

Unc5a Cas9-CKO Strategy

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Overview

Target Gene Name

• *Unc5a*

Project Type

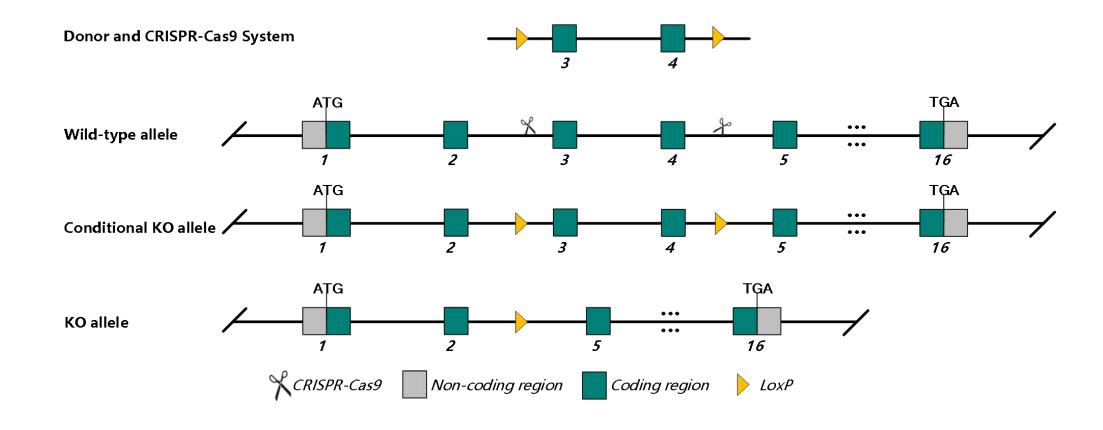
• Cas9-CKO

Genetic Background

• C57BL/6JGpt



Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Unc5a* gene.



Technical Information

- The *Unc5a* gene has 5 transcripts. According to the structure of *Unc5a* gene, exon 3-4 of *Unc5a*-201 (ENSMUST00000026994.14) is recommended as the knockout region. The region contains 187 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 *Unc5a* 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 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.
- 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.



Gene Information

Unc5a unc-5 netrin receptor A [Mus musculus (house mouse)]

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Gene ID: 107448, updated on 5-Mar-2024



△ Genomic context

₹ ?

Location: 13 B1; 13 29.8 cM

See Unc5a in Genome Data Viewer

Exon count: 16

https://www.ncbi.nlm.nih.gov/gene/107448

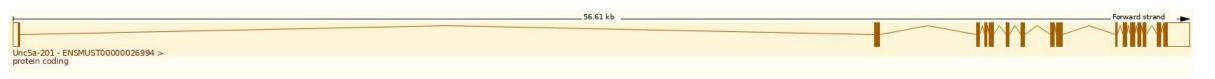


Transcript Information

The gene has 5 transcripts, the transcripts are shown below:

Transcript ID 🍦	Name 🌲	bp 🌲	Protein 🍦	Biotype	CCDS .	UniProt Match A	Flags
ENSMUST00000137967.2	Unc5a-204	593	128aa	Protein coding		F6TGW0₽	TSL:2 CDS 5' incomplete
ENSMUST00000136852.2	Unc5a-203	501	<u>167aa</u>	Protein coding		F7CVI0 ₽	TSL:3 CDS 5' and 3' incomplete
ENSMUST00000026994.14	Unc5a-201	3995	898aa	Protein coding	CCDS26537 ₽	<u>Q8K1S4</u> 굡	Ensembl Canonical GENCODE basic APPRIS P1 TSL
ENSMUST00000109994.9	Unc5a-202	3827	842aa	Protein coding	CCDS79188 ₽	Q8K1S4-2₽	GENCODE basic TSL:1
ENSMUST00000142906.2	Unc5a-205	898	No protein	Retained intron		=:	TSL:3

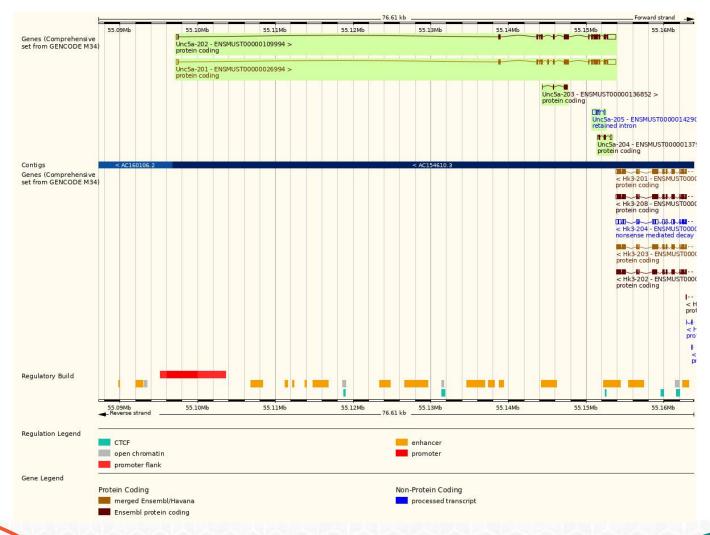
The strategy is based on the design of Unc5a-201 transcript, the transcription is shown below:

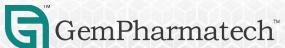




Source: http://asia.ensembl.org/

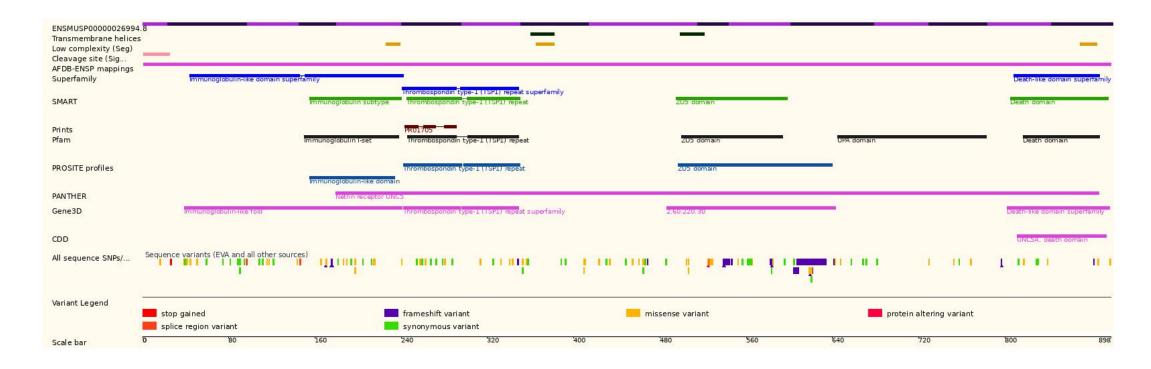
Genomic Information





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

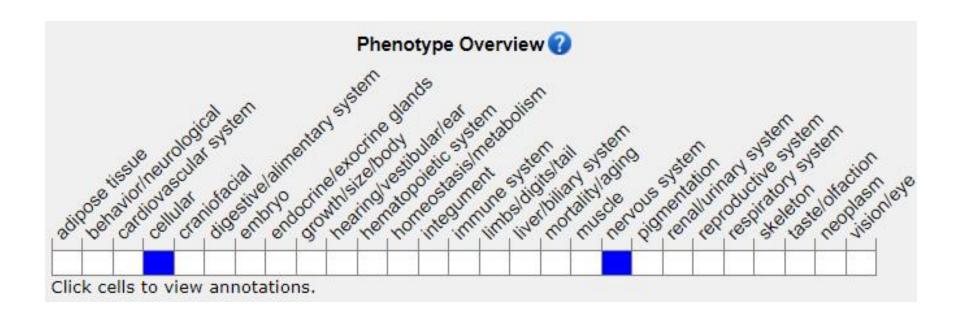
Protein Information





Source: https://www.ensembl.org

Mouse Phenotype Information (MGI)



Homozygous null mice are viable through adulthood but display decreased apoptotic cell death, supernumerary neurons and morphological alterations in the embryonic cervical spinal cord.



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

Important Information

- The intron 4-5 of *Unc5a*-201 is 140 bp, the loxp insertion may affect the regulation of this gene.
- This stratergy may not affect *Unc5a*-203, *Unc5a*-204 and *Unc5a*-205 transcript.
- *Unc5a* is located on Chr 13. 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 risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

