

Trpc5 Cas9-CKO Strategy

Designer: Yang Zeng

Reviewer: Xueting Zhang

Design Date: 2019-10-28

Project Overview



Project Name Trpc5

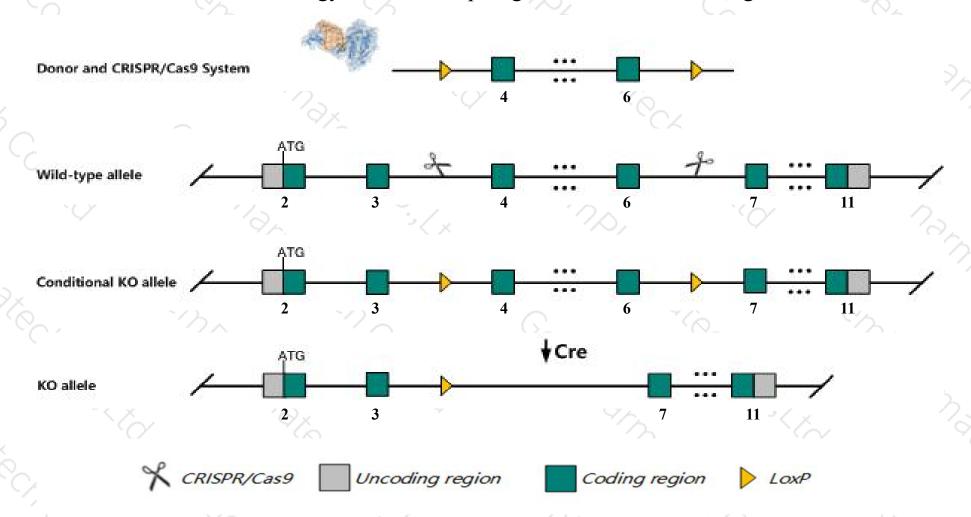
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Trpc5* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Trpc5* gene has 2 transcripts. According to the structure of *Trpc5* gene, exon4-exon6 of *Trpc5-201* (ENSMUST00000040184.3) transcript is recommended as the knockout region. The region contains 800bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Trpc5* 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



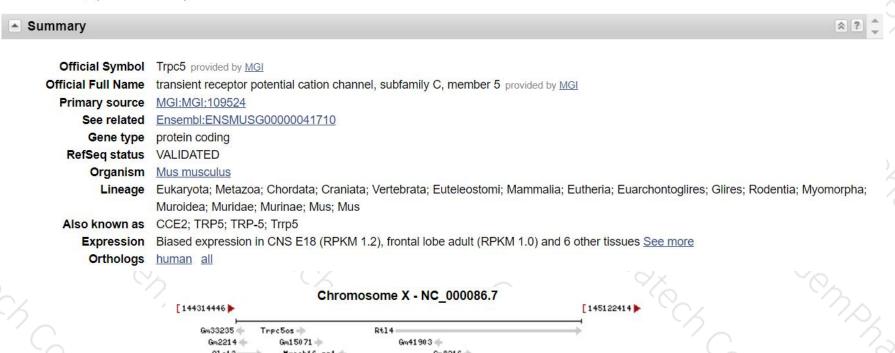
- ➤ According to the existing MGI data, Mice with homozygous null alleles of the gene exhibit diminished innate fear response with reduction in synaptic activation and strength in neurons of the amygdala.
- > The N-terminal of *Trpc5* gene will remain several amino acids ,it may remain the partial function of *Trpc5* gene.
- The *Trpc5* gene is located on the ChrX. 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)



Trpc5 transient receptor potential cation channel, subfamily C, member 5 [Mus musculus (house mouse)]

Gene ID: 22067, updated on 10-Sep-2019



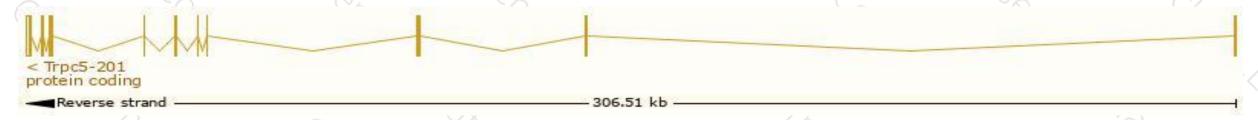
Transcript information (Ensembl)



The gene has 2 transcripts, all transcripts are shown below:

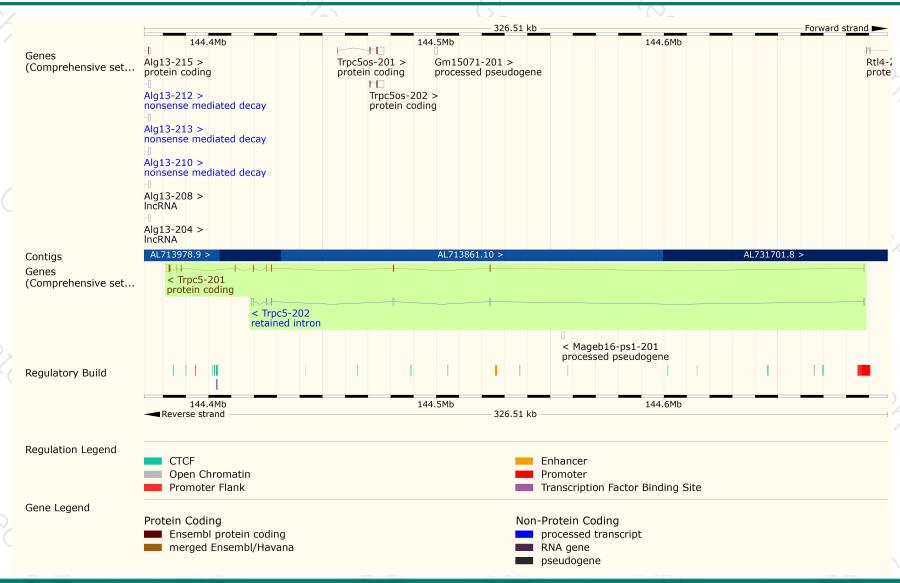
Name 🍦	Transcript ID 👙	bp 🍦	Protein	Translation ID	Biotype	CCDS	UniProt	Flags		
Trpc5-201	ENSMUST00000040184.3	4158	<u>975aa</u>	ENSMUSP00000049063.3	Protein coding	CCDS30457₽	Q2KHN9 & Q9QX29 &	TSL:1	GENCODE basic	APPRIS P1
Trpc5-202	ENSMUST00000148240.1	2782	No protein	18.7	Retained intron	-	-		TSL:1	

The strategy is based on the design of Trpc5-201 transcript, The transcription is shown below



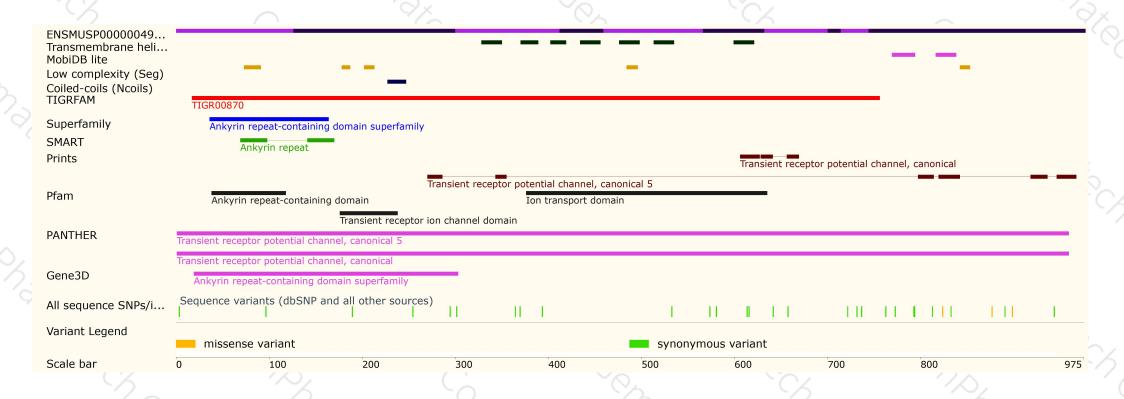
Genomic location distribution





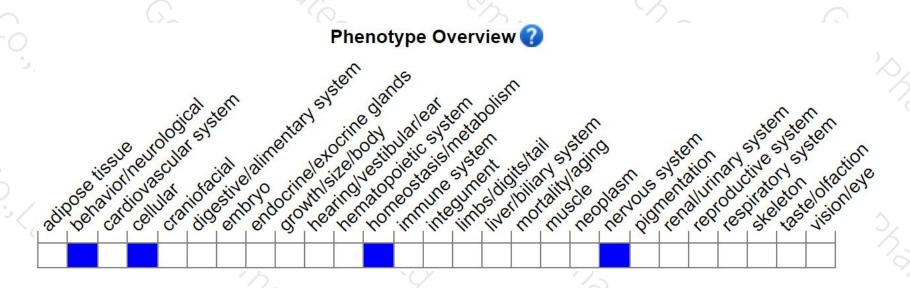
Protein domain





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 with homozygous null alleles of the gene exhibit diminished innate fear response with reduction in synaptic activation and strength in neurons of the amygdala.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





