Mdm2 Cas9-CKO Strategy

Designer: Yanhua Shen
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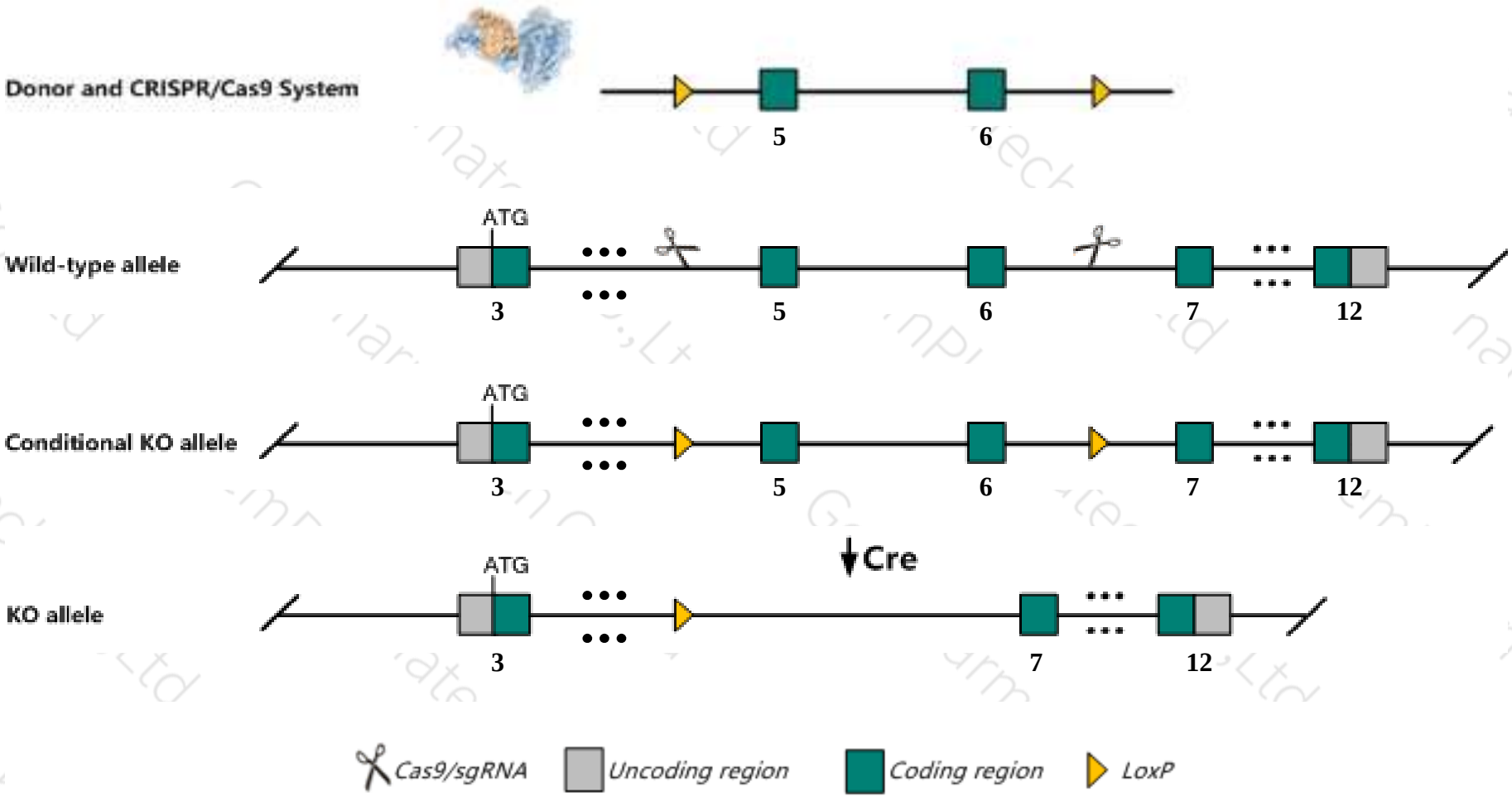
Project Overview

Project Name	***Mdm2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mdm2* gene. The schematic diagram is as follows:



Technical routes

- The *Mdm2* gene has 9 transcripts. According to the structure of *Mdm2* gene, exon5-exon6 of *Mdm2-201* (ENSMUST00000020408.15) transcript is recommended as the knockout region.The region contains 184bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Mdm2* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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.

Notice

➢ According to the existing MGI data,Mice homozygous for a gene trapped allele exhibit embryonic lethality. Mice homozygous for a null allele exhibit prenatal lethality. Mice homozygous for one knock-in allele exhibit embryonic lethality while mice homozygous for a different knock-in allele exhibit alters cell cycle regulation.

➢ The *Mdm2* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Mdm2 transformed mouse 3T3 cell double minute 2 [Mus musculus (house mouse)]

Gene ID: 17246, updated on 9-Apr-2019

Summary

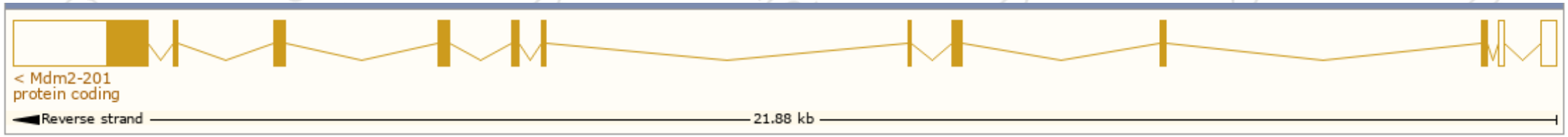
Official Symbol	Mdm2 provided by MGI
Official Full Name	transformed mouse 3T3 cell double minute 2 provided by MGI
Primary source	MGI:MGI:96952
See related	Ensembl:ENSMUSG00000020184
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1700007J15Rik, AA415488, Mdm-2
Expression	Ubiquitous expression in testis adult (RPKM 10.8), CNS E11.5 (RPKM 9.8) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

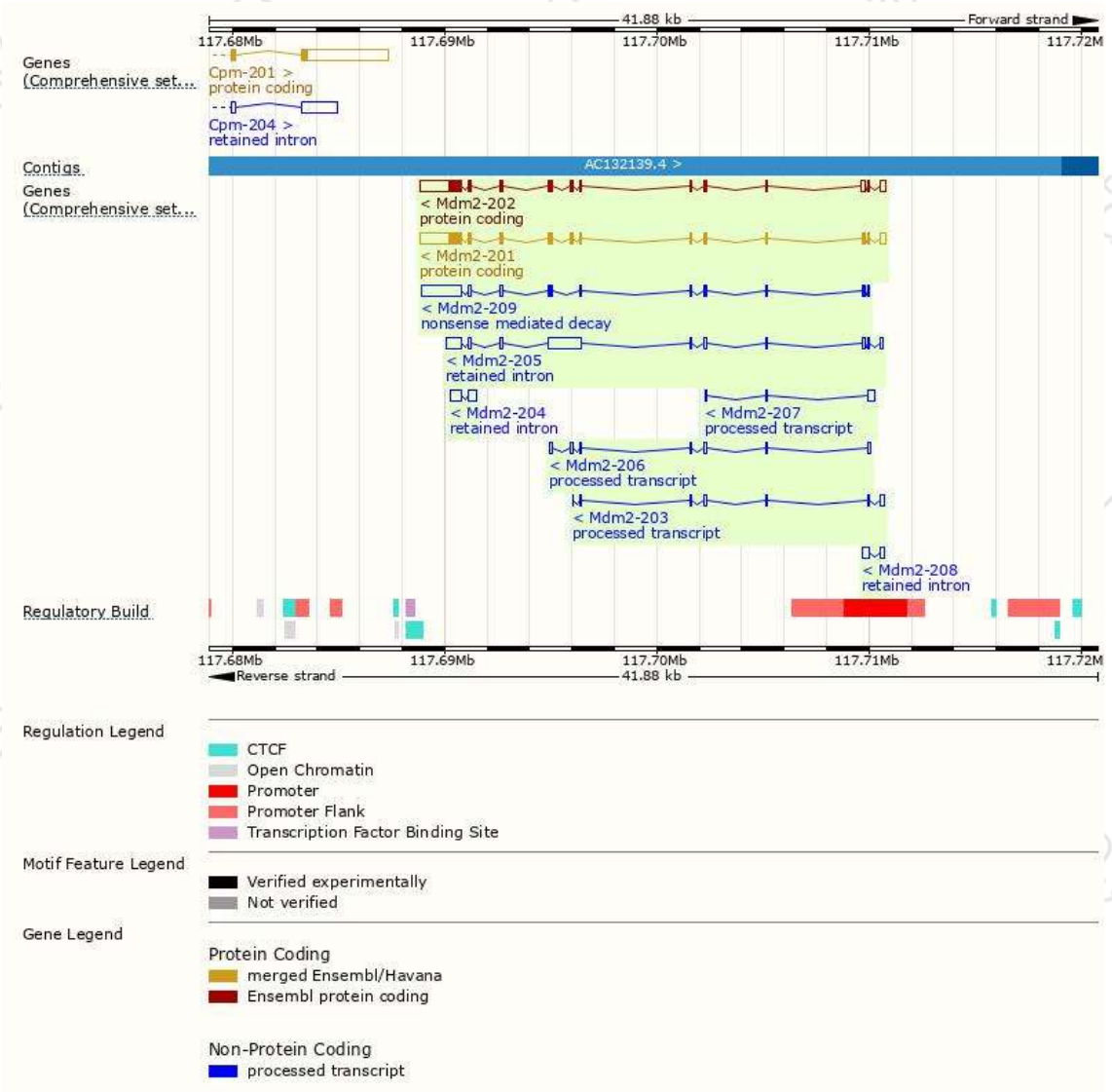
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mdm2-202	ENSMUST00000105263.8	3145	440aa	Protein coding	CCDS70110	P23804	TSL:5 GENCODE basic
Mdm2-201	ENSMUST00000020408.15	3099	489aa	Protein coding	CCDS24194	P23804	TSL:1 GENCODE basic APPRIS P1
Mdm2-209	ENSMUST00000155285.7	2769	162aa	Nonsense mediated decay	-	J3QP04	TSL:1
Mdm2-206	ENSMUST00000137102.7	683	No protein	Processed transcript	-	-	TSL:2
Mdm2-203	ENSMUST00000126022.7	570	No protein	Processed transcript	-	-	TSL:3
Mdm2-207	ENSMUST00000147823.1	499	No protein	Processed transcript	-	-	TSL:2
Mdm2-205	ENSMUST00000132277.7	3007	No protein	Retained intron	-	-	TSL:2
Mdm2-204	ENSMUST00000131627.1	919	No protein	Retained intron	-	-	TSL:2
Mdm2-208	ENSMUST00000151725.1	474	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Mdm2-201* 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.

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd