

Trio Cas9-CKO Strategy

Designer:

Ruirui Zhang

Reviewer:

Huimin Su

Design Date:

2020-2-27

Project Overview

Project Name

Trio

Project type

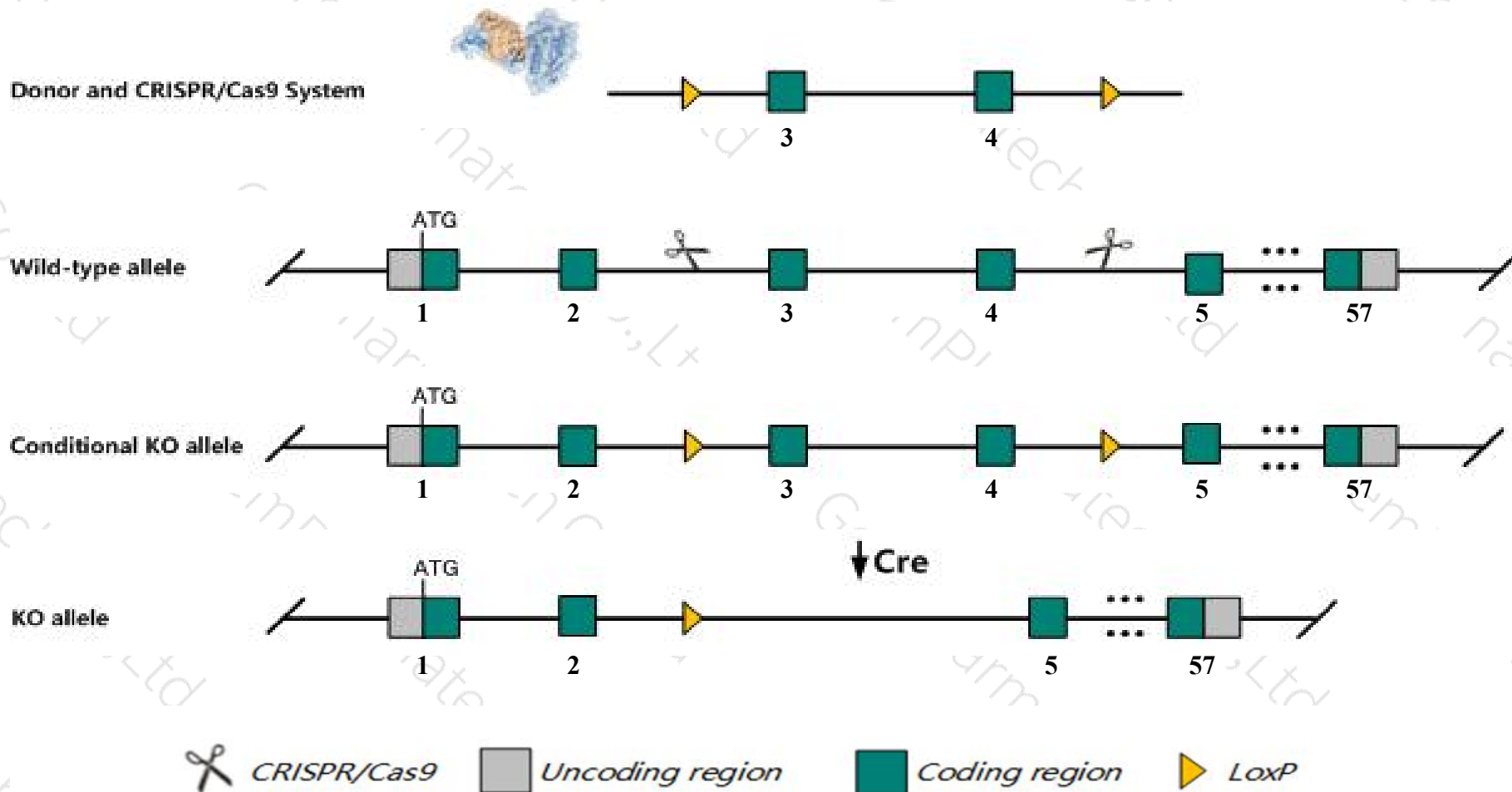
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Trio* gene has 15 transcripts. According to the structure of *Trio* gene, exon3-exon4 of *Trio-201* (ENSMUST00000090247.6) transcript is recommended as the knockout region. The region contains 308bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Trio* 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.

- According to the existing MGI data, homozygous mutant mice die during late embryonic development or shortly after birth. They exhibit abnormal skeletal myogenesis and display aberrant organization within the hippocampus and olfactory bulb.
- Transcript *Trio-203*, *205*, *206* may not be affected.
- The *Trio* 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)

Trio triple functional domain (PTPRF interacting) [*Mus musculus* (house mouse)]

Gene ID: 223435, updated on 31-Dec-2019

Summary

Official Symbol Trio provided by [MGI](#)

Official Full Name triple functional domain (PTPRF interacting) provided by [MGI](#)

Primary source [MGI:MGI:1927230](#)

See related [Ensembl:ENSMUSG000000022263](#)

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 Solo; AA408740; 6720464I07Rik

Expression Broad expression in limb E14.5 (RPKM 17.6), CNS E18 (RPKM 15.5) and 28 other tissues [See more](#)

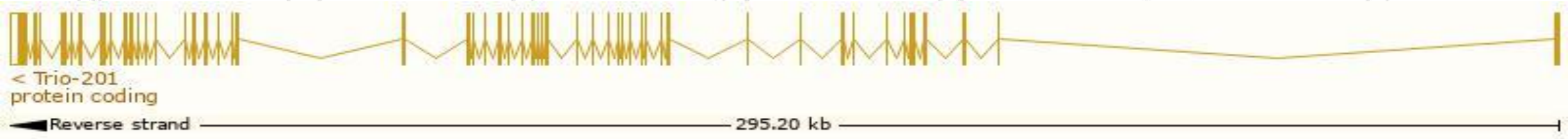
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

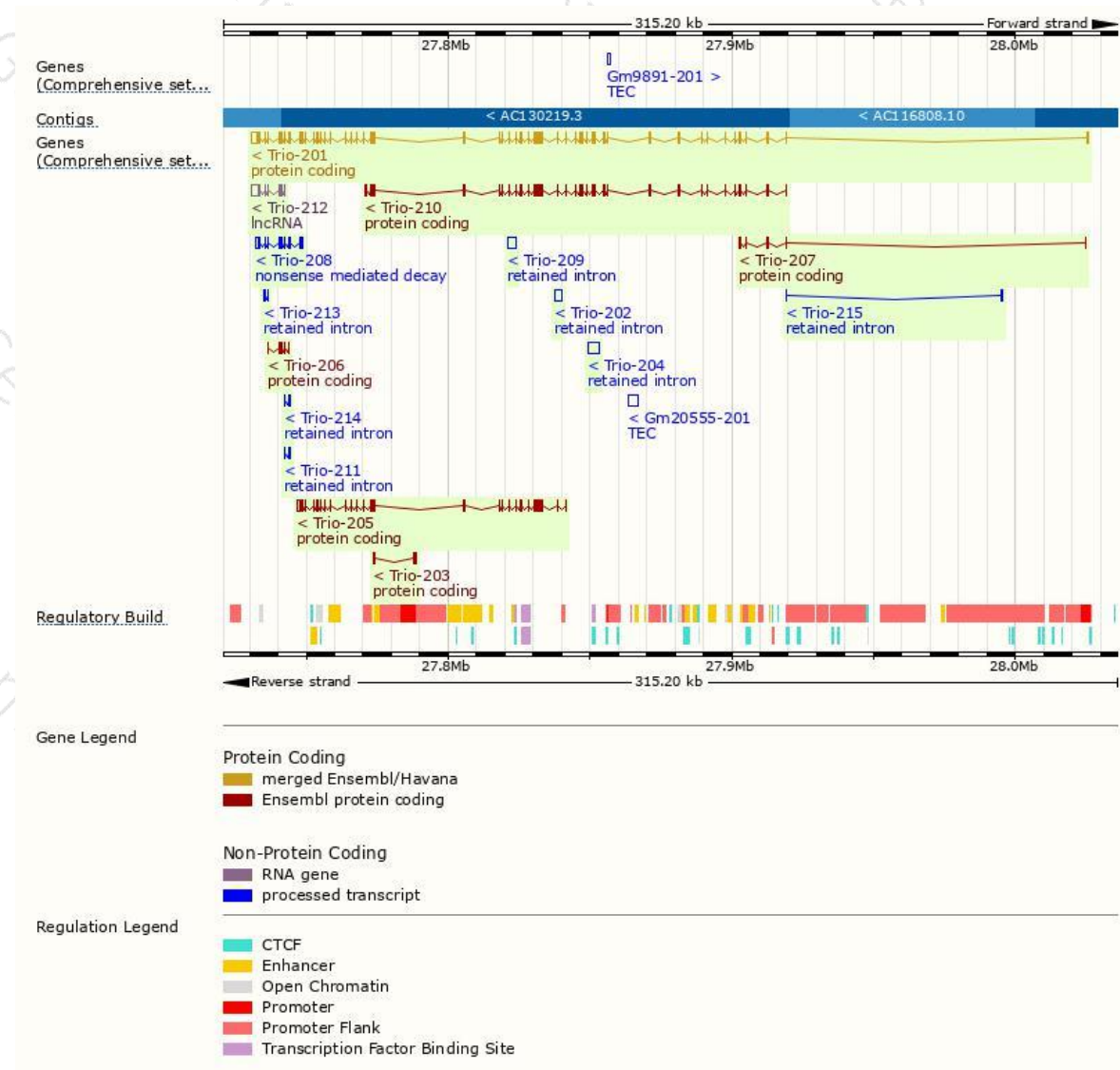
The gene has 15 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trio-201	ENSMUST00000090247.6	11495	3103aa	Protein coding	CCDS49587	Q0KL02	TSL:5 GENCODE basic APPRIS P1
Trio-210	ENSMUST00000227337.1	6375	1849aa	Protein coding	-	A0A2I3BRP6	GENCODE basic
Trio-205	ENSMUST00000226644.1	5233	1424aa	Protein coding	-	A0A2I3BQT2	CDS 5' incomplete
Trio-203	ENSMUST00000226287.1	850	132aa	Protein coding	-	A0A2I3BRF7	CDS 3' incomplete
Trio-207	ENSMUST00000226775.1	712	178aa	Protein coding	-	A0A2I3BRK3	CDS 3' incomplete
Trio-206	ENSMUST00000226713.1	550	184aa	Protein coding	-	A0A2I3BS12	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete
Trio-208	ENSMUST00000227030.1	2335	67aa	Nonsense mediated decay	-	A0A2I3BPR4	CDS 5' incomplete
Trio-204	ENSMUST00000226580.1	3887	No protein	Retained intron	-	-	
Trio-209	ENSMUST00000227044.1	2570	No protein	Retained intron	-	-	
Trio-202	ENSMUST00000226117.1	2495	No protein	Retained intron	-	-	
Trio-213	ENSMUST00000228054.1	606	No protein	Retained intron	-	-	
Trio-211	ENSMUST00000227642.1	477	No protein	Retained intron	-	-	
Trio-214	ENSMUST00000228084.1	457	No protein	Retained intron	-	-	
Trio-215	ENSMUST00000228950.1	442	No protein	Retained intron	-	-	
Trio-212	ENSMUST00000227999.1	3132	No protein	lncRNA	-	-	

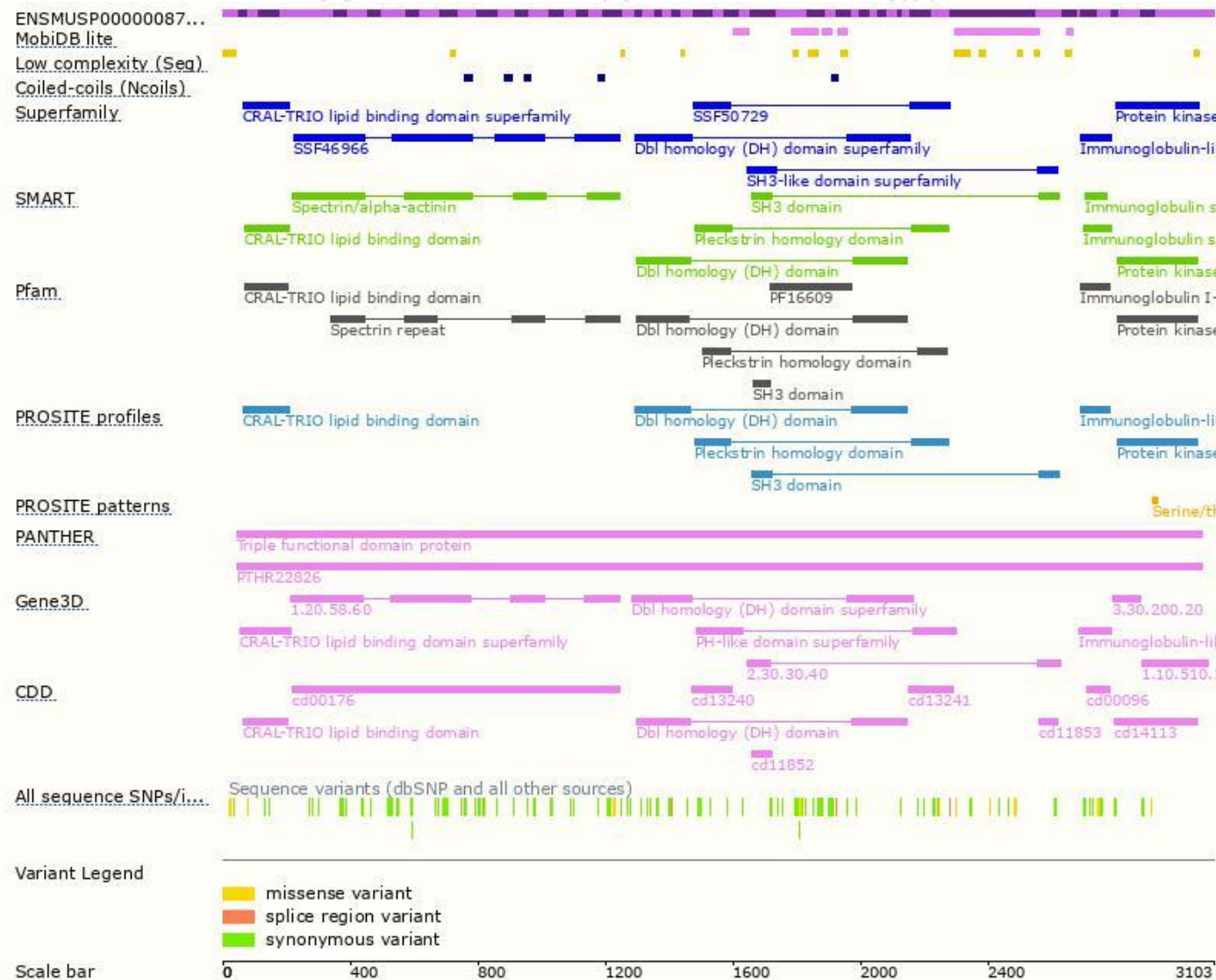
The strategy is based on the design of *Trio-201* transcript,The transcription is shown below



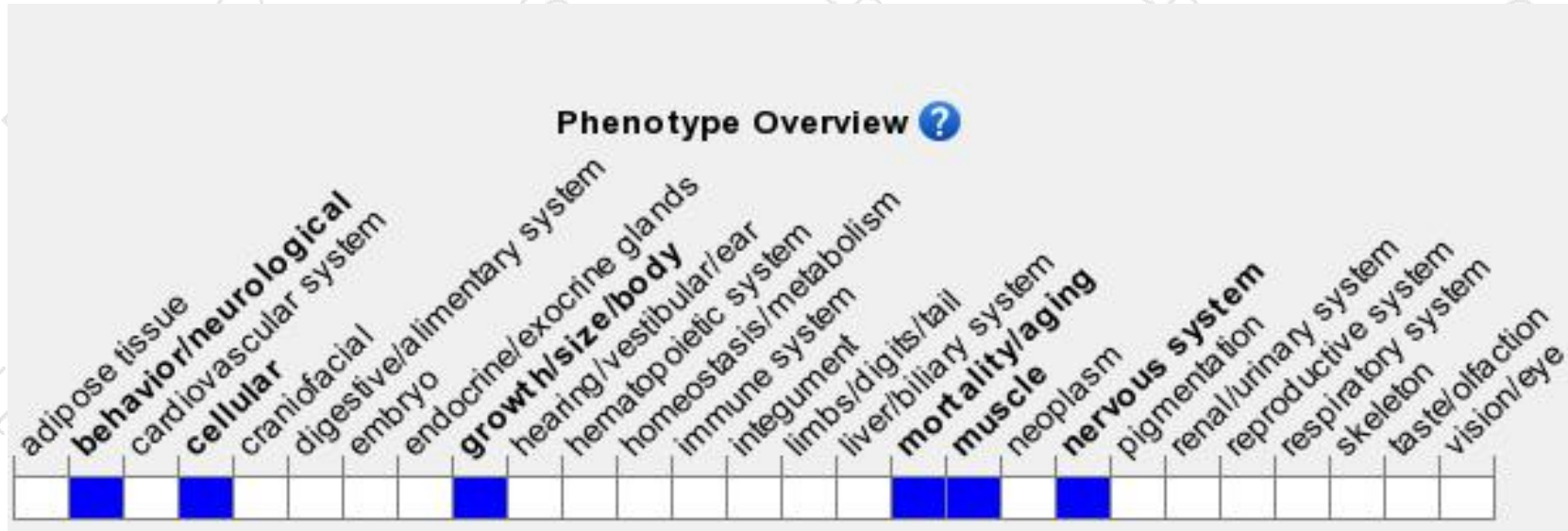
Genomic location distribution



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, homozygous mutant mice die during late embryonic development or shortly after birth.

They exhibit abnormal skeletal myogenesis and display aberrant organization within the hippocampus and olfactory bulb.

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

Tel: 400-9660890

