Unc13a Cas9-CKO Strategy

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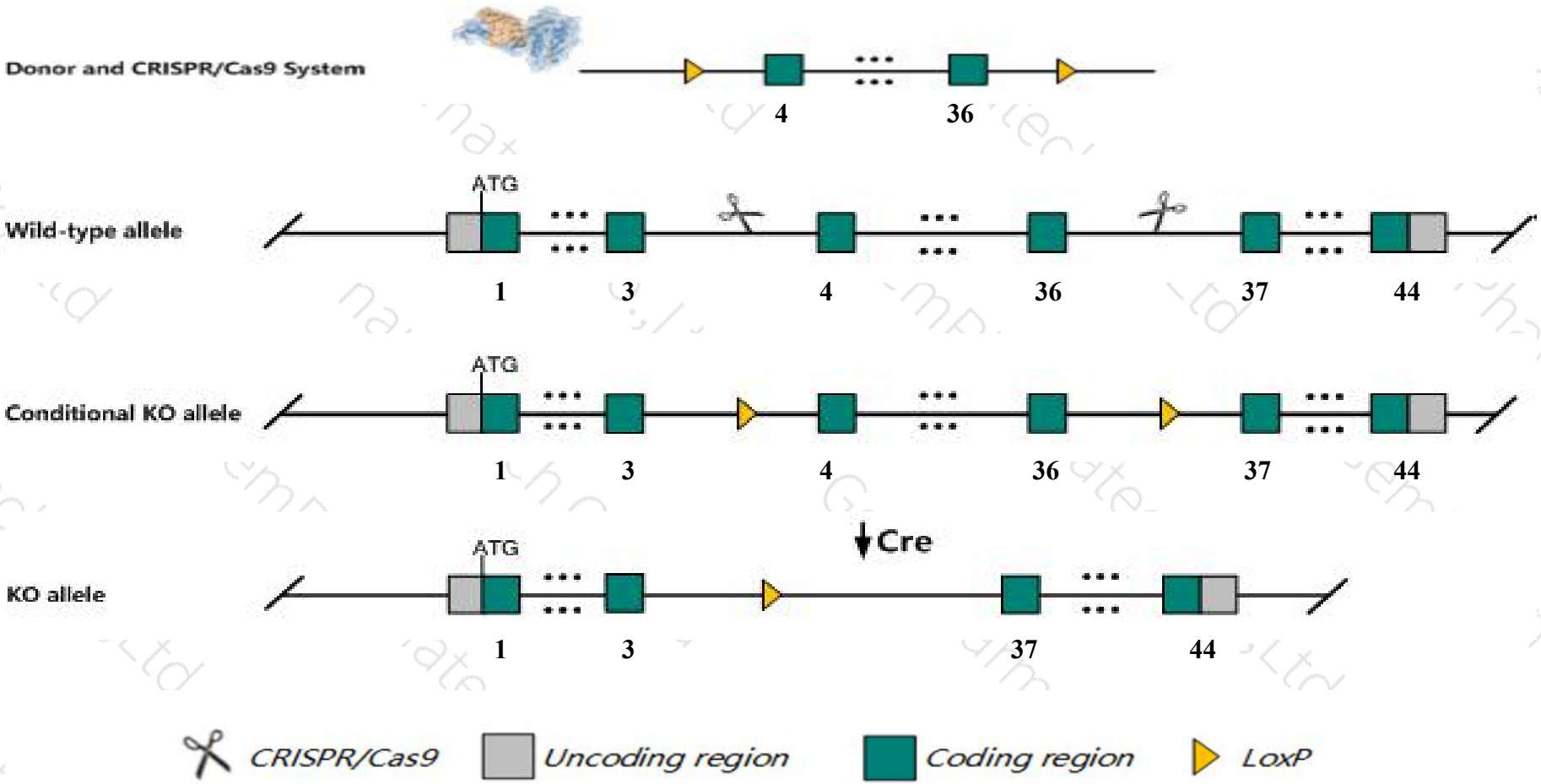
Project Overview

Project Name	***Unc13a***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Unc13a* gene. The schematic diagram is as follows:



Technical routes

- The *Unc13a* gene has 8 transcripts. According to the structure of *Unc13a* gene, exon4-exon36 of *Unc13a-201* (ENSMUST00000030170.14) transcript is recommended as the knockout region.The region contains 4078bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Unc13a* 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.

Notice

- According to the existing MGI data,Homozygous mutant mice do not feed and die within hours of birth and synaptic vesicle maturation is impaired. Mice homozygous for a knock-in allele exhibit slower rate of synaptic vesicle replenishment, aberrant short-term depression and reduced recoveryfrom synaptic depression.

- The *Unc13a* gene is located on the Chr8. 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）

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Unc13a unc-13 homolog A [Mus musculus (house mouse)]

Gene ID: 382018, updated on 5-Feb-2019

Summary

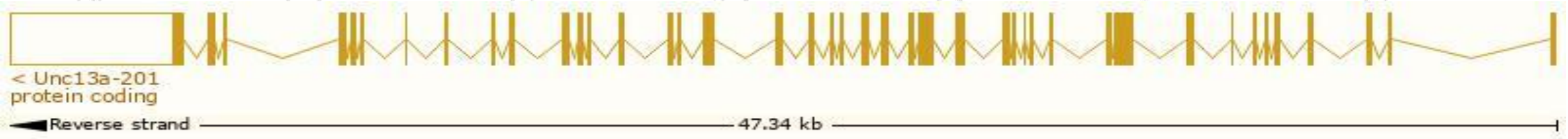
Official Symbol	Unc13a provided by MGI
Official Full Name	unc-13 homolog A provided byMGI
Primary source	MGI:MGI:3051532
See related	Ensembl:ENSMUSG00000034799
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2410078G03Rik, Munc13-1
Expression	Biased expression in cortex adult (RPKM 45.3), frontal lobe adult (RPKM 39.2) and 5 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

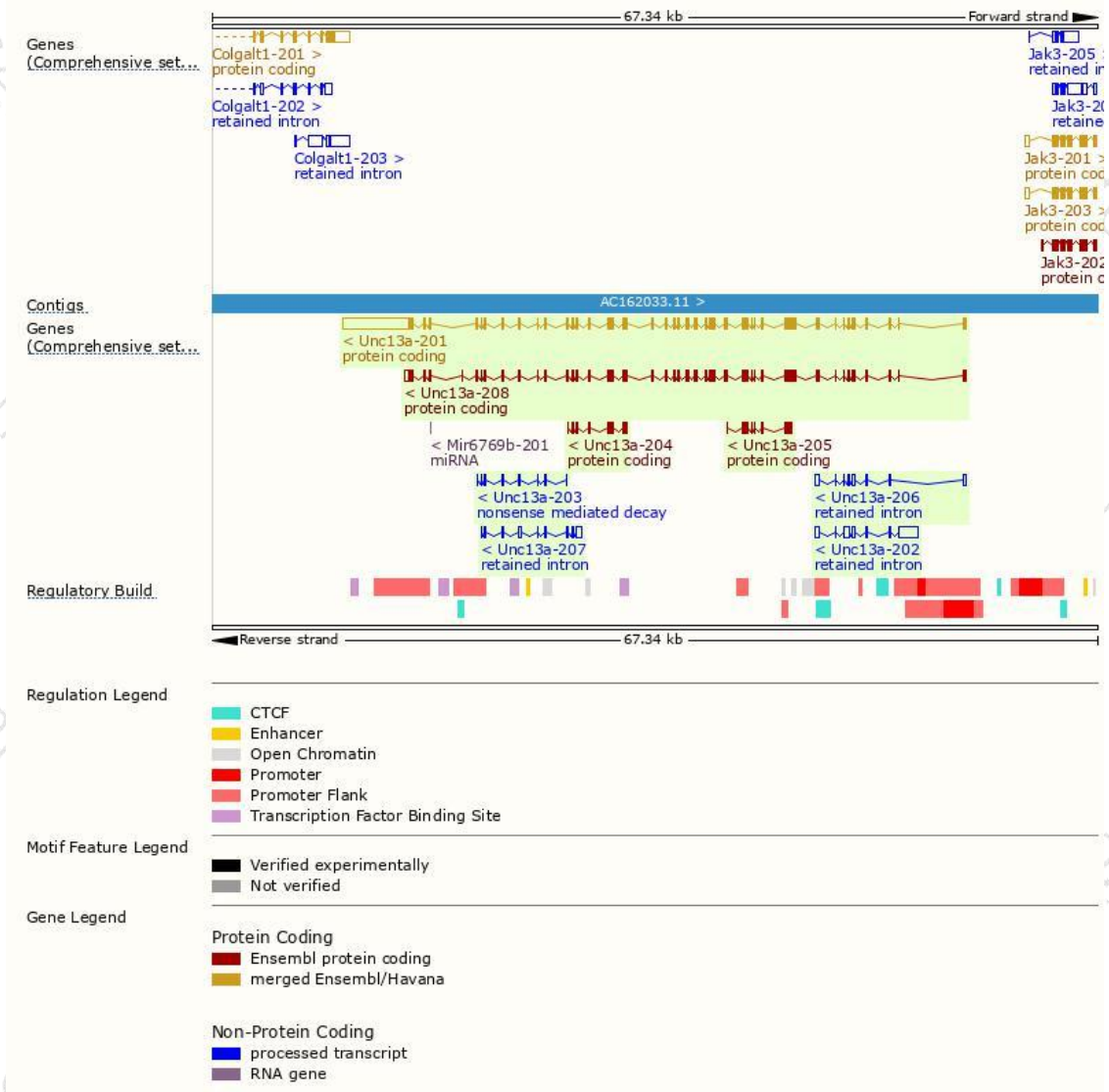
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Unc13a-201	ENSMUST00000030170.14	10255	1712aa	Protein coding	CCDS22402	Q4KUS2	TSL:5 GENCODE basic APPRIS P2
Unc13a-208	ENSMUST00000177517.7	5654	1731aa	Protein coding	-	H3BJZ7	TSL:5 GENCODE basic APPRIS ALT2
Unc13a-205	ENSMUST00000176426.1	761	254aa	Protein coding	-	H3BJL3	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
Unc13a-204	ENSMUST00000176127.1	721	241aa	Protein coding	-	H3BKY4	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
Unc13a-203	ENSMUST00000175909.7	390	17aa	Nonsense mediated decay	-	H3BKU4	CDS 5' incomplete TSL:5
Unc13a-202	ENSMUST00000175780.1	2569	No protein	Retained intron	-	-	TSL:2
Unc13a-207	ENSMUST00000177032.1	1136	No protein	Retained intron	-	-	TSL:3
Unc13a-206	ENSMUST00000176777.7	944	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Unc13a-201* transcript,The transcription is shown below

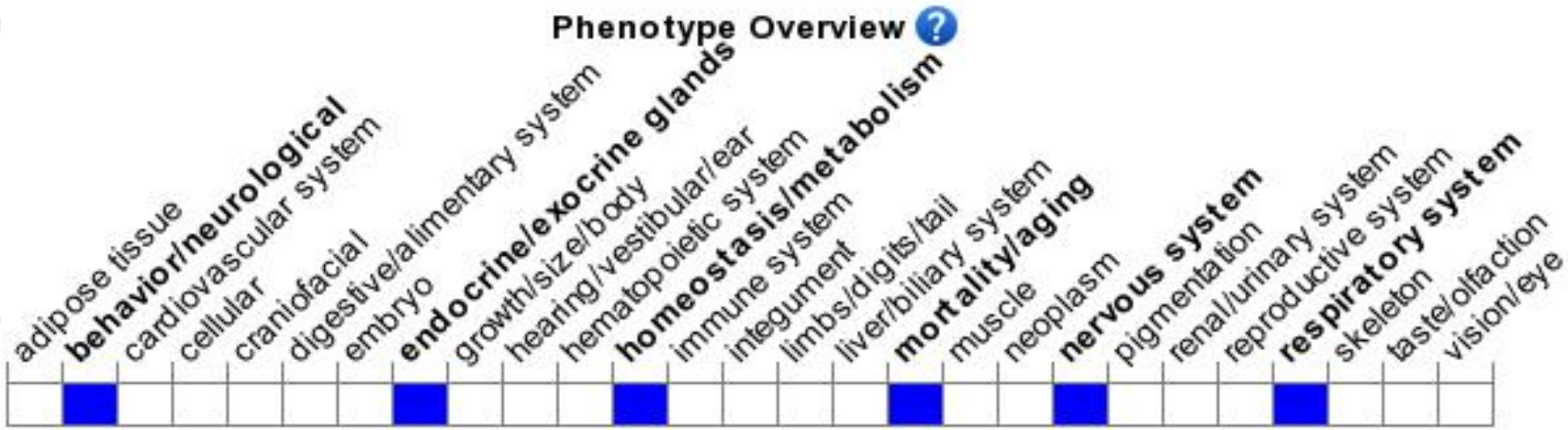


Genomic location distribution



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/).

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

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