Vps13d Cas9-CKO Strategy RONDHAMAKOCH Co. S. C.

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Project Overview



Project Name

Vps13d

Project type

Cas9-CKO

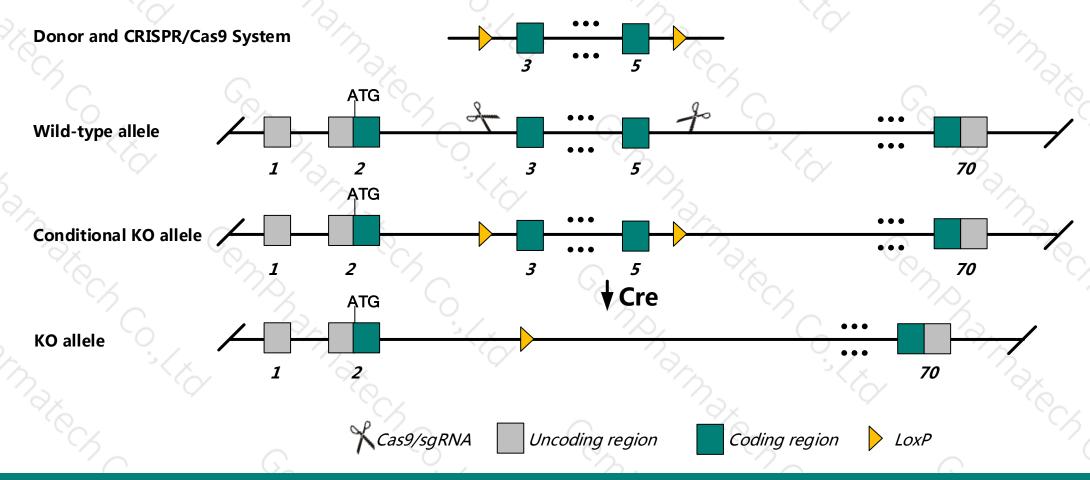
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Vps13d* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Vps13d* gene has 10 transcripts. According to the structure of *Vps13d* gene, exon3-exon5 of *Vps13d*-202 (ENSMUST00000036579.13) transcript is recommended as the knockout region. The region contains 353bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Vps13d* 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 or cell types.

Notice



- > According to the existing MGI data, Mice homozygous for a transgenic gene disruption exhibit embryonic lethality at E7.
- Transcript *Vps13d-210* may not be affected.
- ➤ The *Vps13d* gene is located on the Chr4. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



Vps13d vacuolar protein sorting 13D [Mus musculus (house mouse)]

Gene ID: 230895, updated on 5-Aug-2018

Summary

Official Symbol Vps13d provided by MGI

Official Full Name vacuolar protein sorting 13D provided by MGI

Primary source MGI:MGI:2448530

See related Ensembl:ENSMUSG00000020220 Vega:OTTMUSG00000010745

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

Expression Ubiquitous expression in genital fat pad adult (RPKM 5.8), thymus adult (RPKM 4.6) and 28 other tissues See more

Orthologs human all

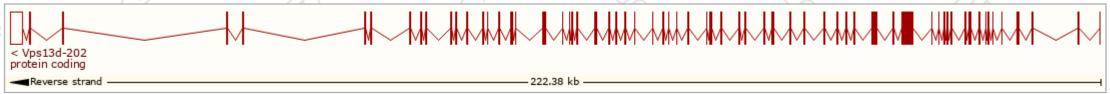
Transcript information (Ensembl)



The gene has 10 transcripts, and all transcripts are shown below:

S	Show/hide columns (1 hidden)							Filter
Na	ame 🌲	Transcript ID	bp 🌲	Protein 🍦	Biotype	CCDS	UniProt 🍦	Flags 🝦
Vps	13d-202	ENSMUST00000036579.13	15849	<u>4390aa</u>	Protein coding	<u>CCDS71517</u> ₽	<u>B1ART2</u> ₽	TSL:5 GENCODE basic APPRIS P1
Vps	13d-201	ENSMUST00000020441.12	15665	<u>4359aa</u>	Protein coding	-	<u>B1ART1</u> ₽	TSL:5 GENCODE basic
Vps	13d-210	ENSMUST00000185113.1	10176	<u>3211aa</u>	Protein coding	-	<u>V9GX23</u> ₽	CDS 5' incomplete TSL:5
Vps	13d-203	ENSMUST00000130704.7	6143	<u>1078aa</u>	Nonsense mediated decay	-	<u>F6XI52</u> ₽	CDS 5' incomplete TSL:1
Vps	13d-206	ENSMUST00000142308.1	4371	No protein	Retained intron	-	-	TSL:1
Vps	13d-205	ENSMUST00000141208.7	3811	No protein	Retained intron	-	-	TSL:1
Vps	13d-204	ENSMUST00000132700.1	2253	No protein	Retained intron	-	-	TSL:1
Vps	13d-209	ENSMUST00000155670.1	358	No protein	Retained intron	-	-	TSL:3
Vps	13d-208	ENSMUST00000147511.1	802	No protein	IncRNA	-	-	TSL:3
Vps	13d-207	ENSMUST00000144835.1	689	No protein	IncRNA	-	-	TSL:3

The strategy is based on the design of *Vps13d*-202 transcript, The transcription is shown below



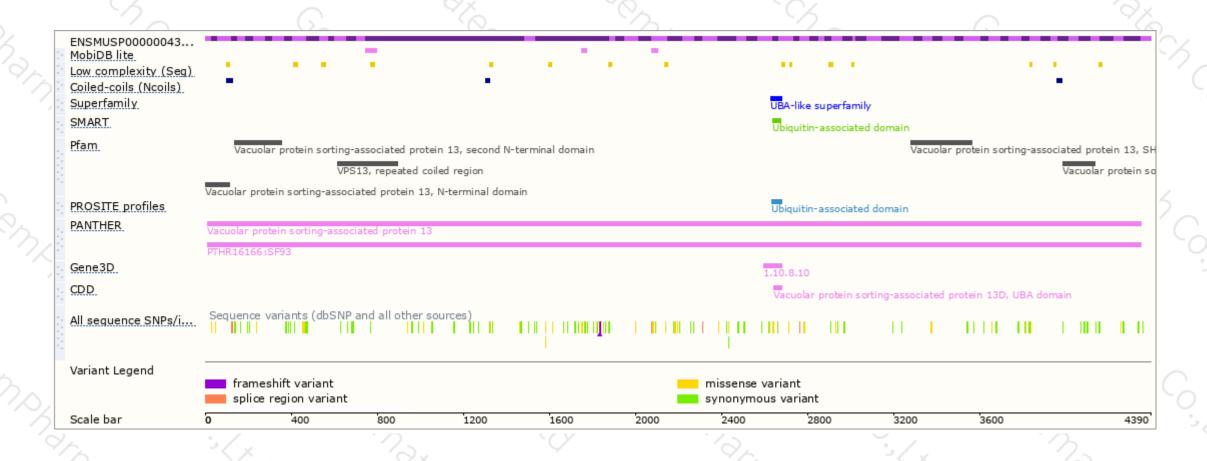
Genomic location distribution





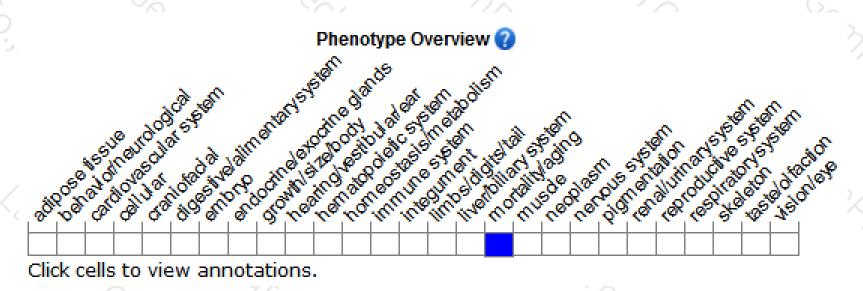
Protein domain





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 transgenic gene disruption exhibit embryonic lethality at E7.

If you have any questions, you are welcome to inquire. Tel: 025-5864 1534





