Tbc1d32 Cas9-KO Strategy

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Reviewer: JiaYu

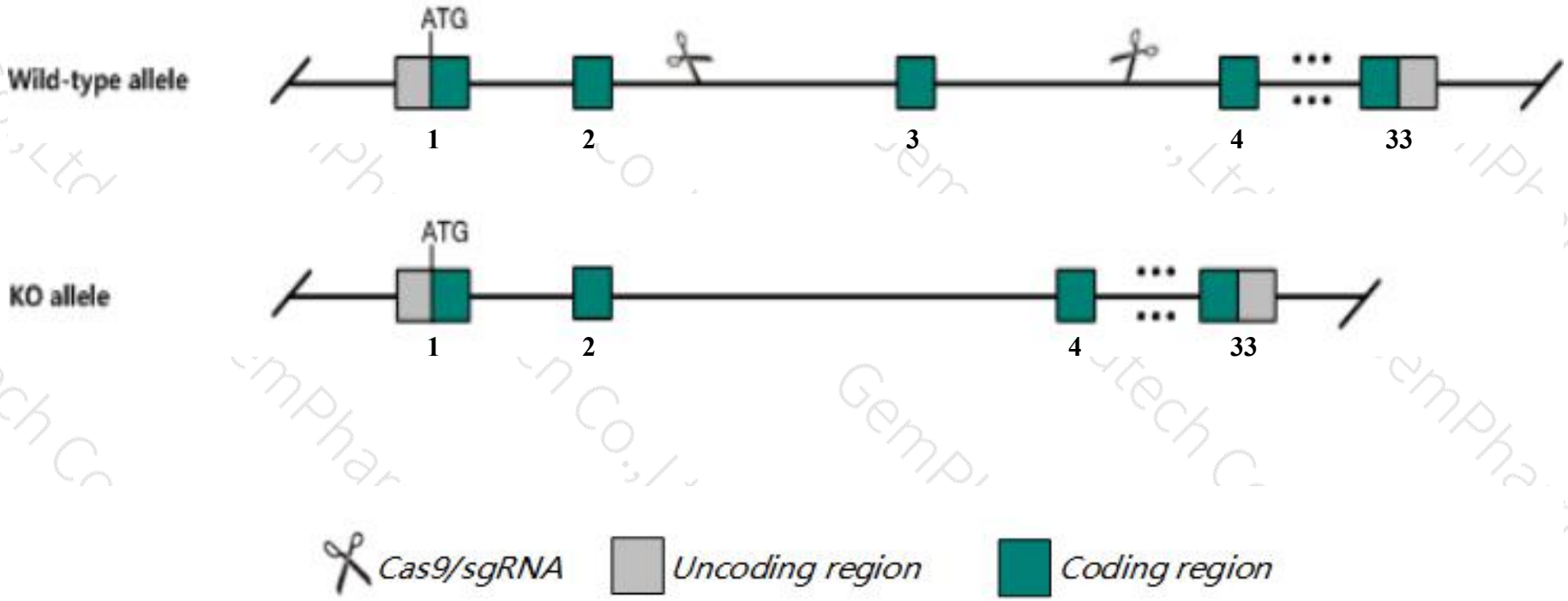
Design Date: 2020-8-17

Project Overview

Project Name	***Tbc1d32***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Tbc1d32* gene. The schematic diagram is as follows:



Technical routes

➢ The *Tbc1d32* gene has 3 transcripts. According to the structure of *Tbc1d32* gene, exon3 of *Tbc1d32-201*(ENSMUST00000099739.4) transcript is recommended as the knockout region.The region contains 169bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Tbc1d32* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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 gene trap allele or ENU induced mutation exhibit exencephaly and poor eye development.

➢ The *Tbc1d32* gene is located on the Chr10. 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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Tbc1d32 TBC1 domain family, member 32 [Mus musculus (house mouse)]

Gene ID: 544696, updated on 13-Mar-2020

Summary

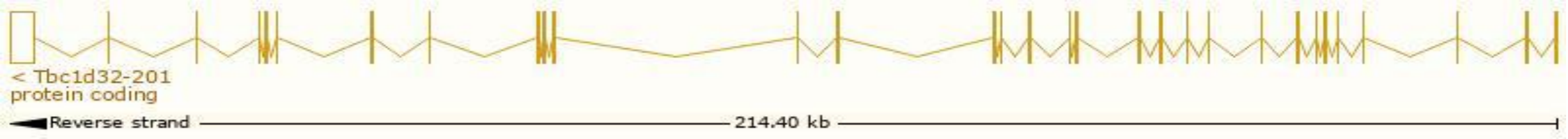
Official Symbol	Tbc1d32 provided by MGI
Official Full Name	TBC1 domain family, member 32 provided byMGI
Primary source	MGI:MGI:2442827
See related	Ensembl:ENSMUSG00000038122
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Bromi, C6orf170, D630037F22Rik, b2b2284Clo
Expression	Ubiquitous expression in bladder adult (RPKM 1.5), CNS E18 (RPKM 1.1) and 26 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

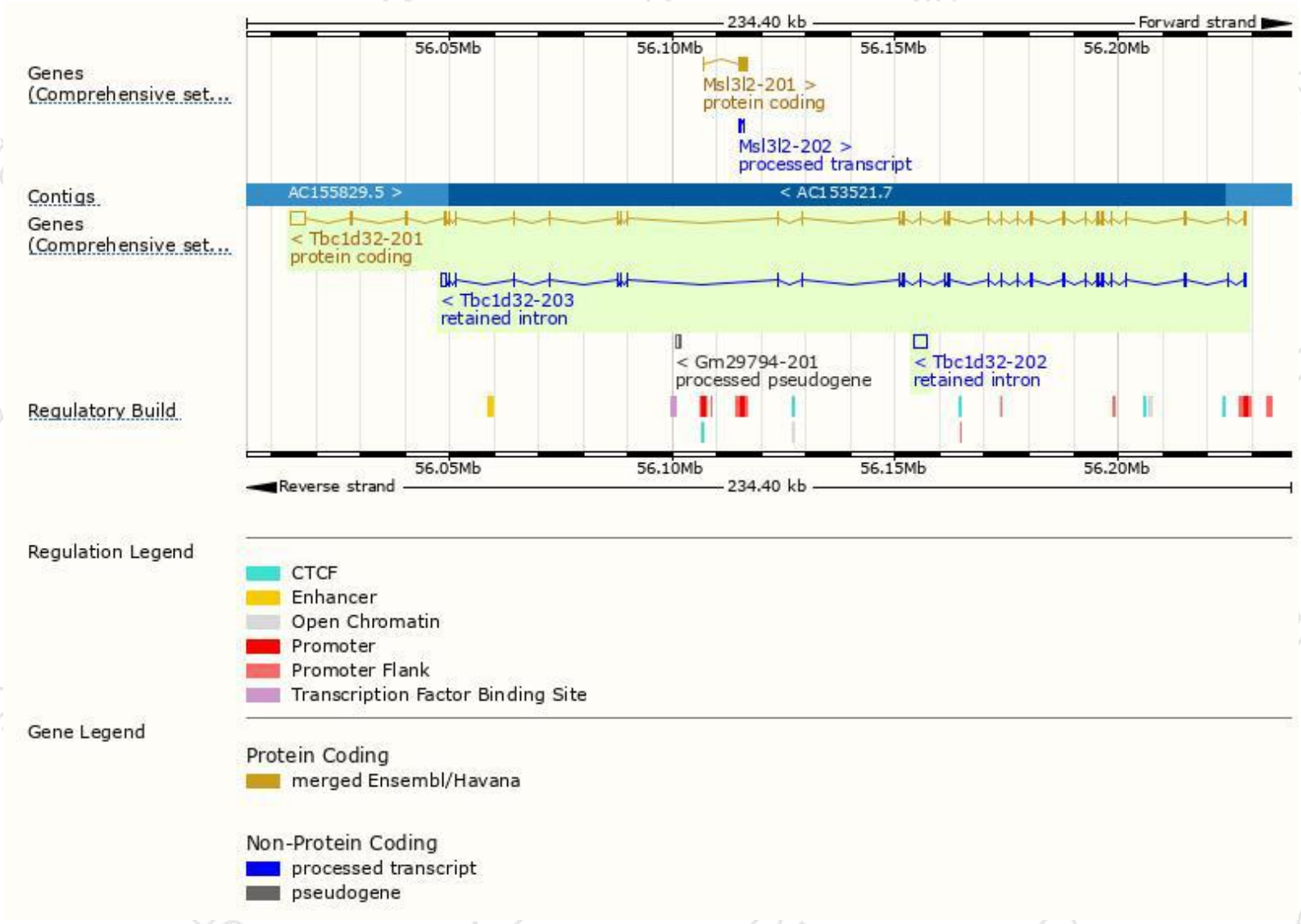
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tbc1d32-201	ENSMUST00000099739.4	7240	1296aa	Protein coding	CCDS48562	Q3URV1	TSL:5 GENCODE basic APPRIS P1
Tbc1d32-203	ENSMUST00000219385.1	4192	No protein	Retained intron	-	-	TSL:5
Tbc1d32-202	ENSMUST00000217792.1	2761	No protein	Retained intron	-	-	TSL:NA

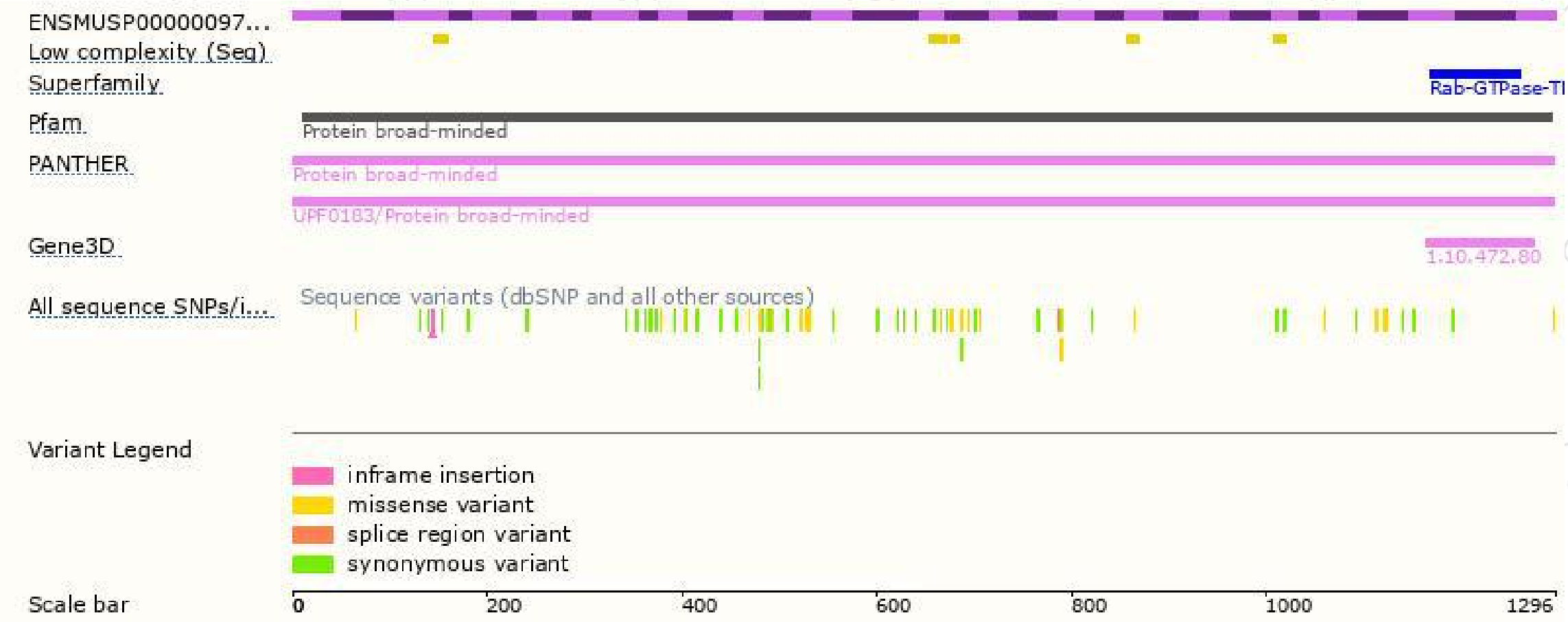
The strategy is based on the design of *Tbc1d32-201* transcript,the transcription is shown below:



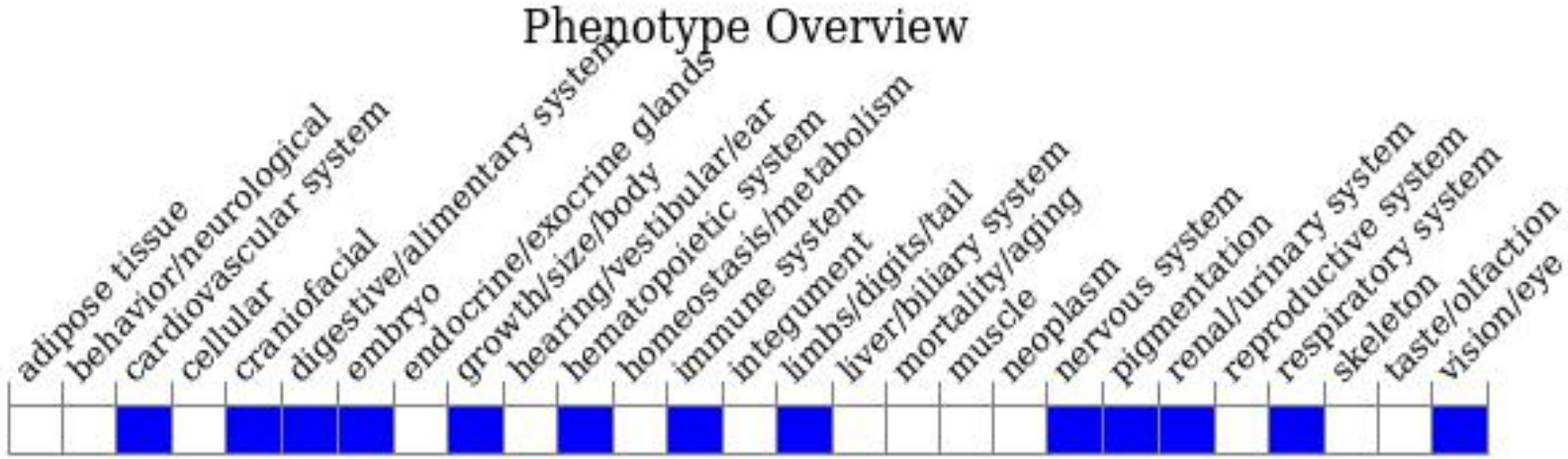
Genomic location distribution



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/).

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

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