

# Tnrc6a Cas9-CKO Strategy

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**Reviewer:** Xueting Zhang

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# **Project Overview**



**Project Name** 

Tnrc6a

**Project type** 

Cas9-CKO

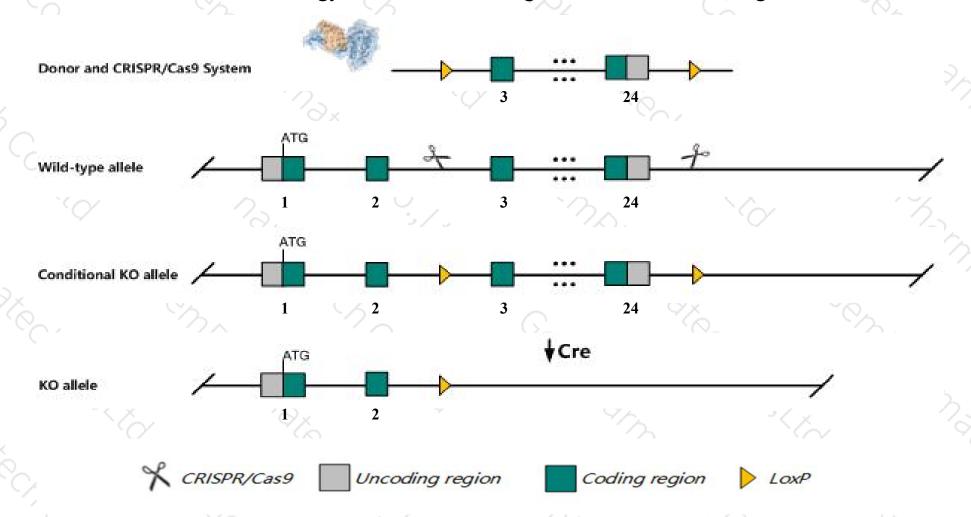
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Tnrc6a* gene. The schematic diagram is as follows:



### Technical routes



- The *Tnrc6a* gene has 11 transcripts. According to the structure of *Tnrc6a* gene, exon3-exon24 of *Tnrc6a-201* (ENSMUST0000094053.6) transcript is recommended as the knockout region. The region contains most of coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Tnrc6a* 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 a gene trap allele exhibit partial embryonic lethality during organogenesis associated with impaired hematopoiesis.
- > *Gm45846*-201 gene may be destroyed.
- ➤ The effect of transcripts 203,205,207 is unknown.
- > The *Tnrc6a* gene is located on the Chr7. 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)



#### Tnrc6a trinucleotide repeat containing 6a [Mus musculus (house mouse)]

Gene ID: 233833, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Tnrc6a provided by MGI

Official Full Name trinucleotide repeat containing 6a provided by MGI

Primary source MGI:MGI:2385292

See related Ensembl:ENSMUSG00000052707

Gene type protein coding
RefSeq status VALIDATED
Organism Mus musculus

Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as 201032105Rik, 3110054G10Rik, AW557223, CAGH26, D130023A07Rik, GW182, Tnrc6

Summary This gene encodes a member of the trinucleotide repeat containing 6 protein family. The protein is highly similar to a human protein that

functions in post-transcriptional gene silencing through the RNA interference (RNAi) and microRNA pathways. The human protein associates

with messenger RNAs and argonaute proteins in cytoplasmic bodies known as GW-bodies or P-bodies, and inhibiting its expression

delocalizes other GW-body proteins and impairs RNAi and microRNA-induced gene silencing. [provided by RefSeq, Jul 2008]

Expression Ubiquitous expression in CNS E11.5 (RPKM 14.3), CNS E14 (RPKM 13.5) and 28 other tissuesSee more

Orthologs human all

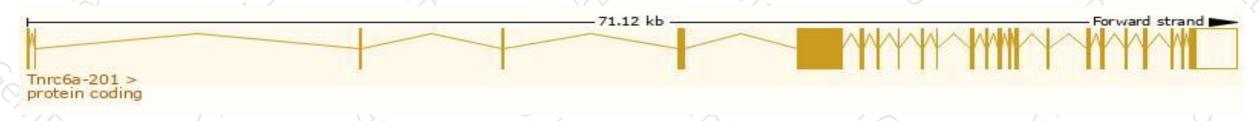
# Transcript information (Ensembl)



The gene has 11 transcripts, all transcripts are shown below:

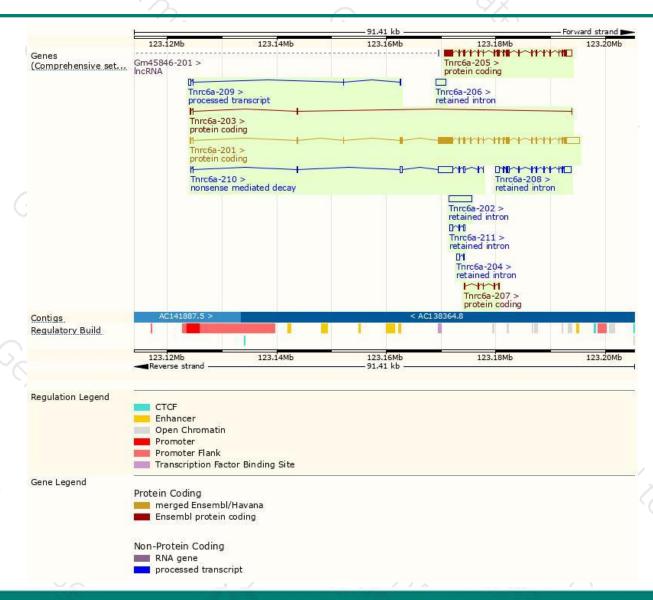
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tnrc6a-201	ENSMUST00000094053.6	8183	1896aa	Protein coding	CCDS57583	Q3UHK8	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Tnrc6a-205	ENSMUST00000205760.1	5223	<u>1398aa</u>	Protein coding		A0A0U1RQ65	CDS 5' incomplete TSL:5
Tnrc6a-207	ENSMUST00000206014.1	463	<u>154aa</u>	Protein coding	140	A0A0U1RQ94	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Tnrc6a-203	ENSMUST00000205514.1	358	<u>69aa</u>	Protein coding	127	A0A0U1RPB4	CDS 3' incomplete TSL:3
Tnrc6a-210	ENSMUST00000206888.1	3625	<u>51aa</u>	Nonsense mediated decay		A0A0U1RPF5	TSL:1
Tnrc6a-209	ENSMUST00000206458.1	688	No protein	Processed transcript			TSL:3
Tnrc6a-202	ENSMUST00000205449.1	4312	No protein	Retained intron	(44)	9	TSL:NA
Tnrc6a-208	ENSMUST00000206126.1	3297	No protein	Retained intron	127	- 4	TSL:1
Tnrc6a-206	ENSMUST00000205789.1	1783	No protein	Retained intron	-	- 5	TSL:NA
Tnrc6a-211	ENSMUST00000206922.1	939	No protein	Retained intron			TSL:1
Tnrc6a-204	ENSMUST00000205732.1	606	No protein	Retained intron	1940	e e	TSL:2

The strategy is based on the design of *Tnrc6a-201* transcript, The transcription is shown below



### Genomic location distribution





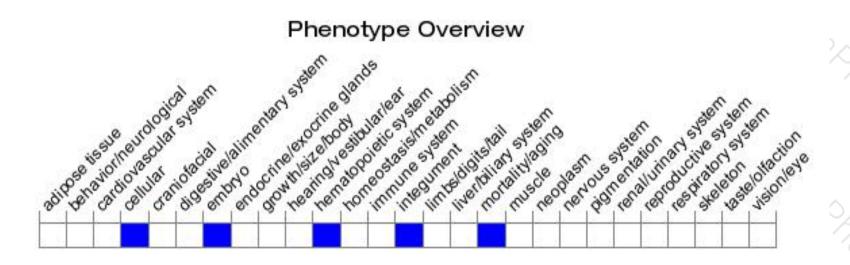
### Protein domain





# 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 exhibit partial embryonic lethality during organogenesis associated with impaired hematopoiesis.



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





