

***Vps9d1* Cas9-CKO Strategy**

Designer: Zihe Cui

Reviewer: Huimin Su

Design Date: 2020-9-11

Project Overview

Project Name

Vps9d1

Project type

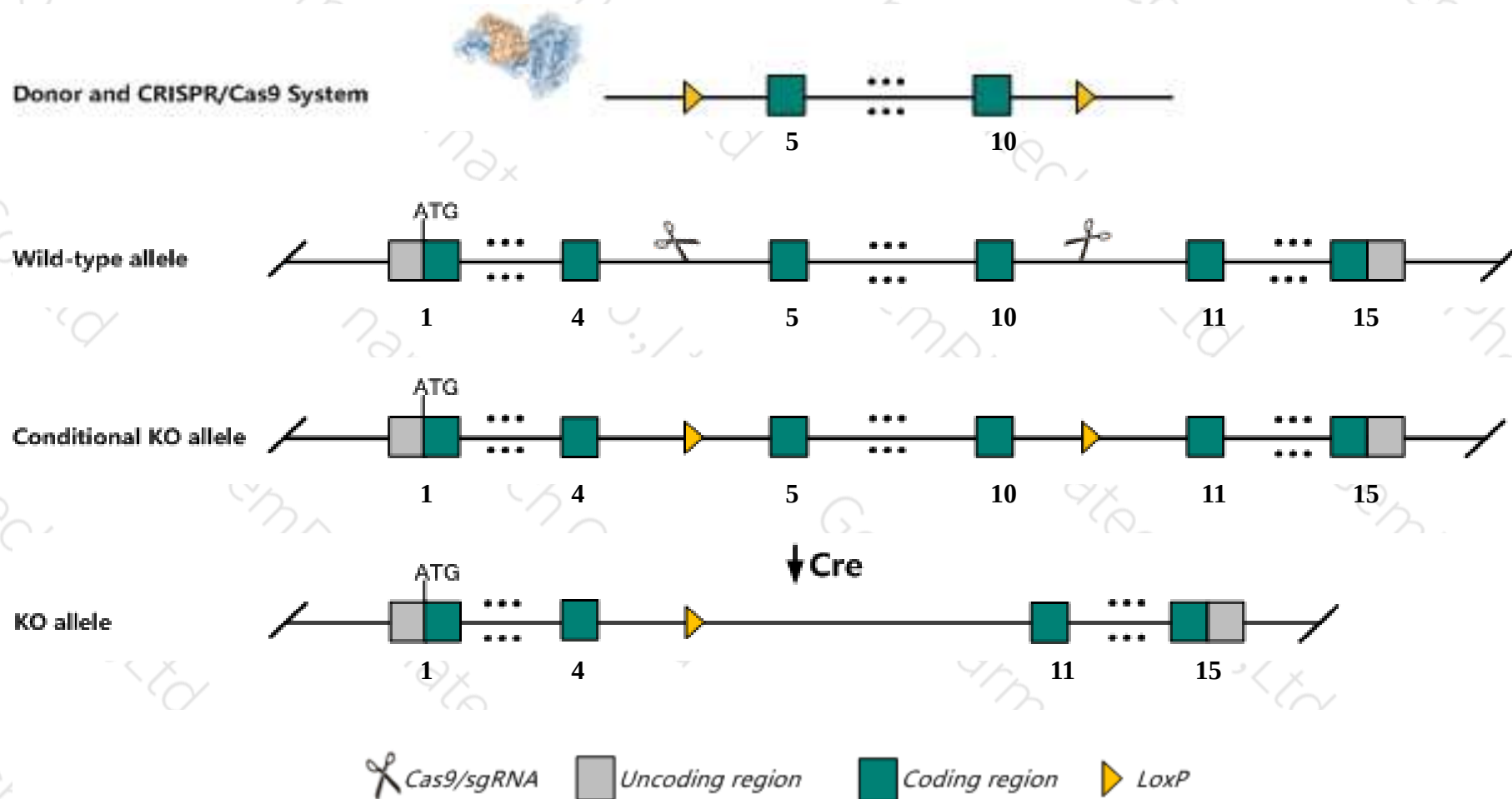
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Vps9d1* gene. The schematic diagram is as follows:



- The *Vps9d1* gene has 13 transcripts. According to the structure of *Vps9d1* gene, exon5-exon10 of *Vps9d1*-202(ENSMUST00000118279.1) transcript is recommended as the knockout region. The region contains 887bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Vps9d1* 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.

- The KO region contains part intron of *Galnt2l-201* .
- The *Vps9d1* gene is located on the Chr8. 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)

Vps9d1 VPS9 domain containing 1 [Mus musculus (house mouse)]

Gene ID: 72325, updated on 13-Mar-2020

Summary



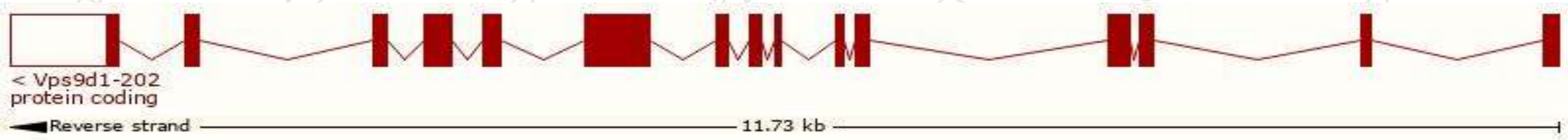
Official Symbol	Vps9d1 provided by MGI
Official Full Name	VPS9 domain containing 1 provided by MGI
Primary source	MGI:MGI:1914143
See related	Ensembl:ENSMUSG00000001062
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1300018I17Rik, 2410004N05Rik, 5DOS1, Vsp9d1
Expression	Ubiquitous expression in whole brain E14.5 (RPKM 24.0), CNS E18 (RPKM 23.7) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

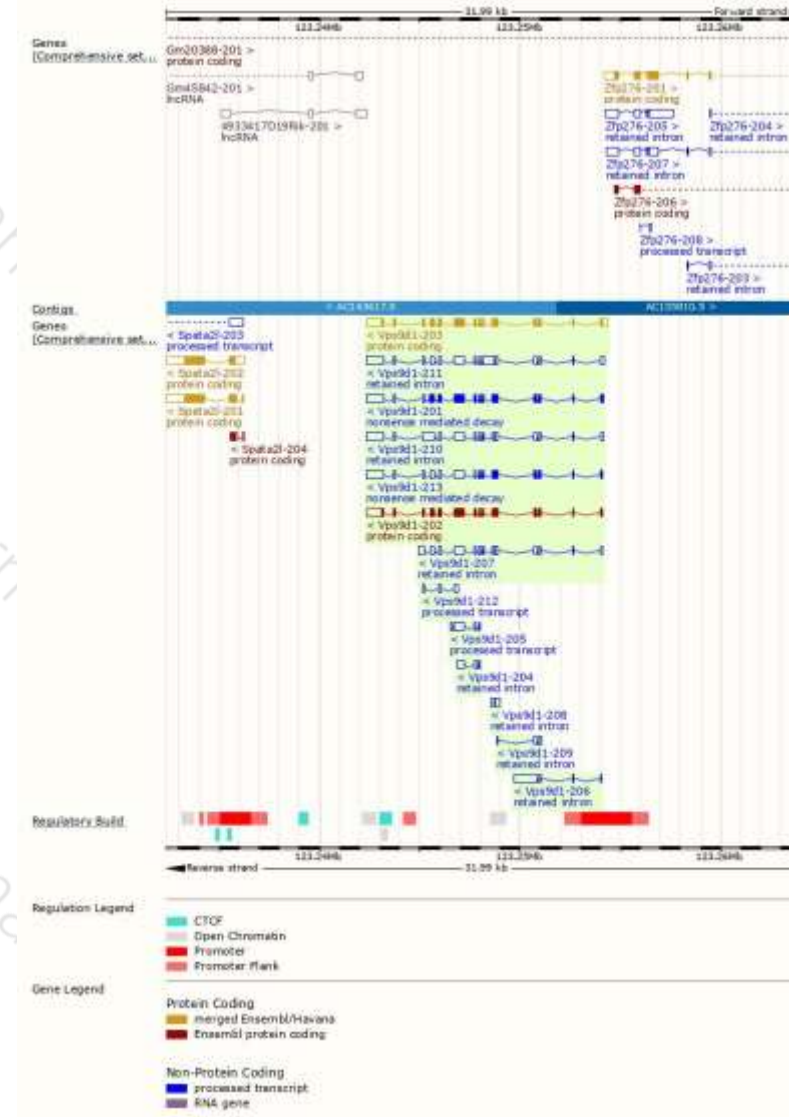
The gene has 13 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Vps9d1-203	ENSMUST00000122363.7	2952	649aa	Protein coding	CCDS52698	Q8C190	TSL:1 GENCODE basic APPRIS P3
Vps9d1-202	ENSMUST00000118279.1	2696	650aa	Protein coding	CCDS80942	Q3U280	TSL:1 GENCODE basic APPRIS ALT2
Vps9d1-201	ENSMUST00000117643.8	2806	555aa	Nonsense mediated decay	-	F7DE99 Q8C190	TSL:1
Vps9d1-213	ENSMUST00000155869.7	2728	233aa	Nonsense mediated decay	-	D6RGB6	TSL:1
Vps9d1-205	ENSMUST00000127978.1	705	No protein	Processed transcript	-	-	TSL:3
Vps9d1-212	ENSMUST00000155853.1	404	No protein	Processed transcript	-	-	TSL:3
Vps9d1-211	ENSMUST00000149490.7	3233	No protein	Retained intron	-	-	TSL:2
Vps9d1-210	ENSMUST00000146136.7	3078	No protein	Retained intron	-	-	TSL:1
Vps9d1-207	ENSMUST00000130275.7	1937	No protein	Retained intron	-	-	TSL:1
Vps9d1-206	ENSMUST00000129591.1	1361	No protein	Retained intron	-	-	TSL:2
Vps9d1-204	ENSMUST00000124508.1	565	No protein	Retained intron	-	-	TSL:2
Vps9d1-209	ENSMUST00000137569.1	354	No protein	Retained intron	-	-	TSL:5
Vps9d1-208	ENSMUST00000130965.1	346	No protein	Retained intron	-	-	TSL:2

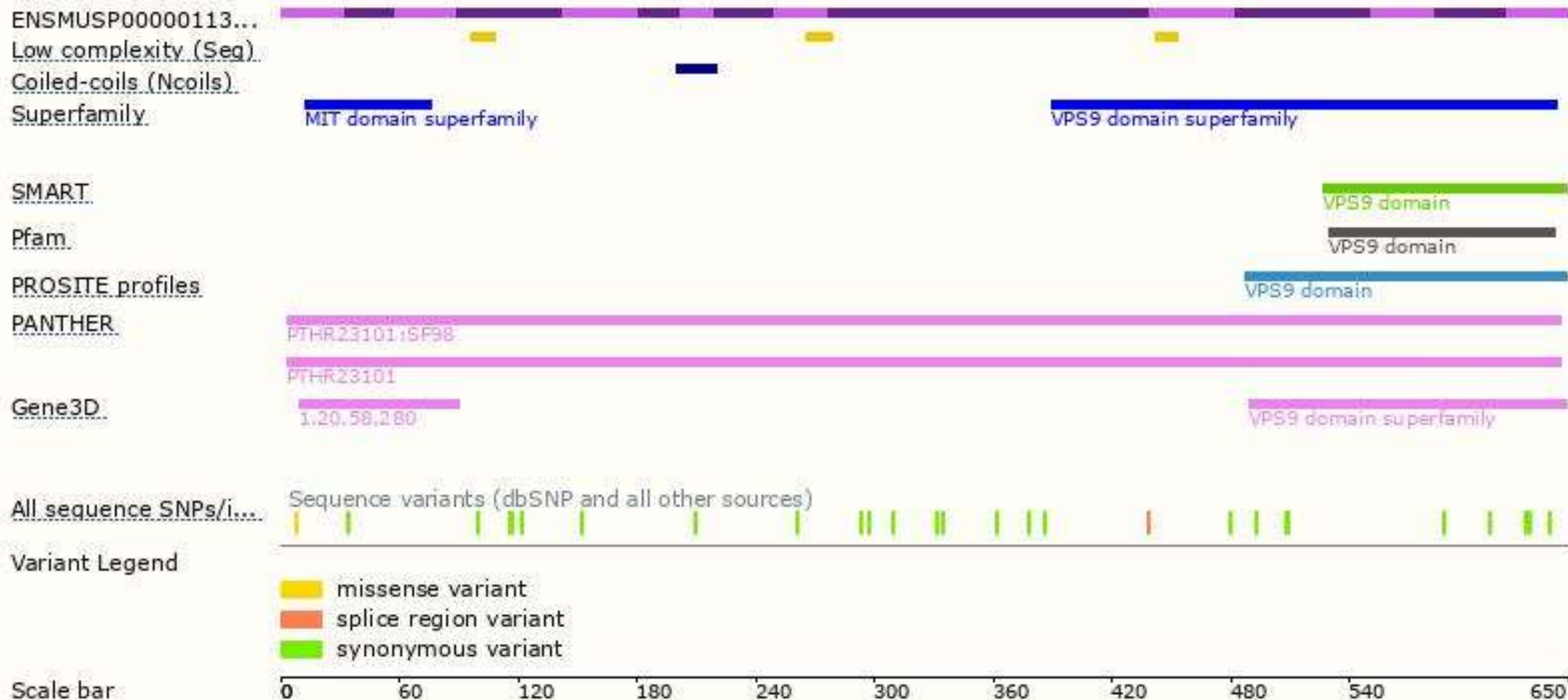
The strategy is based on the design of *Vps9d1-202* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

Tel: 025-5864 1534

