Mink1 Cas9-CKO Strategy

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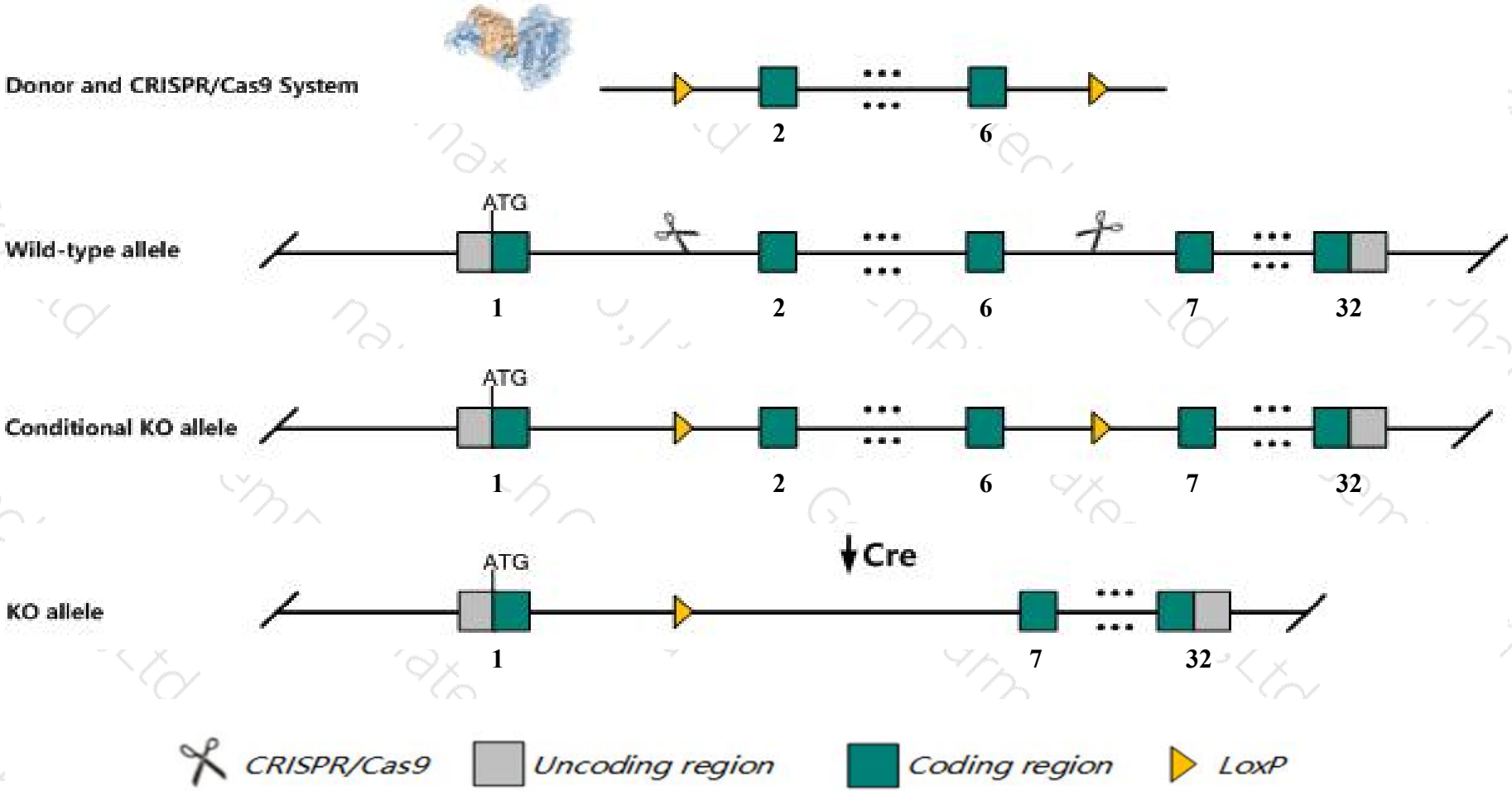
Project Overview

Project Name	***Mink1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mink1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Mink1* gene has 13 transcripts. According to the structure of *Mink1* gene, exon2-exon6 of *Mink1-202*(ENSMUST00000072873.13) transcript is recommended as the knockout region.The region contains 451bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Mink1* gene. The brief process is as follows:CRISPR/Cas9 system 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 will be 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

➢ The *Mink1* gene is located on the Chr11. 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.

➢ Transcript *Mink1-209,213,210,212,211,206* may not be affected.

➢ 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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Mink1 misshapen-like kinase 1 (zebrafish) [Mus musculus (house mouse)]

Gene ID: 50932, updated on 13-Mar-2020

Summary

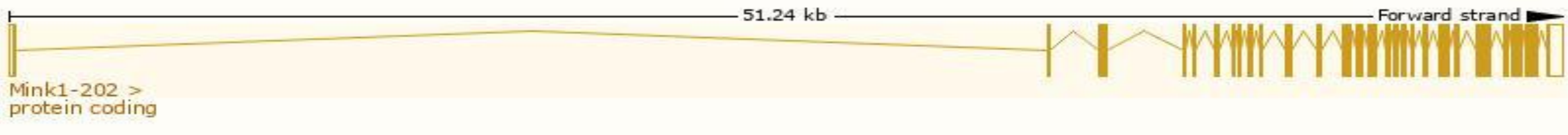
Official Symbol	Mink1 provided by MGI
Official Full Name	misshapen-like kinase 1 (zebrafish) provided by MGI
Primary source	MGI:MGI:1355329
See related	Ensembl:ENSMUSG00000020827
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	B55, MINK, Map4k6, Ysk2
Expression	Ubiquitous expression in cortex adult (RPKM 44.5), frontal lobe adult (RPKM 42.1) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

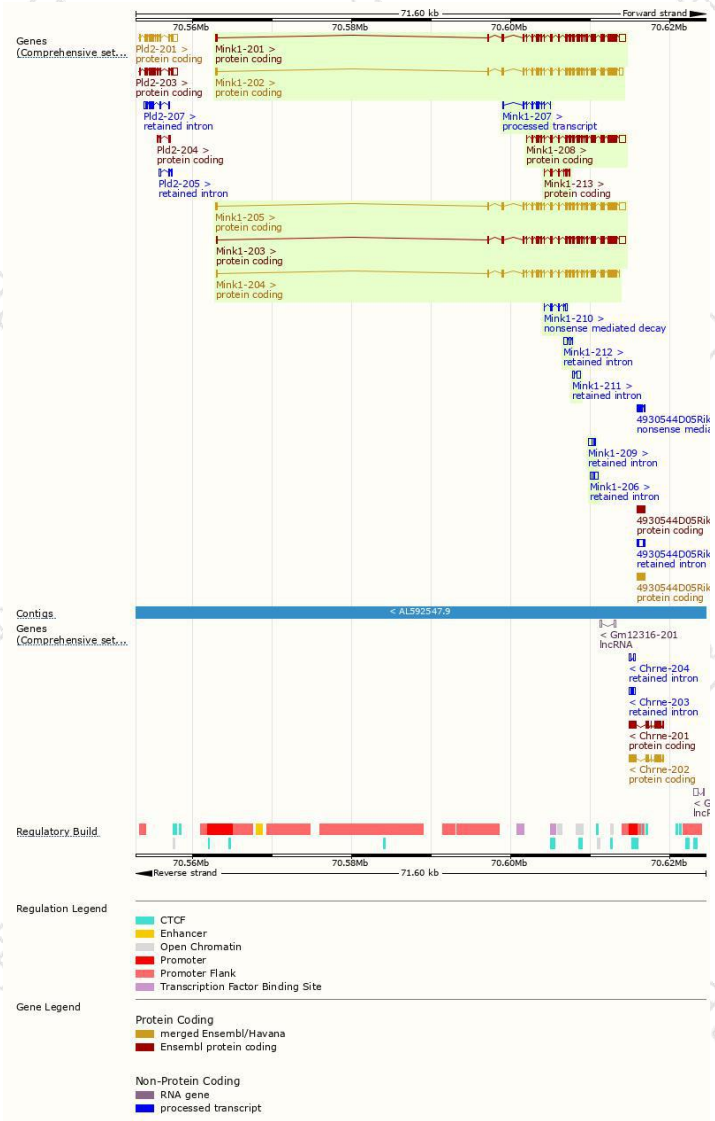
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mink1-201	ENSMUST00000072237.12	4994	1344aa	Protein coding	CCDS48837	G3X9G2	TSL:5 GENCODE basic APPRIS ALT2
Mink1-205	ENSMUST00000102559.10	4865	1308aa	Protein coding	CCDS24955	Q9JM52	TSL:1 GENCODE basic APPRIS P4
Mink1-202	ENSMUST00000072873.13	4610	1337aa	Protein coding	CCDS36205	Q5SXG3	TSL:5 GENCODE basic APPRIS ALT2
Mink1-204	ENSMUST00000102558.10	3903	1300aa	Protein coding	CCDS24954	Q9JM52	TSL:1 GENCODE basic APPRIS ALT2
Mink1-203	ENSMUST00000079244.11	4829	1334aa	Protein coding	-	Q5SXG1	TSL:1 GENCODE basic APPRIS ALT2
Mink1-208	ENSMUST00000136663.7	4367	1198aa	Protein coding	-	F7AMS7	CDS 5' incomplete TSL:1
Mink1-213	ENSMUST00000178764.7	777	259aa	Protein coding	-	J3QM71	CDS 5' and 3' incomplete TSL:3
Mink1-210	ENSMUST00000149845.1	704	90aa	Nonsense mediated decay	-	J3QP32	CDS 5' incomplete TSL:5
Mink1-207	ENSMUST00000133310.1	765	No protein	Processed transcript	-	-	TSL:5
Mink1-211	ENSMUST00000152857.1	697	No protein	Retained intron	-	-	TSL:3
Mink1-209	ENSMUST00000142650.7	693	No protein	Retained intron	-	-	TSL:2
Mink1-206	ENSMUST00000132208.1	650	No protein	Retained intron	-	-	TSL:2
Mink1-212	ENSMUST00000153503.2	635	No protein	Retained intron	-	-	TSL:2

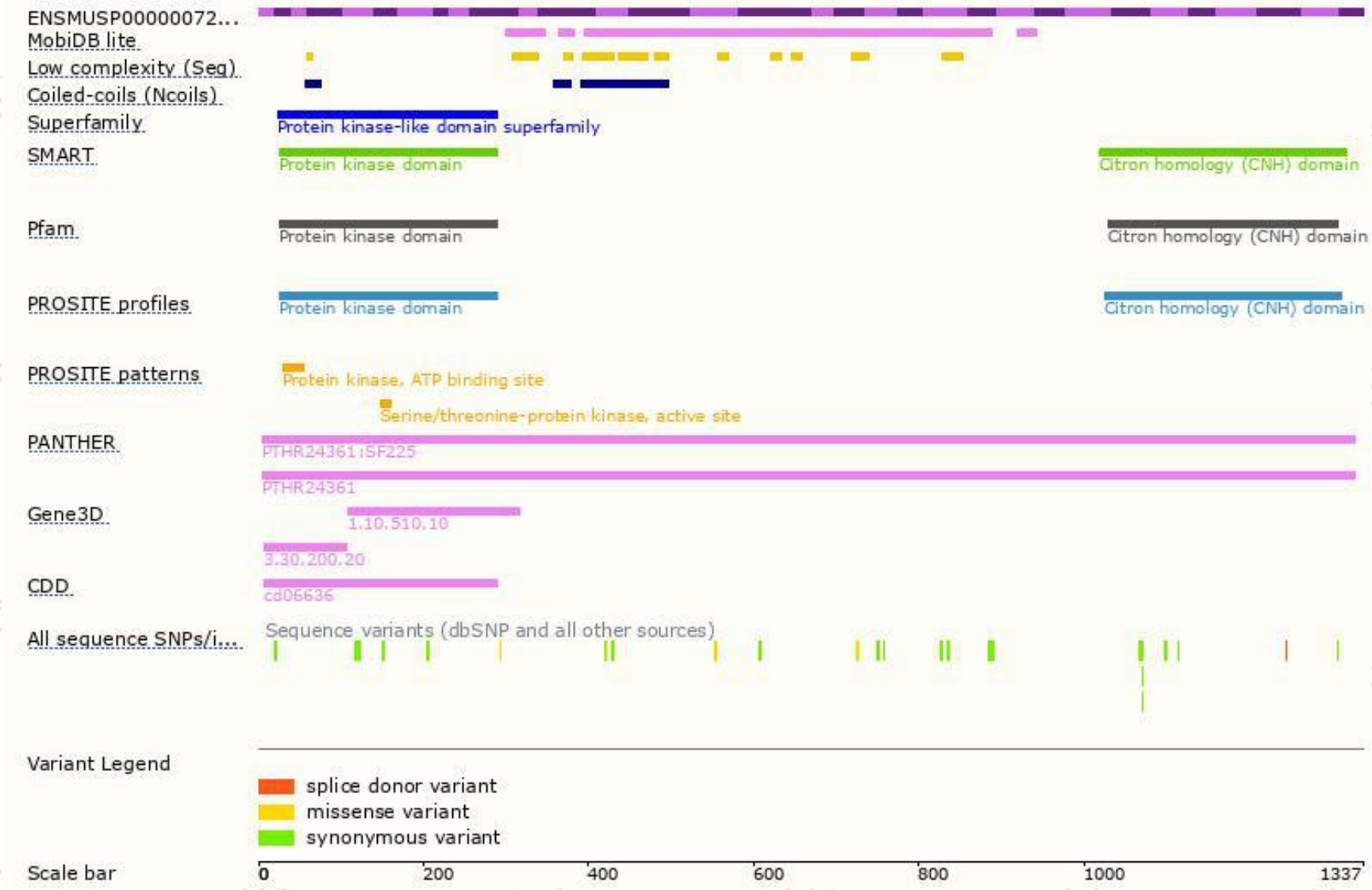
The strategy is based on the design of *Mink1-202* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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