

Unc13b Cas9-CKO Strategy

Designer:

JiaYu

Reviewer:

Xiaojing Li

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Project Overview

Project Name

Unc13b

Project type

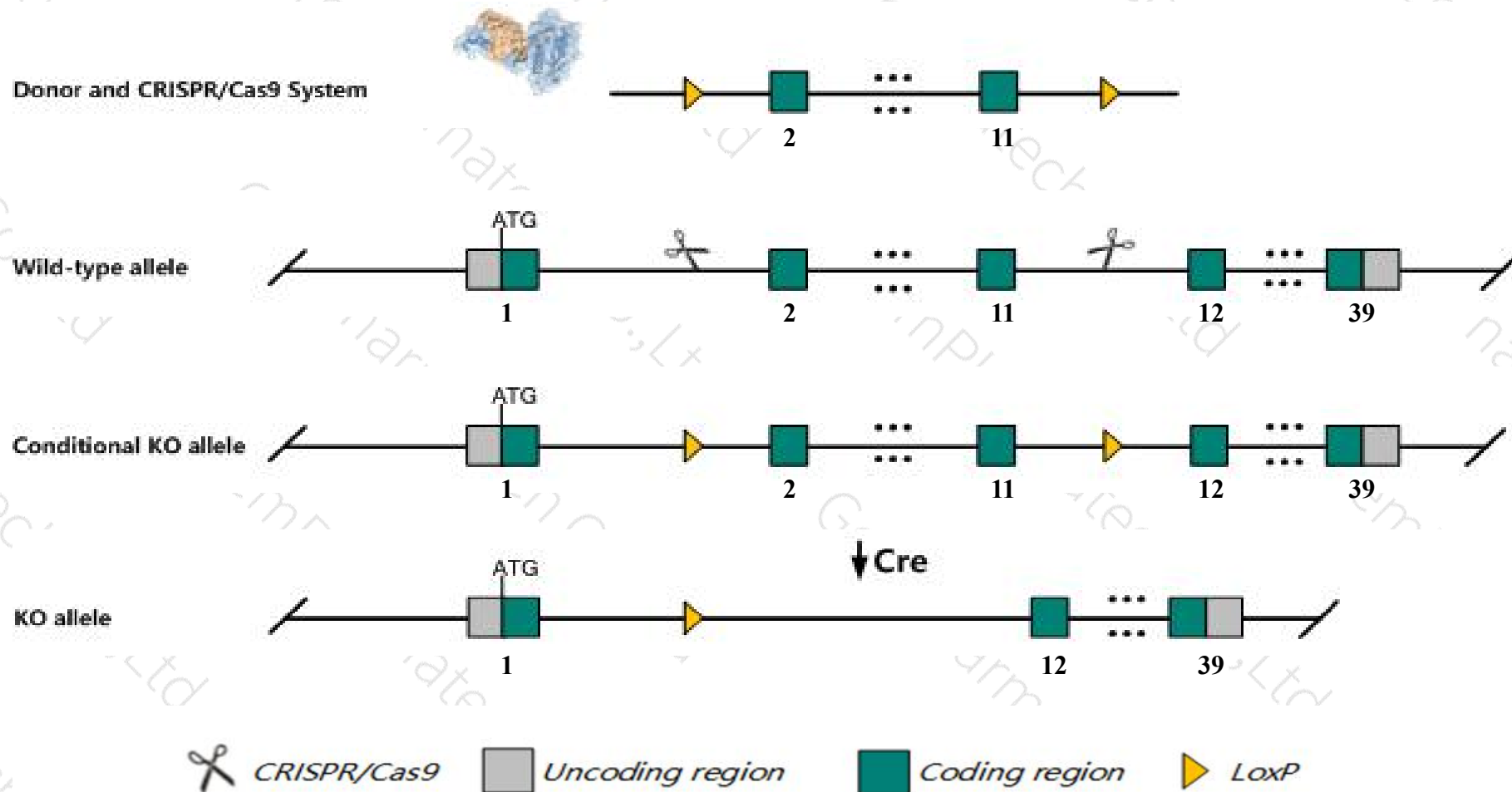
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Unc13b* gene. The schematic diagram is as follows:



- The *Unc13b* gene has 16 transcripts. According to the structure of *Unc13b* gene, exon2-exon11 of *Unc13b-201* (ENSMUST00000079978.12) transcript is recommended as the knockout region. The region contains 1192bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Unc13b* 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- 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.

- According to the existing MGI data, Homozygous mutant mice are grossly phenotypically normal. Mice older than 12 months will exhibit sporadic seizures.
- The KO region contains *Gm23709* and *Gm25010* gene. Knockout the region will delete them.
- The *Unc13b* gene is located on the Chr4. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

Unc13b unc-13 homolog B [Mus musculus (house mouse)]

Gene ID: 22249, updated on 19-Mar-2019

Summary



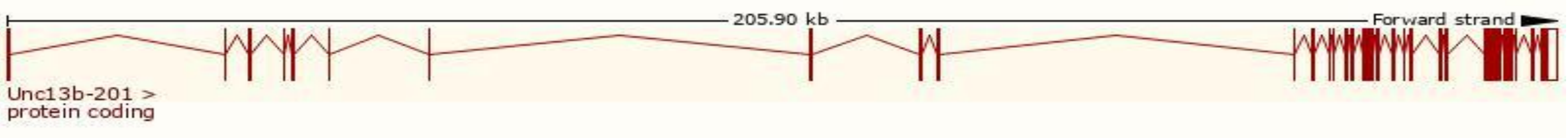
Official Symbol	Unc13b provided by MGI
Official Full Name	unc-13 homolog B provided by MGI
Primary source	MGI:MGI:1342278
See related	Ensembl:ENSMUSG00000028456
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Munc13-1, Munc13-2, Unc13a, Unc13h1, Unc13h2
Expression	Broad expression in CNS E18 (RPKM 6.9), CNS E14 (RPKM 6.8) and 23 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

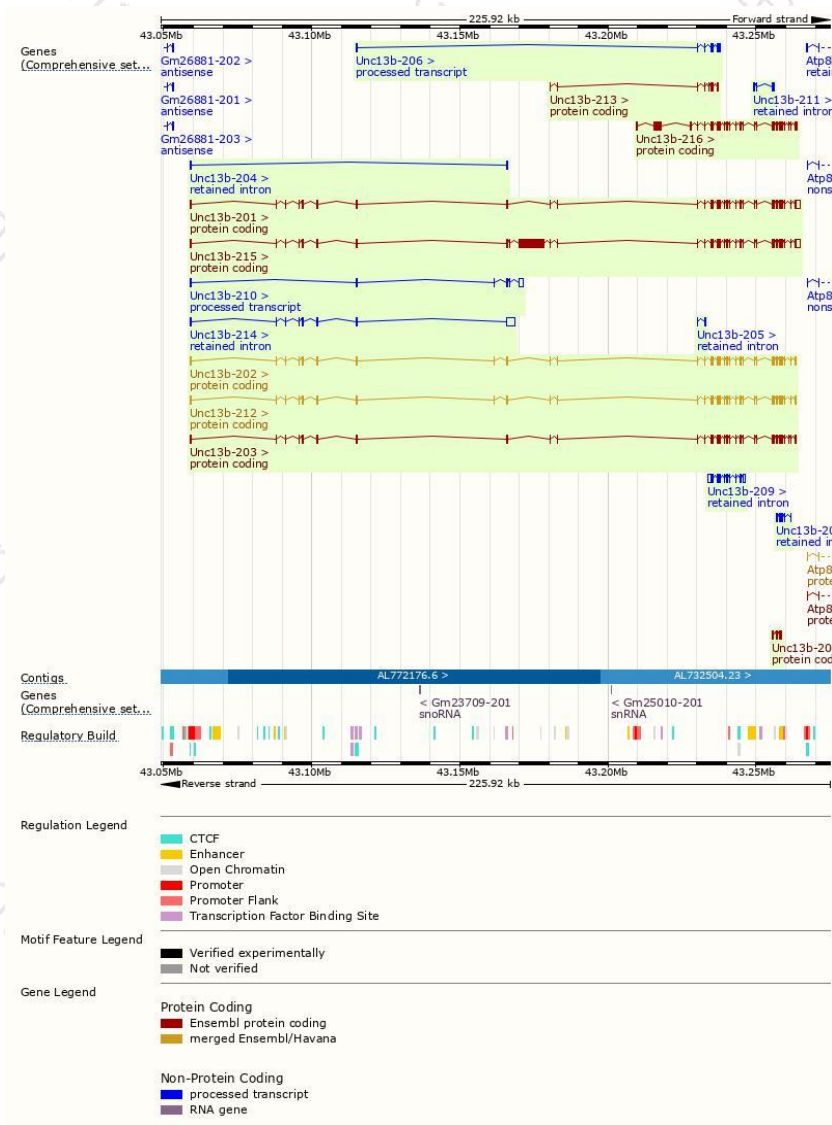
The gene has 16 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Unc13b-201	ENSMUST00000079978.12	6354	1590aa	Protein coding	CCDS80089	Q9Z1N9	TSL:5 GENCODE basic APPRIS P2
Unc13b-202	ENSMUST000000107952.8	5037	1602aa	Protein coding	CCDS51161	Q9Z1N9	TSL:1
Unc13b-212	ENSMUST000000163653.7	5034	1601aa	Protein coding	CCDS38738	Q9Z1N9	TSL:1
Unc13b-215	ENSMUST000000207569.1	14754	4390aa	Protein coding	-	A0A140LJ69	TSL:5 GENCODE basic
Unc13b-216	ENSMUST000000207708.1	6574	1982aa	Protein coding	-	A0A140LHX5	TSL:5 GENCODE basic
Unc13b-203	ENSMUST000000107953.8	5034	1609aa	Protein coding	-	E9Q263	TSL:5 GENCODE basic APPRIS ALT 1
Unc13b-213	ENSMUST000000168032.1	768	256aa	Protein coding	-	F6X605	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Unc13b-208	ENSMUST000000145899.1	569	189aa	Protein coding	-	F7CEK4	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Unc13b-210	ENSMUST000000151611.8	2173	No protein	Processed transcript	-	-	TSL:1
Unc13b-206	ENSMUST000000132310.8	780	No protein	Processed transcript	-	-	TSL:5
Unc13b-214	ENSMUST000000171234.1	3617	No protein	Retained intron	-	-	TSL:1
Unc13b-209	ENSMUST000000149945.2	3258	No protein	Retained intron	-	-	TSL:2
Unc13b-207	ENSMUST000000143653.2	1036	No protein	Retained intron	-	-	TSL:5
Unc13b-211	ENSMUST000000153168.2	856	No protein	Retained intron	-	-	TSL:3
Unc13b-204	ENSMUST000000126878.1	712	No protein	Retained intron	-	-	TSL:3
Unc13b-205	ENSMUST000000127597.1	495	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Unc13b-201* transcript,The transcription is shown below



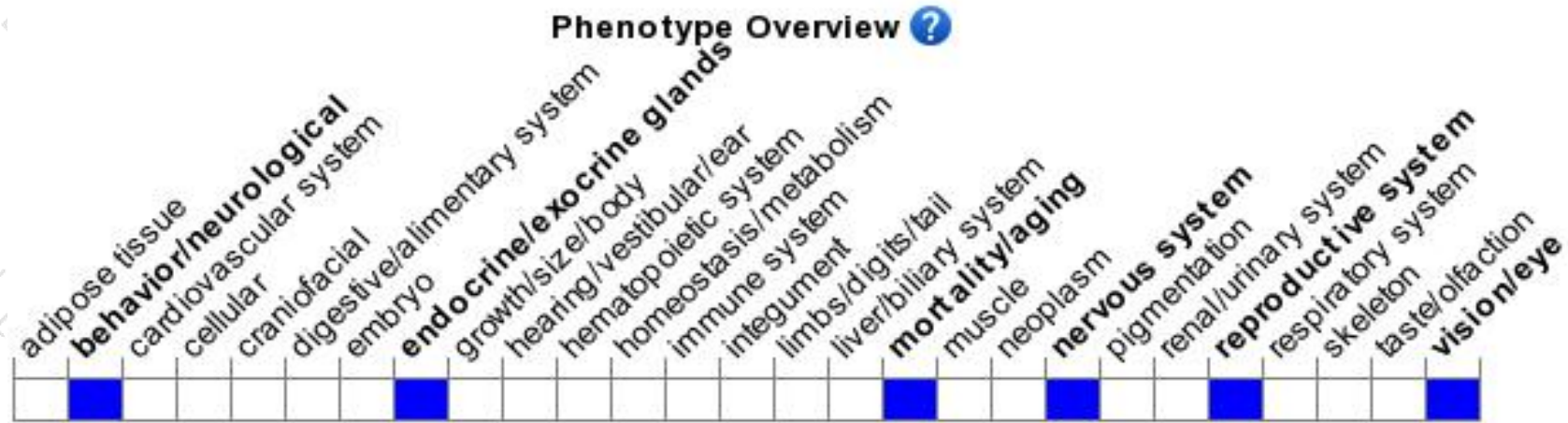
Genomic location distribution



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, Homozygous mutant mice are grossly phenotypically normal. Mice older than 12 months will exhibit sporadic seizures.

If you have any questions, you are welcome to inquire.

Tel: 400-9660890

