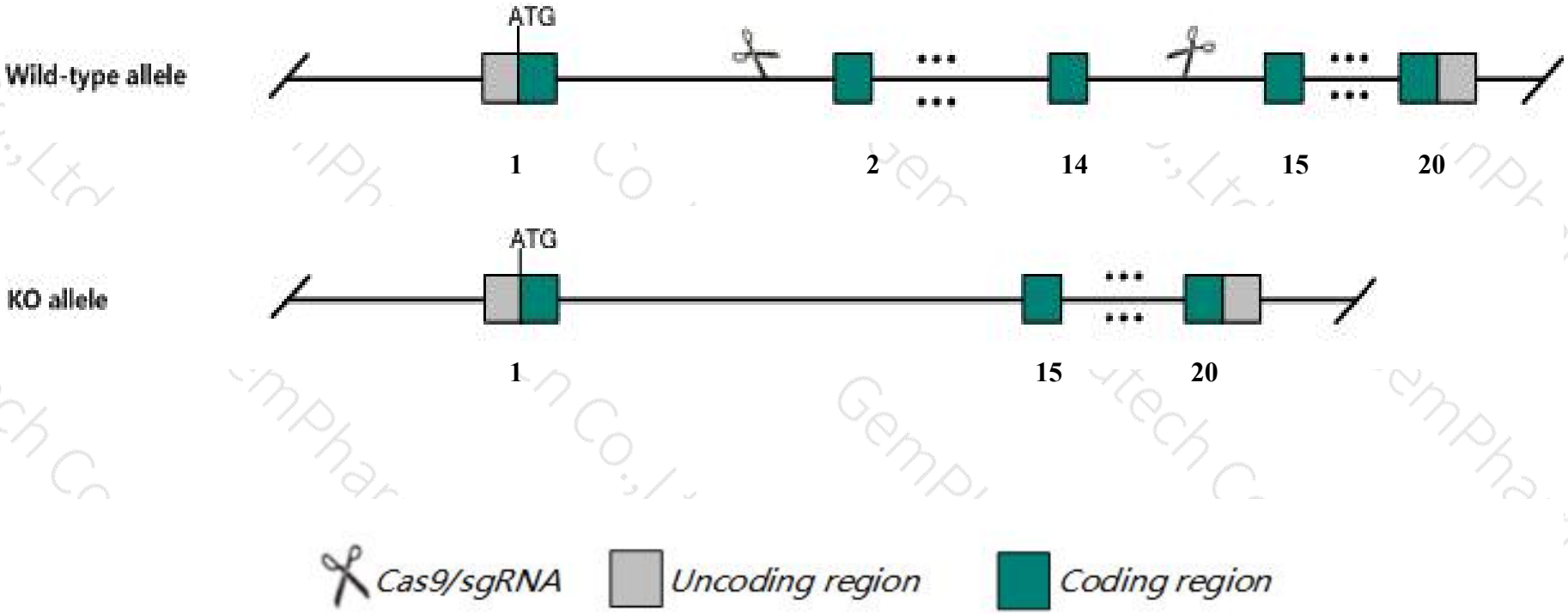
Project Overview

Project Name	***Pygm***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

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



Technical routes

➢ The *Pygm* gene has 4 transcripts. According to the structure of *Pygm* gene, exon2-exon14 of *Pygm-201* (ENSMUST00000035269.14) transcript is recommended as the knockout region.The region contains 1525bp coding sequenc Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Pygm* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

- According to the existing MGI data,Mice homozygous for a null mutation exhibit massive muscle glycogen accumulation, elevated creatine kinase levels in blood, and very poor exercise performance.

- The *Pygm* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Pygm muscle glycogen phosphorylase [Mus musculus (house mouse)]

Gene ID: 19309, updated on 31-Jan-2019

Summary

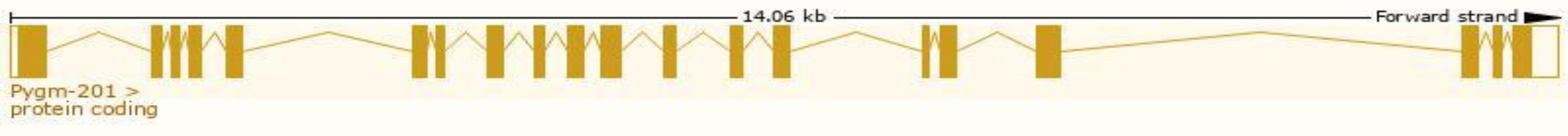
Official Symbol	Pygm provided by MGI
Official Full Name	muscle glycogen phosphorylase provided by MGI
Primary source	MGI:MGI:97830
See related	Ensembl:ENSMUSG00000032648
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI115133, PG
Summary	This gene encodes a glycolysis enzyme found in muscle. Highly similar enzymes encoded by different genes are found in liver and brain. The encoded protein is involved in regulating the breakdown of glycogen to glucose-1-phosphate, which is necessary for ATP generation. [provided by RefSeq, Dec 2015]
Expression	Biased expression in heart adult (RPKM 318.9), mammary gland adult (RPKM 190.6) and 3 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

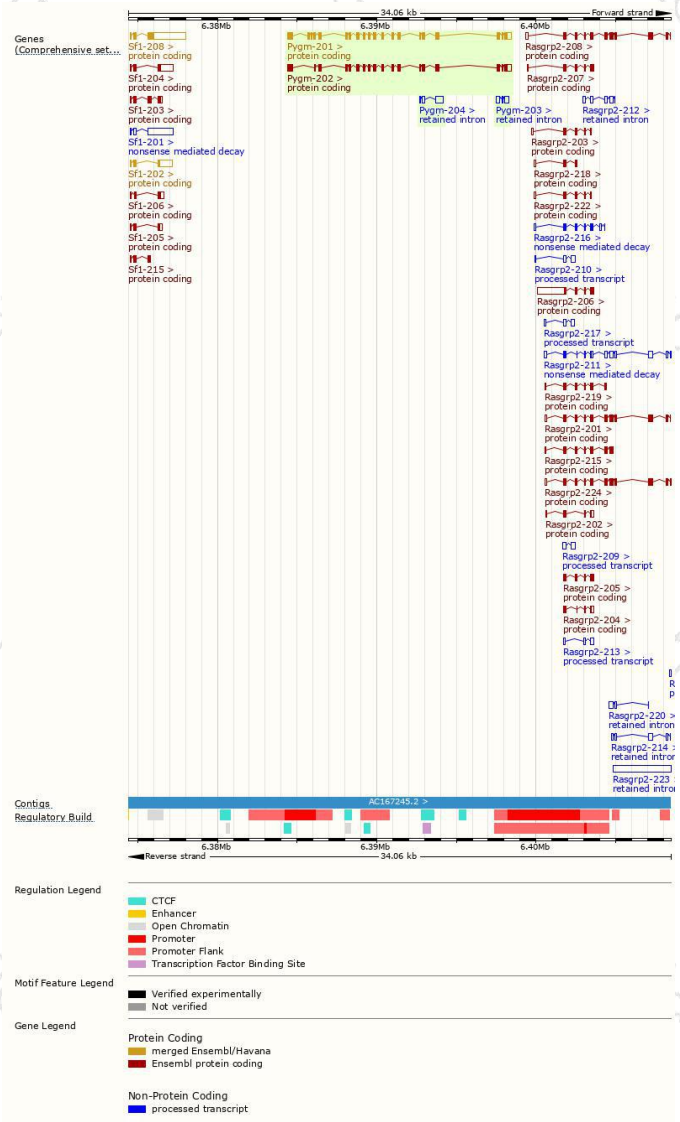
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pygm-201	ENSMUST00000035269.14	2874	842aa	Protein coding	CCDS29504	Q9WUB3	TSL:1 GENCODE basic APPRIS P1
Pygm-202	ENSMUST00000113483.1	2583	754aa	Protein coding	-	E9PUM3	TSL:5 GENCODE basic
Pygm-204	ENSMUST00000142755.1	638	No protein	Retained intron	-	-	TSL:3
Pygm-203	ENSMUST00000139775.1	532	No protein	Retained intron	-	-	TSL:1

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

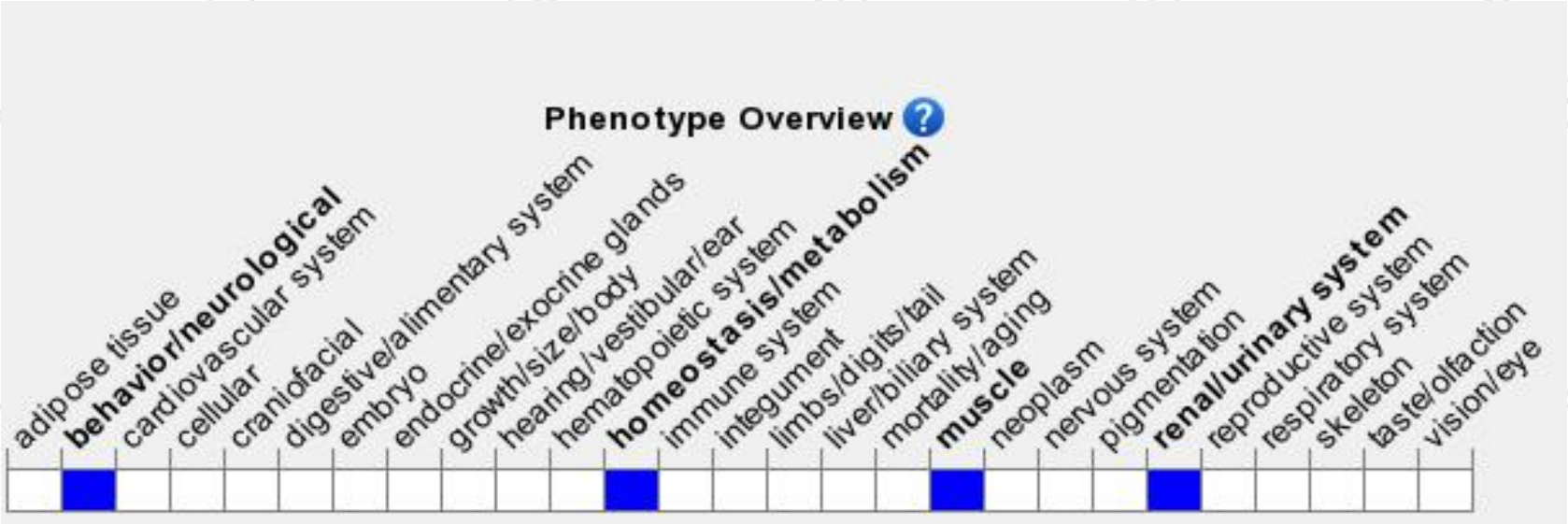


Genomic location distribution



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/).

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

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