Mdm4 Cas9-KO Strategy

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Reviewer: Rui Xiong

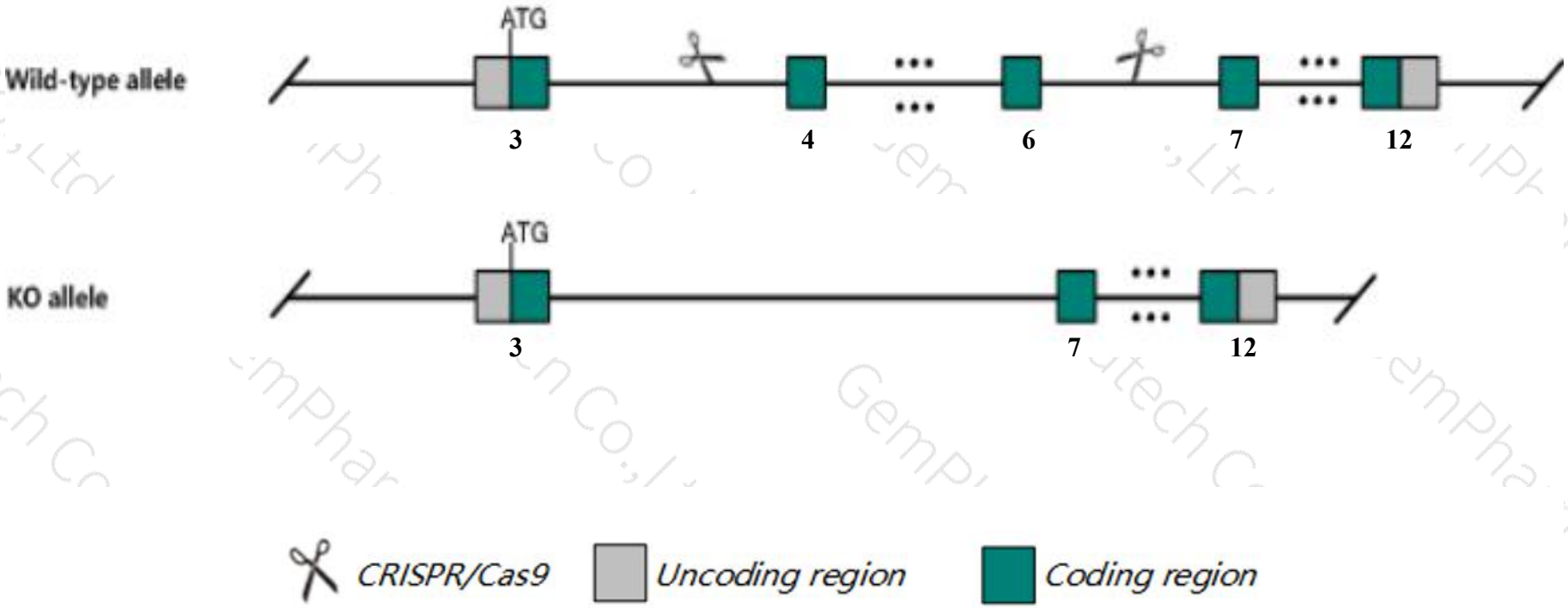
Design Date: 2019-1-18

Project Overview

Project Name	***Mdm4***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mdm4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Mdm4* gene has 14 transcripts. According to the structure of *Mdm4* gene, exon4-exon6 of *Mdm4-202*(ENSMUST00000067429.9) transcript is recommended as the knockout region.The region contains 265bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Mdm4* 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,mice homozygous for a gene trap allele exhibit embryonic lethality, decreased cellular proliferation, and abnormal nervous system development.

➢ The *Mdm4* 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 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

Mdm4 transformed mouse 3T3 cell double minute 4 [Mus musculus (house mouse)]

Gene ID: 17248, updated on 13-Mar-2020

Summary

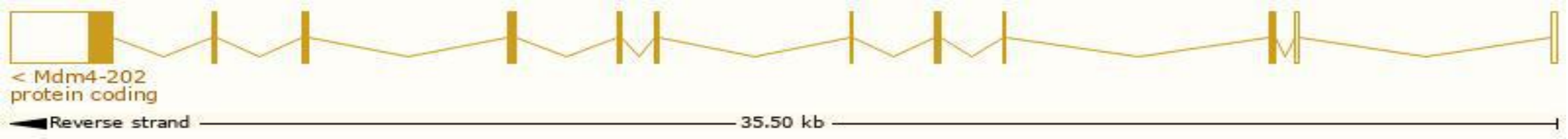
Official Symbol	Mdm4 provided by MGI
Official Full Name	transformed mouse 3T3 cell double minute 4 provided by MGI
Primary source	MGI:MGI:107934
See related	Ensembl:ENSMUSG00000054387
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4933417N07Rik, AA414968, AL023055, AU018793, AU021806, C85810, Mdmx
Summary	This gene encodes a protein that has been shown to negatively regulate the activity of the tumor suppressor protein p53. Homozygous knockout mice exhibit embryonic lethality as a result of p53-dependent apoptosis and cell cycle arrest. Amplification of this gene or overexpression of the encoded protein has been linked to a range of human cancers. A pseudogene has been identified on the X chromosome. Alternative splicing of this gene results in multiple transcript variants. [provided by RefSeq, Nov 2014]
Expression	Ubiquitous expression in limb E14.5 (RPKM 31.1), CNS E14 (RPKM 26.8) and 26 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

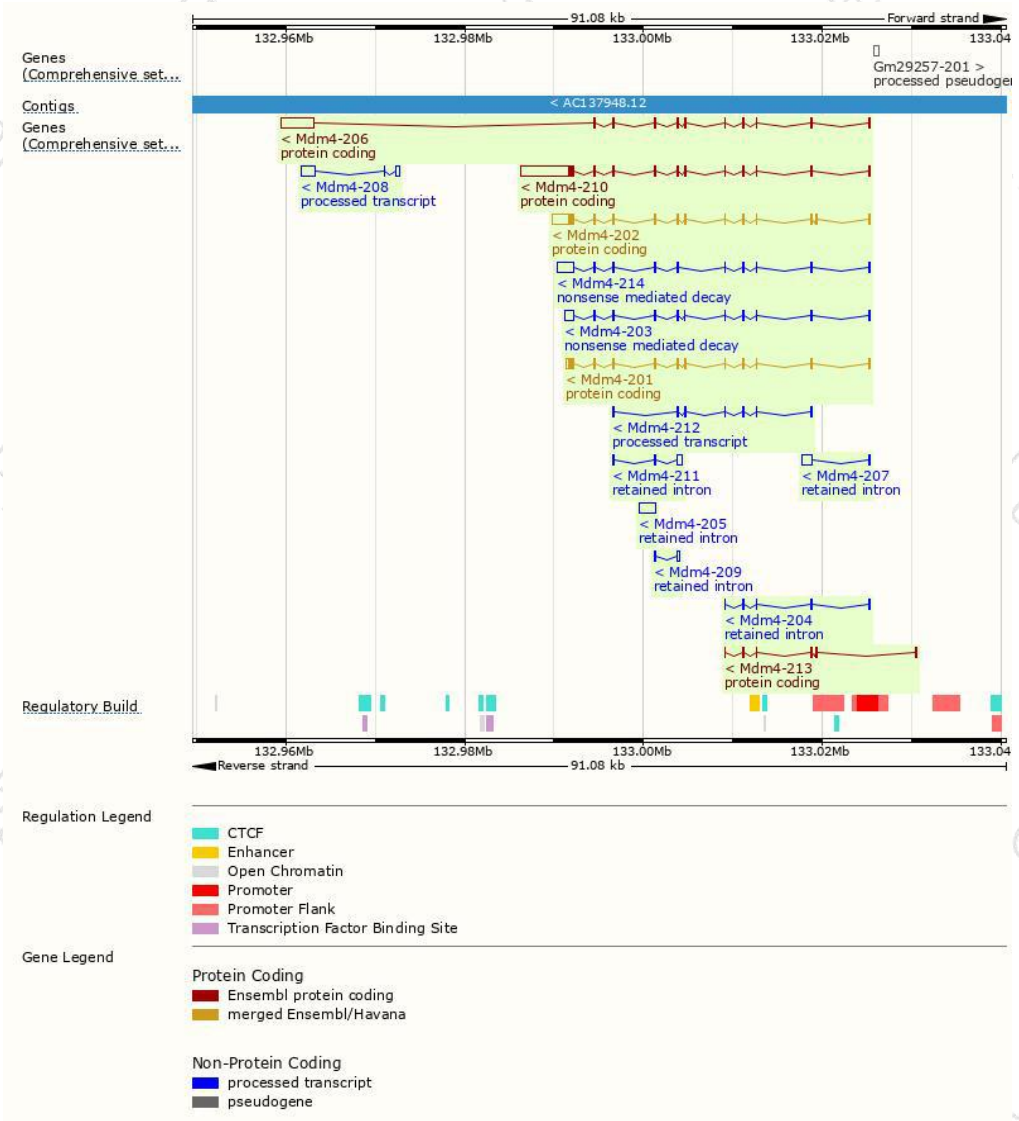
The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mdm4-210	ENSMUST00000188090.6	7105	489aa	Protein coding	CCDS15290	O35618	TSL:1 GENCODE basic APPRIS P3
Mdm4-202	ENSMUST00000067429.9	3490	489aa	Protein coding	CCDS15290	O35618	TSL:1 GENCODE basic APPRIS P3
Mdm4-201	ENSMUST00000067398.12	1914	490aa	Protein coding	CCDS78683	Q3UTC9	TSL:1 GENCODE basic APPRIS ALT1
Mdm4-206	ENSMUST00000186617.6	4694	318aa	Protein coding	-	A0A087WRX7	TSL:1 GENCODE basic
Mdm4-213	ENSMUST00000190807.1	467	110aa	Protein coding	-	A0A087WQP2	CDS 3' incomplete TSL:5
Mdm4-214	ENSMUST00000191212.6	2799	128aa	Nonsense mediated decay	-	A0A087WQ20	TSL:1
Mdm4-203	ENSMUST00000185398.6	2010	141aa	Nonsense mediated decay	-	A0A087WQ90	TSL:1
Mdm4-208	ENSMUST00000187244.1	2054	No protein	Processed transcript	-	-	TSL:5
Mdm4-212	ENSMUST00000190312.6	587	No protein	Processed transcript	-	-	TSL:3
Mdm4-205	ENSMUST00000186513.1	1950	No protein	Retained intron	-	-	TSL:NA
Mdm4-207	ENSMUST00000186645.1	1275	No protein	Retained intron	-	-	TSL:2
Mdm4-211	ENSMUST00000189596.1	762	No protein	Retained intron	-	-	TSL:3
Mdm4-209	ENSMUST00000187529.1	612	No protein	Retained intron	-	-	TSL:2
Mdm4-204	ENSMUST00000185418.1	496	No protein	Retained intron	-	-	TSL:3

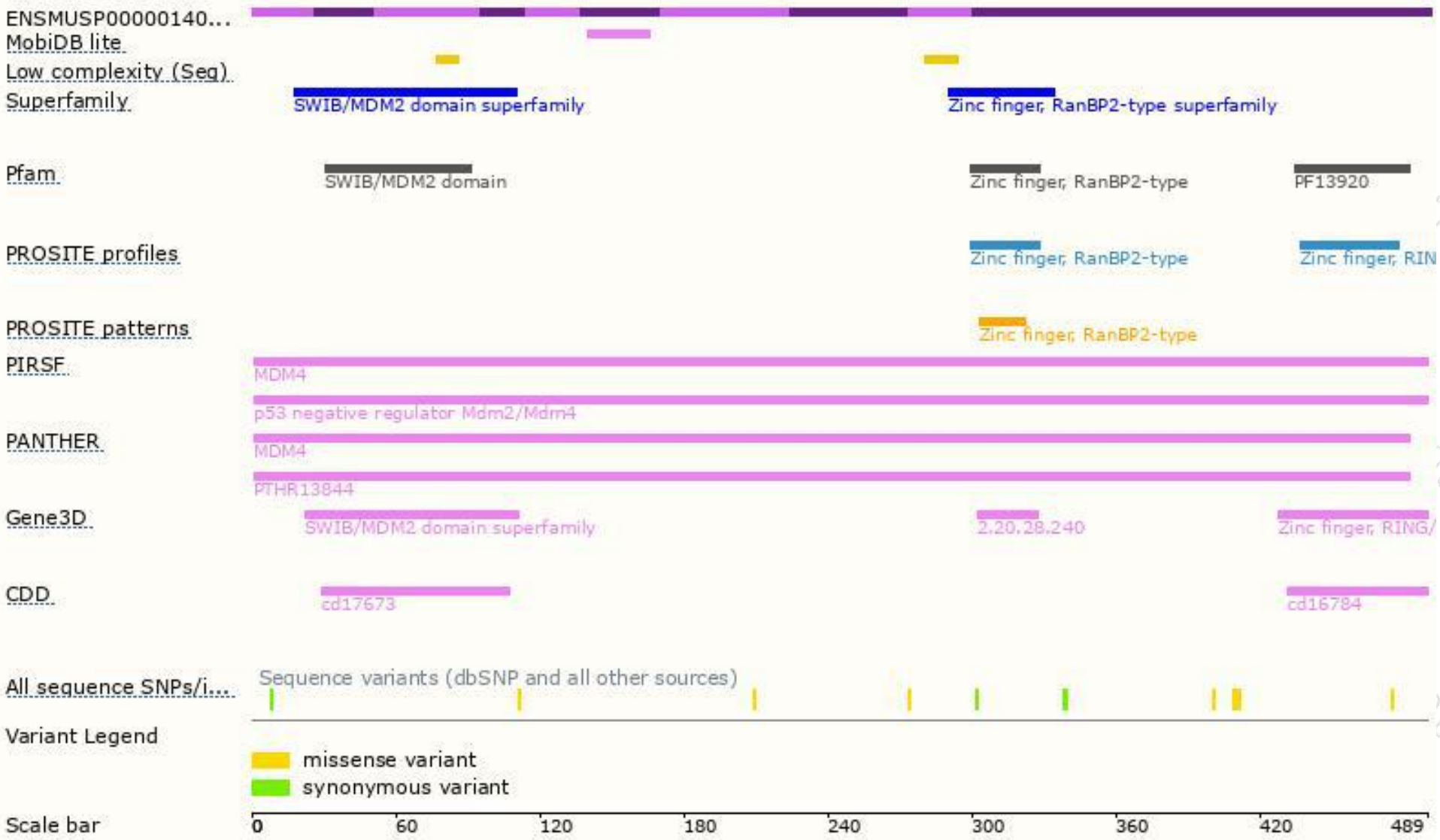
The strategy is based on the design of *Mdm4-202* transcript,the transcription is shown below:



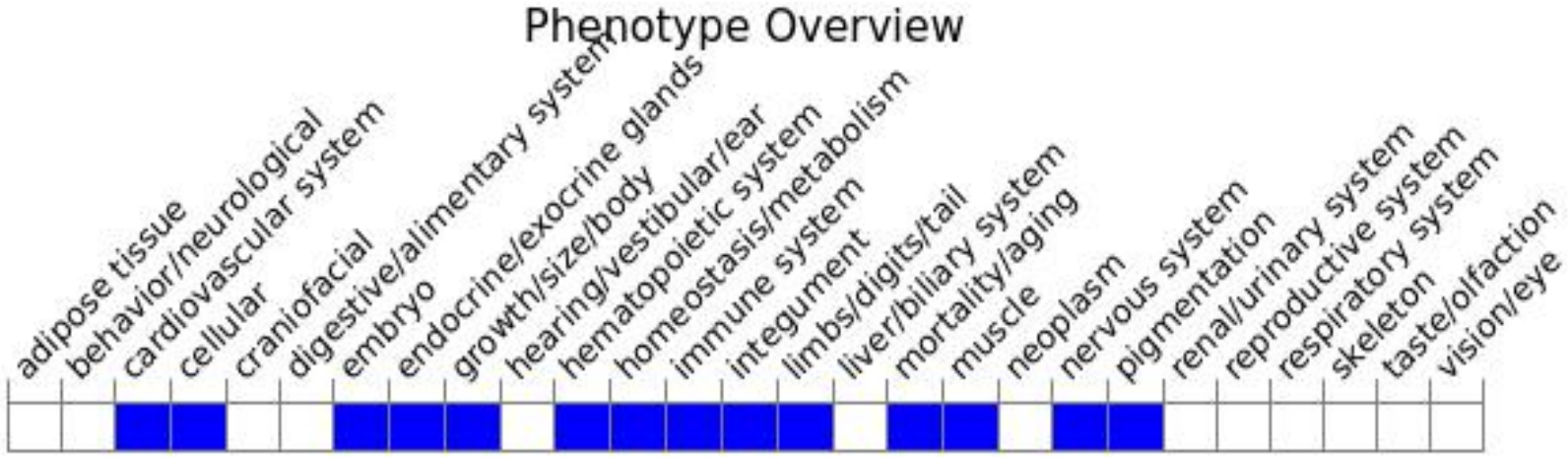
Genomic location distribution



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 gene trap allele exhibit embryonic lethality, decreased cellular proliferation, and abnormal nervous system development.

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

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