Tra2b Cas9-KO Strategy

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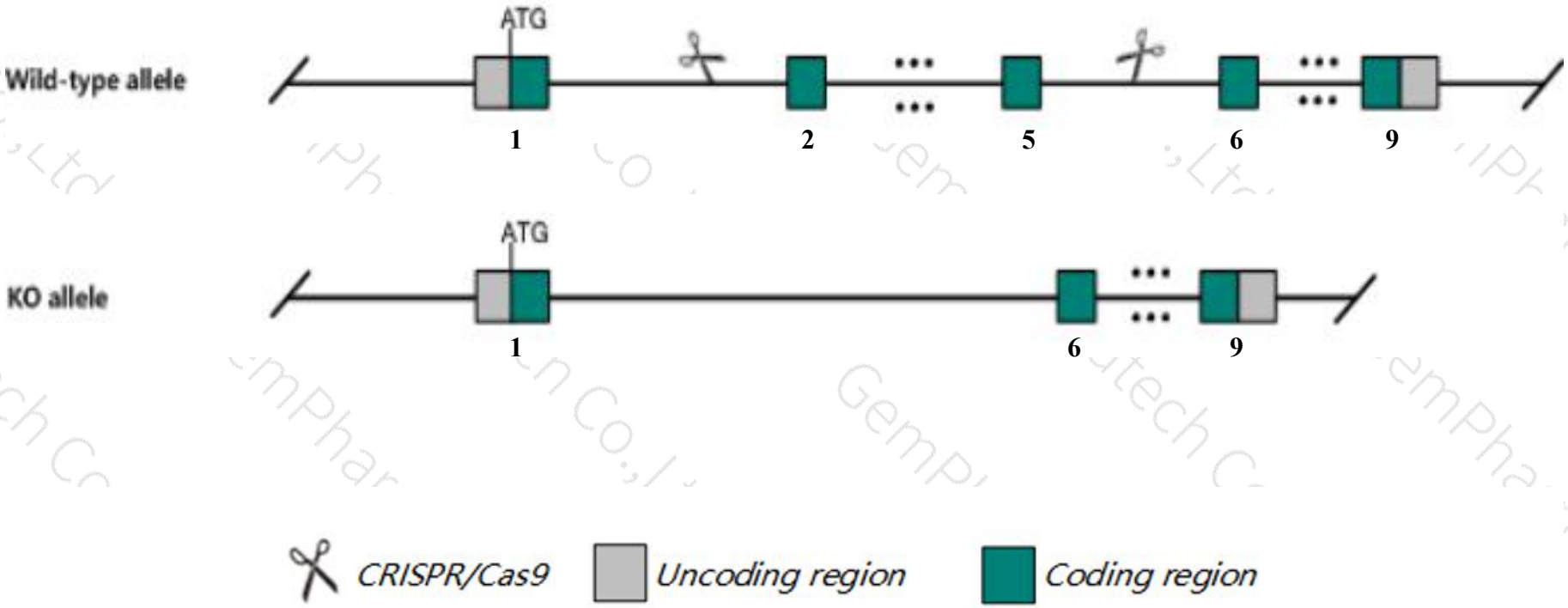
Project Overview

Project Name	***Tra2b***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tra2b* gene. The schematic diagram is as follows:



Technical routes

➢ The *Tra2b* gene has 9 transcripts. According to the structure of *Tra2b* gene, exon2-exon5 of *Tra2b-204*(ENSMUST00000161286.7) transcript is recommended as the knockout region.The region contains 602bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Tra2b* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,mice homozygous for a knock-out allele exhibit reduced embryo size and early embryonic lethality associated with deficient vasculogenesis and abnormal allantois morphology.

➢ The *Tra2b* gene is located on the Chr16. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Tra2b transformer 2 beta [Mus musculus (house mouse)]

Gene ID: 20462, updated on 13-Mar-2020

Summary

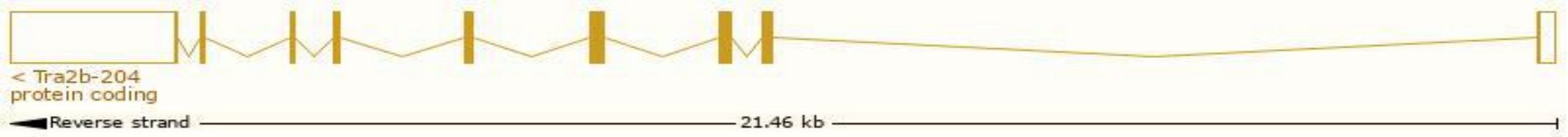
Official Symbol	Tra2b provided by MGI
Official Full Name	transformer 2 beta provided by MGI
Primary source	MGI:MGI:106016
See related	Ensembl:ENSMUSG00000022858
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5730405G21Rik, D16Ertd266e, SIG-41, Sfrs10, Silg41, TRA2beta
Expression	Ubiquitous expression in CNS E11.5 (RPKM 57.4), CNS E14 (RPKM 44.4) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

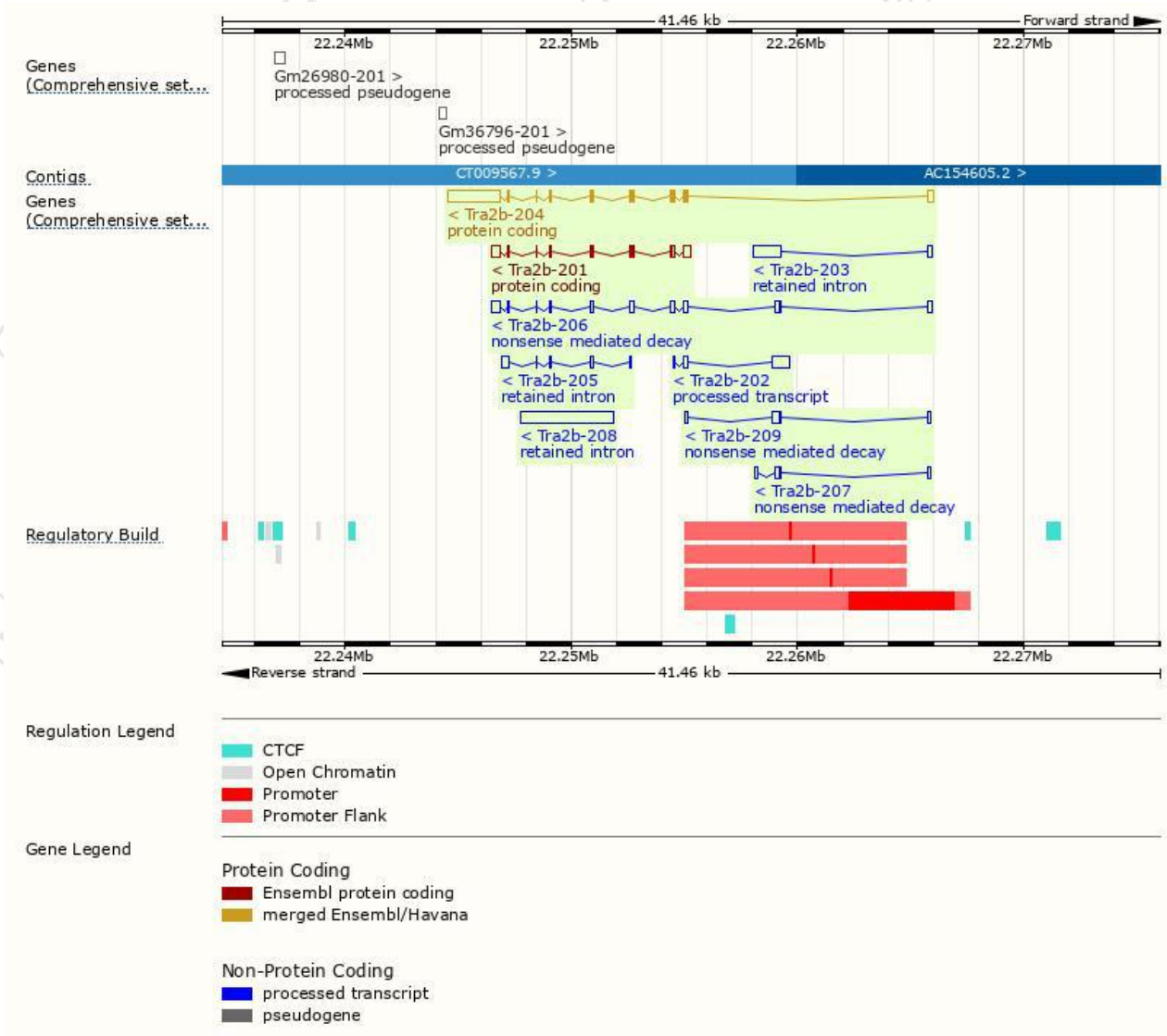
The gene has 9 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tra2b-204	ENSMUST00000161286.7	3403	288aa	Protein coding	CCDS37297	P62996	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 P3
Tra2b-201	ENSMUST00000023564.9	1369	188aa	Protein coding	CCDS84216	F8WJG3	TSL:3 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 ALT1
Tra2b-206	ENSMUST00000162413.8	1687	38aa	Nonsense mediated decay	-	A0A0R4J1X8	TSL:1
Tra2b-209	ENSMUST00000232471.1	632	38aa	Nonsense mediated decay	-	A0A0R4J1X8	
Tra2b-207	ENSMUST00000231312.1	550	38aa	Nonsense mediated decay	-	A0A0R4J1X8	
Tra2b-202	ENSMUST00000159946.1	946	No protein	Processed transcript	-	-	TSL:3
Tra2b-208	ENSMUST00000232411.1	4112	No protein	Retained intron	-	-	
Tra2b-203	ENSMUST00000160579.1	1376	No protein	Retained intron	-	-	TSL:1
Tra2b-205	ENSMUST00000161774.1	668	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Tra2b-204* transcript,the transcription is shown below:

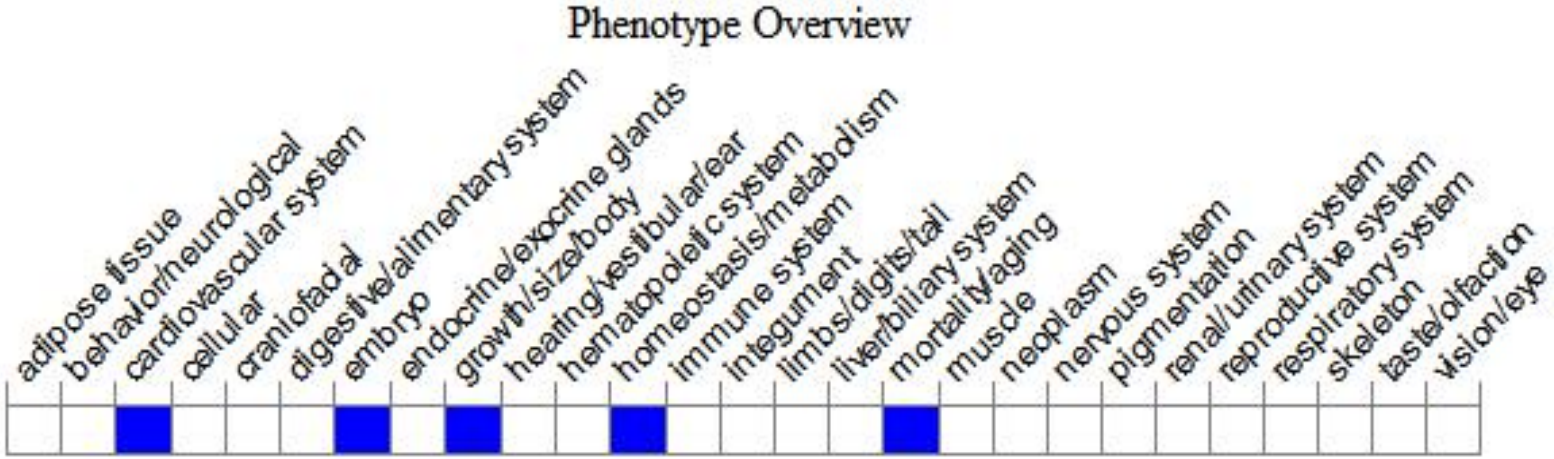


Genomic location distribution



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 knock-out allele exhibit reduced embryo size and early embryonic lethality associated with deficient vasculogenesis and abnormal allantois morphology.

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

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