Myo5a Cas9-KO Strategy

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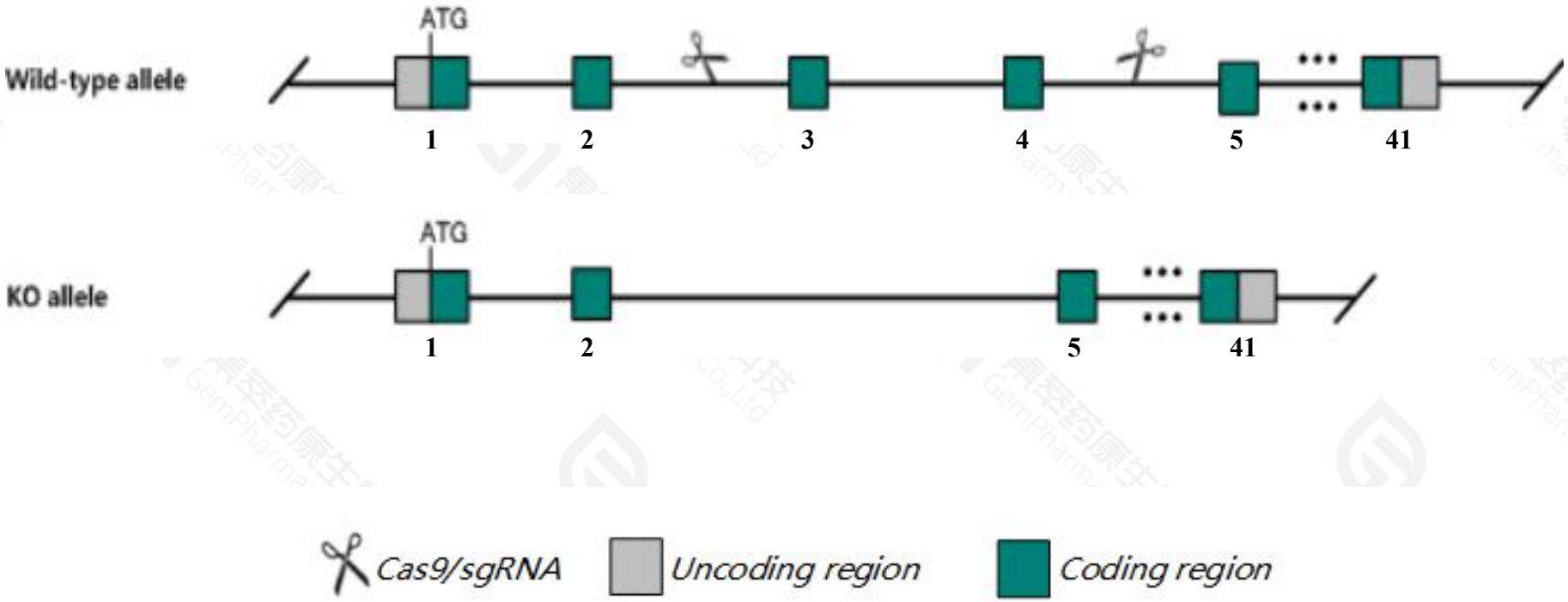
Project Overview

Project Name	***Myo5a***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Myo5a* gene. The schematic diagram is as follows:



Technical routes

➢ The *Myo5a* gene has 14 transcripts. According to the structure of *Myo5a* gene, exon3-exon4 of *Myo5a-201*(ENSMUST00000123128.8) transcript is recommended as the knockout region.The region contains 317bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Myo5a* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

➢ According to the existing MGI data,mutations in this gene result in diluted coat color, behavioral deficits including opisthotonus, and postnatal or premature death.

➢Transcript *Myo5a*-203&204&210 may not be affected.

➢ The *Myo5a* gene is located on the Chr9. 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）

集萃药康 GemPharmatech

Myo5a myosin VA [Mus musculus (house mouse)]

Gene ID: 17918, updated on 17-Dec-2020

Summary

Official Symbol	Myo5a provided by MGI
Official Full Name	myosin VA provided byMGI
Primary source	MGI:MGI:105976
See related	Ensembl:ENSMUSG00000034593
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	9630007J19Rik, AI413174, AI661011, Db, Dbv, M, MVa, My, Myo, Myo5, MyoVA, Sev-1, d, d-120J, f, flail, flr
Expression	Broad expression in cortex adult (RPKM 22.9), frontal lobe adult (RPKM 21.0) and 18 other tissuesSee more
Orthologs	human all

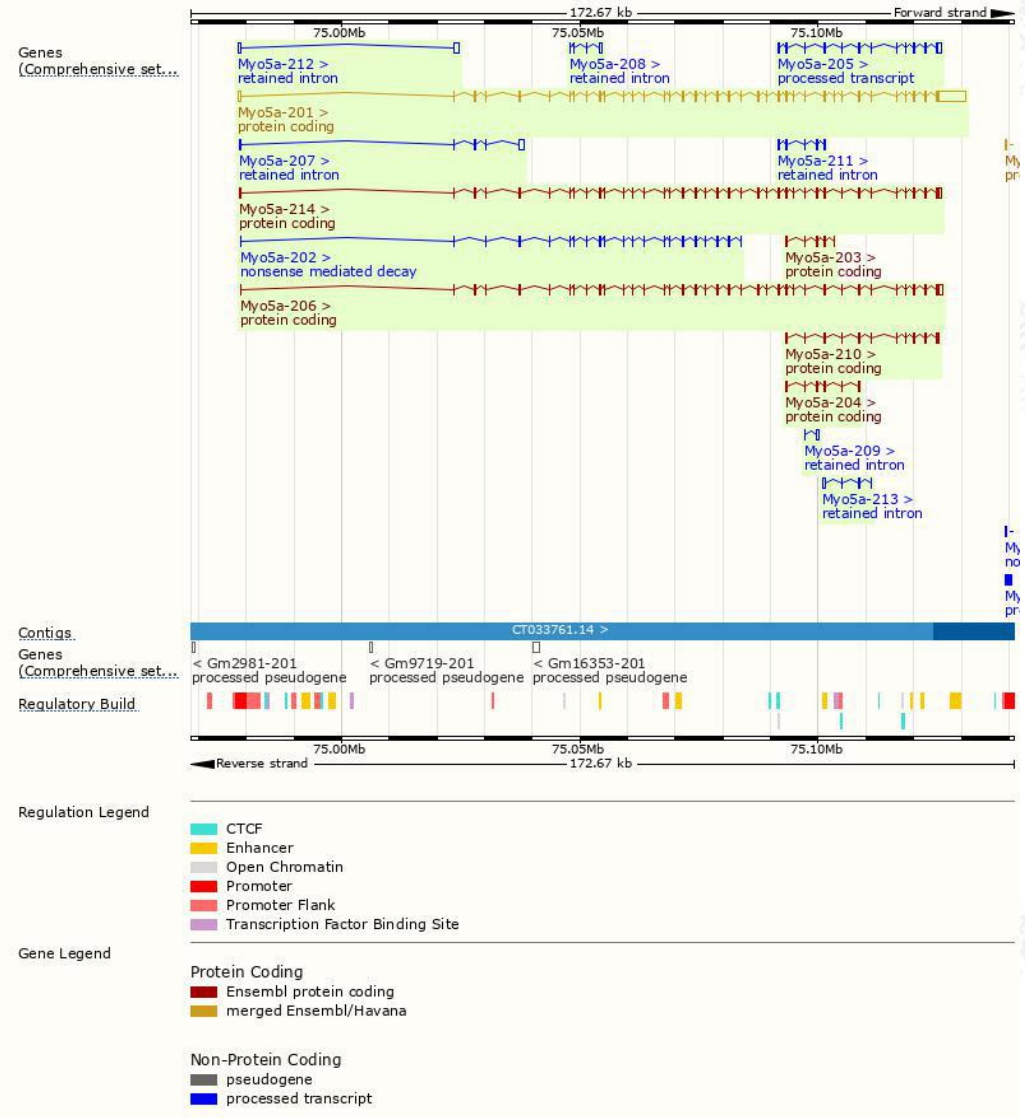
Transcript information（Ensembl）

The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Myo5a-201	ENSMUST00000123128.8	11702	1853aa	Protein coding	CCDS52860		TSL:5 , GENCODE basic ,
Myo5a-206	ENSMUST00000136731.8	6480	1828aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT1 ,
Myo5a-214	ENSMUST00000155282.9	6372	1855aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS P5 ,
Myo5a-210	ENSMUST00000148144.8	1758	585aa	Protein coding	-		CDS 5' incomplete , TSL:5 ,
Myo5a-203	ENSMUST00000129281.8	684	228aa	Protein coding	-		CDS 5' and 3' incomplete , TSL:5 ,
Myo5a-204	ENSMUST00000130384.2	650	217aa	Protein coding	-		CDS 5' and 3' incomplete , TSL:3 ,
Myo5a-202	ENSMUST00000123531.8	3002	47aa	Nonsense mediated decay	-		TSL:5 ,
Myo5a-205	ENSMUST00000136604.8	2640	No protein	Processed transcript	-		TSL:1 ,
Myo5a-207	ENSMUST00000136871.2	1919	No protein	Retained intron	-		TSL:1 ,
Myo5a-212	ENSMUST00000149823.2	1768	No protein	Retained intron	-		TSL:1 ,
Myo5a-211	ENSMUST00000149032.2	801	No protein	Retained intron	-		TSL:3 ,
Myo5a-208	ENSMUST00000142181.2	679	No protein	Retained intron	-		TSL:3 ,
Myo5a-209	ENSMUST00000143355.2	671	No protein	Retained intron	-		TSL:3 ,
Myo5a-213	ENSMUST00000153404.2	651	No protein	Retained intron	-		TSL:3 ,

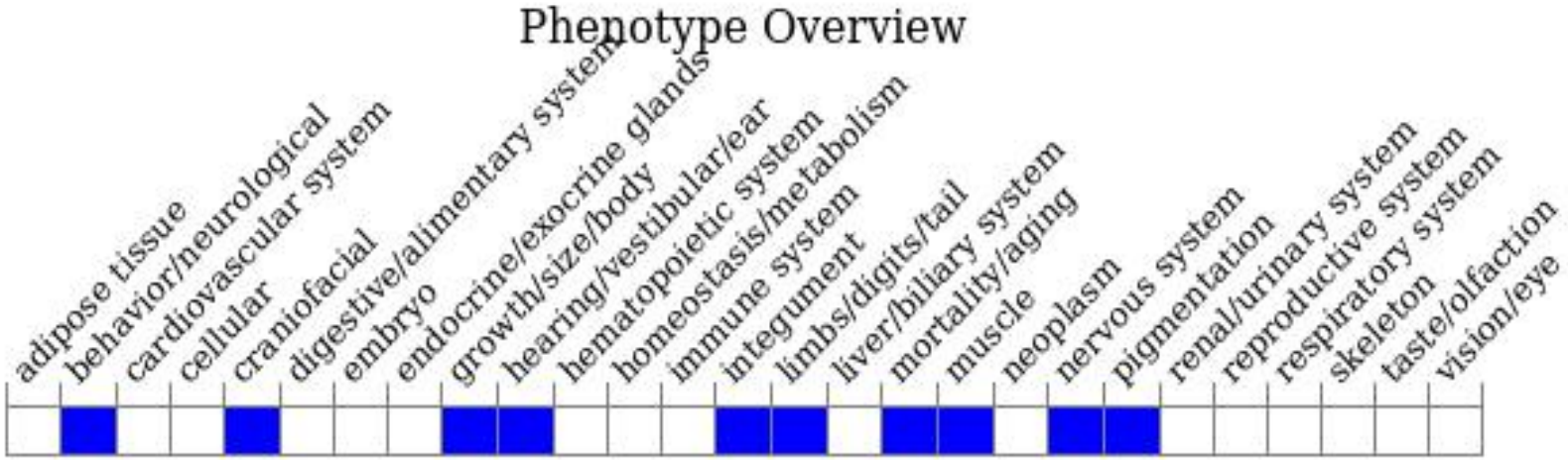
The strategy is based on the design of *Myo5a-201* transcript,the transcription is shown below:

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,mutations in this gene result in diluted coat color, behavioral deficits including opisthotonus, and postnatal or premature death.

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

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