Mip Cas9-KO Strategy

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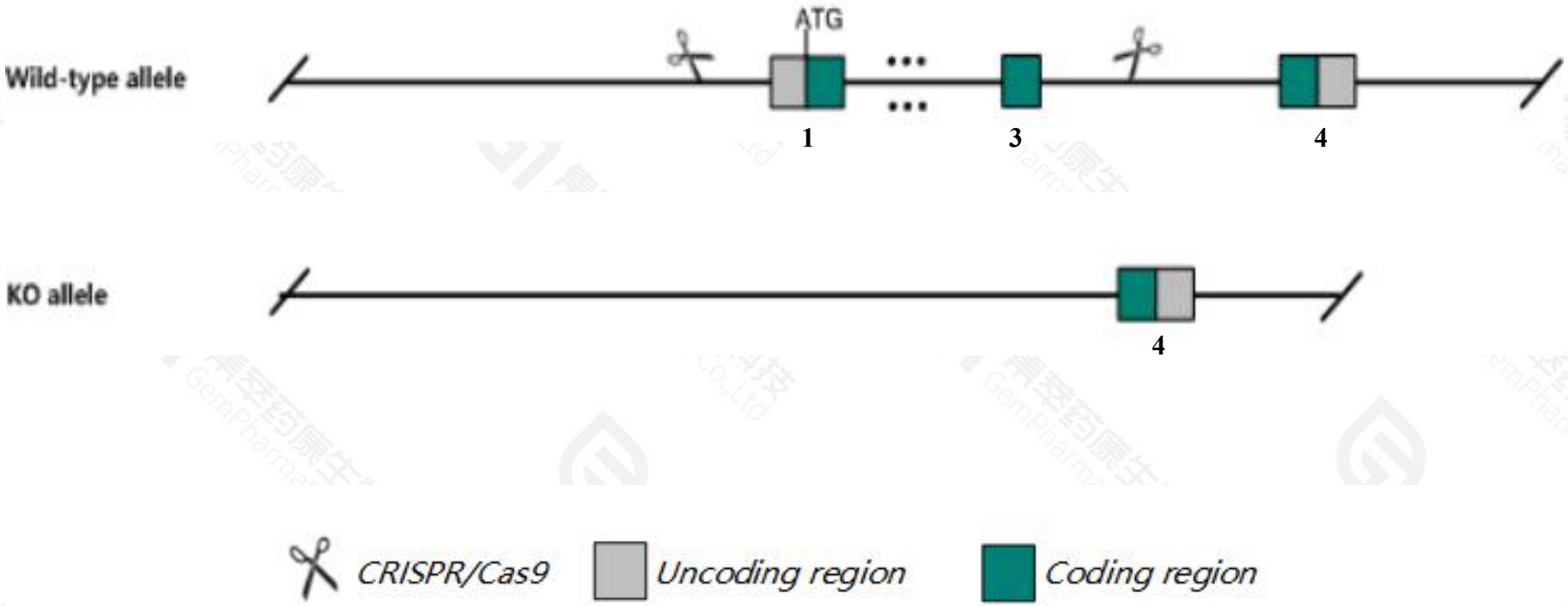
Design Date: 2021-7-27

Project Overview

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

Knockout strategy

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



Technical routes

➢ The *Mip* gene has 1 transcript. According to the structure of *Mip* gene, exon1-exon3 of *Mip-201*(ENSMUST00000026455.8) 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 *Mip* gene. The brief process is as follows: CRISPR/Cas9 system 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,homozygotes have microphthalmia and lens opacity. Other defects may include degeneration of lens fiber cells, vacuolization of lens fibers and reduced gamma:alpha crystallin ratio. Heterozygotes have less severe forms of lens cataract and microphthalmia.

➢ The *Mip* gene is located on the Chr10. 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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Mip major intrinsic protein of lens fiber [Mus musculus (house mouse)]

Gene ID: 17339, updated on 13-Mar-2020

Summary

Official Symbol	Mip provided by MGI
Official Full Name	major intrinsic protein of lens fiber provided by MGI
Primary source	MGI:MGI:96990
See related	Ensembl:ENSMUSG00000025389
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	Aqp0, Cat, Cts, Hfi, Lop, MIP26, MP26, Svl, shrivelled
Expression	Low expression observed in reference dataset See more
Orthologs	human all

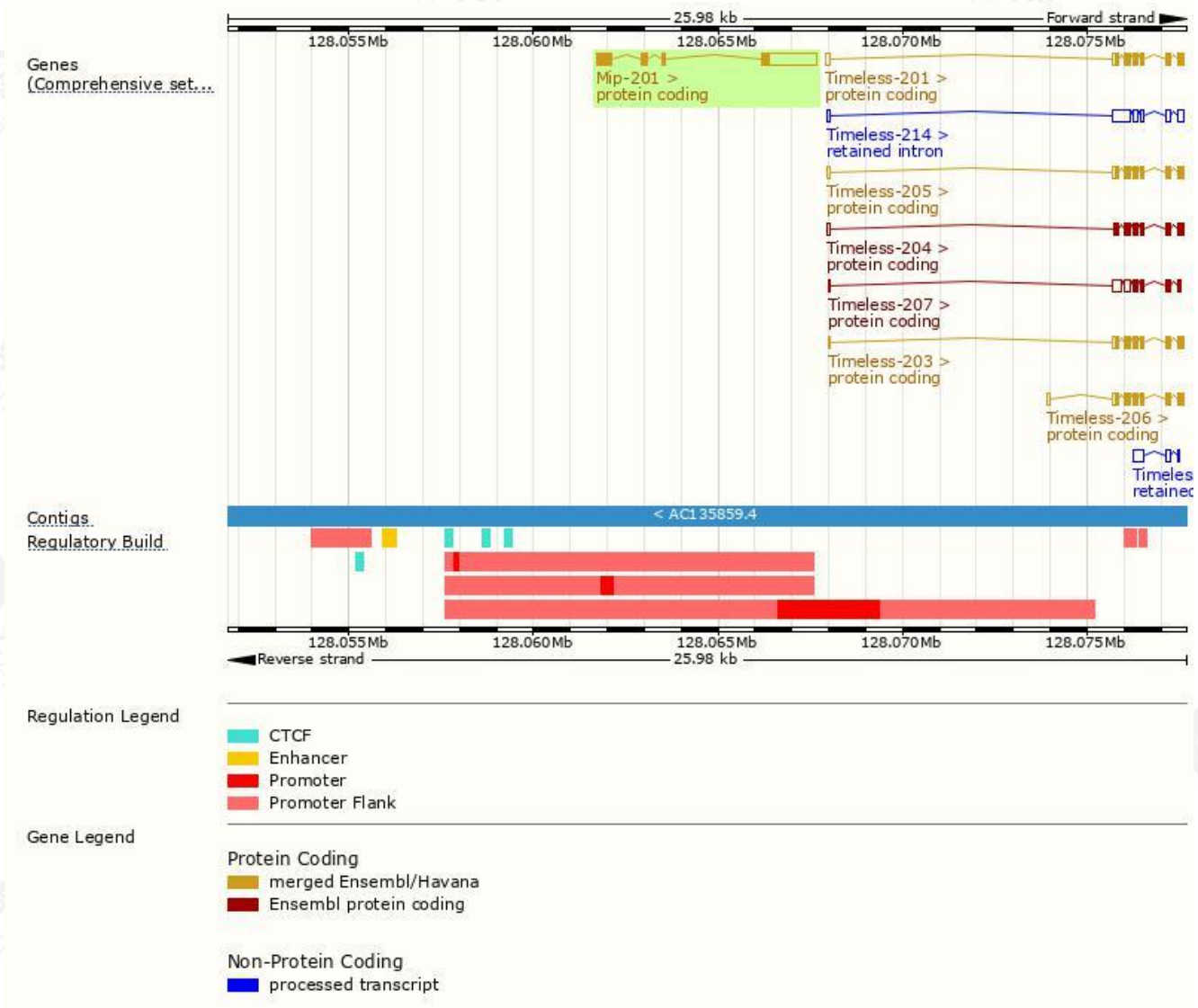
Transcript information（Ensembl）

The gene has 1 transcript,and the transcript is shown below:

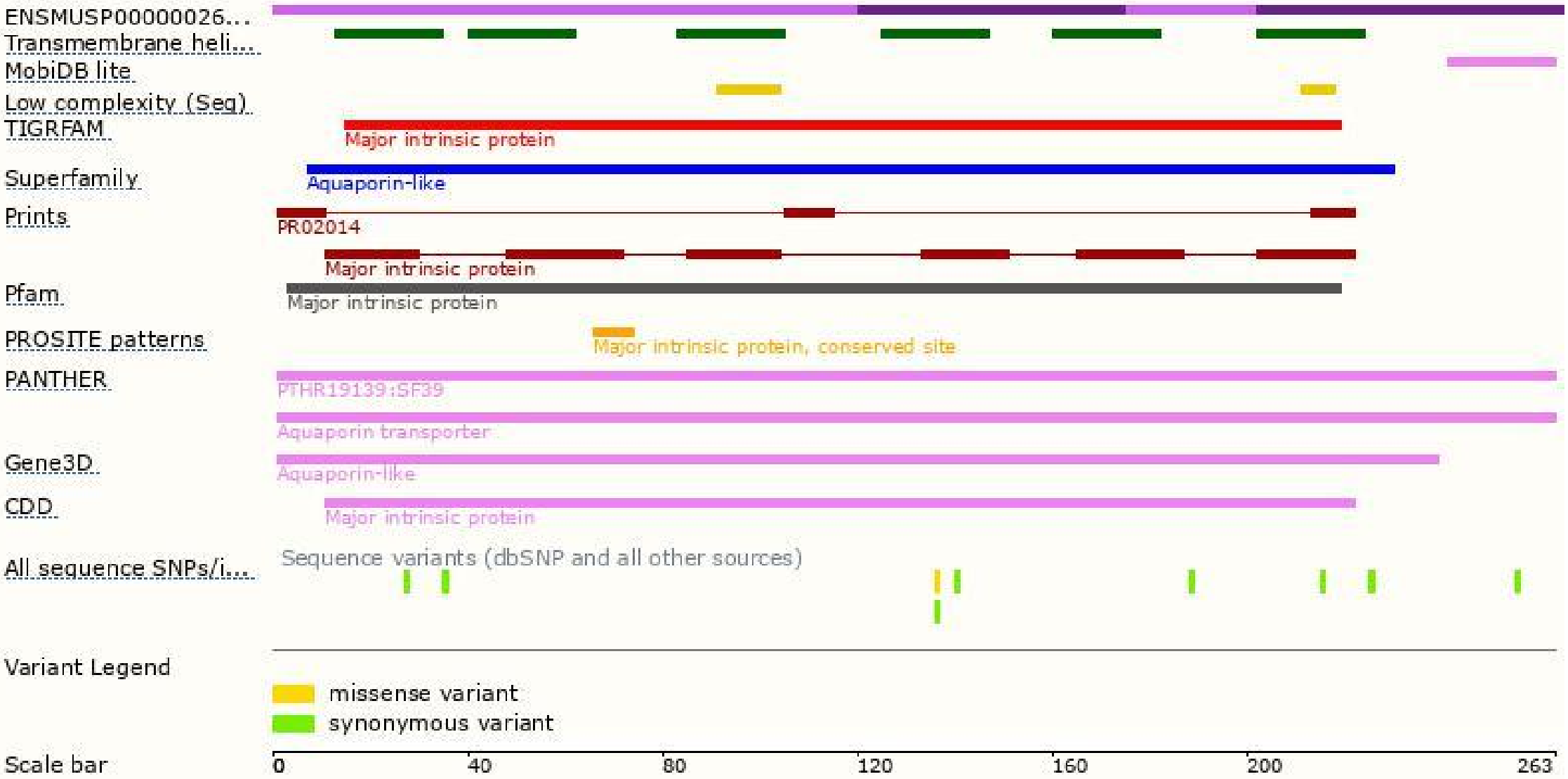
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mip-201	ENSMUST00000026455.7	2144	263aa	Protein coding	CCDS24265	P51180	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1

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

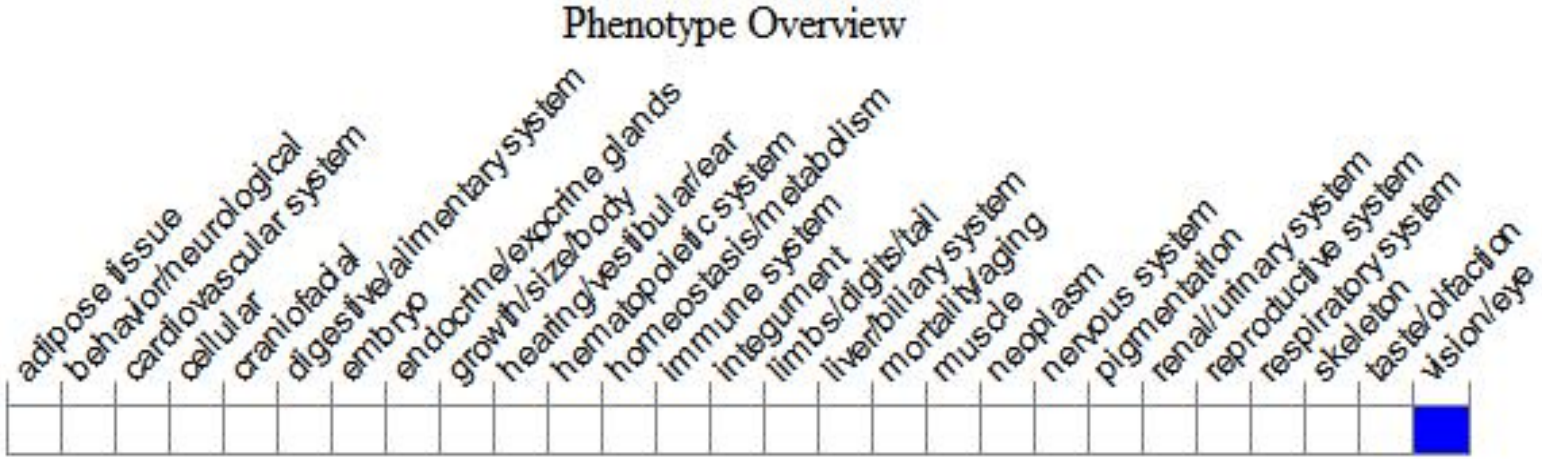
Genomic location distribution



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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