

Trpc6 Cas9-CKO Strategy

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Project Overview

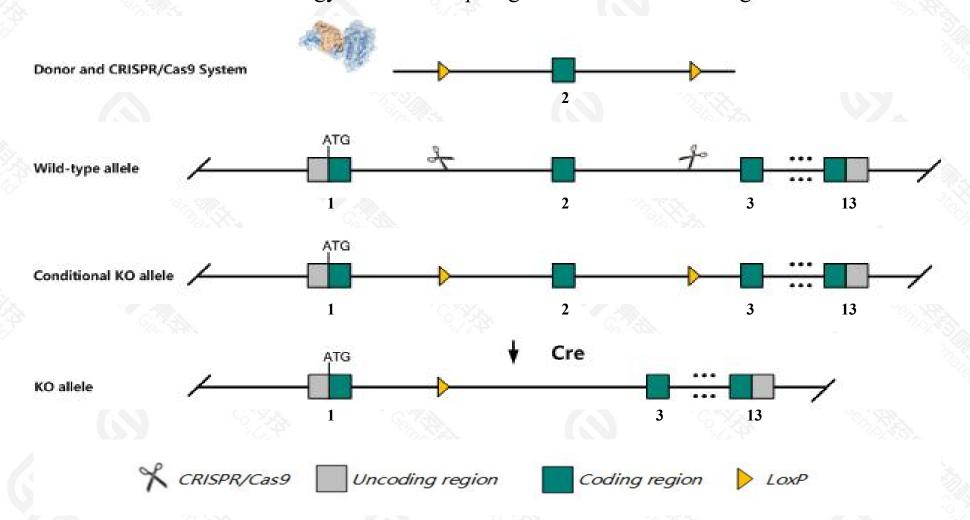


Project Name	Trpc6
Project type	Cas9-CKO
Strain background	C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Trpc6* gene. The schematic diagram is as follows:



Technical routes



- The *Trpc6* gene has 3 transcripts. According to the structure of *Trpc6* gene, exon2 of *Trpc6-201*(ENSMUST00000050433.8) transcript is recommended as the knockout region. The region contains 775bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Trpc6* 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.

Notice



- > According to the existing MGI data, mice homozygous for one null targeted mutation are viable and fertile and exhibit no overt abnormal phenotype. Another knockout results in an increase in thermal nociceptive response latency.
- > The *Trpc6* gene is located on the Chr9. 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)



Trpc6 transient receptor potential cation channel, subfamily C, member 6 [Mus musculus (house mouse)]

Gene ID: 22068, updated on 13-Mar-2020

Summary



Official Symbol Trpc6 provided by MGI

Official Full Name transient receptor potential cation channel, subfamily C, member 6 provided by MGI

Primary source MGI:MGI:109523

See related Ensembl:ENSMUSG00000031997

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 AV025995, LLHWJM002, LLHWJM003, LLHWJM004, TRP-6, Trrp6, mtrp6

Expression Broad expression in lung adult (RPKM 2.0), frontal lobe adult (RPKM 1.1) and 15 other tissuesSee more

Orthologs <u>human all</u>

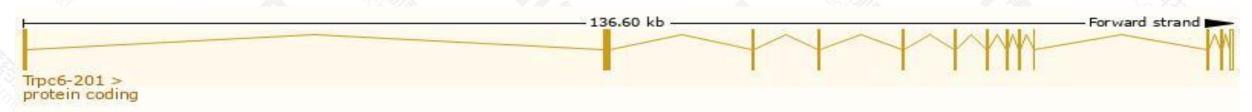
Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

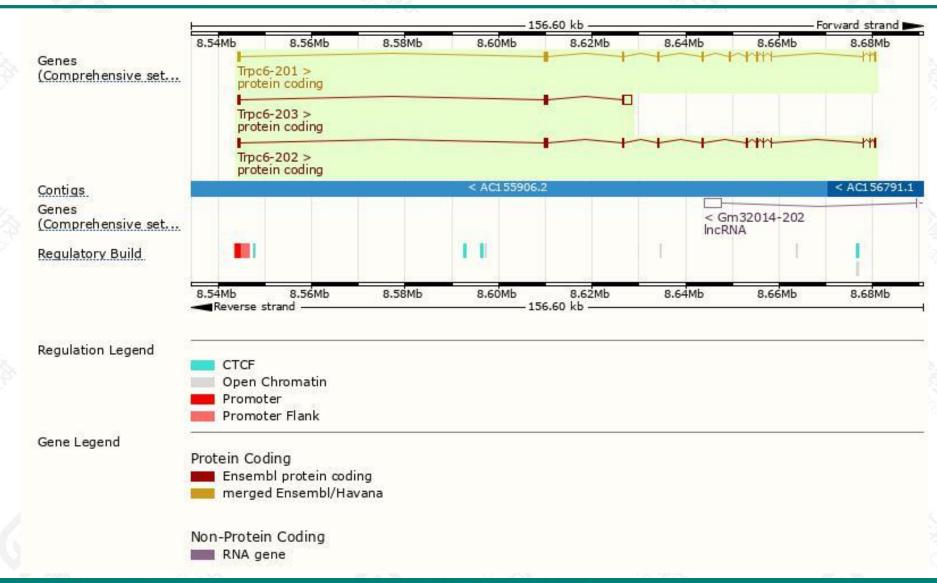
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trpc6-201	ENSMUST00000050433.7	3259	<u>930aa</u>	Protein coding	CCDS22815	Q61143	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Trpc6-203	ENSMUST00000217462.1	3168	<u>406aa</u>	Protein coding	. *8	Q6NV56	TSL:1 GENCODE basic
Trpc6-202	ENSMUST00000214596.1	2913	852aa	Protein coding	20	Q3UZG1	TSL:1 GENCODE basic

The strategy is based on the design of *Trpc6-201* transcript, the transcription is shown below:



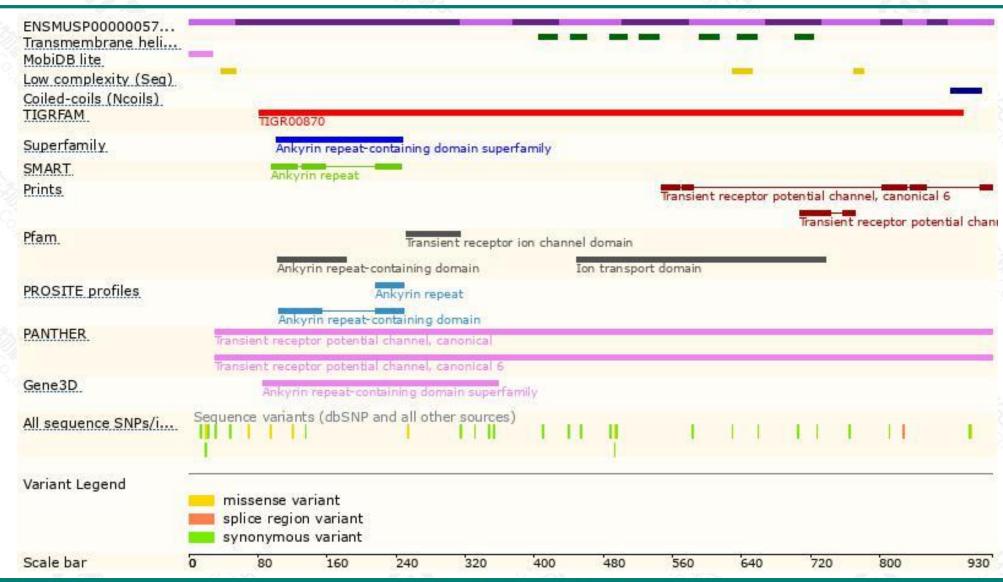
Genomic location distribution





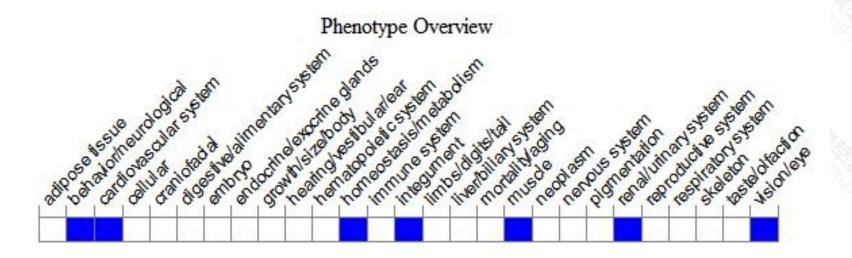
Protein domain





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 one null targeted mutation are viable and fertile and exhibit no overt abnormal phenotype. Another knockout results in an increase in thermal nociceptive response latency.



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

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