

Sema3e Cas9-CKO Strategy

Designer: Ruirui Zhang

Reviewer: Xueting Zhang

Design Date: 2023-11-21

Overview

Target Gene Name

• Sema3e

Project Type

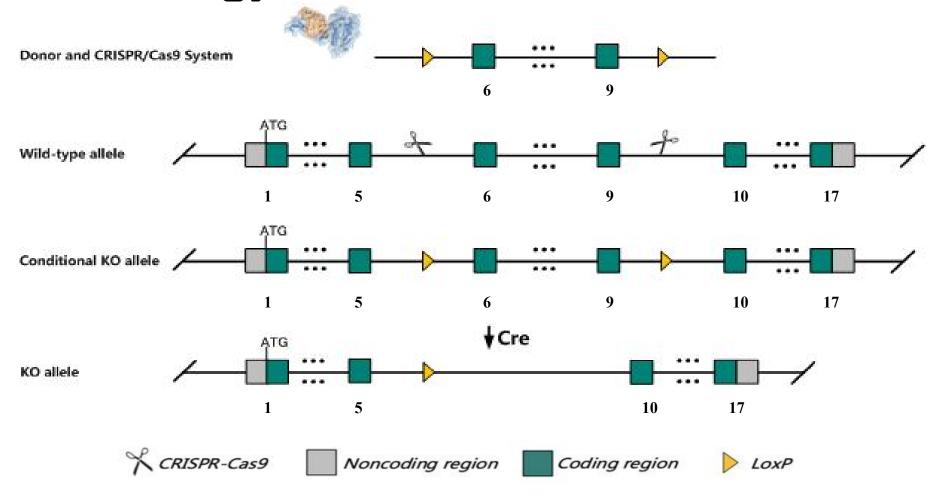
• Cas9-CKO

Genetic Background

• C57BL/6JGpt



Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Sema3e gene.



Technical Information

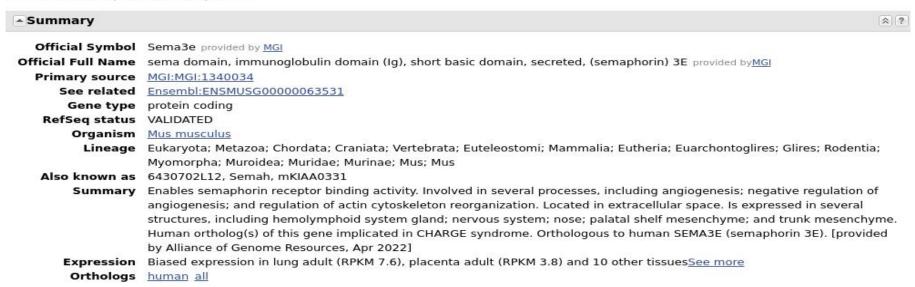
- The *Sema3e* gene has 3 transcripts. According to the structure of *Sema3e* gene, exon6-exon9 of *Sema3e*-201 (ENSMUST00000073957.8) transcript is recommended as the knockout region. The region contains 448bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Sema3e* 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 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.
- 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.



Gene Information

Sema3e sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E [Mus musculus (house mouse)]

Gene ID: 20349, updated on 12-Apr-2023



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

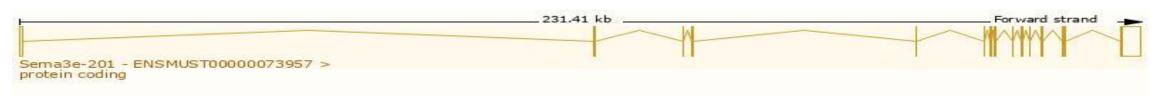


Transcript Information

The gene has 3 transcripts, all transcripts are shown below:

Transcript ID ENSMUST00000073957.8		bp 🍦	7.000 (Cont.)	Biotype Protein coding	UniProt Match P70275 ☑	Flags			
		6861				Ensembl Canonical	GENCODE basic	APPRIS P1	TSL:1
ENSMUST00000130116.2	Sema3e-202	5206	No protein	Retained intron); -	TSL:2			
ENSMUST00000199698.2	Sema3e-203	1849	No protein	Retained intron	(1 4 8)	TSL:NA			

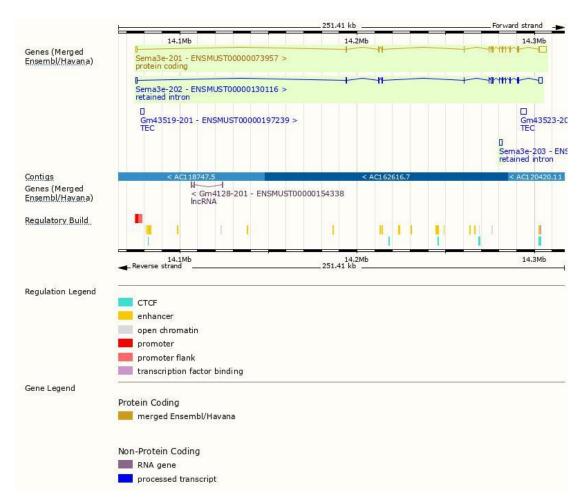
The strategy is based on the design of *Sema3e*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



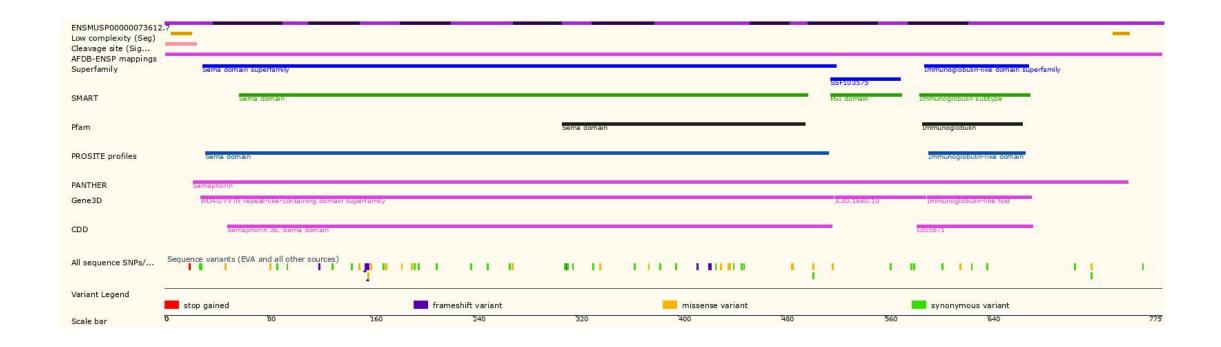
Genomic Information





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

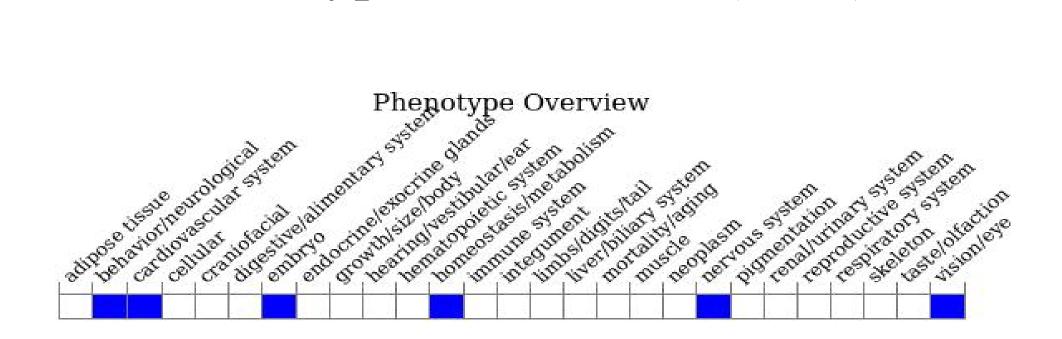
Protein Information





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

Mouse Phenotype Information (MGI)



• Homozygous null mice display abnormal intersomitic vacular development and loss of the normal segmented somite pattern. Homozygous mutants for another allele have Bergmeister papillae on the surface of the optic disc.



Source: https://www.informatics.jax.org

Important Information

- *Sema3e* is located on Chr5. 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 risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

