

Nek2 Cas9-KO Strategy

Designer: Daohua Xu

Reviewer: Huimin Su

Design Date: 2022-8-23

Overview

Target Gene Name

- Nek2

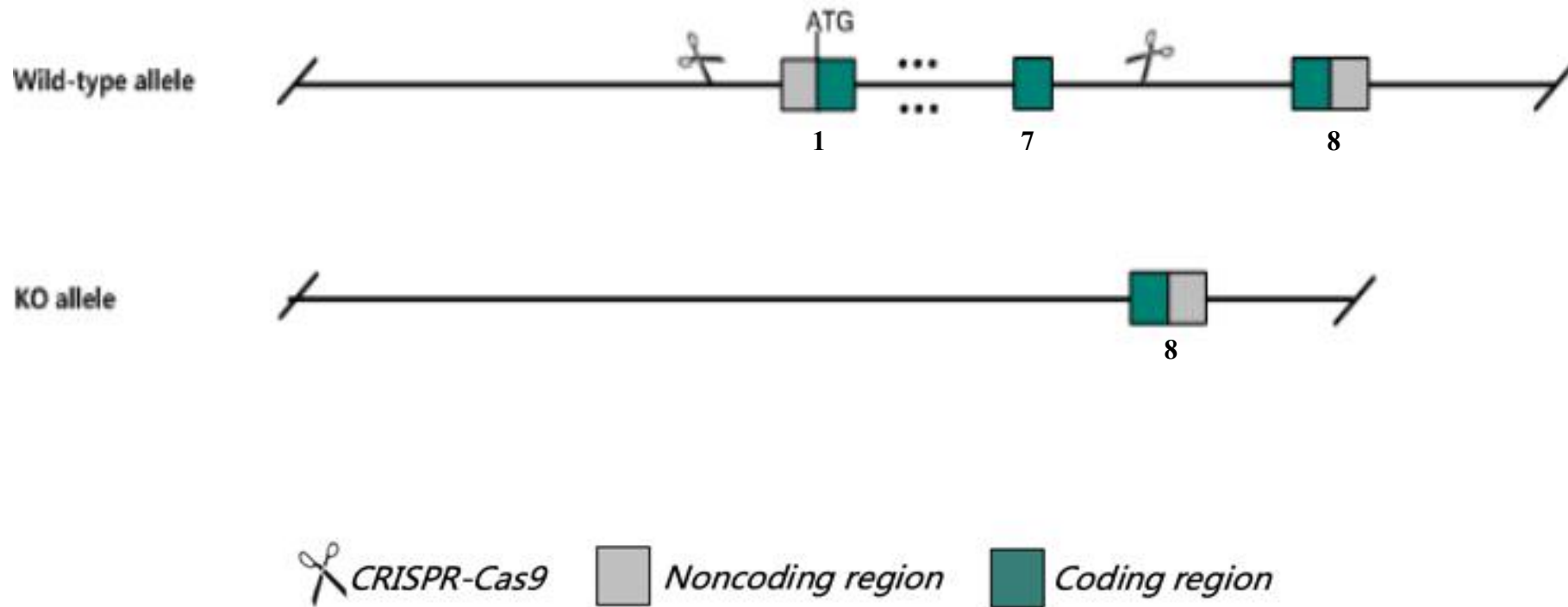
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Nek2* gene.

Technical Information

- The *Nek2* gene has 4 transcripts. According to the structure of *Nek2* gene, exon1-exon7 of *Nek2*-201 (ENSMUST00000027931.8) transcript is recommended as the knockout region. The region contains start codon ATG. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Nek2* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

Gene Information

Nek2 NIMA (never in mitosis gene a)-related expressed kinase 2 [Mus musculus (house mouse)]

Gene ID: 18005, updated on 12-Jul-2022

Summary



Official Symbol	Nek2 provided by MGI
Official Full Name	NIMA (never in mitosis gene a)-related expressed kinase 2 provided by MGI
Primary source	MGI:MGI:109359
See related	Ensembl:ENSMUSG00000026622
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	AA617254, C77054
Expression	Broad expression in testis adult (RPKM 25.4), CNS E11.5 (RPKM 10.9) and 15 other tissues See more
Orthologs	human all

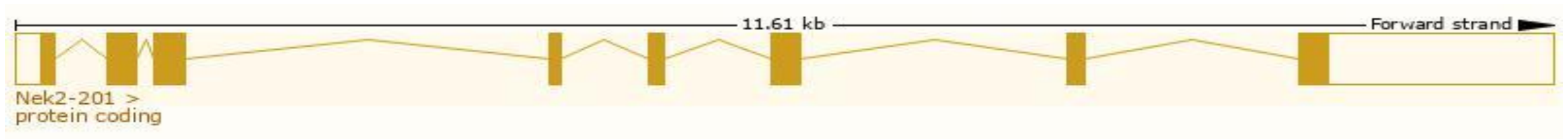
Source: <https://www.ncbi.nlm.nih.gov/>

Transcript Information

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

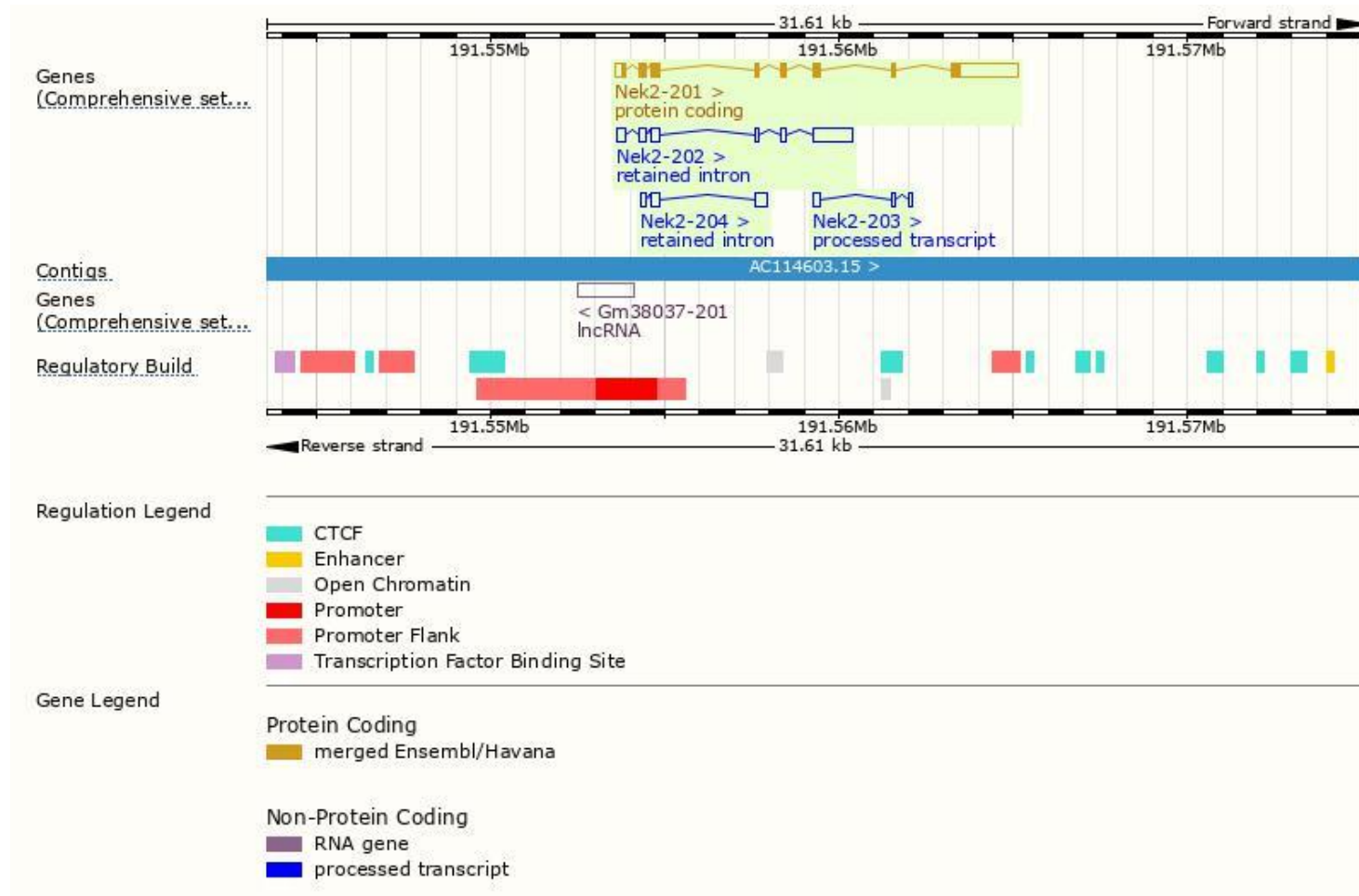
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000027931.8	Nek2-201	3227	443aa	Protein coding	CCDS15623	Q35942	Ensembl Canonical Gencode basic APPRIS P1 TSL:1
ENSMUST00000136733.2	Nek2-203	452	No protein	Processed transcript		-	TSL:3
ENSMUST00000126446.8	Nek2-202	2044	No protein	Retained intron		-	TSL:2
ENSMUST00000150839.2	Nek2-204	767	No protein	Retained intron		-	TSL:3

The strategy is based on the design of Nek2-201 transcript, the transcription is shown below:

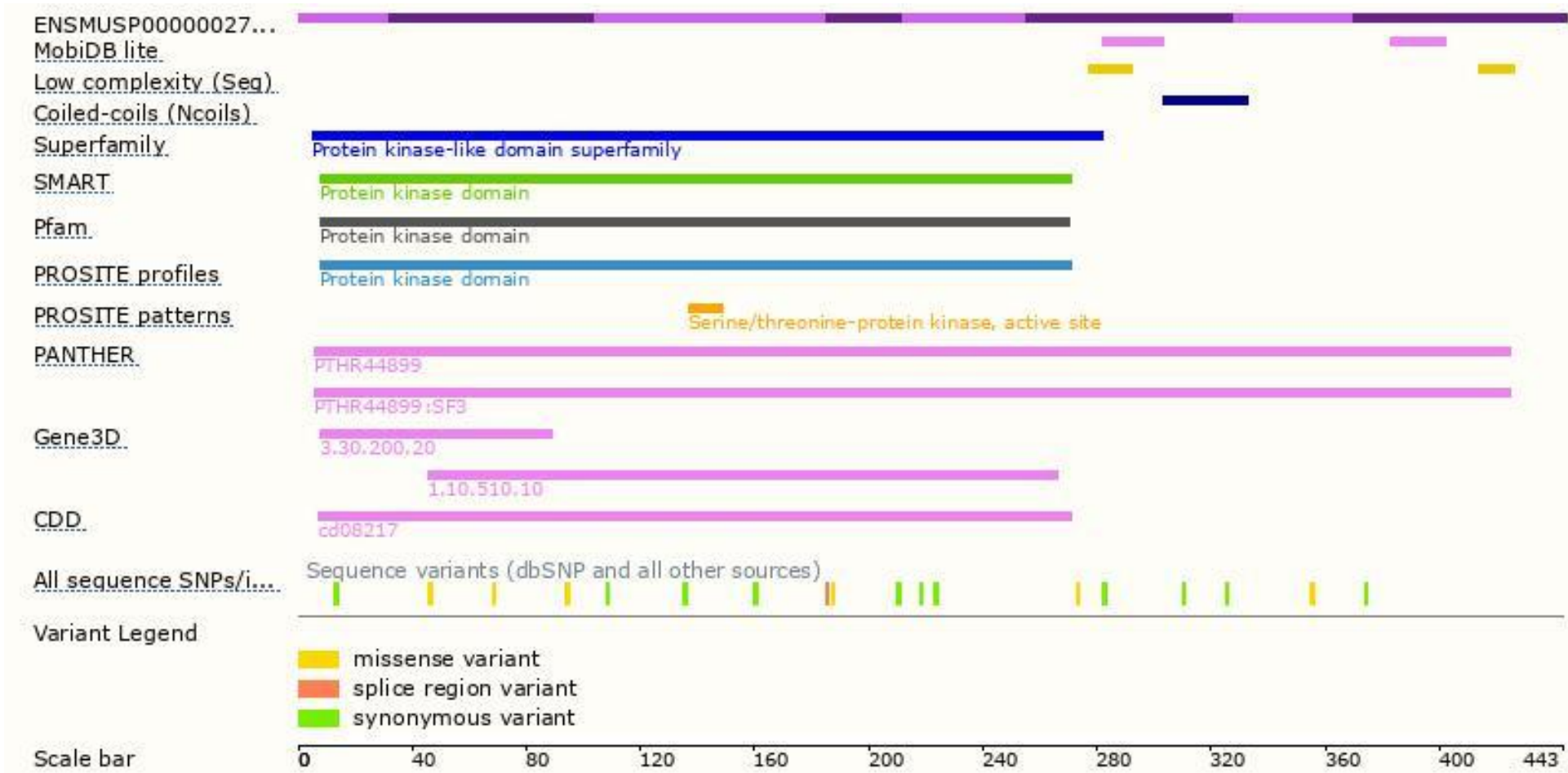


Source: <https://www.ensembl.org>

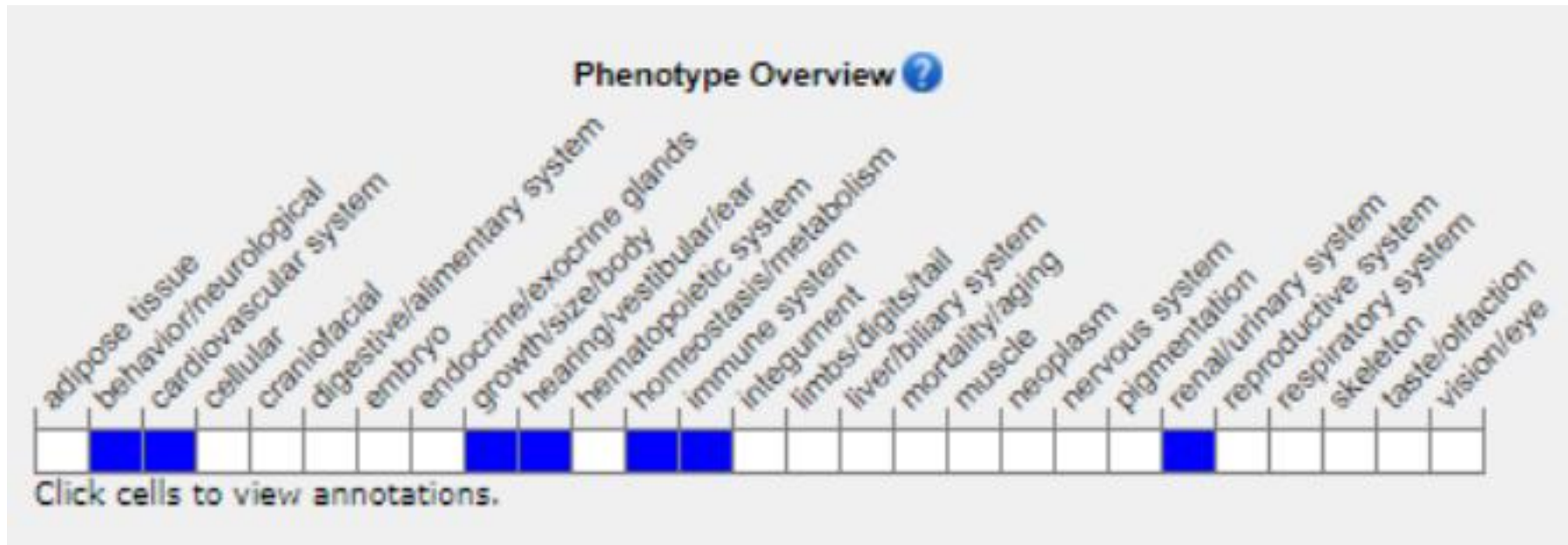
Genomic Information



Protein Information



Mouse Phenotype Information (MGI)



Important Information

- *Nek2* is located on Chr1. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.