

***Tra2b* Cas9-CKO Strategy**

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Project Overview

Project Name

Tra2b

Project type

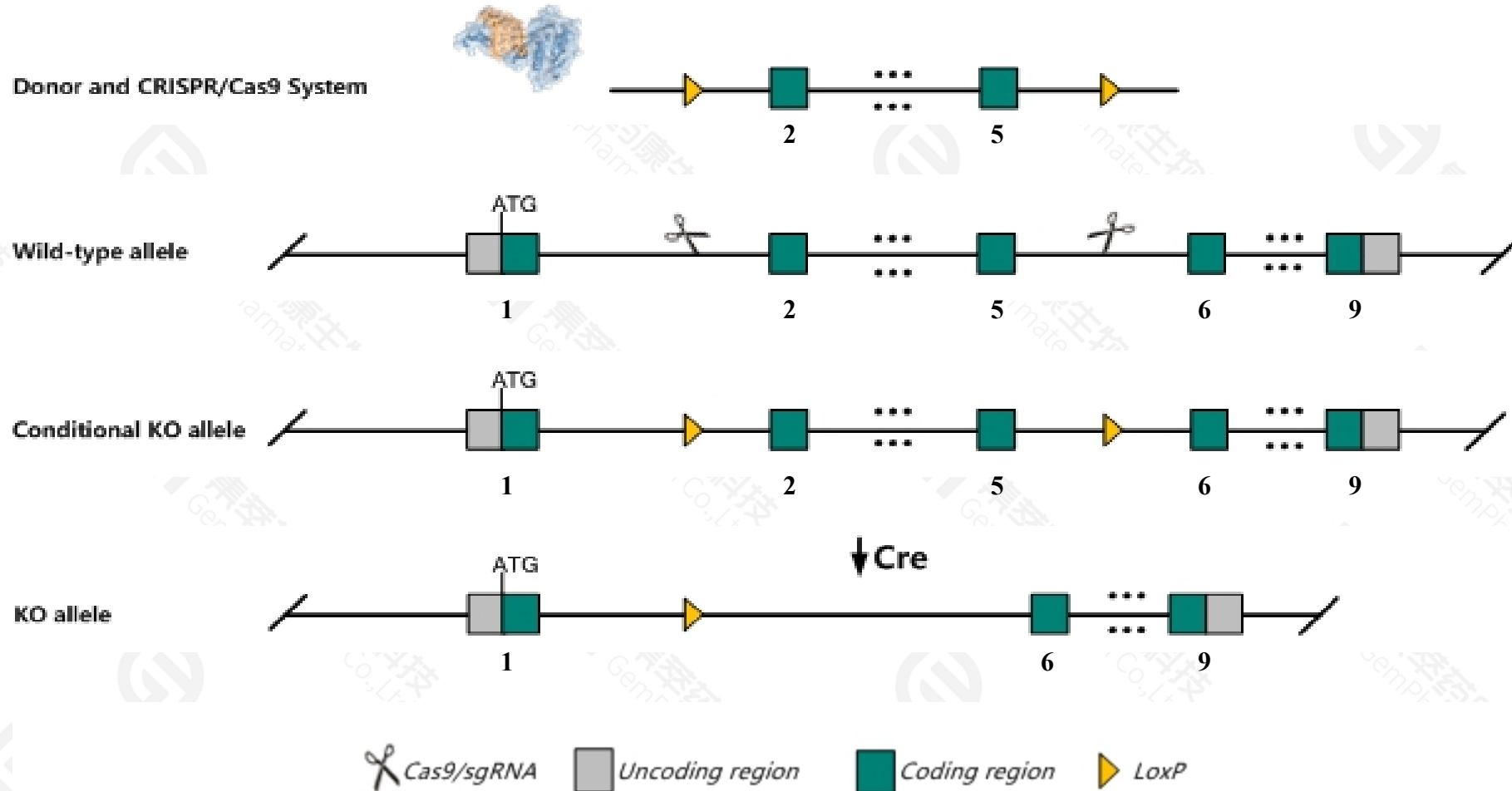
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional 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: sgRNA was transcribed in vitro, donor was constructed. Cas9, sgRNA 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 was 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, 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

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, D16Ert266e, 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](#)

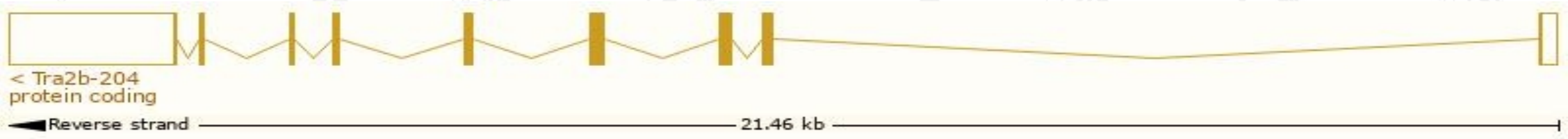
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

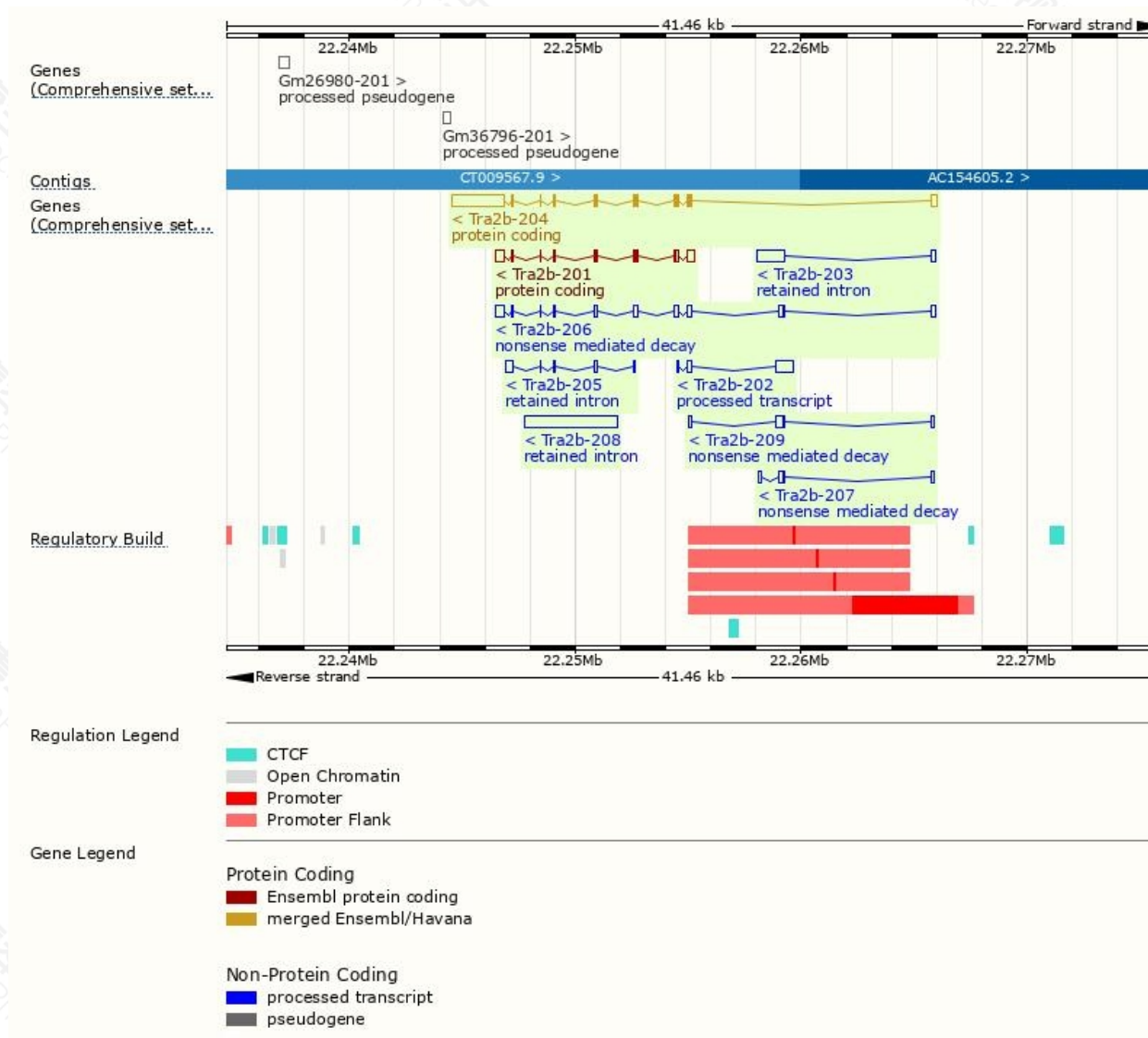
The gene has 9 transcripts,all transcripts are shown below:

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

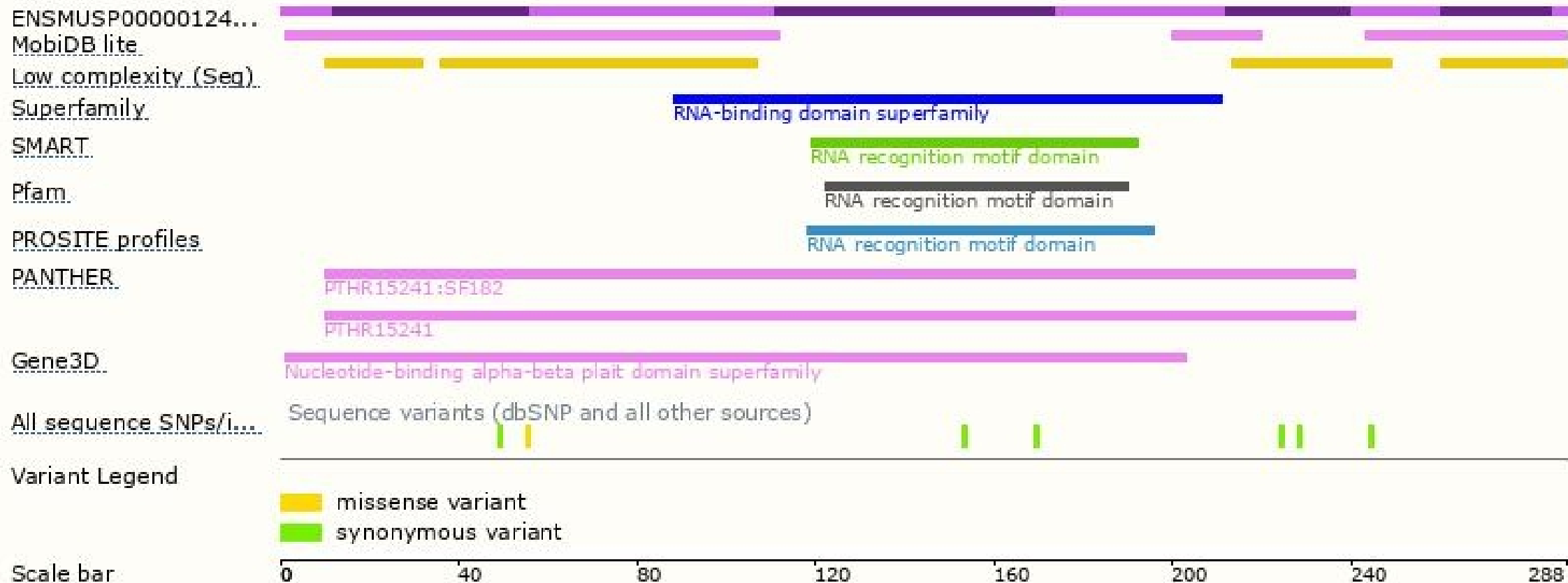
The strategy is based on the design of *Tra2b-204* transcript,the transcription is shown below:



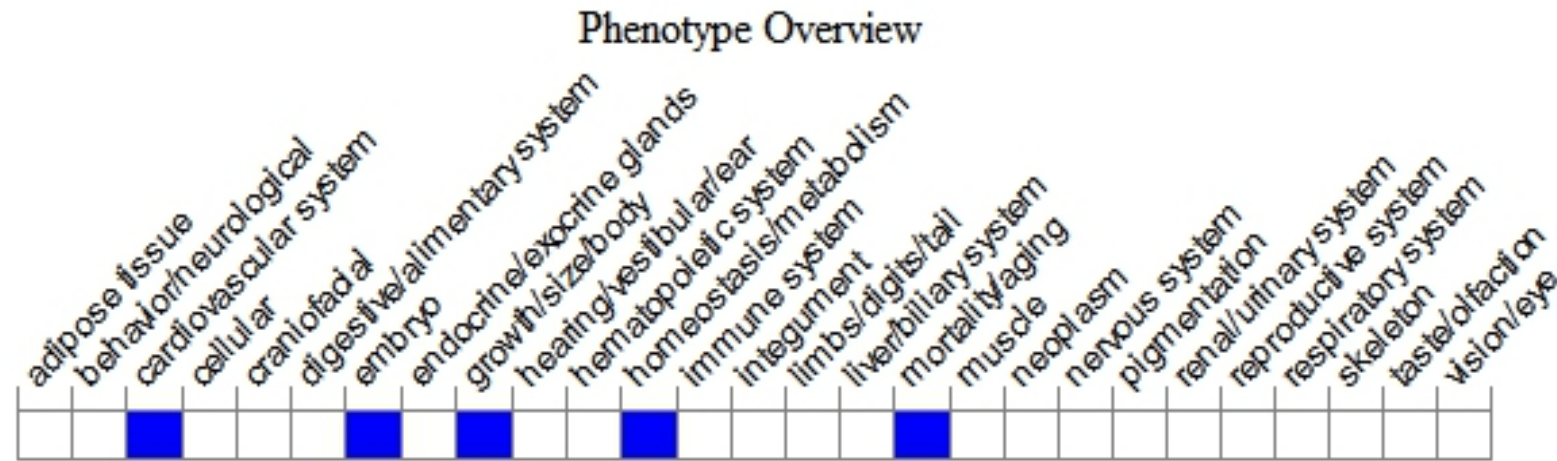
Genomic location distribution



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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