

***Kcnk13* Cas9-CKO Strategy**

Designer: Longyun Hu

Reviewer: Yun Li

Design Date: 2020-11-19

Project Overview

Project Name

Kcnk13

Project type

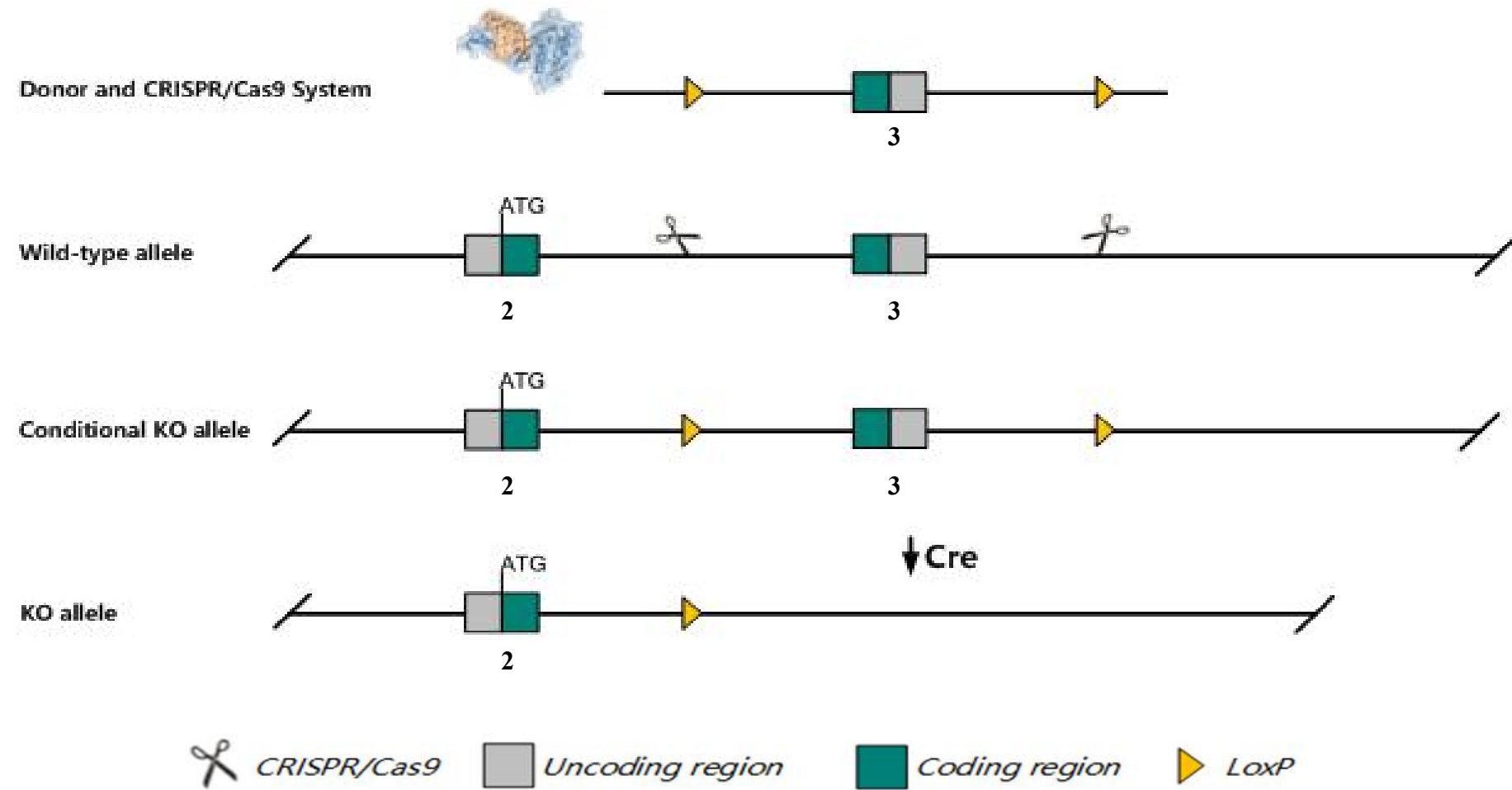
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Kcnk13* gene. The schematic diagram is as follows:



The *Kcnk13* gene has 4 transcripts. According to the structure of *Kcnk13* gene, exon3 of *Kcnk13-201*(ENSMUST00000049788.8) transcript is recommended as the knockout region. The region contains 884bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Kcnk13* 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.

According to the existing MGI data, homozygous knockout reduces the surveillance activity of microglial cells in the brain.

The *Kcnk13* gene is located on the Chr12. 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.

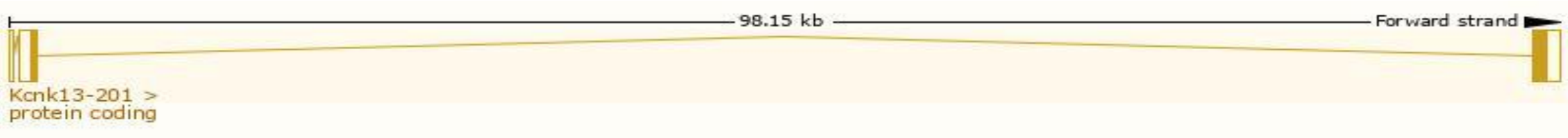
Transcript information Ensembl



The gene has 4 transcripts,all transcripts are shown below:

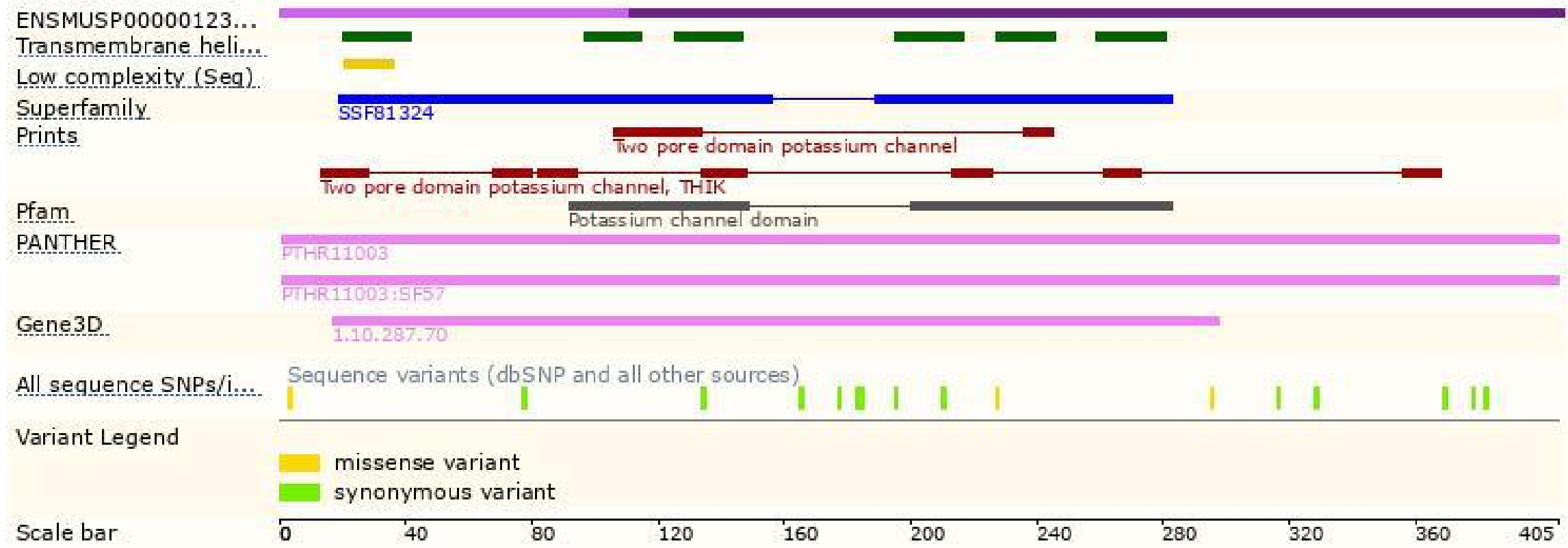
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kcnk13-201	ENSMUST00000049788.8	3078	405aa	Protein coding	CCDS26106	Q3TYG8 Q8R1P5	TSL:1 GENCODE basic APPRIS P1
Kcnk13-204	ENSMUST00000177549.7	3075	405aa	Protein coding	CCDS26106	Q3TYG8 Q8R1P5	TSL:5 GENCODE basic APPRIS P1
Kcnk13-202	ENSMUST00000160413.7	3061	405aa	Protein coding	CCDS26106	Q3TYG8 Q8R1P5	TSL:1 GENCODE basic APPRIS P1
Kcnk13-203	ENSMUST00000162221.2	834	No protein	Processed transcript	-	-	TSL:2

The strategy is based on the design of *Kcnk13-201* transcript,the transcription is shown below:

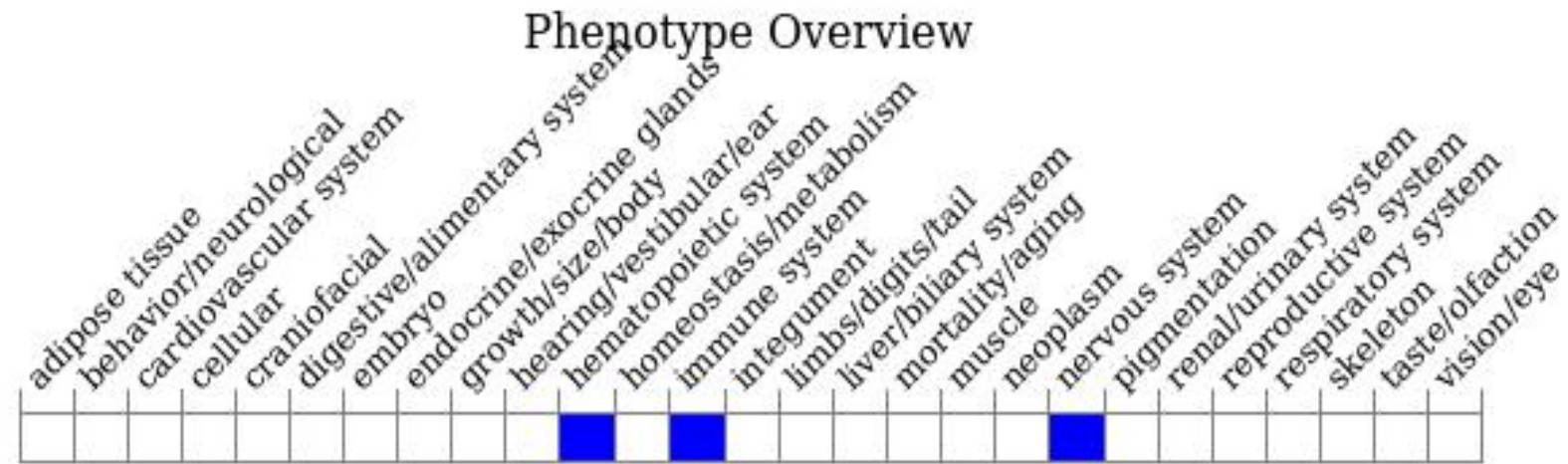


Genomic location distribution

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/>).

According to the existing MGI data, homozygous knockout reduces the surveillance activity of microglial cells in the brain.

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

Tel: 400-9660890

