

# Pank4 Cas9-KO Strategy

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### Overview

#### Target Gene Name

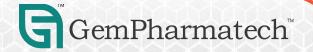
• Pank4

### Project Type

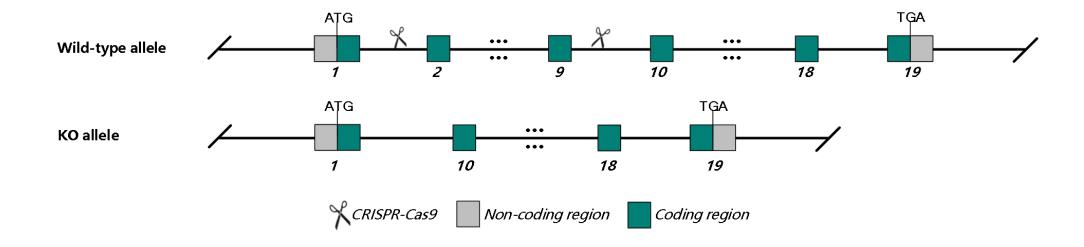
• Cas9-KO

#### Genetic Background

• C57BL/6JGpt



### Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Pank4* gene.



#### **Technical Information**

- The *Pank4* gene has 7 transcripts. According to the structure of *Pank4* gene, exon 2-9 of *Pank4*-201 (ENSMUST00000030931.11) is recommended as the knockout region. The region contains 1094 bp of coding sequence. Knocking out the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Pank4* 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 ontarget 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.



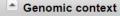
#### Gene Information

Pank4 pantothenate kinase 4 [ Mus musculus (house mouse) ]

**≛** Download Datasets

Gene ID: 269614, updated on 7-Sep-2023



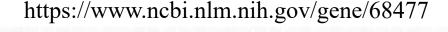


☆ ?

Location: 4; 4 E2

See Pank4 in Genome Data Viewer

Exon count: 21



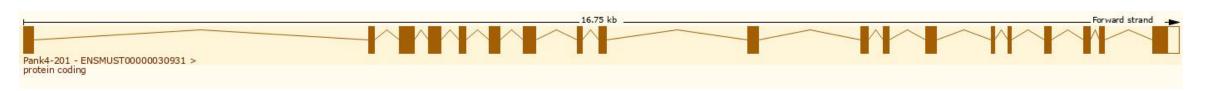


# Transcript Information

The gene has 7 transcripts, all transcripts are shown below:

Transcript ID 🔷	Name 🝦	bp 🛊	Protein ▼	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000070953.11	Pank4-202	2706	820aa	Protein coding	CCDS84834 ₺	Q80YV4-1@	Ensembl Canonical GENCODE basic TSL:1
ENSMUST00000030931.11	Pank4-201	2508	<u>773aa</u>	Protein coding	CCDS19019 &	Q80YV4-2 ₺	GENCODE basic   APPRIS P1   TSL:1
ENSMUST00000148934.8	Pank4-207	2042	<u>473aa</u>	Nonsense mediated decay		F7B6K4₺	TSL:1 CDS 5' incomplete
ENSMUST00000129386.2	Pank4-205	1532	No protein	Retained intron		-	TSL:2
ENSMUST00000105632.8	Pank4-203	1431	No protein	Retained intron		-	TSL:5
ENSMUST00000148299.8	Pank4-206	892	No protein	Retained intron		2	TSL:3
ENSMUST00000128297.8	Pank4-204	737	No protein	Retained intron		-	TSL:3

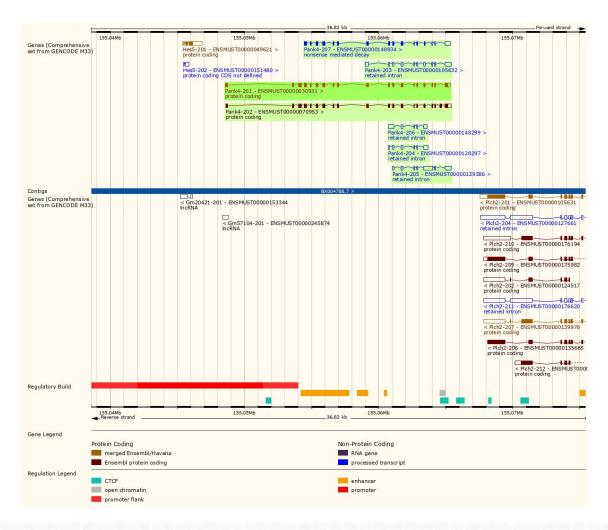
The strategy is based on the design of *Pank4*-201 transcript, the transcription is shown below:

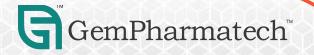




Source: http://asia.ensembl.org/

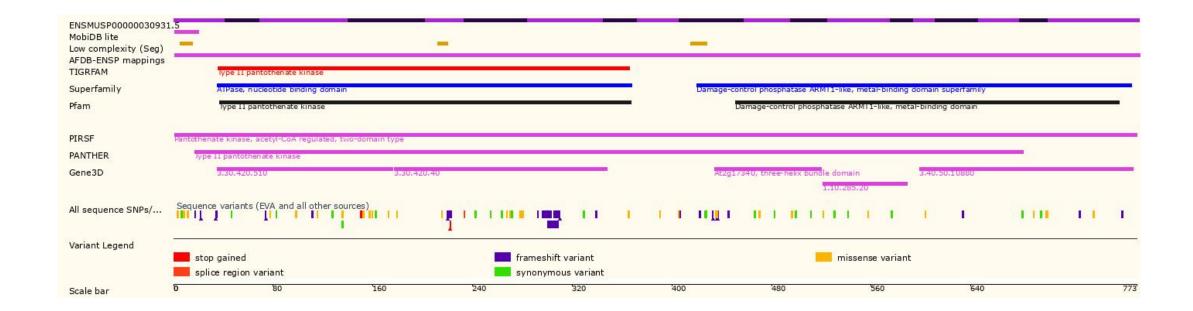
#### Genomic Information





Source: http://asia.ensembl.org/

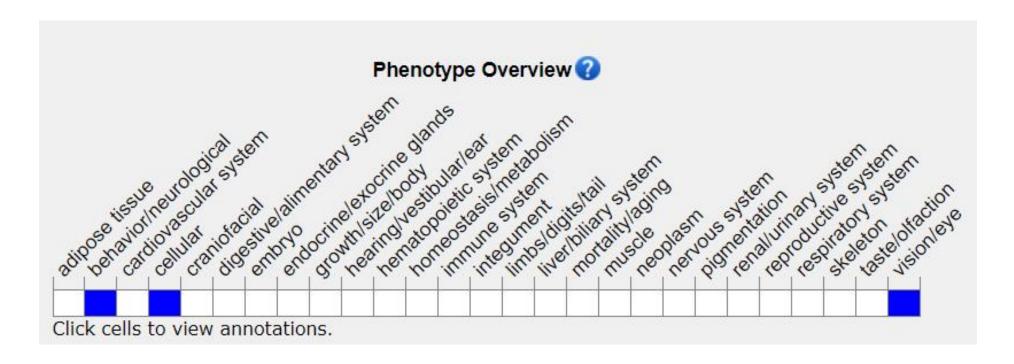
#### Protein Information





Source: https://www.ensembl.org

# Mouse Phenotype Information (MGI)



Mice homozygous for a null allele develop cataracts, with increased lens epithelial cell apoptosis, decreased lens epithelium thickness, and lens fiber abnormalities.



Source: https://www.informatics.jax.org

### Important Information

- The knockout region is 4.4 kb away from the 5' of *Gm57104* gene, which may affect the 5' regulation of *Gm57104* gene.
- The knockout region is 6.9 kb away from the 5' of *Gm20421* gene, which may affect the 5' regulation of *Gm20421* gene.
- This stratergy may not affect Pank4-203, Pank4-204, Pank4-205 and Pank4-206 transcript.
- *Pank4* is located on Chr 4. 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.

