Sema3f Cas9-KO Strategy

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Project Overview

Project Name	*Sema3f*
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sema3f* gene. The schematic diagram is as follows:

Technical routes

➢ The *Sema3f* gene has 12 transcripts. According to the structure of *Sema3f* gene, exon3-exon11 of *Sema3f-201* (ENSMUST00000080560.8) transcript is recommended as the knockout region.The region contains 1028bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Sema3f* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice

➢ According to the existing MGI data,Inactivation of this locus results in neuronal defects including impaired CNS axon pathfinding, and PNS and limbic system circuitry. Mice homozygous for a knock-out allele exhibit increased lymphatic branching complexity and LEC numbers.

➢ The effect on transcript *Sema3f*-205&206&211 is unknown.

➢ Transcript *Sema3f*-202&210&212 may not be affected.

➢ The knockout region is near to the N-terminal of *Gnat1* gene,this strategy may influence the regulatory function of the N-terminal of *Gnat1* gene.

➢ The *Sema3f* gene is located on the Chr9. 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）

Sema3f sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F [*Mus musculus* (house mouse)]

Gene ID: 20350, updated on 5-Nov-2019

▲ Summary

Official Symbol	Sema3f provided by MGI
Official Full Name	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3F provided by MGI
Primary source	MGI:MGI:1096347
See related	Ensembl:ENSMUSG00000034684
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	Sema4; Semak
Expression	Broad expression in lung adult (RPKM 77.6), limb E14.5 (RPKM 39.8) and 19 other tissues See more
Orthologs	human all

▲ Genomic context

Location: 9; 9 F1

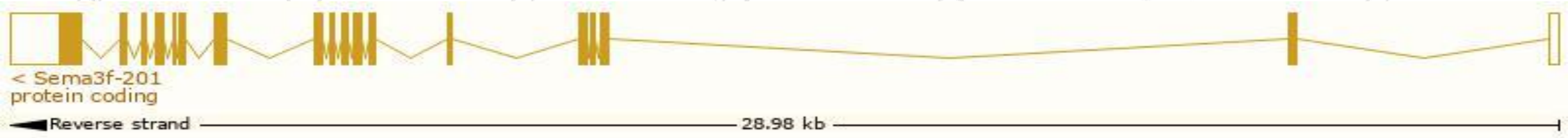See Sema3f in Genome Data Viewer

Exon count: 21

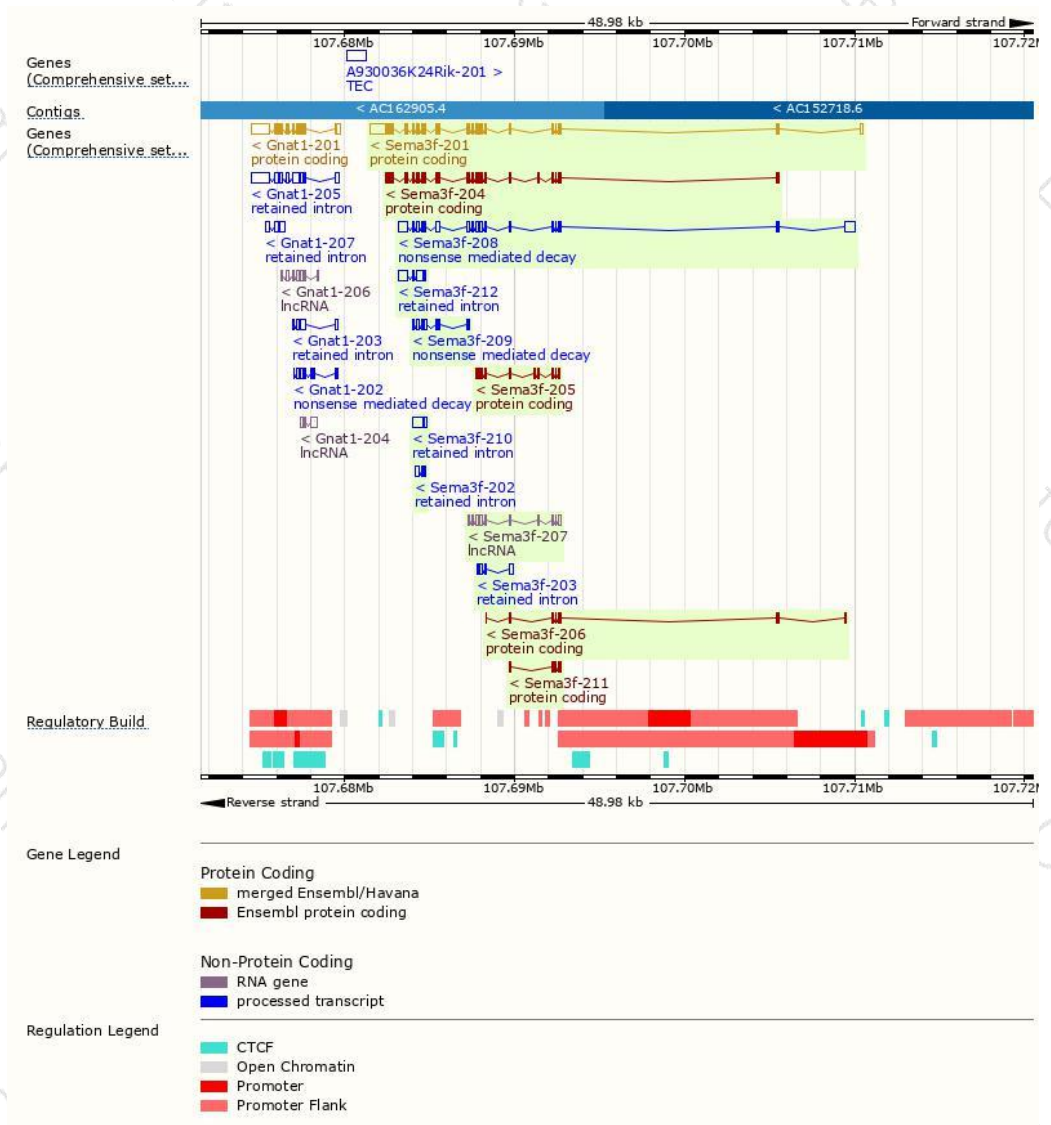| Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	9	NC_000075.6 (107681499..107710475, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	9	NC_000075.5 (107583833..107612806, complement)

Transcript information（Ensembl）

The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sema3f-201	ENSMUST00000080560.8	3395	754aa	Protein coding	CCDS23505	O88632	TSL:1 GENCODE basic APPRIS P3
Sema3f-204	ENSMUST00000192727.5	2383	785aa	Protein coding	CCDS81069	O88632	TSL:1 GENCODE basic APPRIS ALT2
Sema3f-205	ENSMUST00000192783.5	906	284aa	Protein coding	-	A0A0A6YWS0	CDS 5' incomplete TSL:3
Sema3f-206	ENSMUST00000193108.5	685	191aa	Protein coding	-	A0A0A6YX80	CDS 3' incomplete TSL:5
Sema3f-211	ENSMUST00000195023.2	443	148aa	Protein coding	-	A0A0A6YW11	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
Sema3f-208	ENSMUST00000194039.5	2972	185aa	Nonsense mediated decay	-	A0A0A6YY06	TSL:1
Sema3f-209	ENSMUST00000194424.5	711	116aa	Nonsense mediated decay	-	A0A0A6YXX2	CDS 5' incomplete TSL:3
Sema3f-212	ENSMUST00000195267.5	1084	No protein	Retained intron	-	-	TSL:3
Sema3f-210	ENSMUST00000194846.1	695	No protein	Retained intron	-	-	TSL:3
Sema3f-203	ENSMUST00000192712.1	581	No protein	Retained intron	-	-	TSL:3
Sema3f-202	ENSMUST00000192157.1	376	No protein	Retained intron	-	-	TSL:3
Sema3f-207	ENSMUST00000193665.5	1034	No protein	lncRNA	-	-	TSL:5

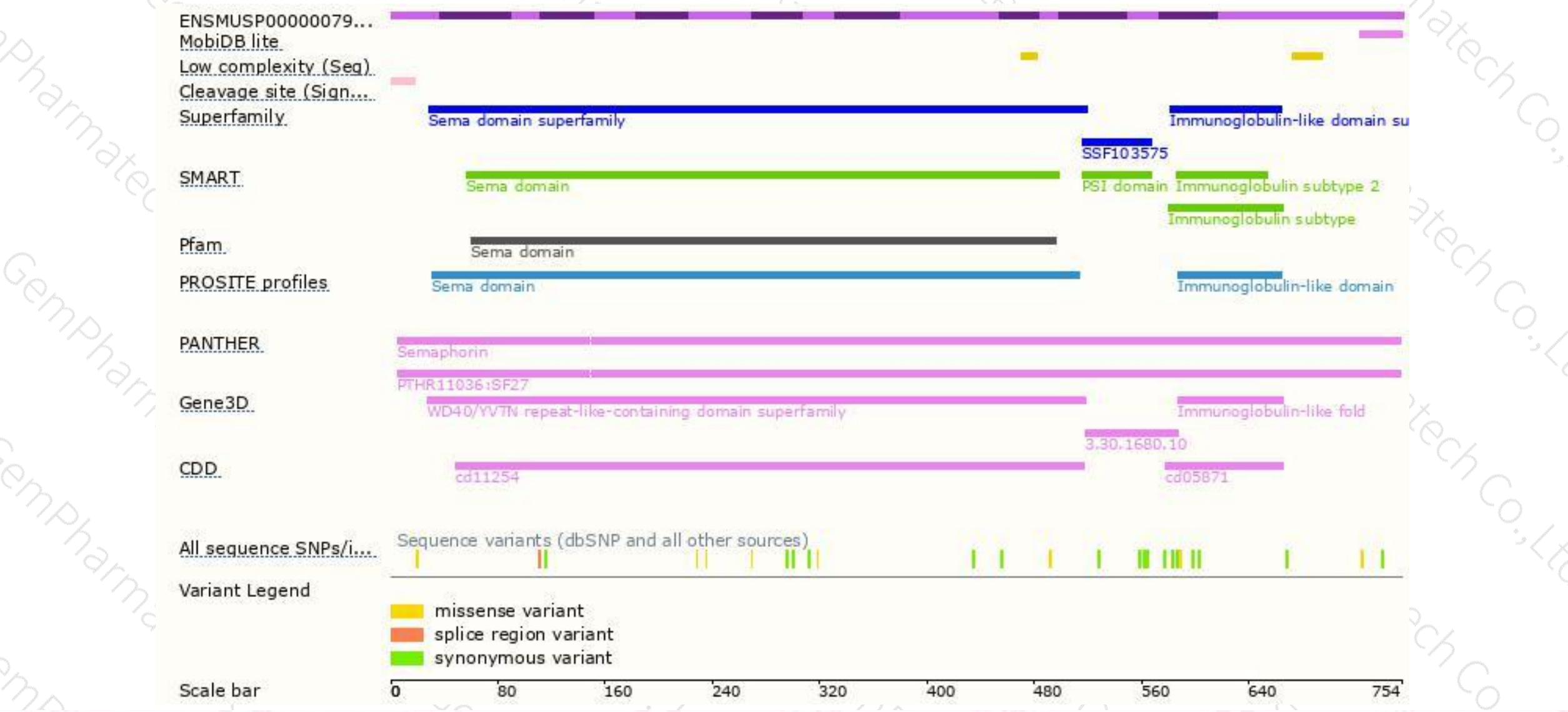The strategy is based on the design of *Sema3f-201* transcript,The transcription is shown below

< Sema3f-201
protein coding

◄— Reverse strand

28.98 kb

Genomic location distribution

Protein domain

ENSMUSP00000079...	
MobiDB lite	
Low complexity (Seg)	
Cleavage site (Sign...	
Superfamily	Sema domain superfamily Immunoglobulin-like domain su
	SSF103575
SMART	Sema domain PSI domain Immunoglobulin subtype 2
	Immunoglobulin subtype
Pfam	Sema domain
PROSITE profiles	Sema domain Immunoglobulin-like domain
PANTHER	Semaphorin
	PTHR11036:SF27
Gene3D	WD40/YVTN repeat-like-containing domain superfamily Immunoglobulin-like fold
	3.30.1680.10
CDD	cd11254 cd05871
All sequence SNPs/i...	Sequence variants (dbSNP and all other sources)
Variant Legend	

Variant Legend

- ▬ missense variant
- ▬ splice region variant
- ▬ synonymous variant

Scale bar

| 0 | 80 | 160 | 240 | 320 | 400 | 480 | 560 | 640 | 754 |

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,Inactivation of this locus results in neuronal defects including impaired CNS axon pathfinding, and PNS and limbic system circuitry. Mice homozygous for a knock-out allele exhibit increased lymphatic branching complexity and LEC numbers.

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