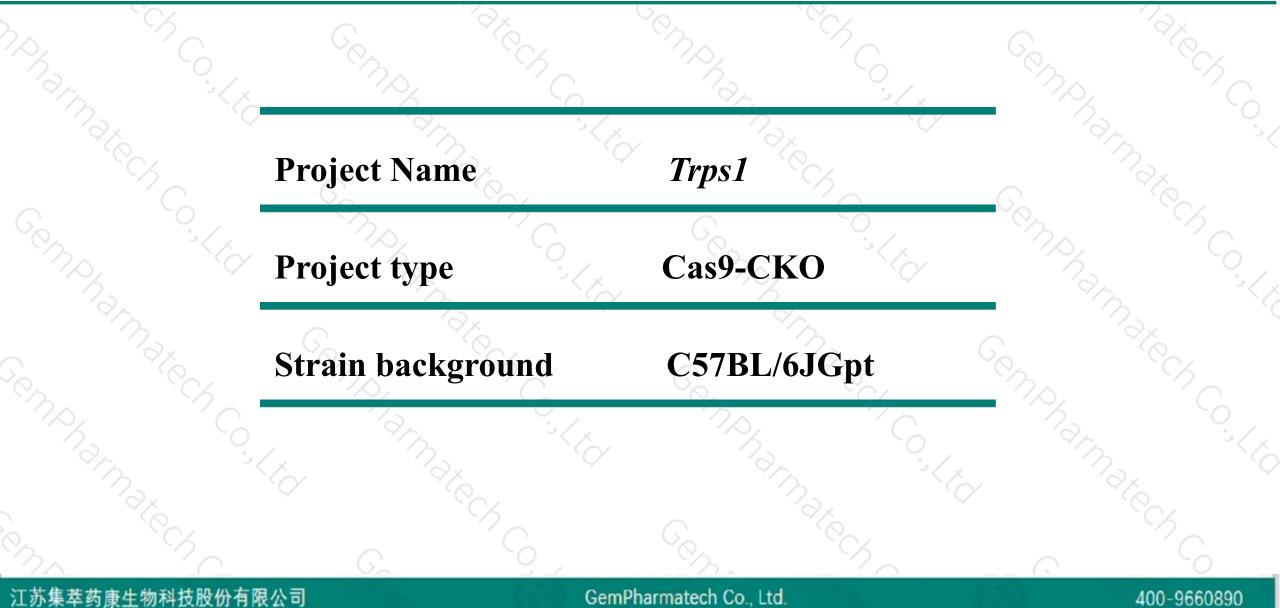


Trps1 Cas9-CKO Strategy

Designer: Reviewer: Design Date: Ruirui Zhang Huimin Su 2019-9-21

Project Overview

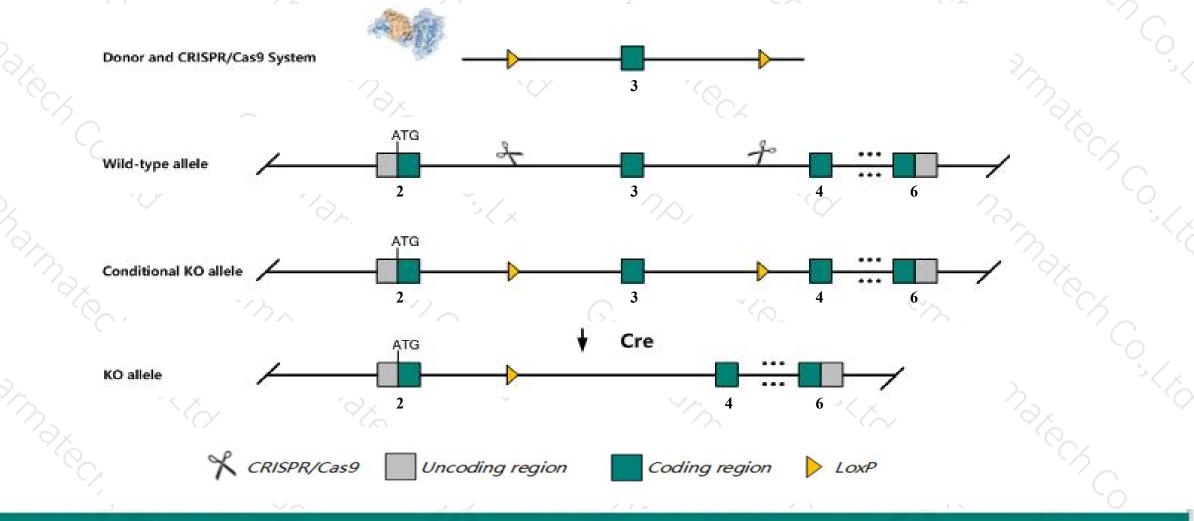




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Trps1* gene. The schematic diagram is as follows:



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The *Trps1* gene has 8 transcripts. According to the structure of *Trps1* gene, exon3 of *Trps1-204* (ENSMUST00000183757.7) transcript is recommended as the knockout region. The region contains 1130bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Trps1* 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, Newborn mice homozygous for a knock-out allele exhibit craniofacial and hair anomalies and die of respiratory failure due to thoracic spine and rib defects. Mice homozygous for a reporter allele show additional defects in chondrocyte proliferation and apoptosis as well as reduced nephron formation.
- Transcript *Trps1-208* may not be affected.
- The Trps1 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)



\$?

Trps1 transcriptional repressor GATA binding 1 [Mus musculus (house mouse)]

Gene ID: 83925, updated on 11-Sep-2019

Summary

Official Symbol	Trps1 provided by MGI
Official Full Name	transcriptional repressor GATA binding 1 provided by MGI
Primary source	MGI:MGI:1927616
See related	Ensembl:ENSMUSG0000038679
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	AI115454; AI447310; D15Ertd586e
Expression	Broad expression in limb E14.5 (RPKM 5.4), bladder adult (RPKM 4.0) and 17 other tissues See more
Orthologs	human all

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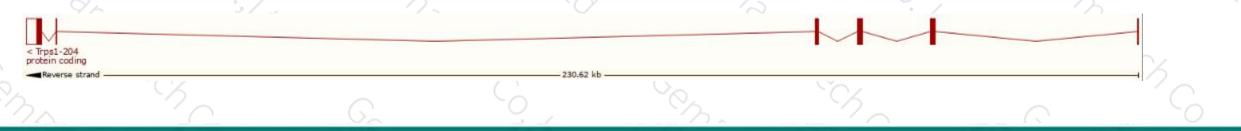
Transcript information (Ensembl)



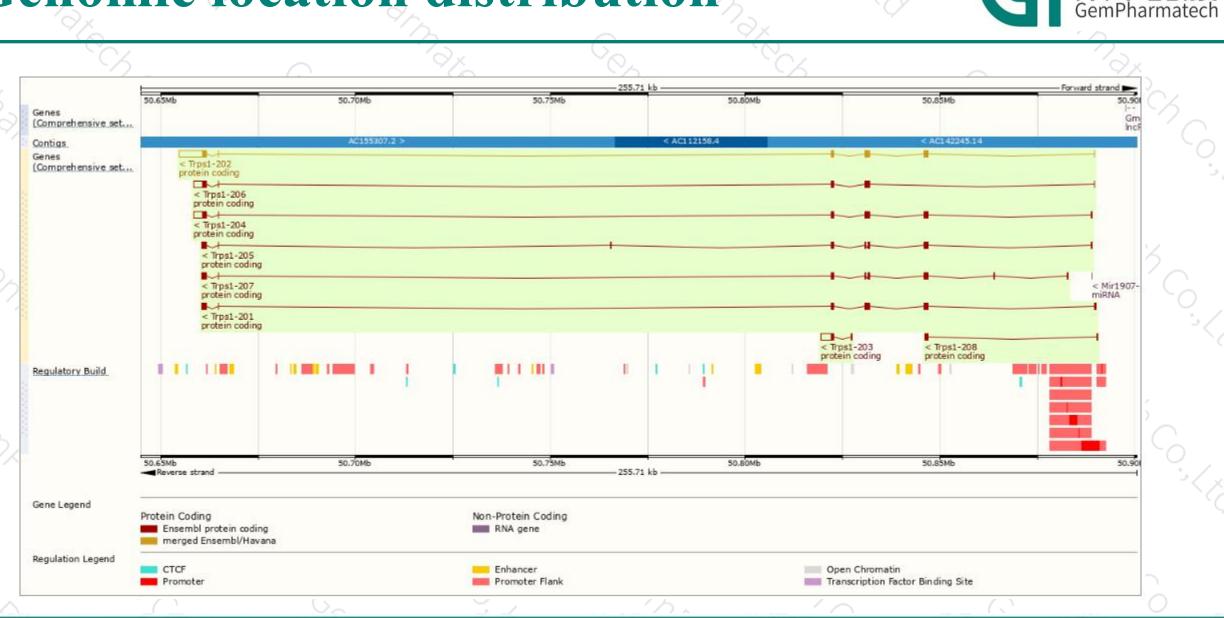
The gene has 8 transcripts, all transcripts are shown below:

Name 🛊	Transcript ID 💧	bp 🛊	Protein	Biotype 🕴	CCDS 🕴	UniProt 🕴	Flags
Trps1-202	ENSMUST00000165201.8	9859	<u>1281aa</u>	Protein coding	CCDS27460@	<u>G3UW90</u> @	TSL:1 GENCODE basic APPRIS P3
Trps1-204	ENSMUST00000183757.7	6202	<u>1285aa</u>	Protein coding	CCDS79370@	V9GX74@	TSL:1 GENCODE basic APPRIS ALT2
Trps1-201	ENSMUST00000077935.5	4436	<u>1281aa</u>	Protein coding	CCDS27460@	<u>G3UW90</u> @	TSL:1 GENCODE basic APPRIS P3
Trps1-205	ENSMUST00000183997.7	3627	<u>1096aa</u>	Protein coding	CCDS79371@	V9GXE9	TSL:1 GENCODE basic
Trps1-206	ENSMUST00000184458.7	5293	<u>994aa</u>	Protein coding		V9GXA5@	TSL:1 GENCODE basic
Trps1-207	ENSMUST00000184885.7	3492	<u>1035aa</u>	Protein coding	850	<u>Q80V18</u> @	TSL:1 GENCODE basic
Trps1-203	ENSMUST00000183421.1	3446	<u>229aa</u>	Protein coding	254-3	<u>Q8BZ62</u> @	TSL:1 GENCODE basic
Trps1-208	ENSMUST00000185183.1	887	<u>255aa</u>	Protein coding	1.000	<u>V9GX39</u> ₽	CDS 3' incomplete TSL:2
144 000 000 000 000 000 000 000 000 000			/ Alternative Alternative	-	i i i i i i i i i i i i i i i i i i i		

The strategy is based on the design of *Trps1-204* transcript, The transcription is shown below



Genomic location distribution



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Protein domain



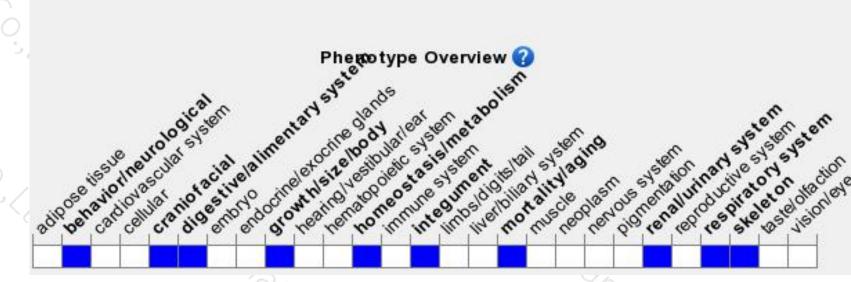
ENSMUSP00000139	0		~~~		2			°Х - С
Low complexity (Seg) Superfamily			· • •		-	SSF57716	Zinc finger	a 20
SMART Prints		Zinc finger C2H2-type				Zinc finger GATA-type		2
Pfam PROSITE profiles				Zinc fi	nger C2H2-type	Zinc finger, GATA-type Zinc finger, GATA-type Zinc finger, GATA-type		;
PROSITE patterns PANTHER	Transcription factor TRP51					Zinc finger: GATA-type	Zinc finger	
Gene3D						Zinc finger, NHR/GATA-type	3,30,160,60	
All sequence SNPs/i	Sequence variants (dbSNP and	f all other sources)	u i	1	à î		1 11	-
	frameshift variant synonymous variant	200	missense variant	600	800	splice region variant	12	
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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, Newborn mice homozygous for a knock-out allele exhibit craniofacial and hair anomalies and die of respiratory failure due to thoracic spine and rib defects. Mice homozygous for a reporter allele show additional defects in chondrocyte proliferation and apoptosis as well as reduced nephron formation.

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



