

Unc5c Cas9-KO Strategy

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Design Date: 2023-3-29

Overview

Target Gene Name

• Unc5c

Project Type

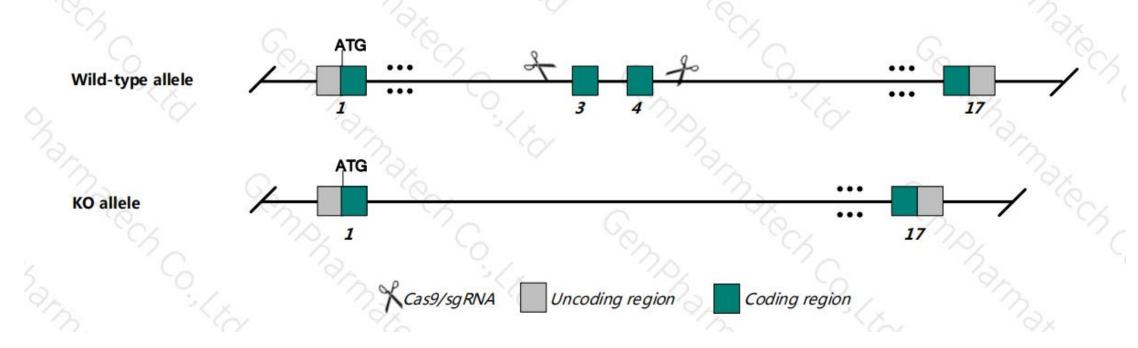
• Cas9-KO

Genetic Background

• C57BL/6JGpt



Strain Strategy

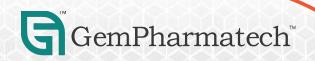


Schematic representation of CRISPR-Cas9 engineering used to edit the *Unc5c* gene.

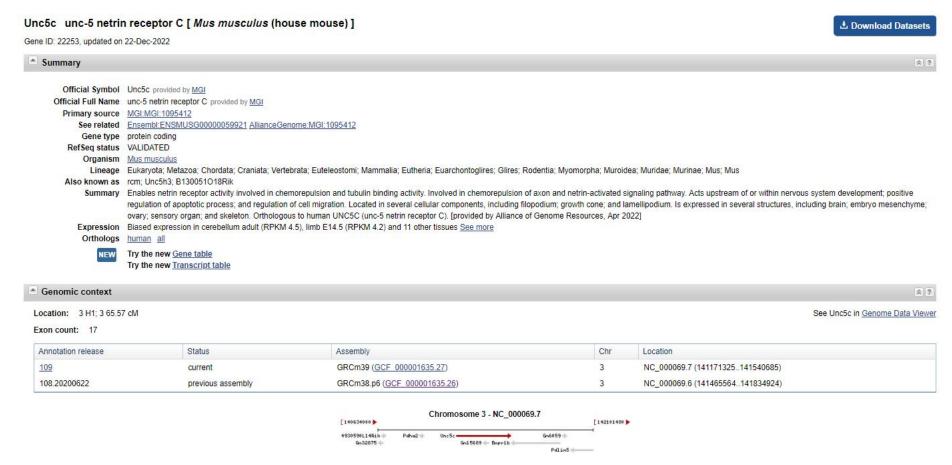


Technical Information

- The *Unc5c* gene has 8 transcripts. According to the structure of *Unc5c* gene, exon3-4 of *Unc5c-201* (ENSMUST00000075282.10) transcript is recommended as the knockout region. The region contains 248bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Unc5c* 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



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



Transcript Information

The gene has 8 transcripts, all transcripts are shown below:

Transcript ID	Name	bp 🍦	Protein	Biotype	CCDS	UniProt Match 🍦	Flags
ENSMUST00000075282.10	Unc5c-201	3890	950aa	Protein coding	CCDS80034 ₺	<u>008747-2</u> ₽	Ensembl Canonical GENCODE basic APPRIS P4 TSL:1
ENSMUST00000106236.9	Unc5c-202	9646	931aa	Protein coding	CCDS17873 ₺	<u>008747-1</u> ₽	GENCODE basic APPRIS ALT1 TSL:1
ENSMUST00000130636.8	Unc5c-204	3595	876aa	Protein coding		E9PVI4 ₽	GENCODE basic TSL:1
ENSMUST00000142762.2	Unc5c-205	3498	950aa	Nonsense mediated decay	CCDS80034 €	<u>008747-2</u> ₽	TSL:1
ENSMUST00000151007.6	Unc5c-206	357	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000200114.2	Unc5c-208	5330	No protein	Retained intron			TSL:NA
ENSMUST00000155416.5	Unc5c-207	4577	No protein	Retained intron			TSL:2
ENSMUST00000123631.2	Unc5c-203	417	No protein	Retained intron		-	TSL:3

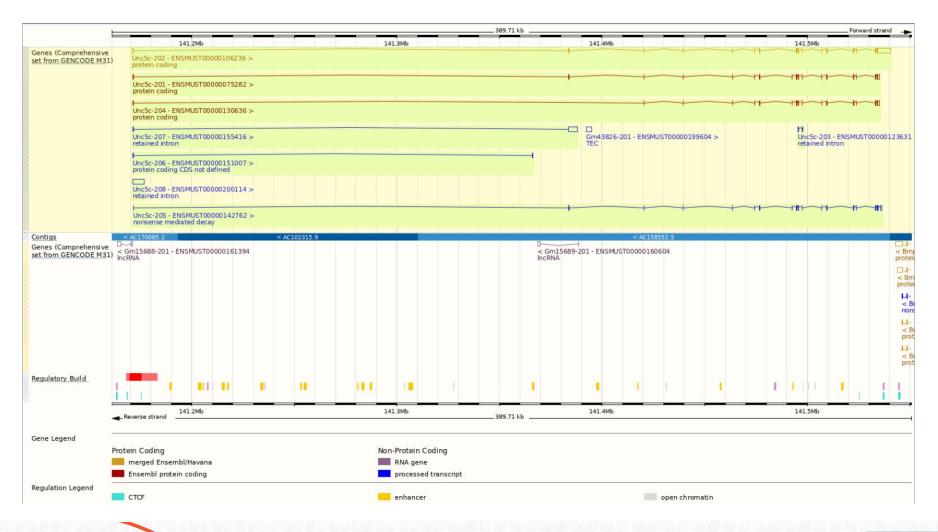
The strategy is based on the design of *Unc5c-201* transcript, the transcription is shown below:



Source: https://www.ensembl.org



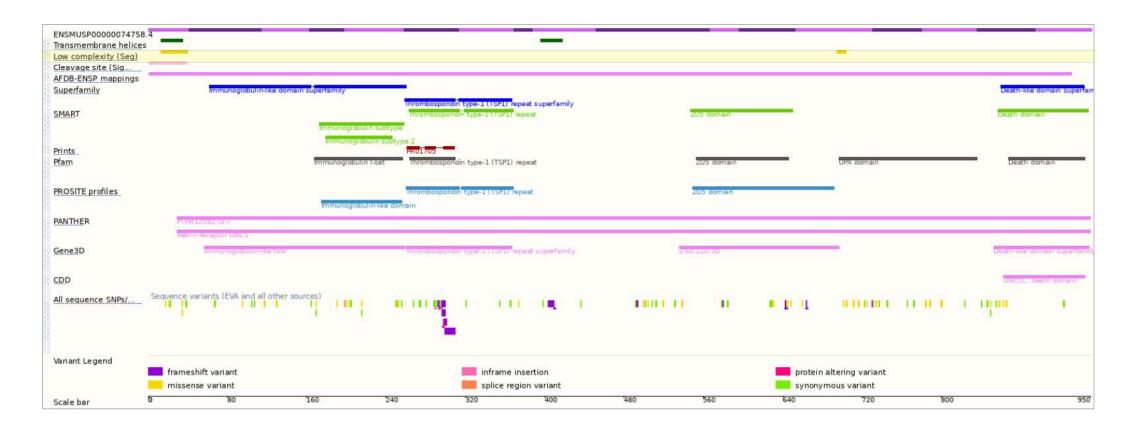
Genomic Information





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

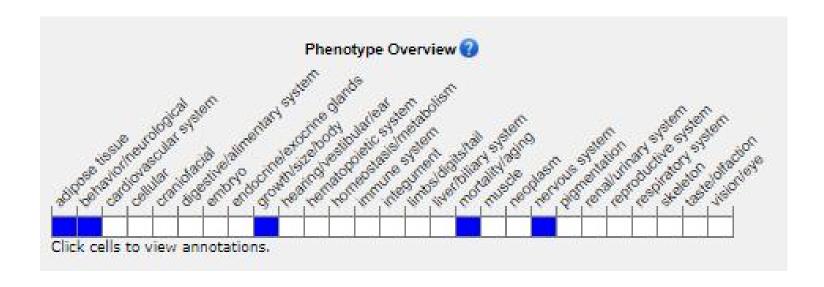
Protein Information





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

Mouse Phenotype Information (MGI)



• Mutants exhibit ataxia, and reduced size early in life. Mutants exhibit cerebellar defects including reduced size and ectopic cerebellar cells in the midbrain.



Important Information

- According to the MGI information and breeding data, the gene knockout homozygote dies during the embryonic stage.
- *Unc5c* is located on Chr3. 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.

