

***Klhl8* Cas9-CKO Strategy**

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Project Overview

Project Name

Klhl8

Project type

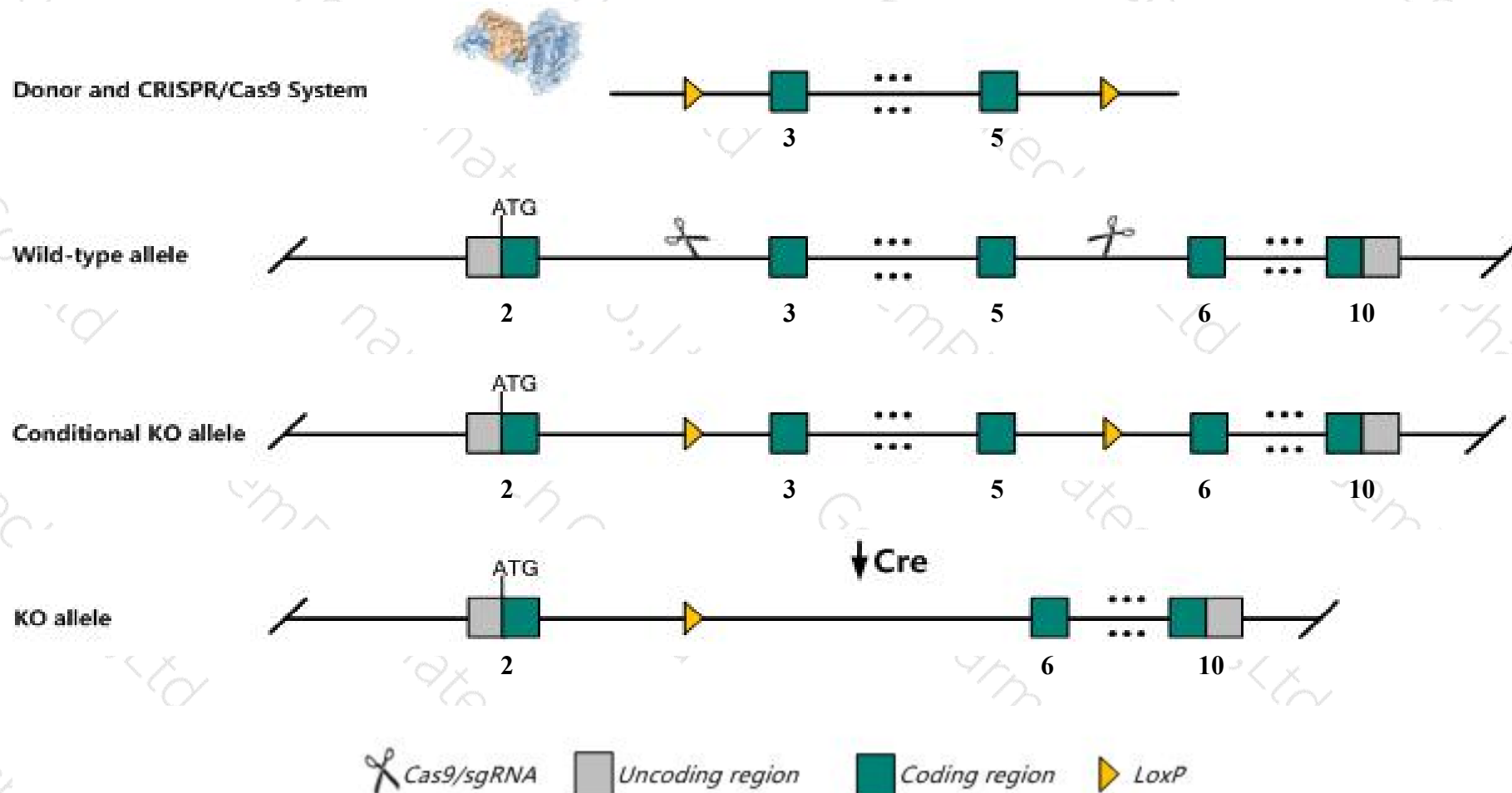
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Klhl8* gene. The schematic diagram is as follows:



Technical routes

- The *Klhl8* gene has 5 transcripts. According to the structure of *Klhl8* gene, exon3-exon5 of *Klhl8*-201(ENSMUST00000031254.8) transcript is recommended as the knockout region. The region contains 880bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Klhl8* 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.

- The *Klhl8* gene is located on the Chr5. 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)

Klhl8 kelch-like 8 [Mus musculus (house mouse)]

Gene ID: 246293, updated on 13-Mar-2020

Summary



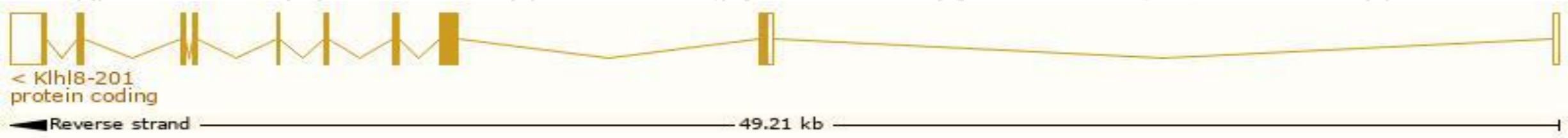
Official Symbol	Klhl8 provided by MGI
Official Full Name	kelch-like 8 provided by MGI
Primary source	MGI:MGI:2179430
See related	Ensembl:ENSMUSG00000029312
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2310001P09Rik, D5Ert431e
Expression	Broad expression in CNS E18 (RPKM 14.2), whole brain E14.5 (RPKM 10.2) and 23 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

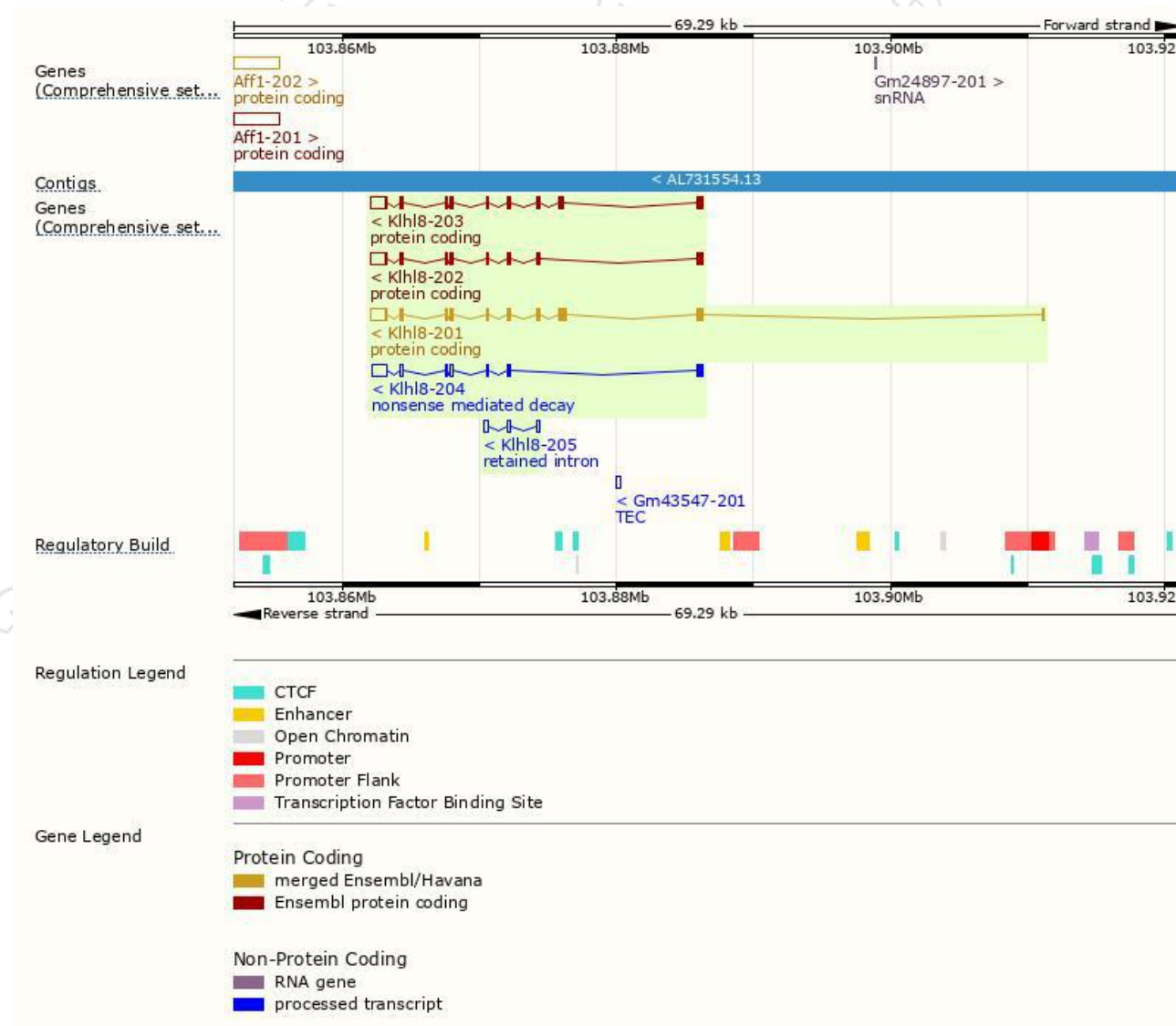
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Klhl8-201	ENSMUST00000031254.8	3193	629aa	Protein coding	CCDS19479	P59280	TSL:1 GENCODE basic APPRIS P1
Klhl8-203	ENSMUST00000112815.7	2898	553aa	Protein coding	-	A2AH60	TSL:5 GENCODE basic
Klhl8-202	ENSMUST00000112811.7	2577	446aa	Protein coding	-	A2AH59	TSL:5 GENCODE basic
Klhl8-204	ENSMUST00000131843.1	2283	115aa	Nonsense mediated decay	-	D6REH9	TSL:5
Klhl8-205	ENSMUST00000147184.1	642	No protein	Retained intron	-	-	TSL:3

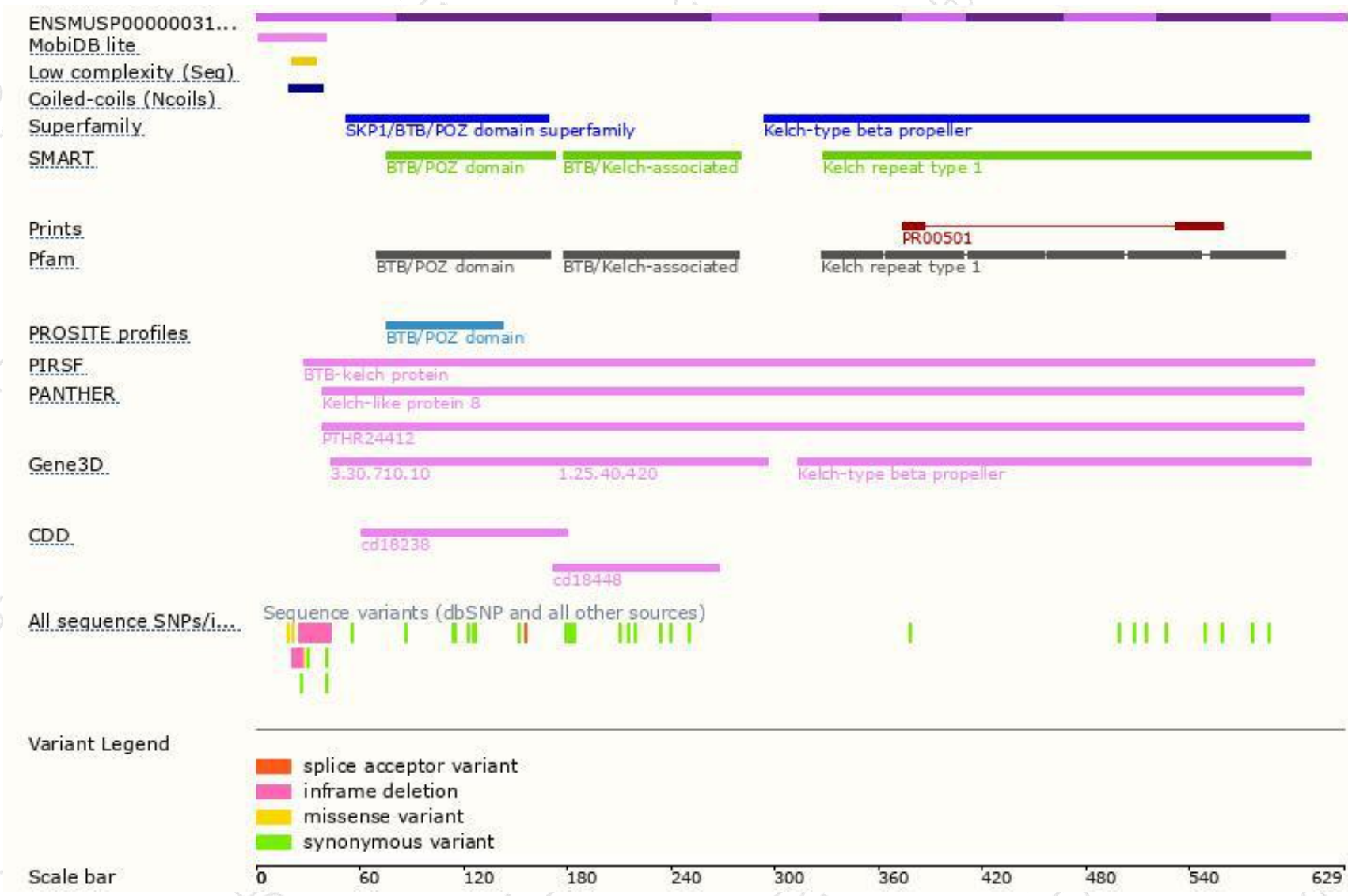
The strategy is based on the design of *Klhl8-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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