Susd4 Cas9-CKO Strategy

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Project Overview

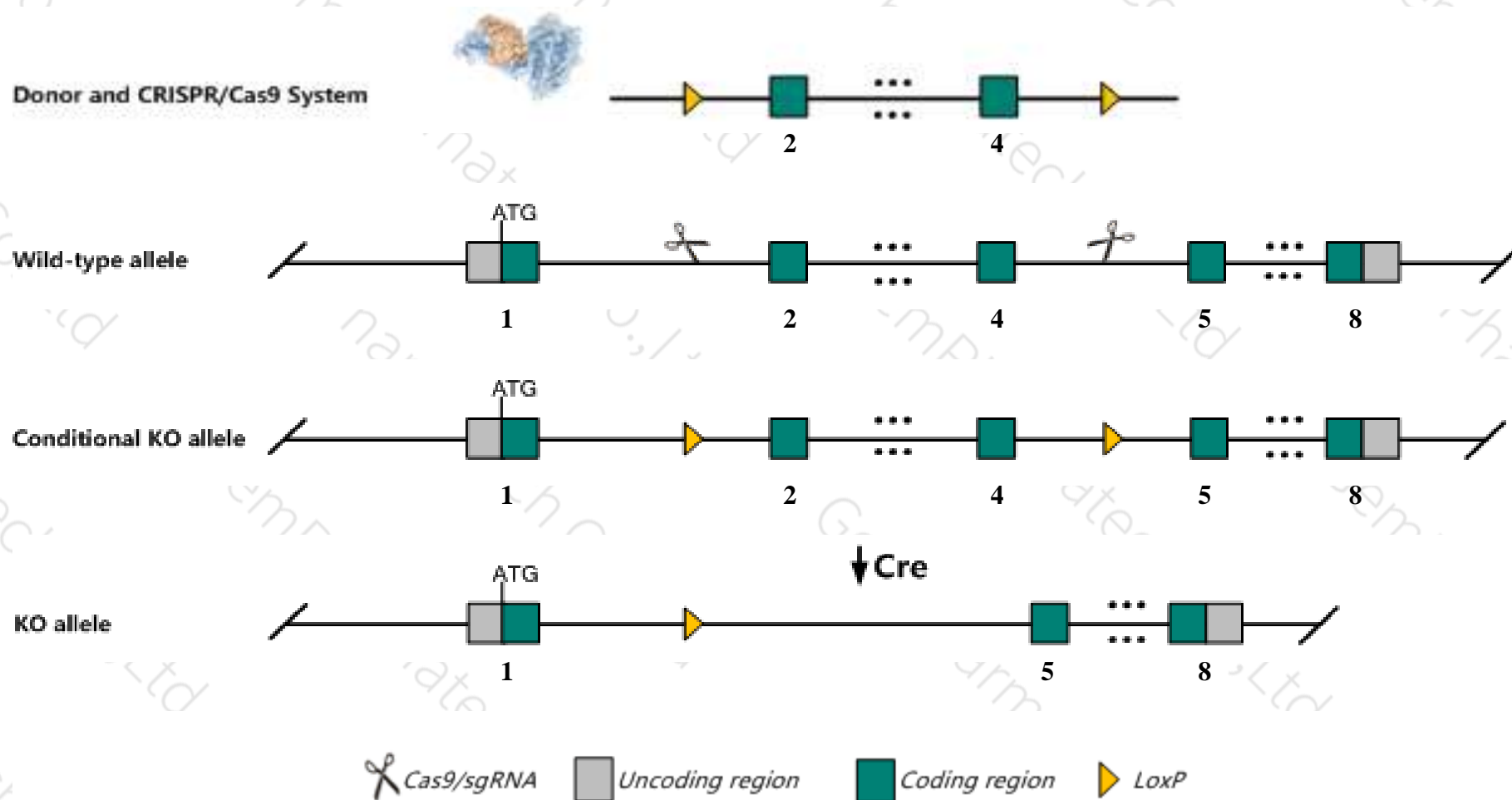|	
Project Name	*Susd4*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Susd4* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele

Cas9/sgRNA   Uncoding region   Coding region   LoxP

Technical routes

➢ The *Susd4* gene has 5 transcripts. According to the structure of *Susd4* gene, exon2-exon4 of *Susd4-201*(ENSMUST00000085724.4) transcript is recommended as the knockout region.The region contains 576bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Susd4* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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.

Notice

➢ The KO region deletes parts of the coding sequence, but does not result in frameshift.

➢ The *Susd4* gene is located on the Chr1. 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）

Susd4 sushi domain containing 4 [Mus musculus (house mouse)]

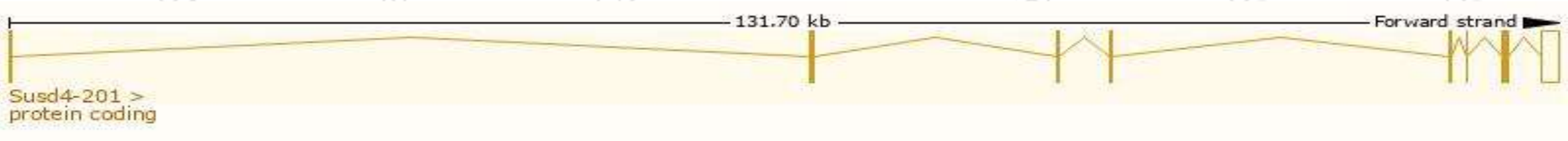Gene ID: 96935, updated on 13-Mar-2020

▲ Summary

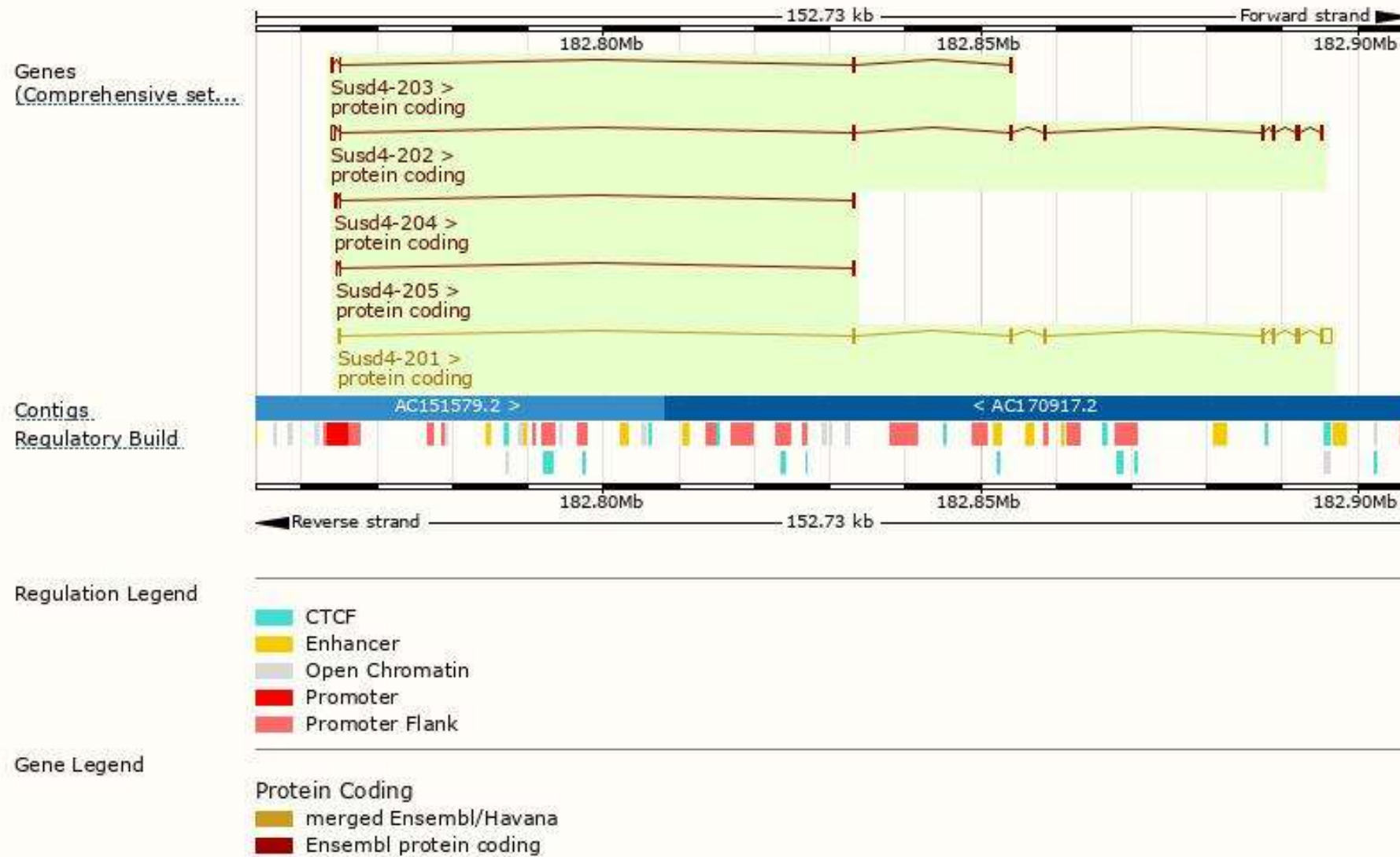|	
Official Symbol	Susd4 provided by MGI
Official Full Name	sushi domain containing 4 provided by MGI
Primary source	MGI:MGI:2138351
See related	Ensembl:ENSMUSG00000038576
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI848994, E430021N18Rik, N28096
Expression	Biased expression in frontal lobe adult (RPKM 29.8), cortex adult (RPKM 26.8) and 13 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Susd4-201	ENSMUST00000085724.4	2983	490aa	Protein coding	CCDS15590	Q8BH32	TSL:1 GENCODE basic APPRIS P1
Susd4-202	ENSMUST00000153348.7	1898	490aa	Protein coding	CCDS15590	Q8BH32	TSL:5 GENCODE basic APPRIS P1
Susd4-203	ENSMUST00000155229.6	734	148aa	Protein coding	-	A0A0A6YY90	CDS 3' incomplete TSL:3
Susd4-204	ENSMUST0000193660.5	559	104aa	Protein coding	-	A0A0A6YX15	CDS 3' incomplete TSL:5
Susd4-205	ENSMUST0000194981.1	554	113aa	Protein coding	-	A0A0A6YWB1	CDS 3' incomplete TSL:5

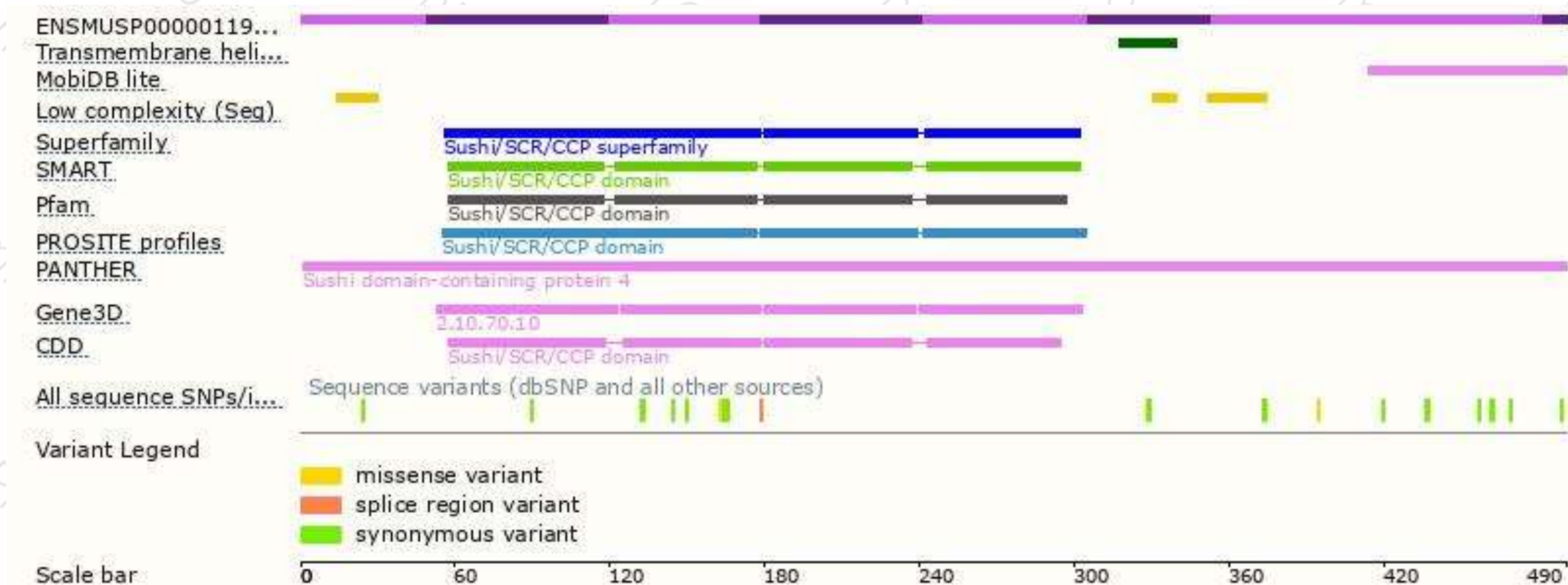The strategy is based on the design of *Susd4-201* transcript,the transcription is shown below:

- 131.70 kb -

Forward strand

Susd4-201 >
protein coding

Genomic location distribution

Protein domain

ENSMUSP00000119...	
Transmembrane heli...	
MobiDB lite	
Low complexity (Seg)	
Superfamily	Sushi/SCR/CCP superfamily
SMART	Sushi/SCR/CCP domain
Pfam	Sushi/SCR/CCP domain
PROSITE profiles	Sushi/SCR/CCP domain
PANTHER	Sushi domain-containing protein 4
Gene3D	2.10.70.10
CDD	Sushi/SCR/CCP domain
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 60 120 180 240 300 360 420 490

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