Vps35 Cas9-CKO Strategy

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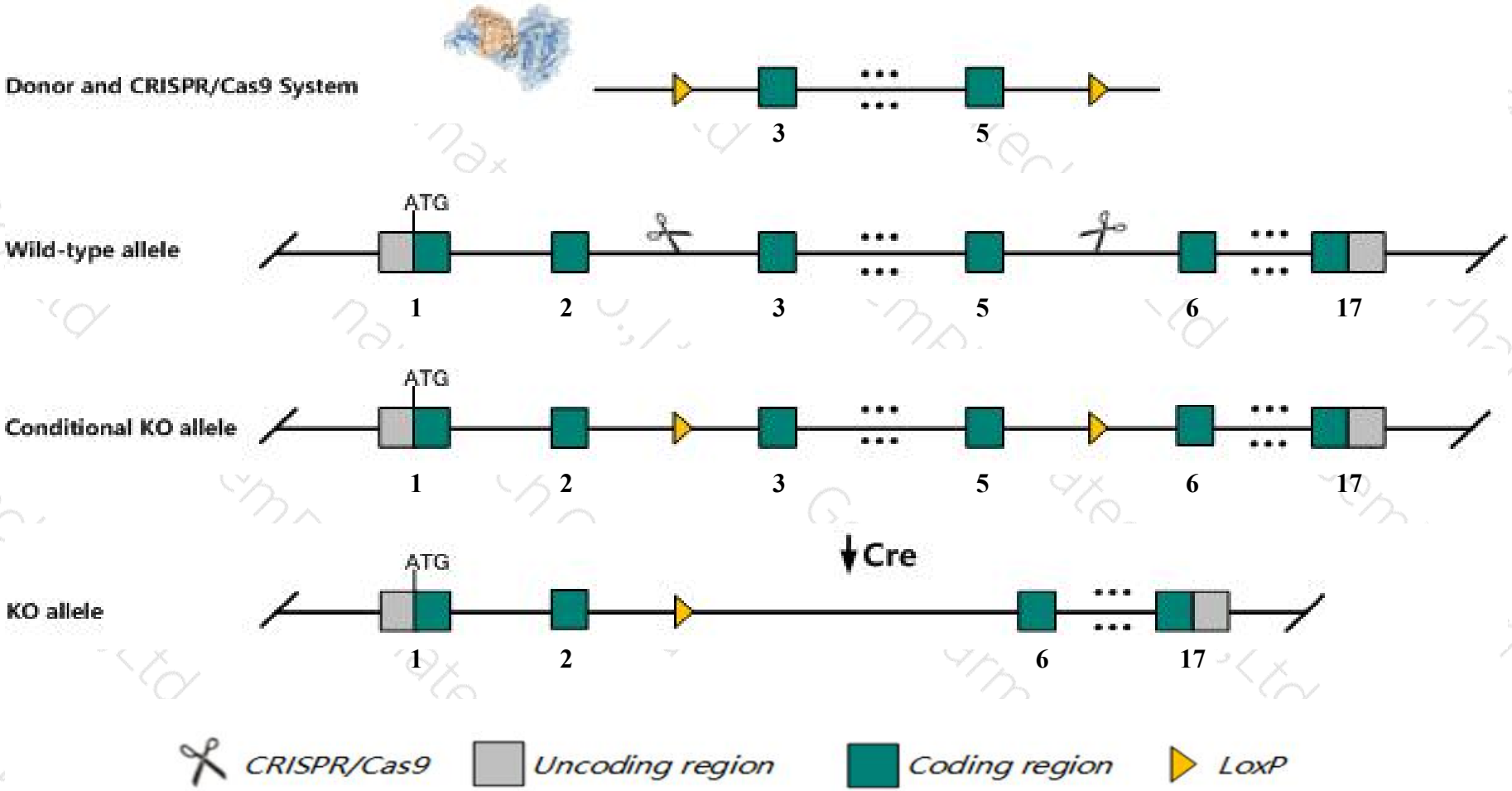
Project Overview

Project Name	***Vps35***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Vps35* gene. The schematic diagram is as follows:



Technical routes

➢ The *Vps35* gene has 5 transcripts. According to the structure of *Vps35* gene, exon3-exon5 of *Vps35-201*(ENSMUST00000034131.9) transcript is recommended as the knockout region.The region contains 404bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Vps35* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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.

Notice

➢ According to the existing MGI data,mice homozygous for a gene trap allele die prior to E10.

➢ The *Vps35* 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）

集萃药康 GemPharmatech

Vps35 VPS35 retromer complex component [Mus musculus (house mouse)]

Gene ID: 65114, updated on 13-Mar-2020

Summary

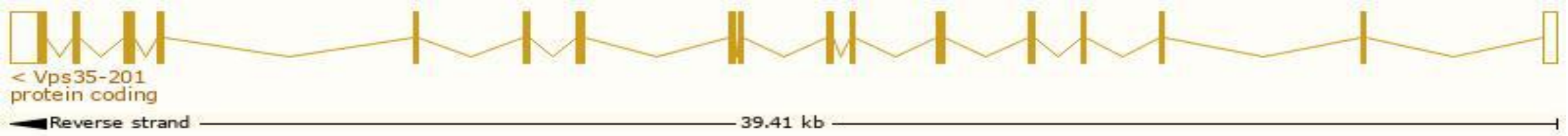
Official Symbol	Vps35 provided by MGI
Official Full Name	VPS35 retromer complex component provided byMGI
Primary source	MGI:MGI:1890467
See related	Ensembl:ENSMUSG00000031696
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI647796, Mem3
Expression	Broad expression in placenta adult (RPKM 65.1), bladder adult (RPKM 38.4) and 21 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

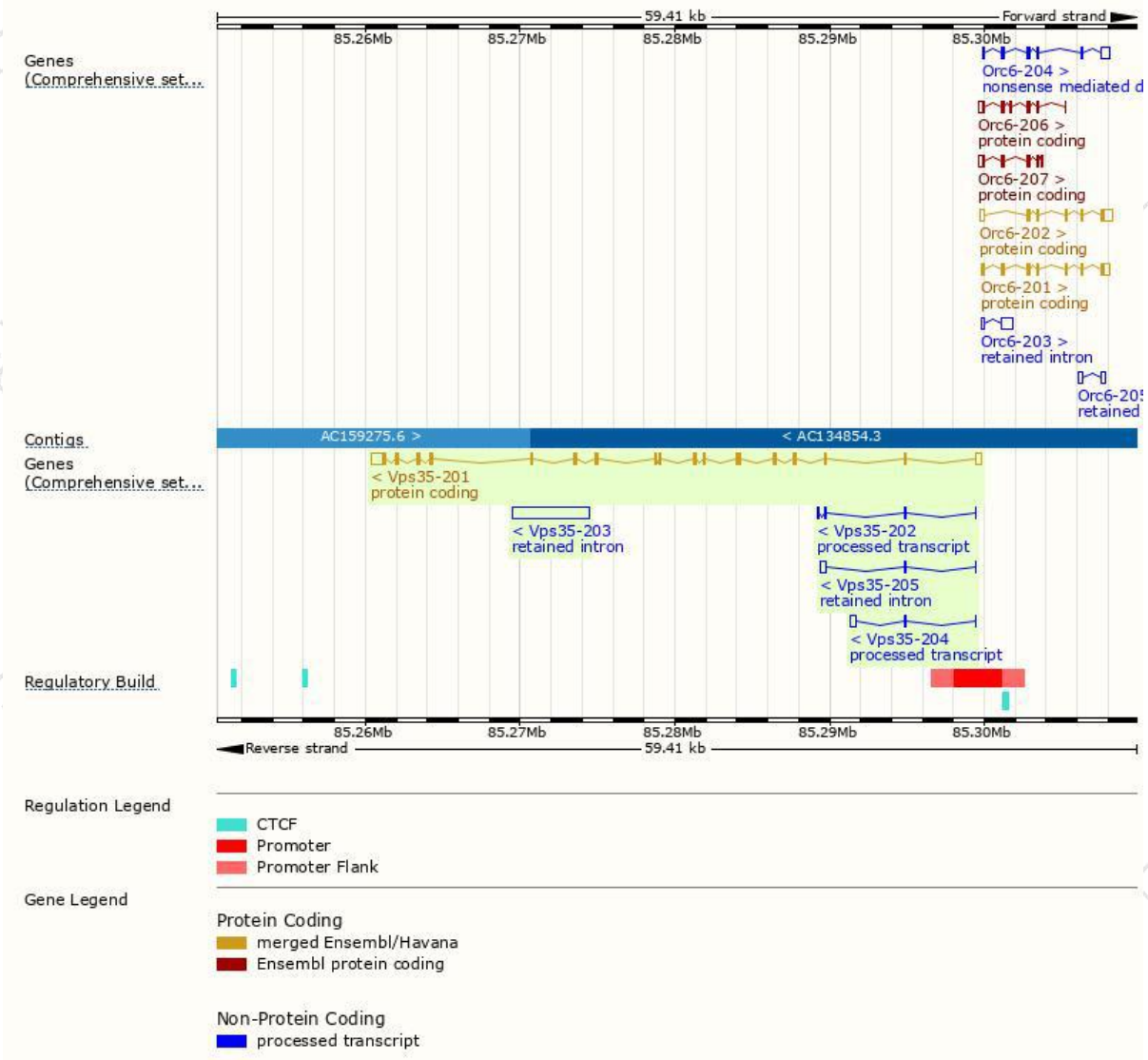
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Vps35-201	ENSMUST00000034131.9	3474	796aa	Protein coding	CCDS40421	Q3TRJ1 Q9EQH3	TSL:1 GENCODE basic APPRIS P1
Vps35-204	ENSMUST00000211154.1	448	No protein	Processed transcript	-	-	TSL:5
Vps35-202	ENSMUST00000209228.1	308	No protein	Processed transcript	-	-	TSL:5
Vps35-203	ENSMUST00000209277.1	5002	No protein	Retained intron	-	-	TSL:NA
Vps35-205	ENSMUST00000211479.1	473	No protein	Retained intron	-	-	TSL:2

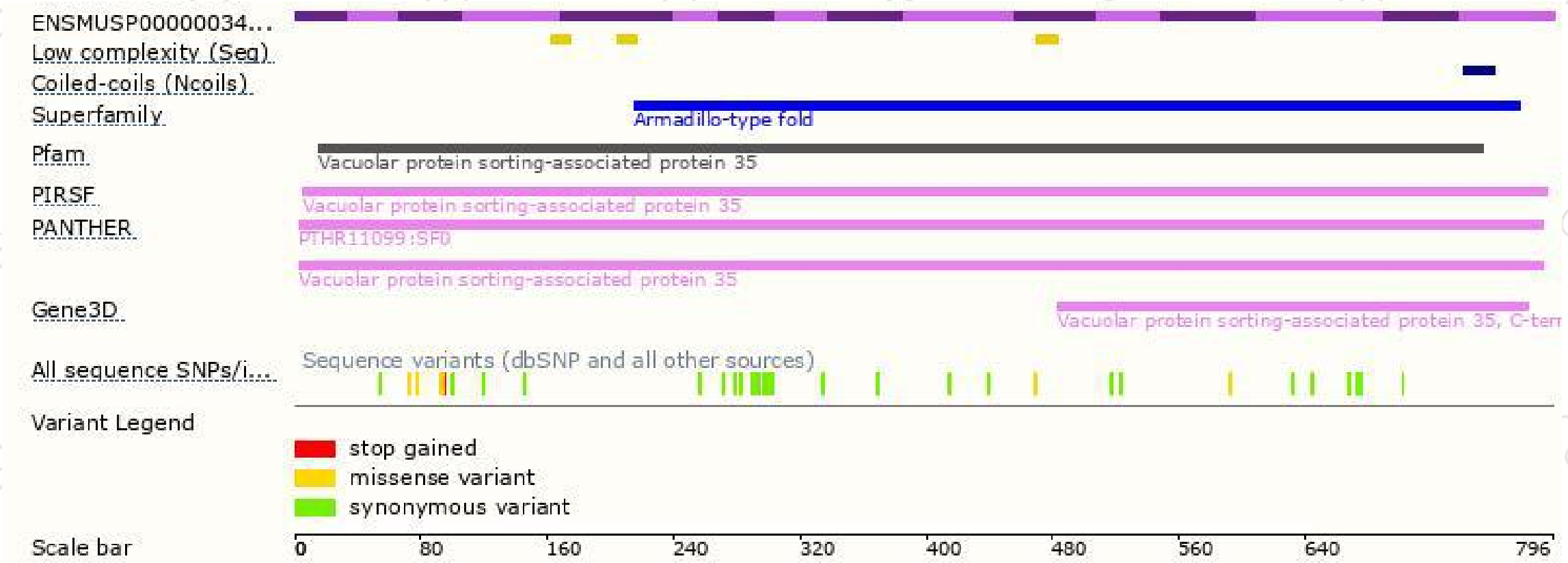
The strategy is based on the design of *Vps35-201* transcript,the transcription is shown below:



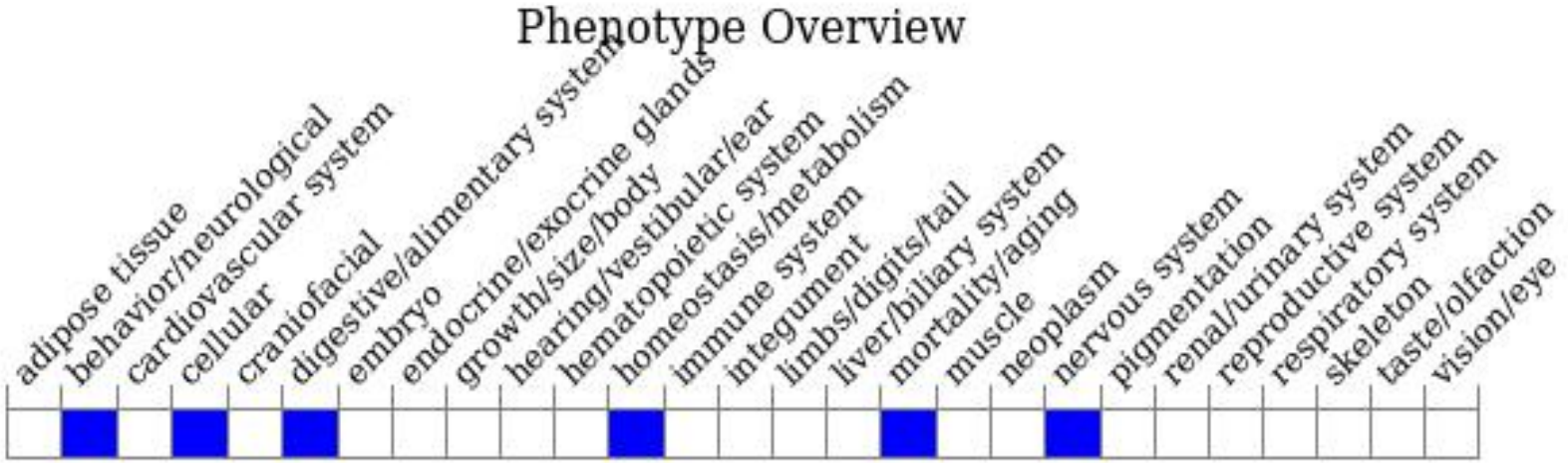
Genomic location distribution



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 a gene trap allele die prior to E10.

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

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