Sema3a Cas9-KO *Strategy*

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Project Overview

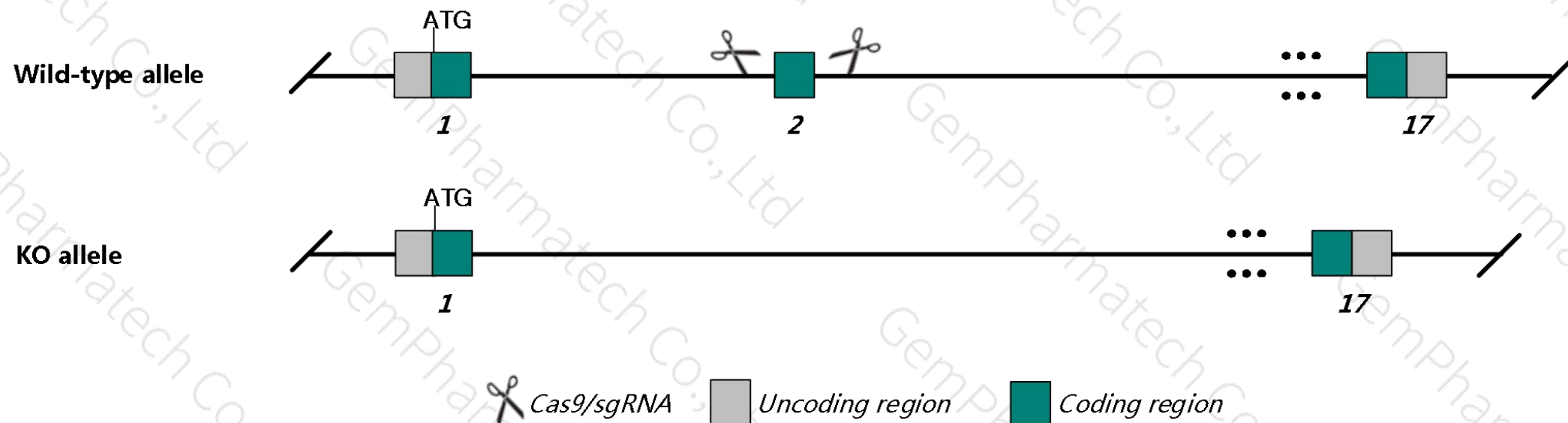|	
Project Name	*Sema3a*
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Sema3a* gene. The schematic diagram is as follows:

Technical routes

➢ The *Sema3a* gene has 9 transcripts.According to the structure of *Sema3a* gene, exon2 of *Sema3a*-201 (ENSMUST00000030714.7)transcript is recommended as the knockout region.The region contains 158bp coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Sema3a* 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，Homozygotes for targeted null mutations exhibit patterning abnormalities of sensory and sympathetic neurons, abnormal embryonic bones and cartilaginous structures, cardiac defects, and high postnatal mortality.

➢ The *Sema3a* gene is located on the Chr5. 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）

Sema3a sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A [*Mus musculus* (house mouse)]

Gene ID: 20346, updated on 13-Mar-2020

▲ Summary

|	
Official Symbol	Sema3a provided by MGI
Official Full Name	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A provided by MGI
Primary source	MGI:MGI:107558
See related	Ensembl:ENSMUSG00000028883
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	SemD; SEMA1; Semad; coll-1; Hsema-I
Expression	Broad expression in limb E14.5 (RPKM 3.0), bladder adult (RPKM 3.0) and 16 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

The gene has 9 transcripts, and all transcripts are shown below:

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags		
Sema3a-201	ENSMUST00000030714.7	5930	772aa	Protein coding	CCDS19092	O08665	TSL:1	GENCODE basic	APPRIS P1
Sema3a-202	ENSMUST00000095012.9	4085	772aa	Protein coding	CCDS19092	O08665	TSL:1	GENCODE basic	APPRIS P1
Sema3a-204	ENSMUST00000137798.7	2285	688aa	Protein coding	-	E9Q668	CDS 3' incomplete	TSL:1	
Sema3a-203	ENSMUST00000125629.7	400	72aa	Protein coding	-	D3Z2A7	CDS 3' incomplete	TSL:2	
Sema3a-205	ENSMUST00000141968.1	312	62aa	Protein coding	-	D3Z4Q3	CDS 3' incomplete	TSL:3	
Sema3a-209	ENSMUST00000200073.1	2593	No protein	Processed transcript	-	-	TSL:1		
Sema3a-207	ENSMUST00000196514.4	1954	No protein	Processed transcript	-	-	TSL:1		
Sema3a-206	ENSMUST00000195907.4	2624	No protein	Retained intron	-	-	TSL:1		
Sema3a-208	ENSMUST00000197582.4	2593	No protein	Retained intron	-	-	TSL:1		

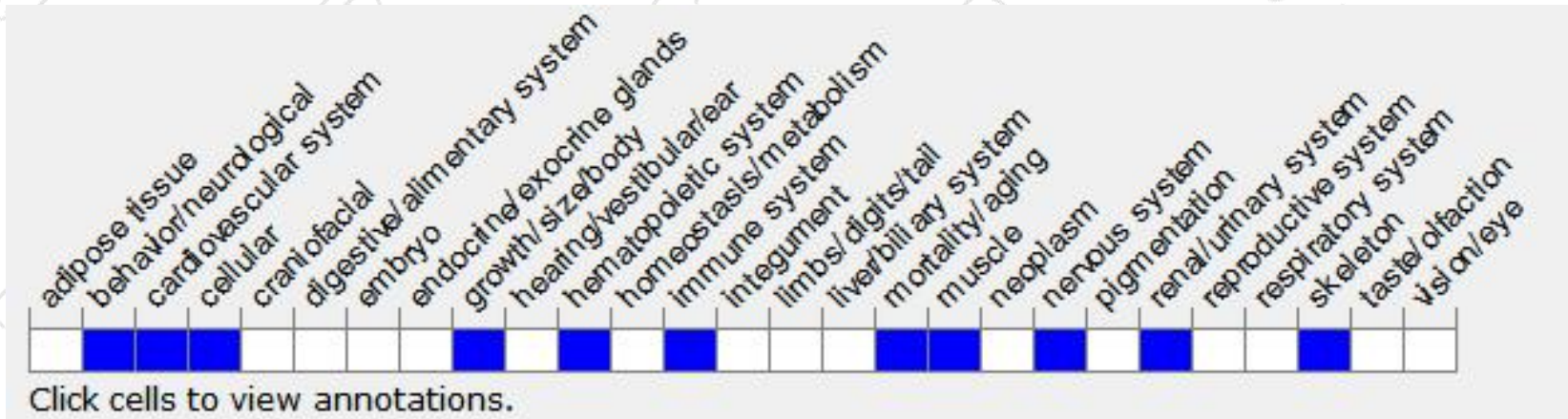The strategy is based on the design of *Sema3a*-201 transcript,The transcription is shown below
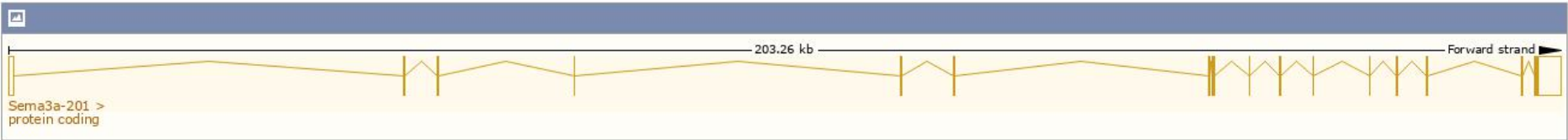
Genomic location distribution
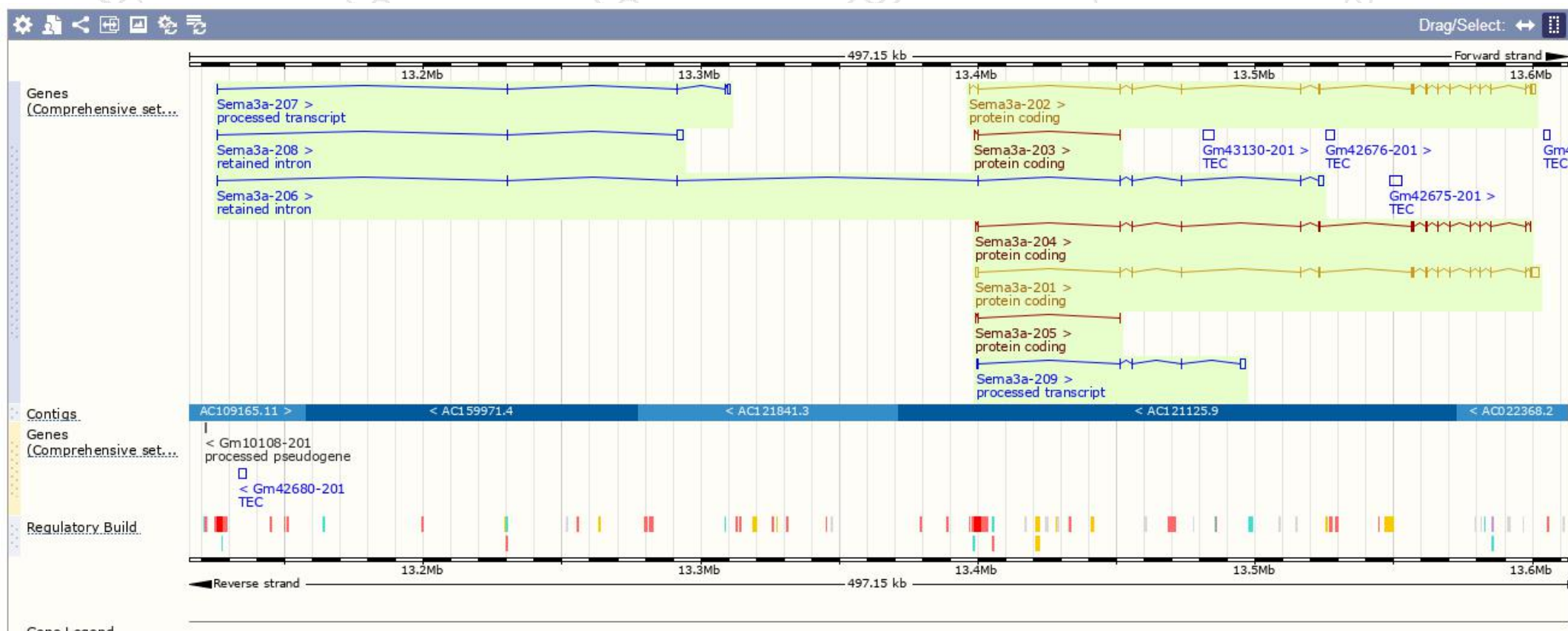
Protein domain
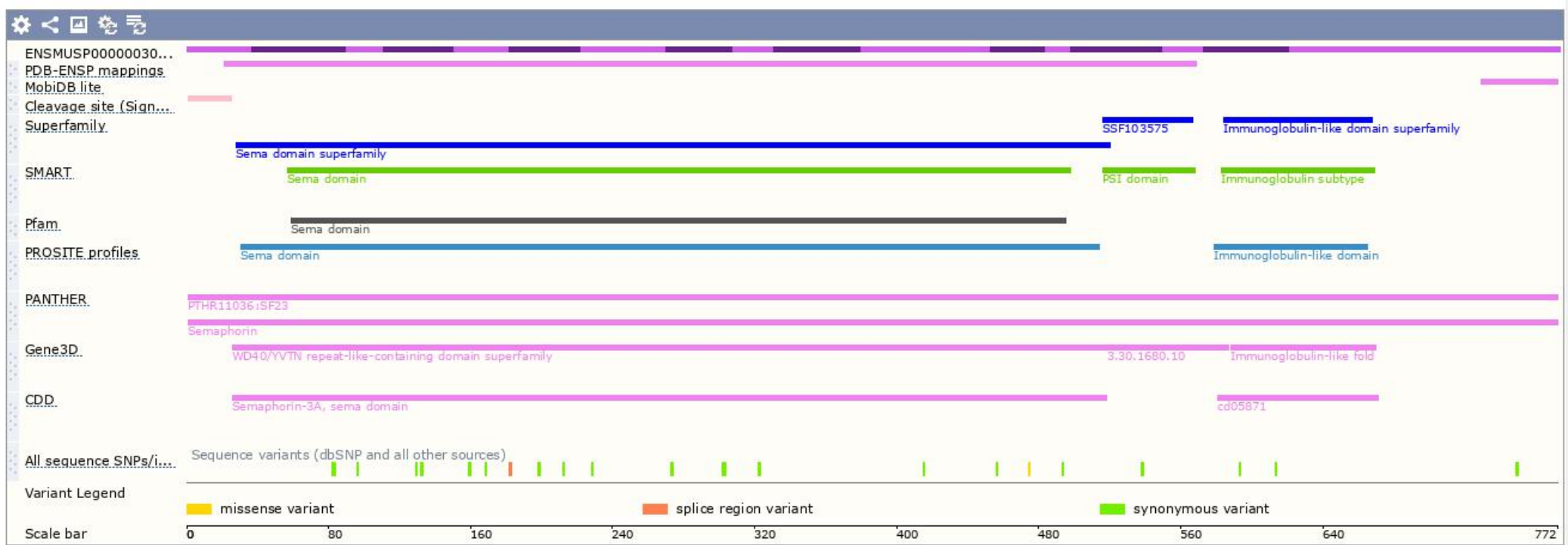
Mouse phenotype description(MGI)

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,Mutations in this locus affect cell-cycle regulation and apoptos is. Null homozygotes show high, early-onset tumor incidence; some have persistent hyaloid vasculature and cataracts. Truncated or temperature-sensitive alleles cause early aging phenotypes.

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