

Vps28 Cas9-CKO Strategy

Designer: Shilei Zhu

Reviewer: Longyun Hu

Design Date: 2020-7-24

Project Overview

Project Name

Vps28

Project type

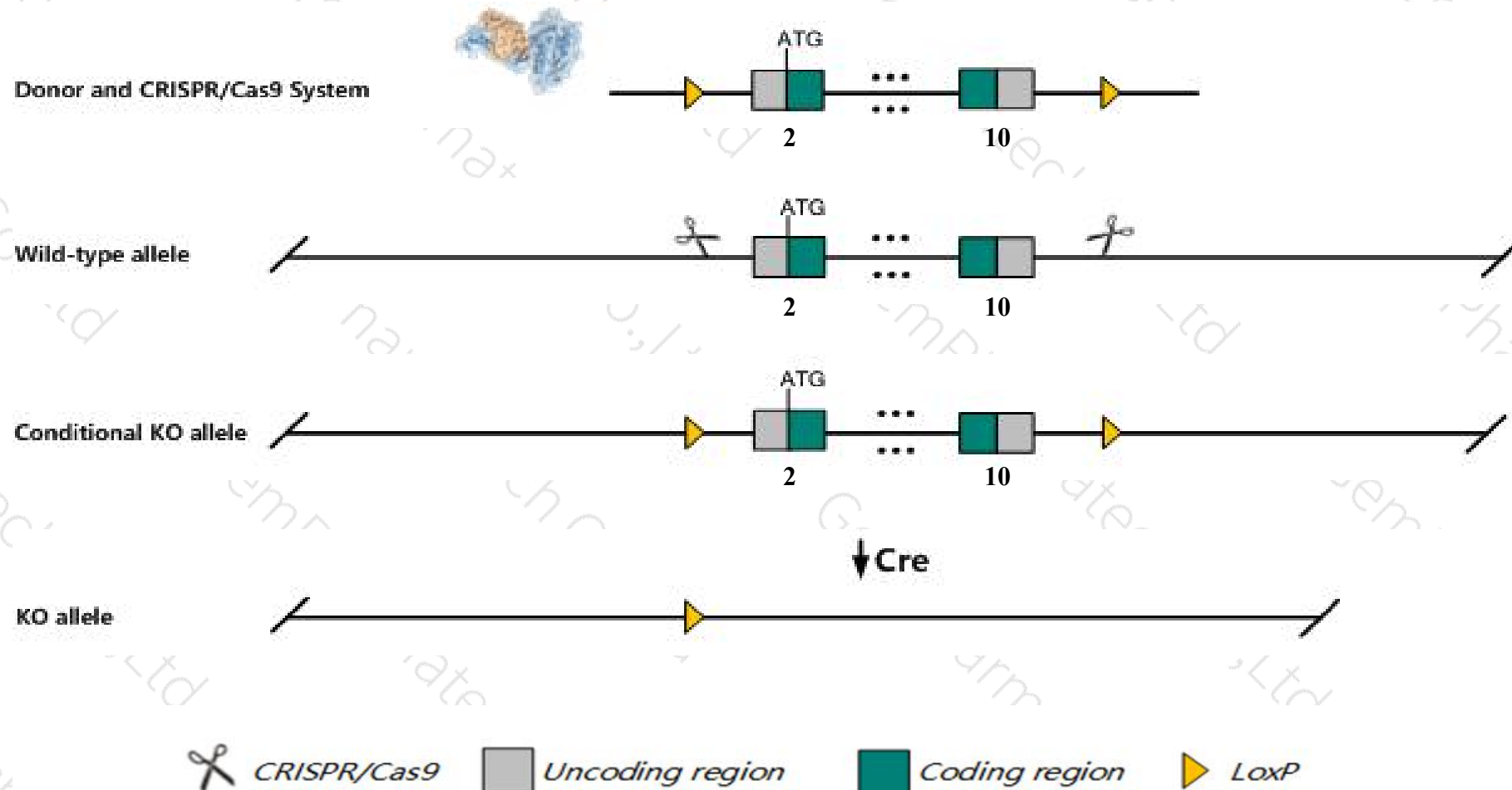
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Vps28* gene. The schematic diagram is as follows:



Technical routes

- The *Vps28* gene has 5 transcripts. According to the structure of *Vps28* gene, exon2-exon10 of *Vps28*-201(ENSMUST00000078803.4) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Vps28* 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.

- The *Vps28* gene is located on the Chr15. 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)

Vps28 vacuolar protein sorting 28 [Mus musculus (house mouse)]

Gene ID: 66914, updated on 13-Mar-2020

Summary



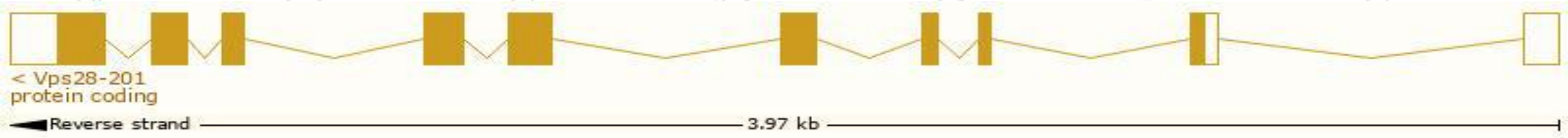
Official Symbol	Vps28 provided by MGI
Official Full Name	vacuolar protein sorting 28 provided by MGI
Primary source	MGI:MGI:1914164
See related	Ensembl:ENSMUSG00000115987
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1110014J03Rik, CIIA, D730005C08Rik
Summary	This gene encodes a protein which is thought to be a subunit of the ESCRT-I complex (endosomal complexes required for transport), which functions in the transport and sorting of proteins into subcellular vesicles. This complex can also be hijacked to facilitate the budding of enveloped viruses from the cell membrane. Alternative splicing results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Mar 2015]
Expression	Ubiquitous expression in large intestine adult (RPKM 132.5), duodenum adult (RPKM 102.1) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

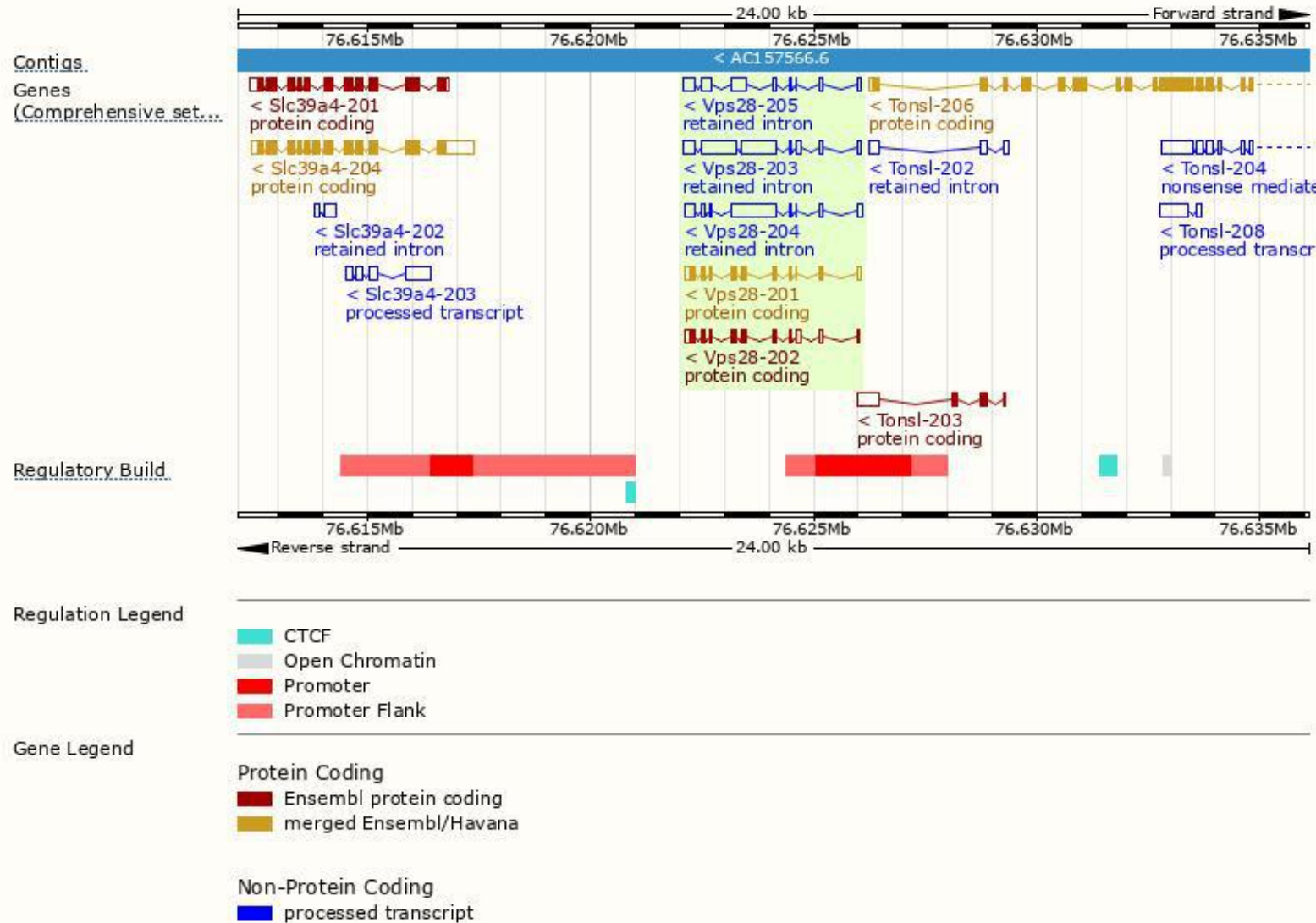
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Vps28-201	ENSMUST00000078803.4	911	221aa	Protein coding	CCDS27579	Q9D1C8	TSL:1 GENCODE basic APPRIS P1
Vps28-202	ENSMUST00000229019.1	941	183aa	Protein coding	-	A0A2R8VKS6	GENCODE basic
Vps28-203	ENSMUST00000229052.1	2148	No protein	Retained intron	-	-	
Vps28-204	ENSMUST00000229261.1	1631	No protein	Retained intron	-	-	
Vps28-205	ENSMUST00000229545.1	1136	No protein	Retained intron	-	-	

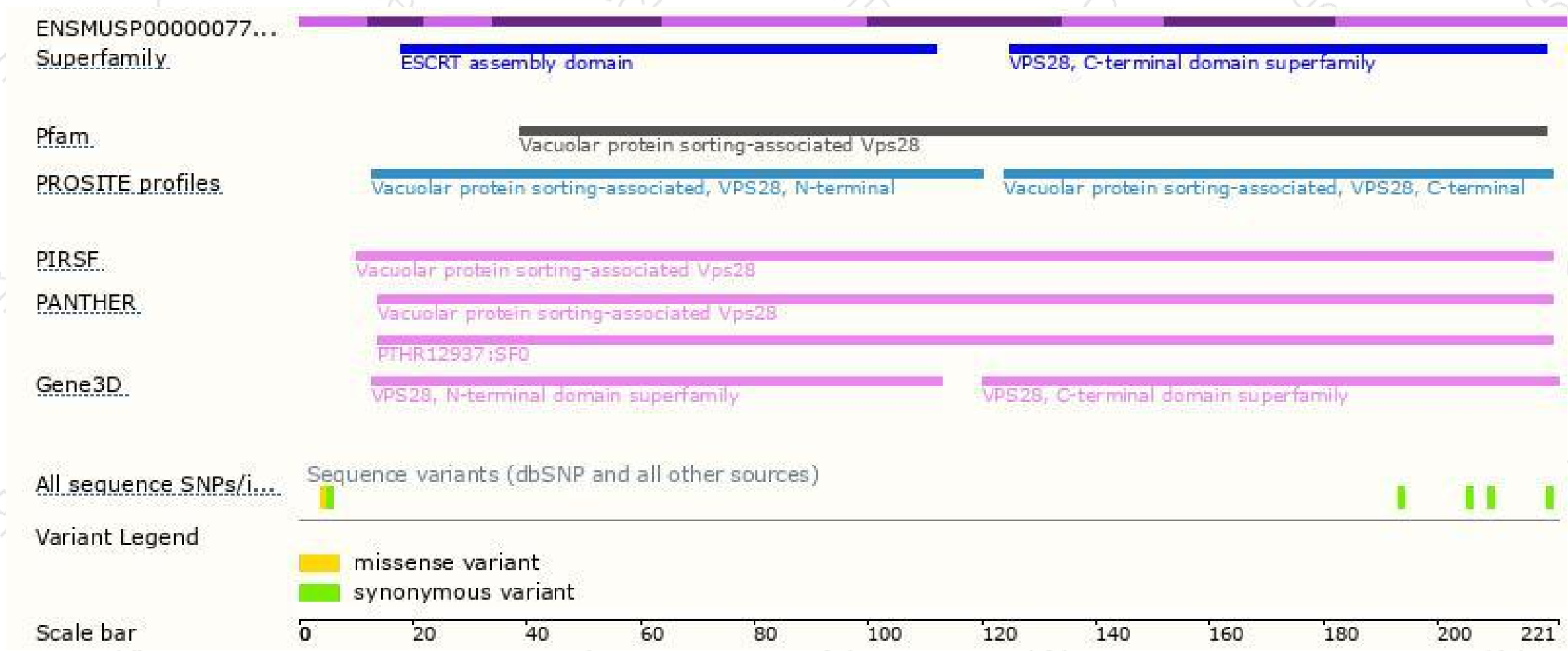
The strategy is based on the design of *Vps28-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

Tel: 400-9660890

