Grk4 Cas9-KO Strategy

Designer: Huan Wang

Reviewer: Yumeng Wang

Design Date: 2021-7-26

Project Overview

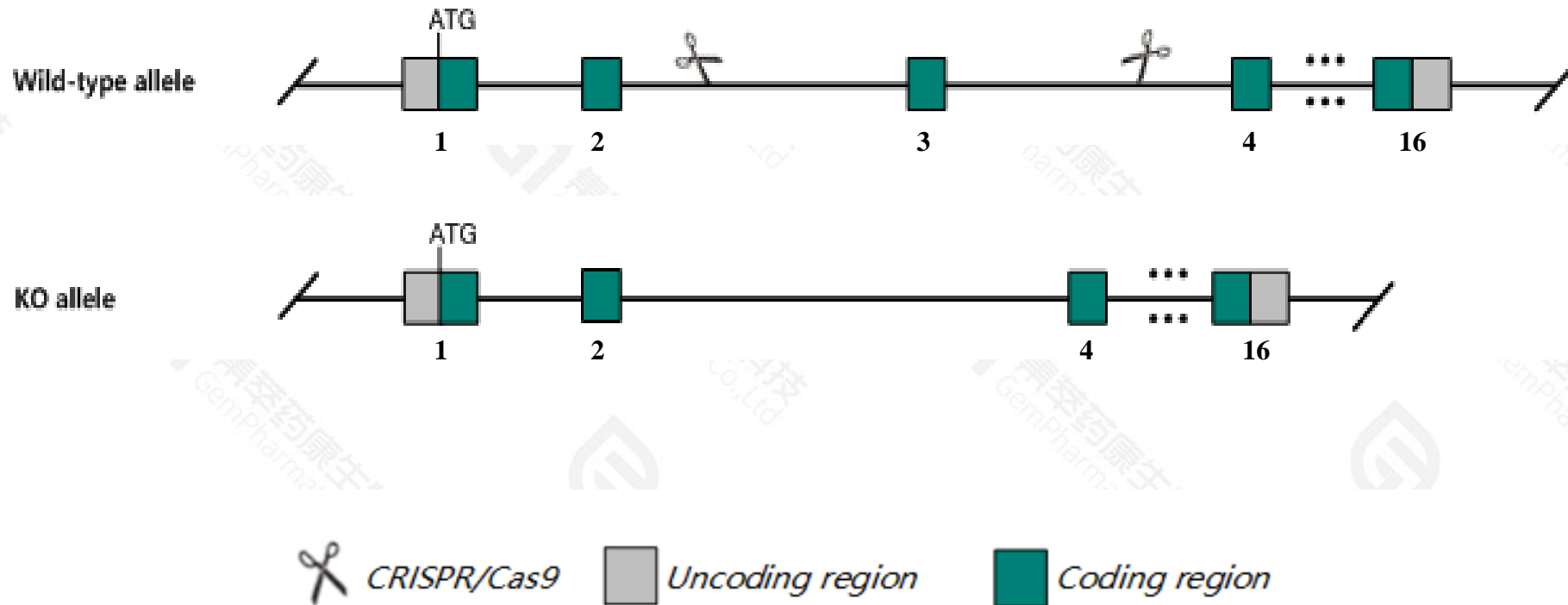|	
Project Name	*Grk4*
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Grk4* gene. The schematic diagram is as follows:

➢ The *Grk4* gene has 5 transcripts. According to the structure of *Grk4* gene, exon3 of *Grk4-201*(ENSMUST00000001112.14) transcript is recommended as the knockout region.The region contains 113bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Grk4* 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

➢ According to the existing MGI data,mice heterozygous for a knock-out allele are viable, fertile and overtly normal.

➢ The *Grk4* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

Grk4 G protein-coupled receptor kinase 4 [Mus musculus (house mouse)]

Gene ID: 14772, updated on 13-Mar-2020

▲ Summary

|	
Official Symbol	Grk4 provided by MGI
Official Full Name	G protein-coupled receptor kinase 4 provided by MGI
Primary source	MGI:MGI:95801
See related	Ensembl:ENSMUSG00000052783
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	A830025H08Rik, GRK, Gprk2l, Gprk4
Expression	Biased expression in testis adult (RPKM 7.6), bladder adult (RPKM 0.8) and 10 other tissuesSee 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
Grk4-202	ENSMUST00000074651.10	3234	177aa	Protein coding	CCDS39070	Q8BP13	TSL:1 GENCODE basic
Grk4-201	ENSMUST00000001112.13	2408	574aa	Protein coding	CCDS19219	O70291	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Grk4-204	ENSMUST00000148588.1	305	96aa	Protein coding	-	F7BEP0	CDS 5' incomplete TSL:2
Grk4-205	ENSMUST00000153323.1	892	No protein	Processed transcript	-	-	TSL:3
Grk4-203	ENSMUST00000134217.1	809	No protein	Processed transcript	-	-	TSL:3

The strategy is based on the design of *Grk4-201* transcript,the transcription is shown below:
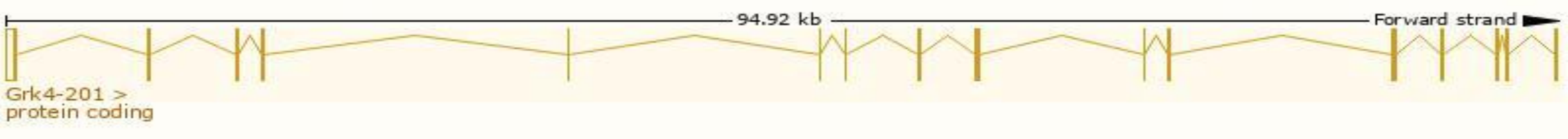
Genomic location distribution
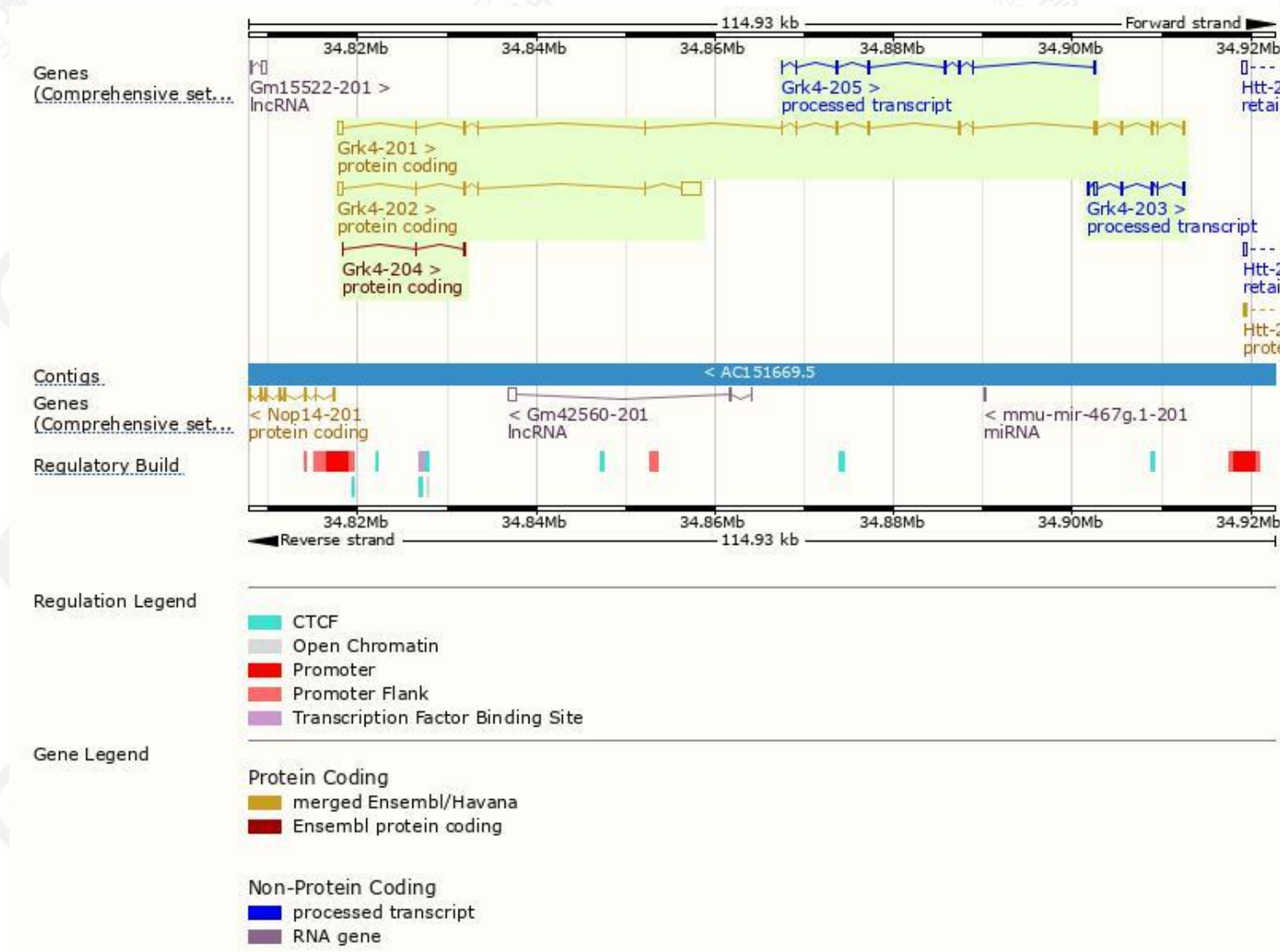
Protein domain
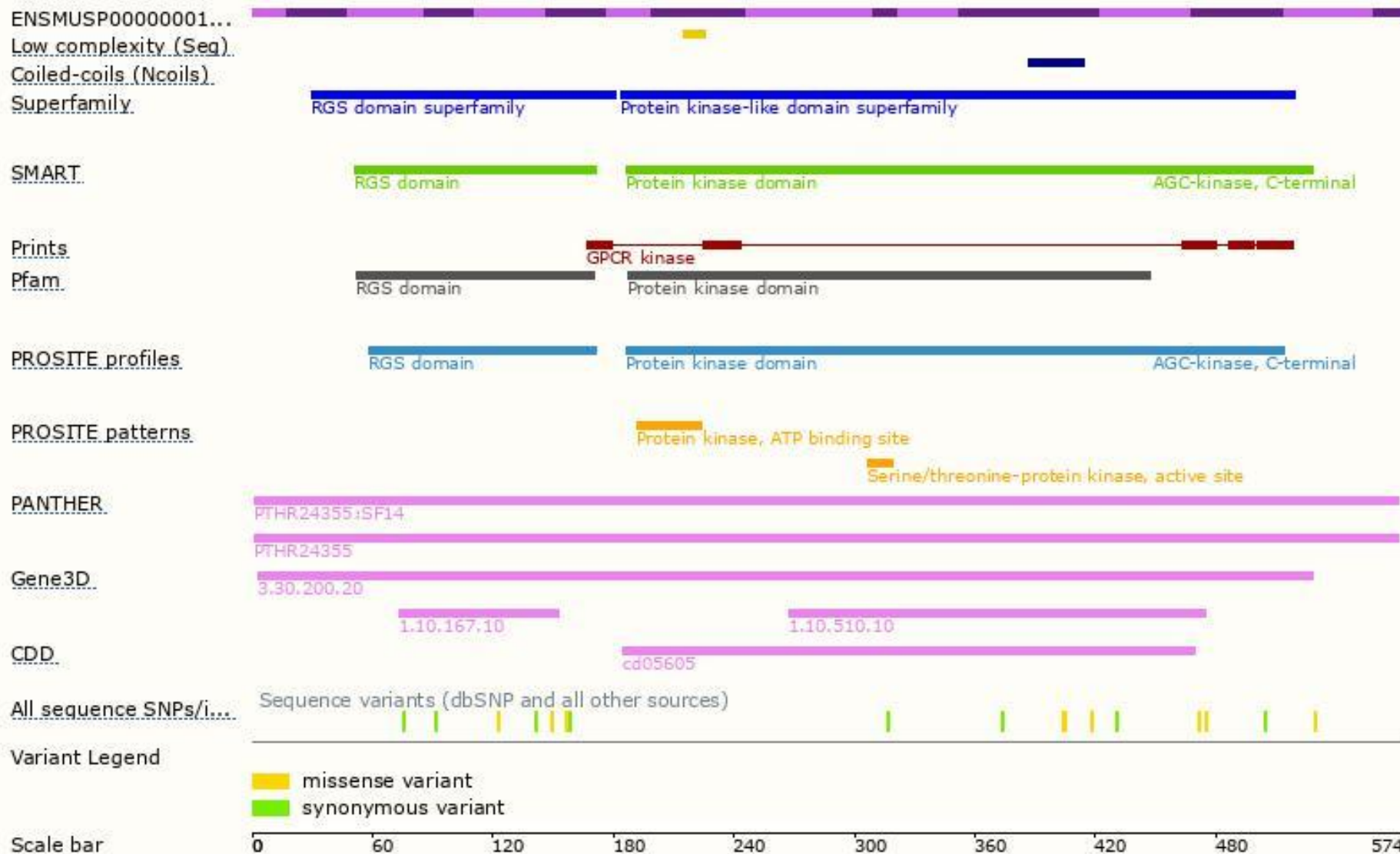
If you have any questions, you are welcome to inquire.
Tel: 400-9660890