

Sema4d Cas9-CKO Strategy

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Reviewer:

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Design Date:

2019-9-28

Project Overview

Project Name

Sema4d

Project type

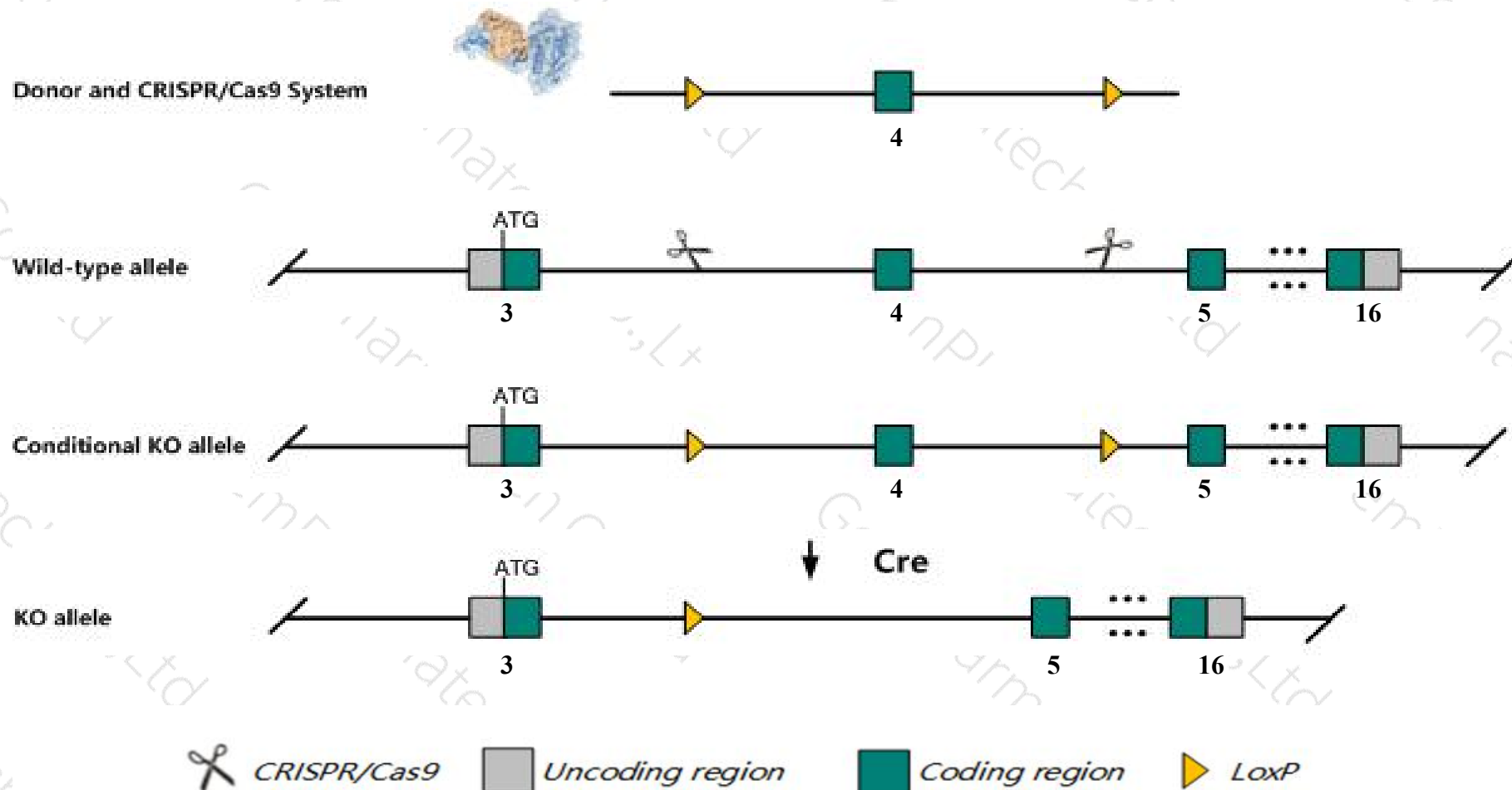
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sema4d* gene. The schematic diagram is as follows:



Technical routes

- The *Sema4d* gene has 14 transcripts. According to the structure of *Sema4d* gene, exon4 of *Sema4d-201* (ENSMUST00000021900.13) transcript is recommended as the knockout region. The region contains 146bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Sema4d* 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 disruptions in this gene display functional defects in their immune system but are normal in other systems of the body.
- The *Sema4d* gene is located on the Chr13. 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)

Sema4d sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D [Mus musculus (house mouse)]

Gene ID: 20354, updated on 9-Mar-2019

Summary



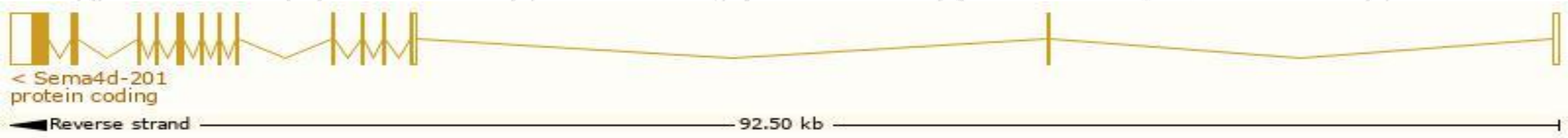
Official Symbol	Sema4d provided by MGI
Official Full Name	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D provided by MGI
Primary source	MGI:MGI:109244
See related	Ensembl:ENSMUSG000000021451
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CD100, Semacl2, Semaj, Semcl2, coll-4
Expression	Broad expression in thymus adult (RPKM 32.3), spleen adult (RPKM 22.4) and 23 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

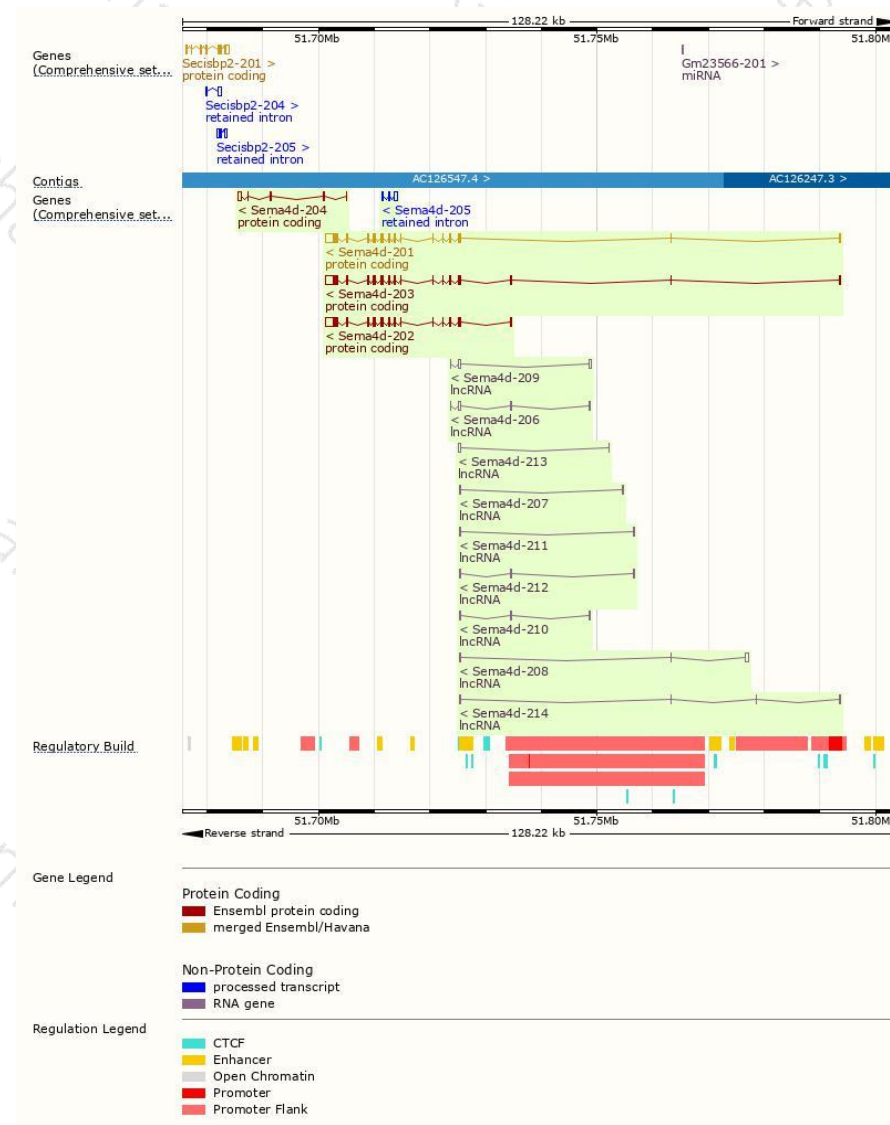
The gene has 14 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Sema4d-201	ENSMUST00000021900.13	4482	861aa	Protein coding	CCDS26514	Q09126	TSL:1 GENCODE basic APPRIS P1
Sema4d-203	ENSMUST00000110040.8	4472	861aa	Protein coding	CCDS26514	Q09126	TSL:1 GENCODE basic APPRIS P1
Sema4d-202	ENSMUST00000110039.1	4415	861aa	Protein coding	CCDS26514	Q09126	TSL:1 GENCODE basic APPRIS P1
Sema4d-204	ENSMUST00000110042.2	1249	185aa	Protein coding	-	D3Z3P4	CDS 5' incomplete TSL:5
Sema4d-205	ENSMUST00000125511.1	745	No protein	Retained intron	-	-	TSL:2
Sema4d-208	ENSMUST00000139858.7	642	No protein	lncRNA	-	-	TSL:3
Sema4d-209	ENSMUST00000139865.7	591	No protein	lncRNA	-	-	TSL:3
Sema4d-206	ENSMUST00000138228.7	551	No protein	lncRNA	-	-	TSL:5
Sema4d-211	ENSMUST00000146238.1	498	No protein	lncRNA	-	-	TSL:2
Sema4d-214	ENSMUST00000155896.1	443	No protein	lncRNA	-	-	TSL:3
Sema4d-207	ENSMUST00000139018.1	442	No protein	lncRNA	-	-	TSL:3
Sema4d-210	ENSMUST00000143396.1	441	No protein	lncRNA	-	-	TSL:3
Sema4d-212	ENSMUST00000146922.7	405	No protein	lncRNA	-	-	TSL:2
Sema4d-213	ENSMUST00000150662.1	385	No protein	lncRNA	-	-	TSL:3

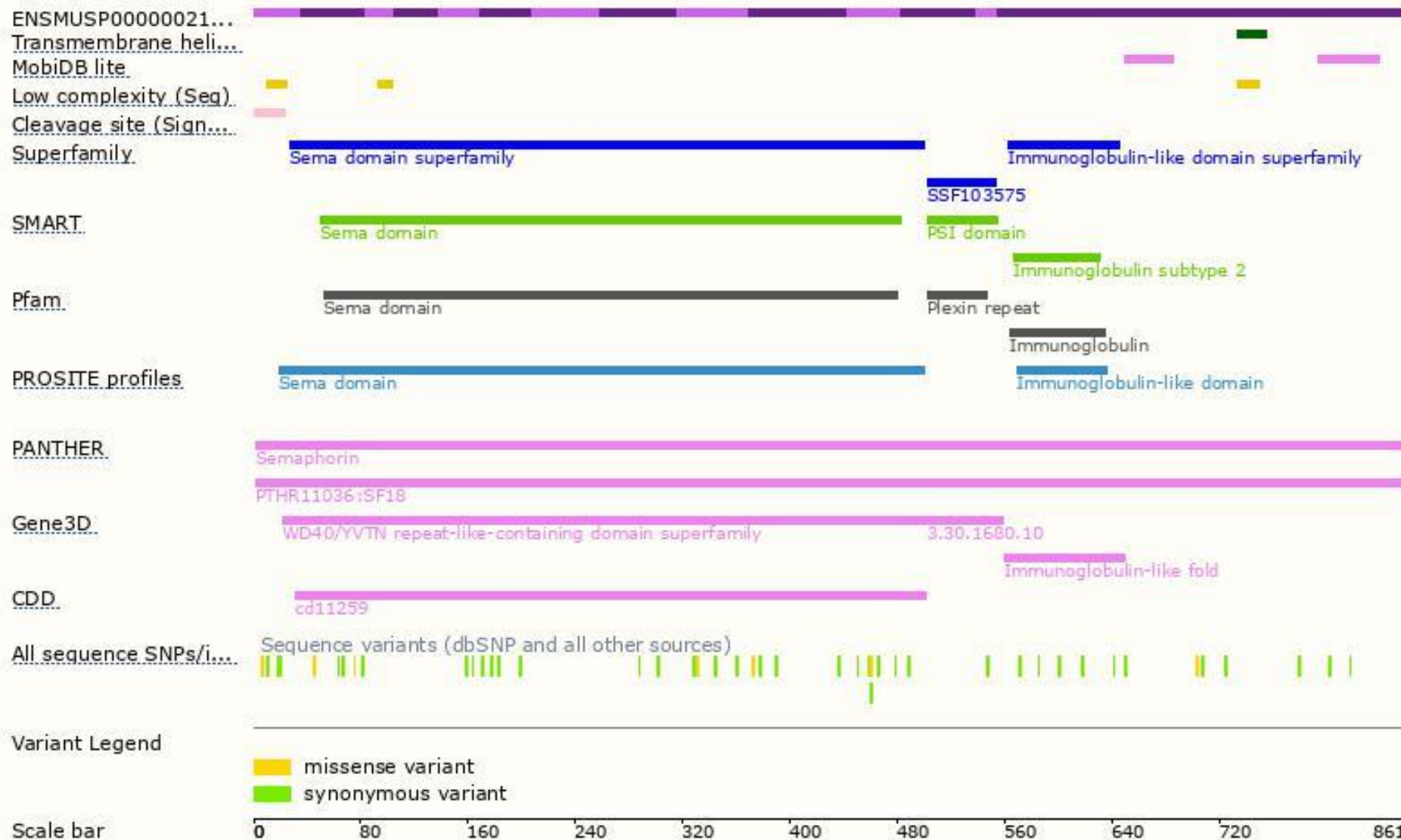
The strategy is based on the design of *Sema4d-201* transcript,The transcription is shown below



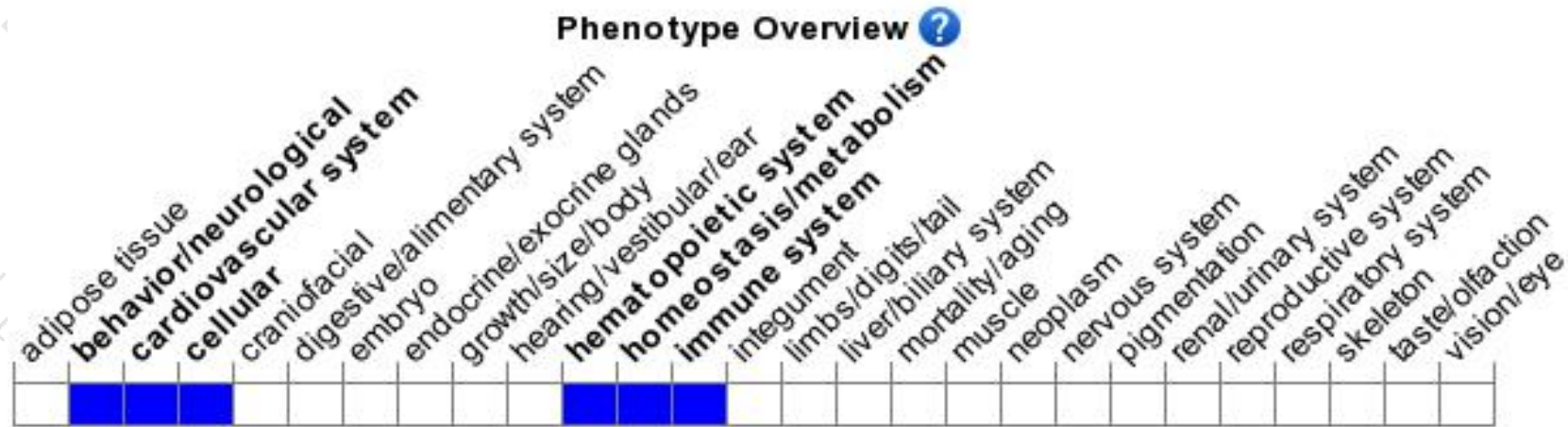
Genomic location distribution



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 disruptions in this gene display functional defects in their immune system but are normal in other systems of the body.

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

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