Mindy3 Cas9-KO Strategy

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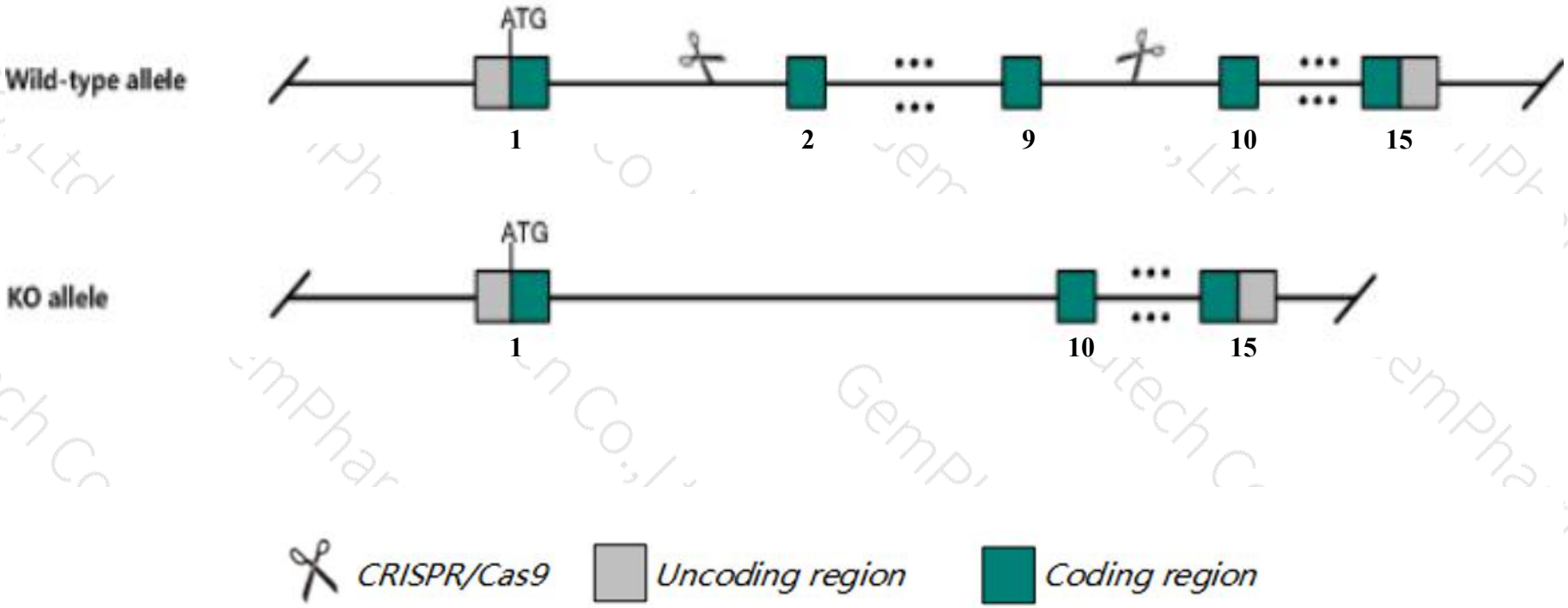
Project Overview

Project Name	***Mindy3***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mindy3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Mindy3* gene has 20 transcripts. According to the structure of *Mindy3* gene, exon2-exon9 of *Mindy3-201*(ENSMUST00000028105.12) transcript is recommended as the knockout region.The region contains 704bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Mindy3* 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

➢ The knockout region contains *Gm38014*.

➢ The *Mindy3* gene is located on the Chr2. 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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Mindy3 MINDY lysine 48 deubiquitinase 3 [Mus musculus (house mouse)]

Gene ID: 66960, updated on 20-Mar-2020

Summary

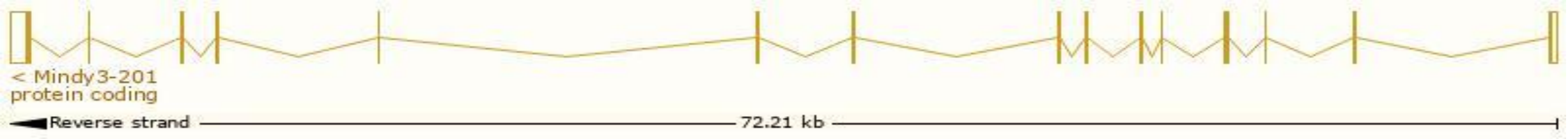
Official Symbol	Mindy3 provided by MGI
Official Full Name	MINDY lysine 48 deubiquitinase 3 provided byMGI
Primary source	MGI:MGI:1914210
See related	Ensembl:ENSMUSG00000026767
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1810041E18Rik, 2310047O13Rik, 5830410F13Rik, AI447827, AW111958, Fam188a
Expression	Ubiquitous expression in CNS E18 (RPKM 3.8), CNS E14 (RPKM 3.4) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

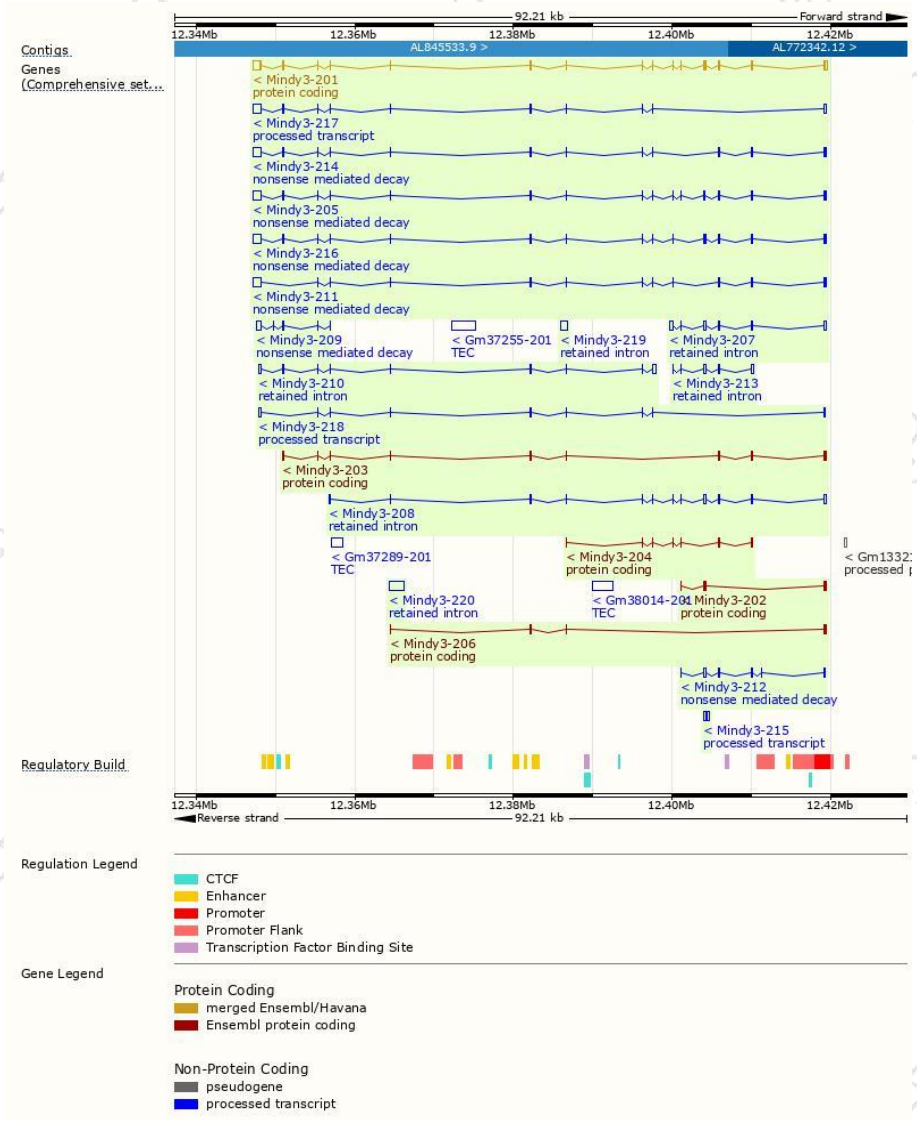
The gene has 20 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mindy3-201	ENSMUST00000028105.12	2344	444aa	Protein coding	CCDS15690	Q9CV28	TSL:1 GENCODE basic APPRIS P1
Mindy3-203	ENSMUST00000124603.7	850	230aa	Protein coding	-	D6REU0	CDS 3' incomplete TSL:5
Mindy3-204	ENSMUST00000129348.1	480	160aa	Protein coding	-	F6S1I0	CDS 5' and 3' incomplete TSL:5
Mindy3-206	ENSMUST00000129993.2	457	86aa	Protein coding	-	A0A0A6YWB5	CDS 3' incomplete TSL:5
Mindy3-202	ENSMUST00000124515.1	422	94aa	Protein coding	-	D3Z0Z1	CDS 3' incomplete TSL:5
Mindy3-205	ENSMUST00000129489.7	2316	90aa	Nonsense mediated decay	-	D6RG90	TSL:1
Mindy3-216	ENSMUST00000155530.7	2257	135aa	Nonsense mediated decay	-	D6RGT9	TSL:1
Mindy3-211	ENSMUST00000144645.7	2028	88aa	Nonsense mediated decay	-	D6RGU8	TSL:1
Mindy3-214	ENSMUST00000154899.6	1984	80aa	Nonsense mediated decay	-	D6RCI2	TSL:5
Mindy3-209	ENSMUST00000135397.7	875	64aa	Nonsense mediated decay	-	F6Z9V3	CDS 5' incomplete TSL:5
Mindy3-212	ENSMUST00000151529.7	468	43aa	Nonsense mediated decay	-	F7BWU4	CDS 5' incomplete TSL:5
Mindy3-217	ENSMUST00000194533.5	1843	No protein	Processed transcript	-	-	TSL:5
Mindy3-218	ENSMUST00000195084.5	908	No protein	Processed transcript	-	-	TSL:5
Mindy3-215	ENSMUST00000154952.1	463	No protein	Processed transcript	-	-	TSL:2
Mindy3-220	ENSMUST00000195758.1	1845	No protein	Retained intron	-	-	TSL:NA
Mindy3-208	ENSMUST00000130225.6	1324	No protein	Retained intron	-	-	TSL:1
Mindy3-210	ENSMUST00000137082.7	1221	No protein	Retained intron	-	-	TSL:5
Mindy3-207	ENSMUST00000130097.7	1126	No protein	Retained intron	-	-	TSL:1
Mindy3-219	ENSMUST00000195125.1	836	No protein	Retained intron	-	-	TSL:NA
Mindy3-213	ENSMUST00000153690.7	582	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Mindy3-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain

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

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