

Sema6a Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Sema6a

Project type

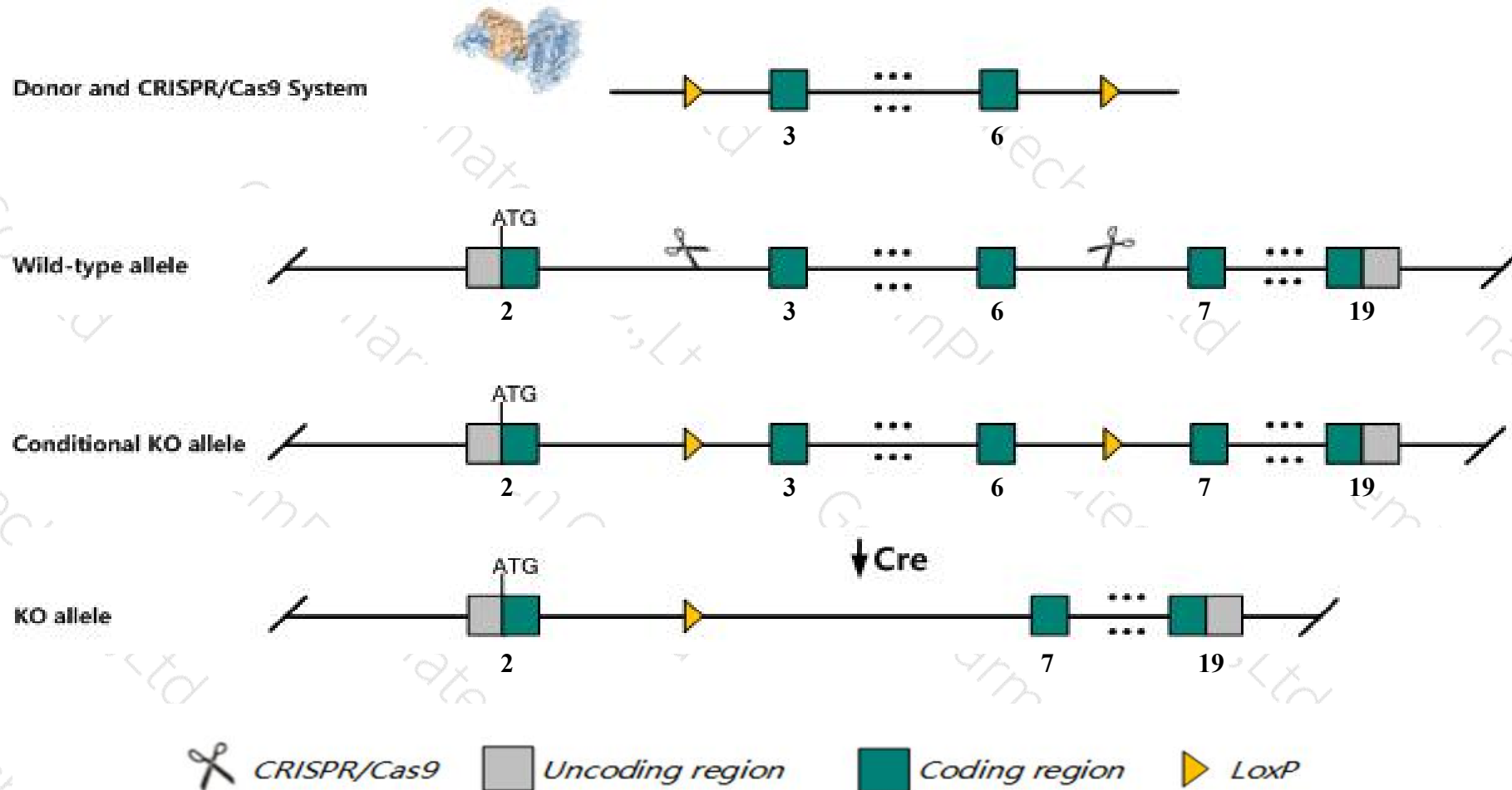
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sema6a* gene. The schematic diagram is as follows:



Technical routes

- The *Sema6a* gene has 10 transcripts. According to the structure of *Sema6a* gene, exon3-exon6 of *Sema6a-201* (ENSMUST00000019791.13) transcript is recommended as the knockout region. The region contains 344bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Sema6a* 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, Mice homozygous for a gene trapped allele exhibit defects in lamina-specific neurite stratification of specific retinal neuron subtypes and disruption of the dendritic plexus organization of On but not Off starburst amacrine cells.
- Transcript *Sema6a*-204 CDS is incomplete, whether it will be affected is unknown.
- The *Sema6a* gene is located on the Chr18. 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.

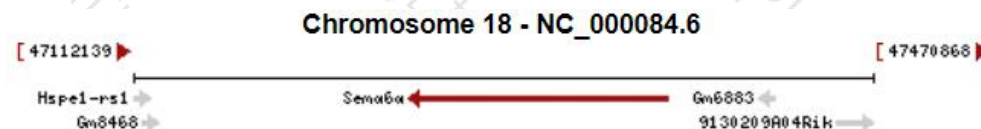
Gene information (NCBI)

Sema6a sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A [*Mus musculus* (house mouse)]

Gene ID: 20358, updated on 22-Oct-2019

Summary

Official Symbol	Sema6a provided by MGI
Official Full Name	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A provided by MGI
Primary source	MGI:MGI:1203727
See related	Ensembl:ENSMUSG00000019647
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	Vla; Semaq; Al851735; Sema6A-1; A730020P05Rik
Expression	Broad expression in limb E14.5 (RPKM 13.8), lung adult (RPKM 13.8) and 23 other tissues See more
Orthologs	human all

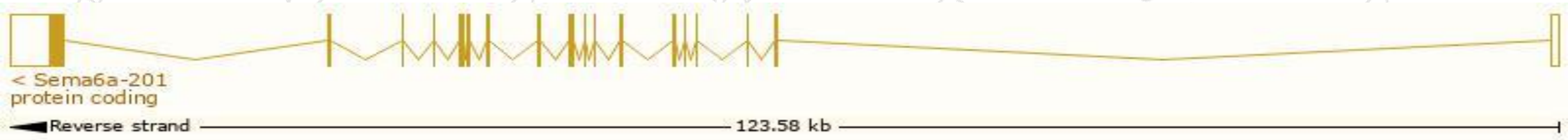


Transcript information (Ensembl)

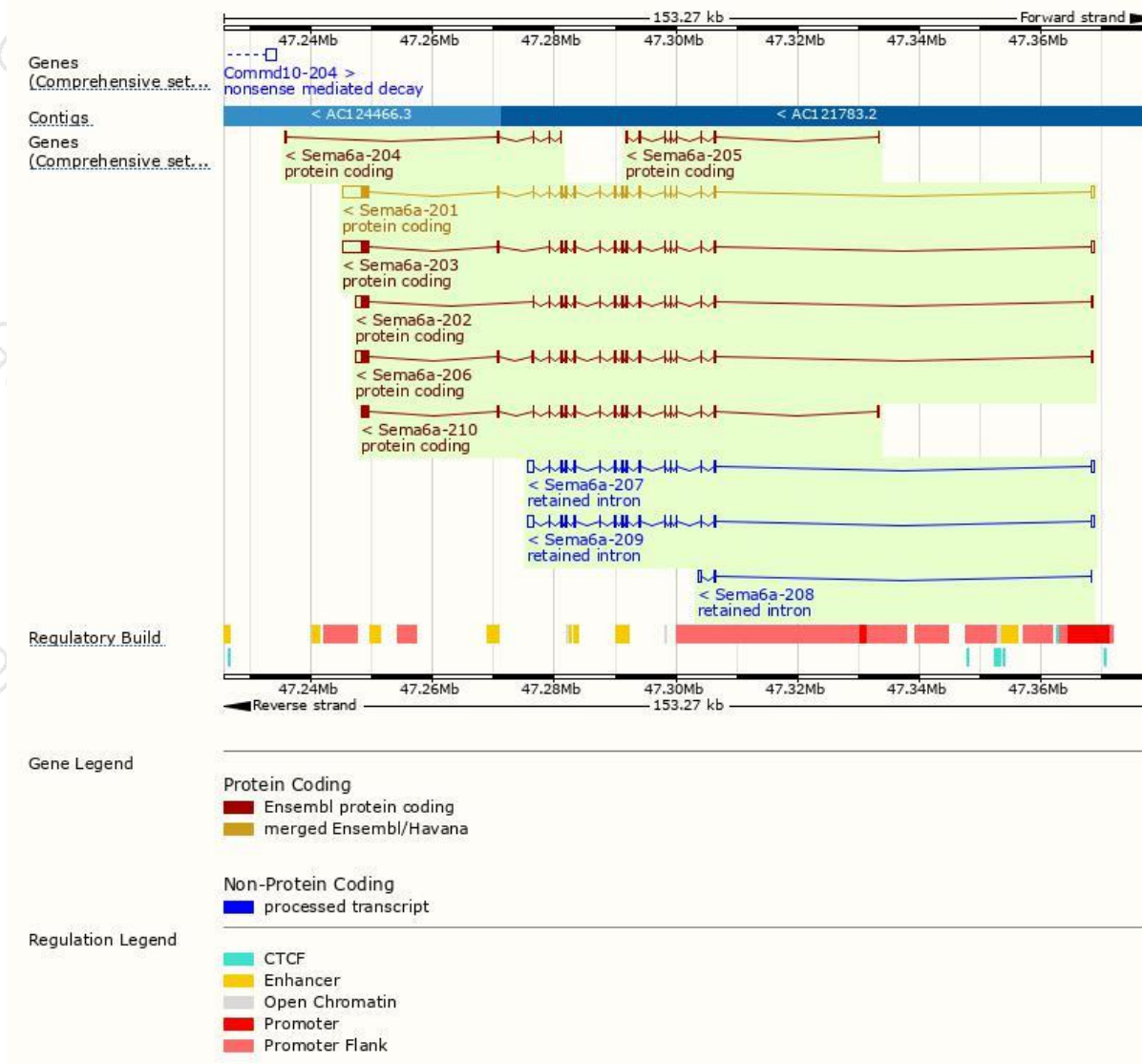
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sema6a-201	ENSMUST00000019791.13	6856	1031aa	Protein coding	CCDS37813	O35464	TSL:1 GENCODE basic APPRIS P3
Sema6a-202	ENSMUST00000076043.12	4123	976aa	Protein coding	CCDS79642	O35464	TSL:1 GENCODE basic APPRIS ALT1
Sema6a-210	ENSMUST00000156422.7	3291	1031aa	Protein coding	CCDS37813	O35464	TSL:5 GENCODE basic APPRIS P3
Sema6a-203	ENSMUST00000115449.8	6815	1005aa	Protein coding	-	K3W4S5	TSL:5 GENCODE basic
Sema6a-206	ENSMUST00000135790.7	4339	1048aa	Protein coding	-	D3YWM8	TSL:5 GENCODE basic APPRIS ALT1
Sema6a-205	ENSMUST00000126684.1	740	216aa	Protein coding	-	D3YZJ8	CDS 3' incomplete TSL:3
Sema6a-204	ENSMUST00000123228.7	652	111aa	Protein coding	-	F6WLW8	CDS 5' incomplete TSL:5
Sema6a-209	ENSMUST00000151382.7	3328	No protein	Retained intron	-	-	TSL:1
Sema6a-207	ENSMUST00000141224.7	3225	No protein	Retained intron	-	-	TSL:1
Sema6a-208	ENSMUST00000144223.1	717	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Sema6a-201* transcript,The transcription is shown below



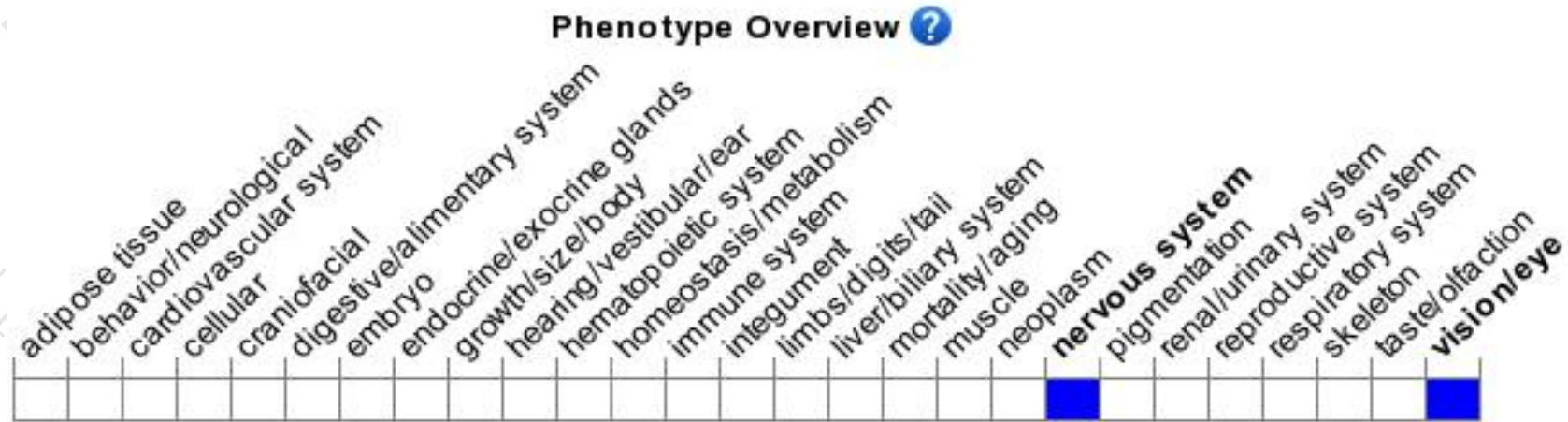
Genomic location distribution



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, Mice homozygous for a gene trapped allele exhibit defects in lamina-specific neurite stratification of specific retinal neuron subtypes and disruption of the dendritic plexus organization of On but not Off starburst amacrine cells.

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

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