

Xkr4 Cas9-CKO Strategy

Designer: Miaomiao Cui

Reviewer: Lingyan Wu

Design Date: 2021-7-8

Project Overview

Project Name

Xkr4

Project type

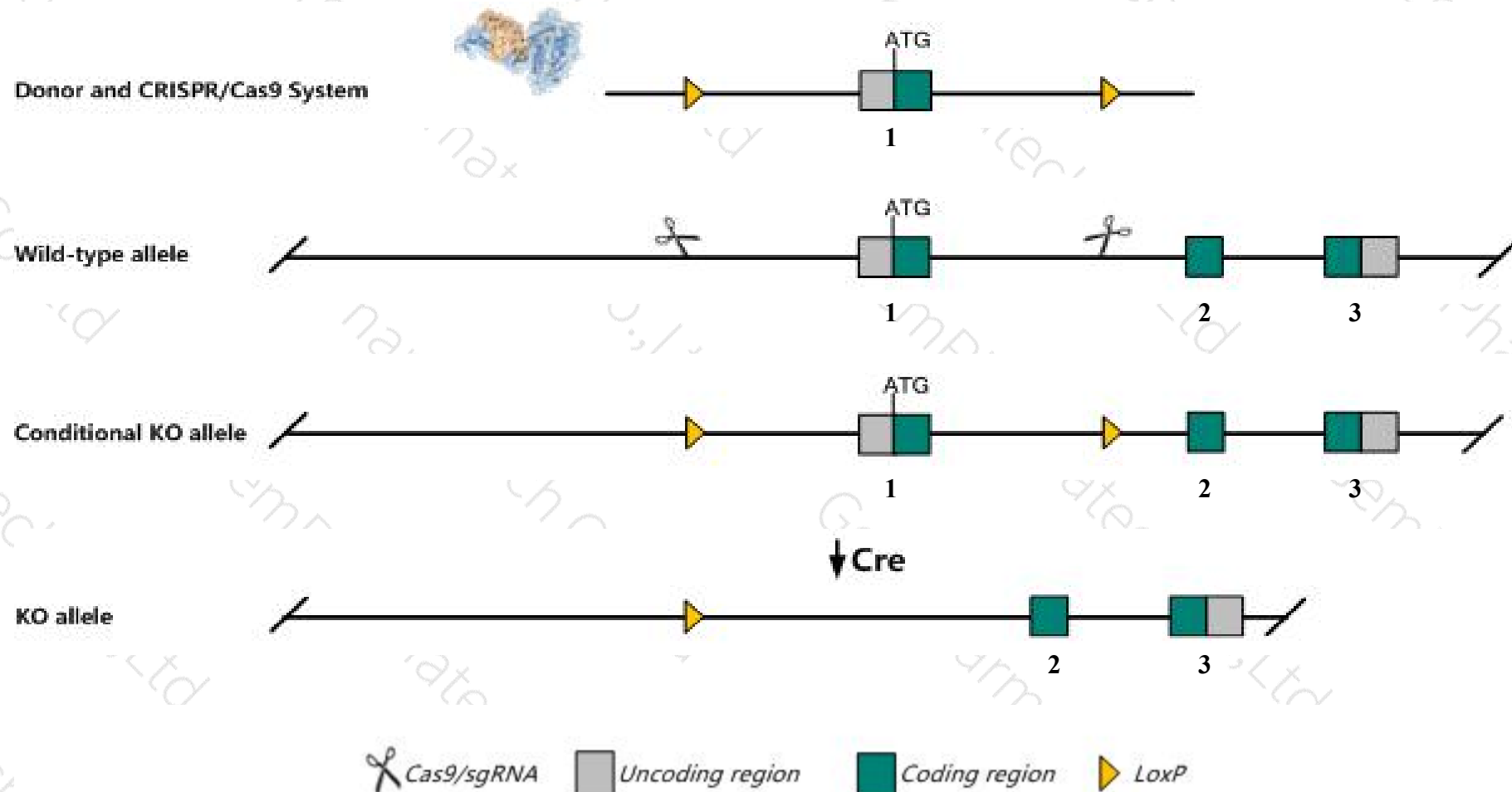
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Xkr4* gene. The schematic diagram is as follows:



Technical routes

- The *Xkr4* gene has 3 transcripts. According to the structure of *Xkr4* gene, exon1 of *Xkr4-201*(ENSMUST00000070533.4) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Xkr4* 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 *Xkr4* gene is located on the Chr1. 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)

Xkr4 X-linked Kx blood group related 4 [Mus musculus (house mouse)]

Gene ID: 497097, updated on 13-Mar-2020

Summary



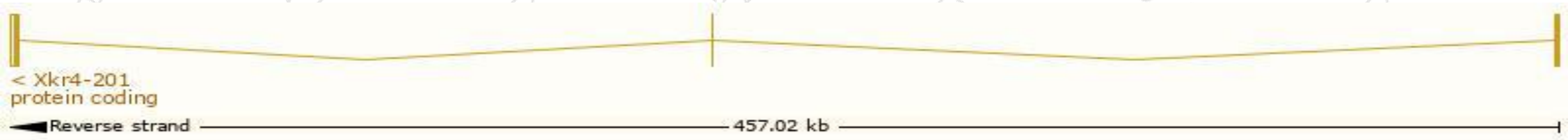
Official Symbol	Xkr4 provided by MGI
Official Full Name	X-linked Kx blood group related 4 provided by MGI
Primary source	MGI:MGI:3528744
See related	Ensembl:ENSMUSG00000051951
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AY534250, Gm210, XRG4, mKIAA1889
Expression	Biased expression in CNS E18 (RPKM 4.4), frontal lobe adult (RPKM 2.6) and 5 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Xkr4-201	ENSMUST00000070533.4	3634	647aa	Protein coding	CCDS14803	Q5GH67	TSL:1 GENCODE basic APPRIS P1
Xkr4-203	ENSMUST00000162897.1	4153	No protein	Processed transcript	-	-	TSL:1
Xkr4-202	ENSMUST00000159265.1	2989	No protein	Processed transcript	-	-	TSL:1

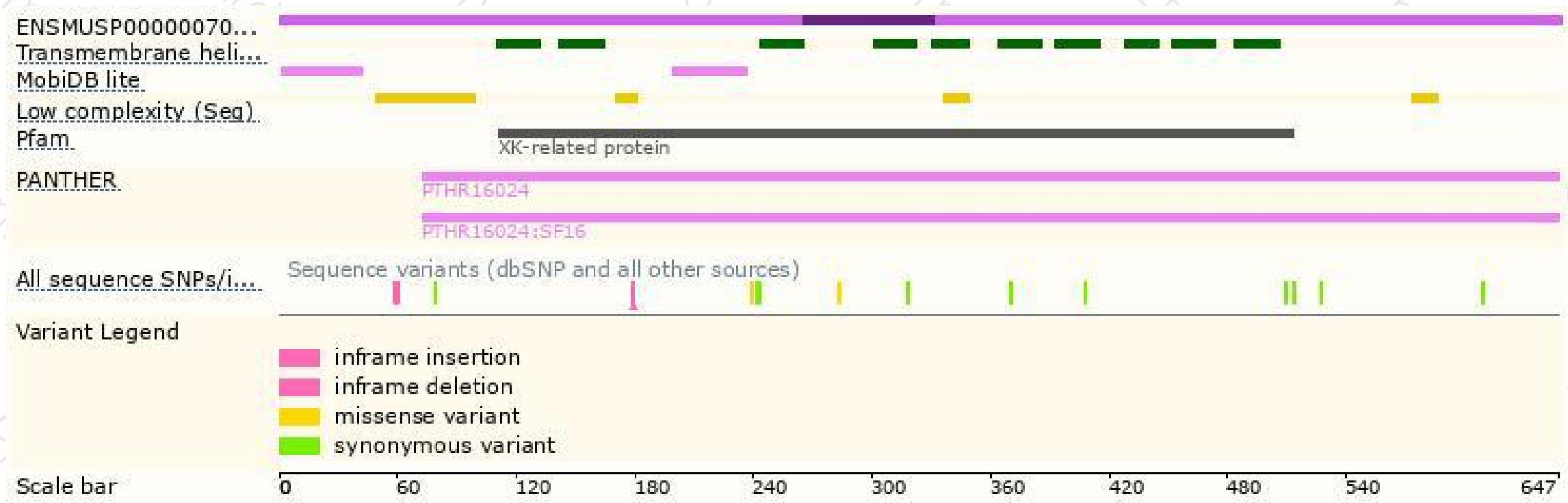
The strategy is based on the design of *Xkr4-201* transcript,the transcription is shown below:



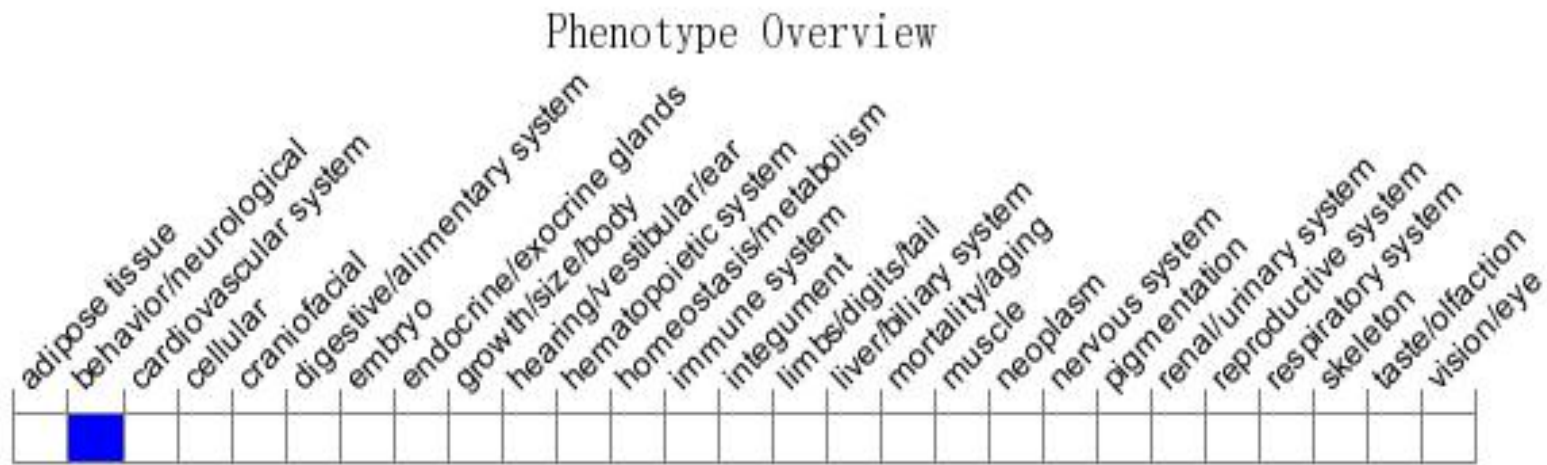
Genomic location distribution



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/>).

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

Tel: 025-5864 1534

