

Unc13d Cas9-KO Strategy

Designer:Xueting Zhang

Reviewer; Yanhua Shen

Date:2020-1-13

Project Overview



Project Name

Unc13d

Project type

Cas9-KO

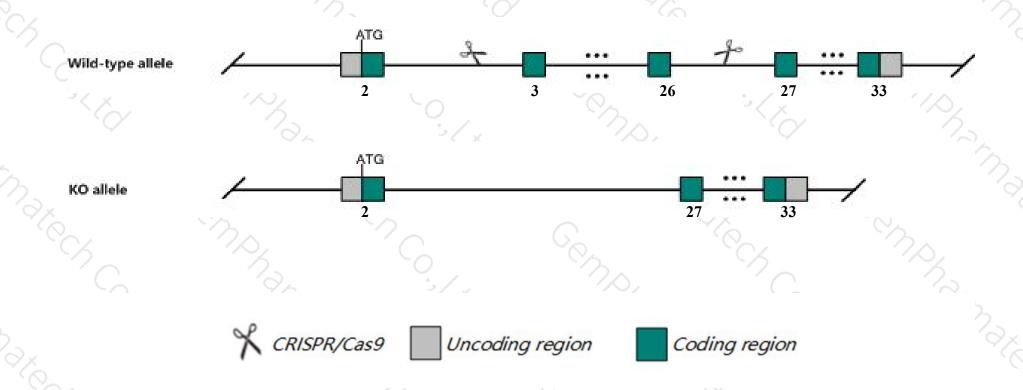
Strain background

C57BL/6JGpt

Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Unc13d* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Unc13d* gene has 9 transcripts. According to the structure of *Unc13d* gene, exon3-exon26 of *Unc13d-201*(ENSMUST00000075036.8) transcript is recommended as the knockout region. The region contains 2324bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Unc13d* gene. The brief process is as follows: CRISPR/Cas9 system

Notice



- ➤ According to the existing MGI data, Targeted deletion of this gene leads to defective hemostasis, abrogated thrombus formation and protection of homozygotes from ischemic stroke in the absence of intracranial bleeding. Homozygous ENU mutant mice are sensitive to infection by mouse cytomegalovirus.
- The *Unc13d* gene is located on the Chr11. 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)



Unc13d unc-13 homolog D [Mus musculus (house mouse)]

Gene ID: 70450, updated on 10-Dec-2019

Summary

2 ?

Official Symbol Unc13d provided by MGI

Official Full Name unc-13 homolog D provided by MGI

Primary source MGI:MGI:1917700

See related Ensembl: ENSMUSG00000057948

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 Jinx; Munc13-4; mFLJ00067; 2610108D09Rik

Expression Broad expression in thymus adult (RPKM 20.5), spleen adult (RPKM 18.5) and 15 other tissues See more

Orthologs human all

- Genomic context



Location: 11; 11 E2

See Unc13d in Genome Data Viewer

Exon count: 35

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	11	NC_000077.6 (116062095116078495, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	11	NC_000077.5 (115923410115939275, complement)

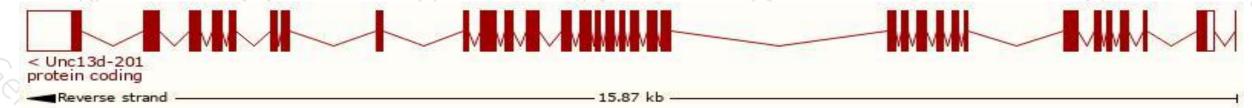
Transcript information (Ensembl)



The gene has 9 transcripts, all transcripts are shown below:

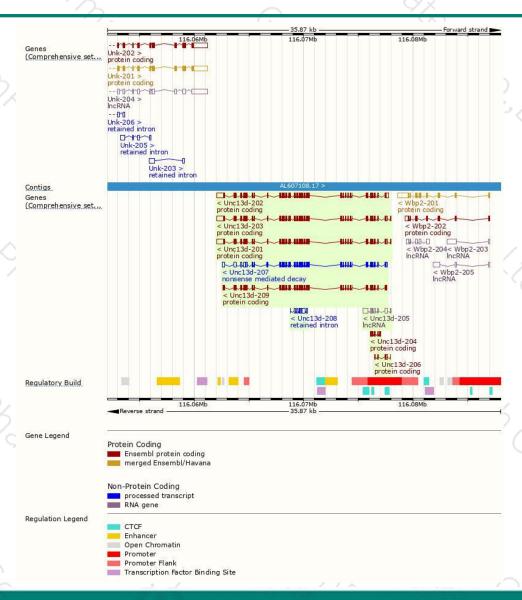
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Unc13d-201	ENSMUST00000075036.8	3967	<u>1083aa</u>	Protein coding	CCDS25656	B2RUP2	TSL:5 GENCODE basic APPRIS P2
Unc13d-209	ENSMUST00000174822.7	3337	<u>1083aa</u>	Protein coding	CCDS25656	B2RUP2	TSL:1 GENCODE basic APPRIS P2
Unc13d-202	ENSMUST00000106450.7	4046	<u>1085aa</u>	Protein coding	20	B2RUP2	TSL:5 GENCODE basic APPRIS ALT2
Unc13d-203	ENSMUST00000106451.7	3973	1085aa	Protein coding	29	B2RUP2	TSL:5 GENCODE basic APPRIS ALT2
Jnc13d-204	ENSMUST00000153408.7	416	<u>117aa</u>	Protein coding	56	A2A855	CDS 3' incomplete TSL:3
Unc13d-206	ENSMUST00000156545.1	353	<u>76aa</u>	Protein coding	+8	A2A858	CDS 3' incomplete TSL:5
Unc13d-207	ENSMUST00000173345.7	3514	834aa	Nonsense mediated decay	20	A0A0R4J257	TSL:1
Jnc13d-208	ENSMUST00000173943.1	766	No protein	Retained intron	29	2	TSL:5
Unc13d-205	ENSMUST00000155120.1	991	No protein	IncRNA	56	ā	TSL:1
					N 7	1 V	

The strategy is based on the design of *Unc13d-201* transcript, The transcription is shown below



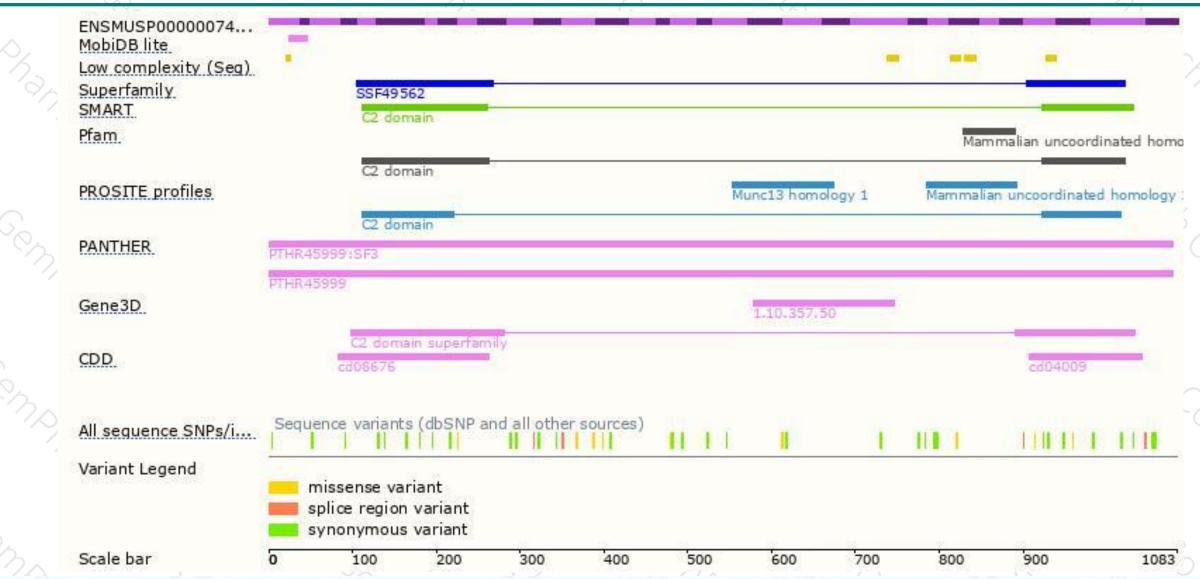
Genomic location distribution





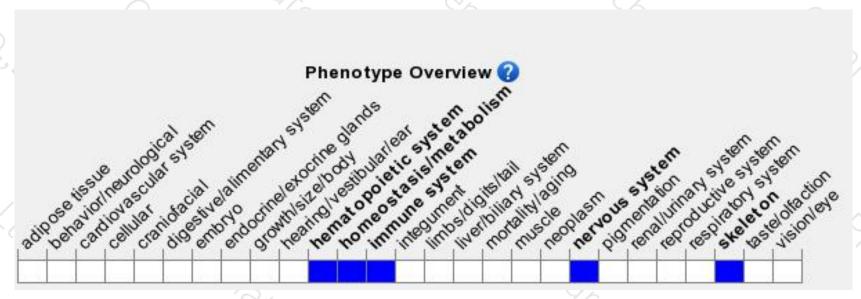
Protein domain





Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

According to the existing MGI data, Targeted deletion of this gene leads to defective hemostasis, abrogated thrombus formation and protection of homozygotes from ischemic stroke in the absence of intracranial bleeding. Homozygous ENU mutant mice are sensitive to infection by mouse cytomegalovirus.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





