Rbx1 Cas9-KO Strategy

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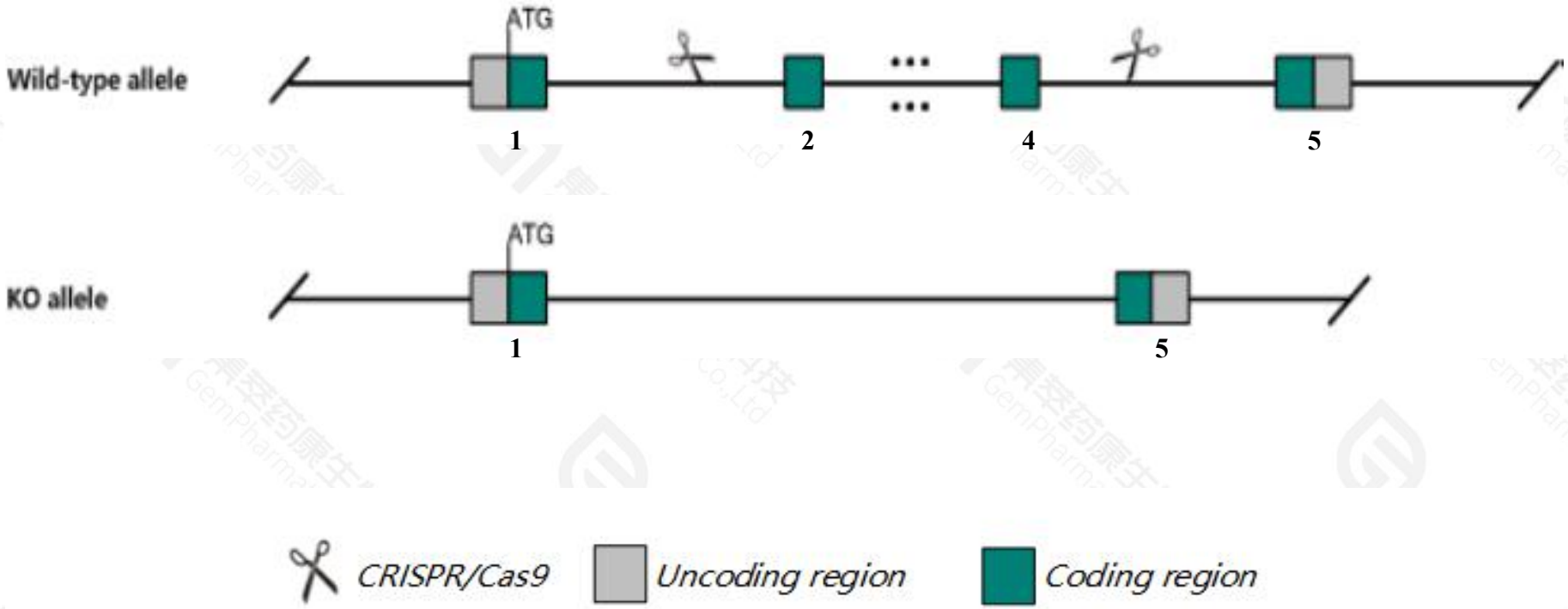
Design Date: 2021-9-6

Project Overview

Project Name	***Rbx1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rbx1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Rbx1* gene has 6 transcripts. According to the structure of *Rbx1* gene, exon2-exon4 of *Rbx1-201*(ENSMUST00000023036.7) transcript is recommended as the knockout region.The region contains 236bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Rbx1* 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 null mutation display embryonic lethality before somite formation with reduced embryo size and decreased cell proliferation. Embryonic fibroblasts from heterozygous mice display decreased cell proliferation and cell cycle abnormalities.

➢ The *Rbx1* 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 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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Rbx1 ring-box 1 [Mus musculus (house mouse)]

Gene ID: 56438, updated on 19-Feb-2019

Summary

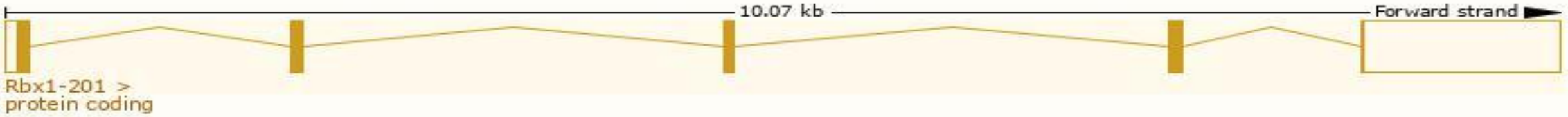
Official Symbol	Rbx1 provided by MGI
Official Full Name	ring-box 1 provided by MGI
Primary source	MGI:MGI:1891829
See related	Ensembl:ENSMUSG00000022400
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1500002P15Rik, AA517855, ROC1
Expression	Ubiquitous expression in CNS E11.5 (RPKM 43.0), CNS E14 (RPKM 40.8) and 26 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

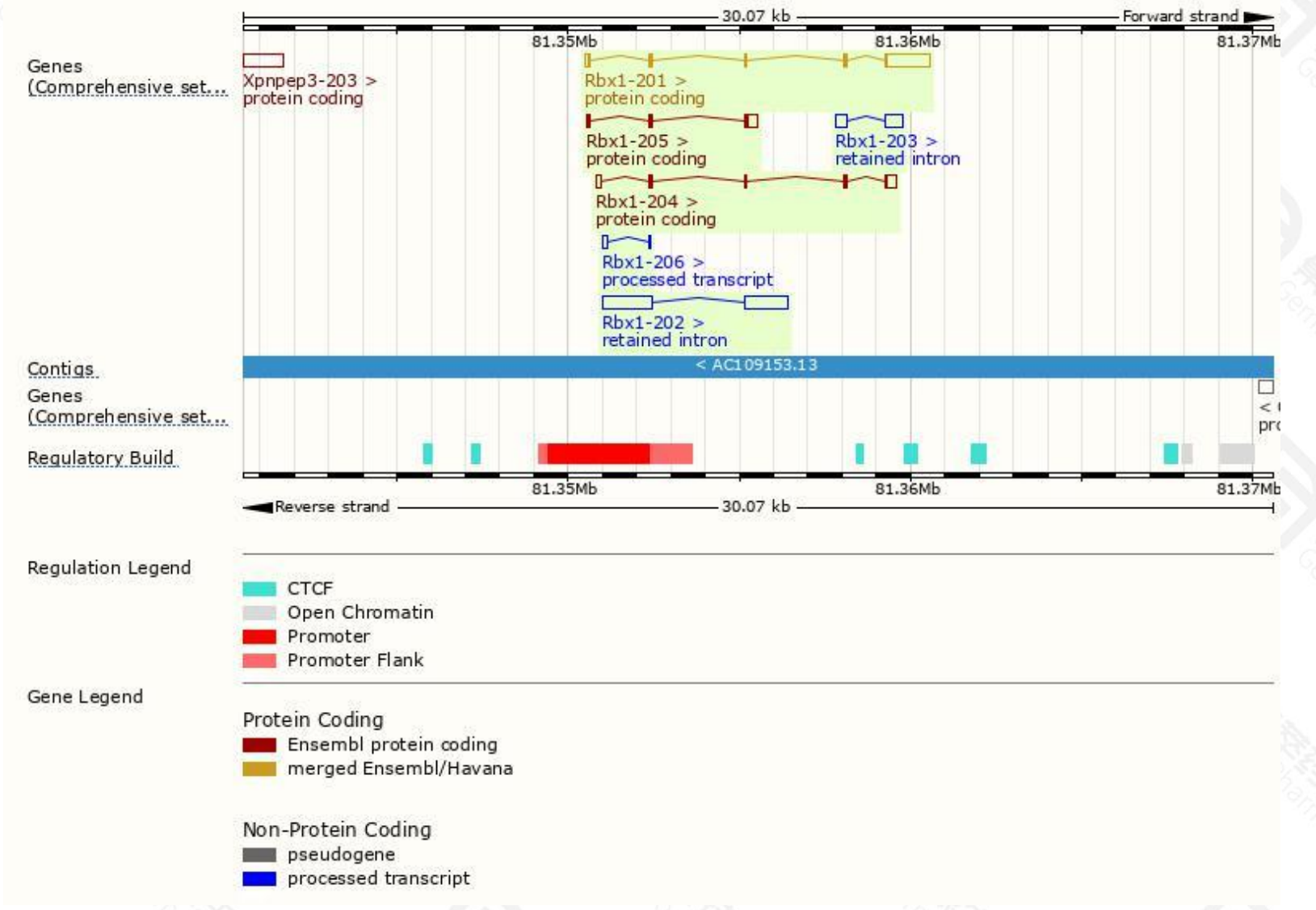
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rbx1-201	ENSMUST00000023036.6	1675	108aa	Protein coding	CCDS49676	P62878	TSL:1 GENCODE basic APPRIS P1
Rbx1-204	ENSMUST00000230062.1	651	59aa	Protein coding	-	A0A2R8W6G1	GENCODE basic
Rbx1-205	ENSMUST00000230219.1	555	83aa	Protein coding	-	A0A2R8W6R3	GENCODE basic
Rbx1-206	ENSMUST00000231027.1	218	No protein	Processed transcript	-	-	
Rbx1-202	ENSMUST00000229880.1	2669	No protein	Retained intron	-	-	
Rbx1-203	ENSMUST00000229991.1	794	No protein	Retained intron	-	-	

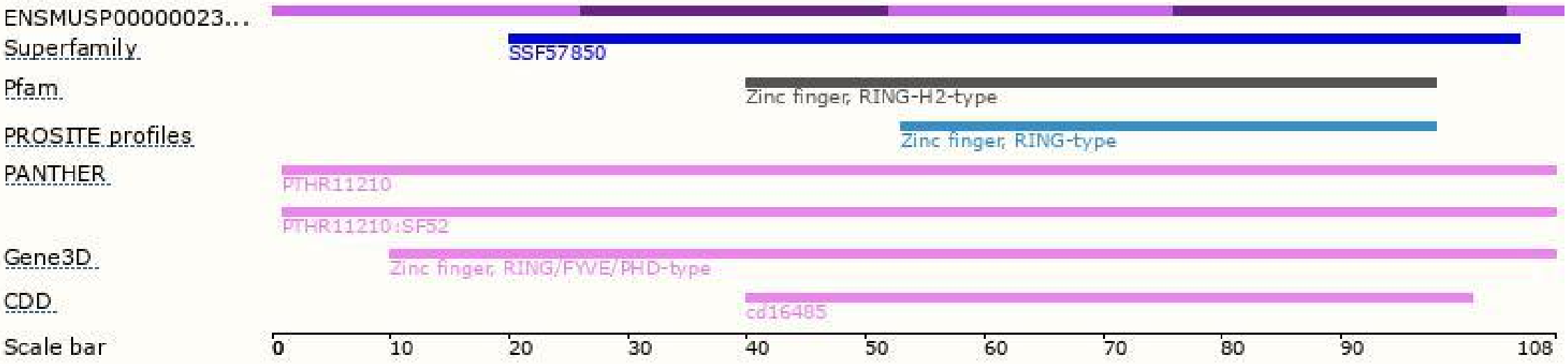
The strategy is based on the design of *Rbx1-201* transcript,the transcription is shown below:



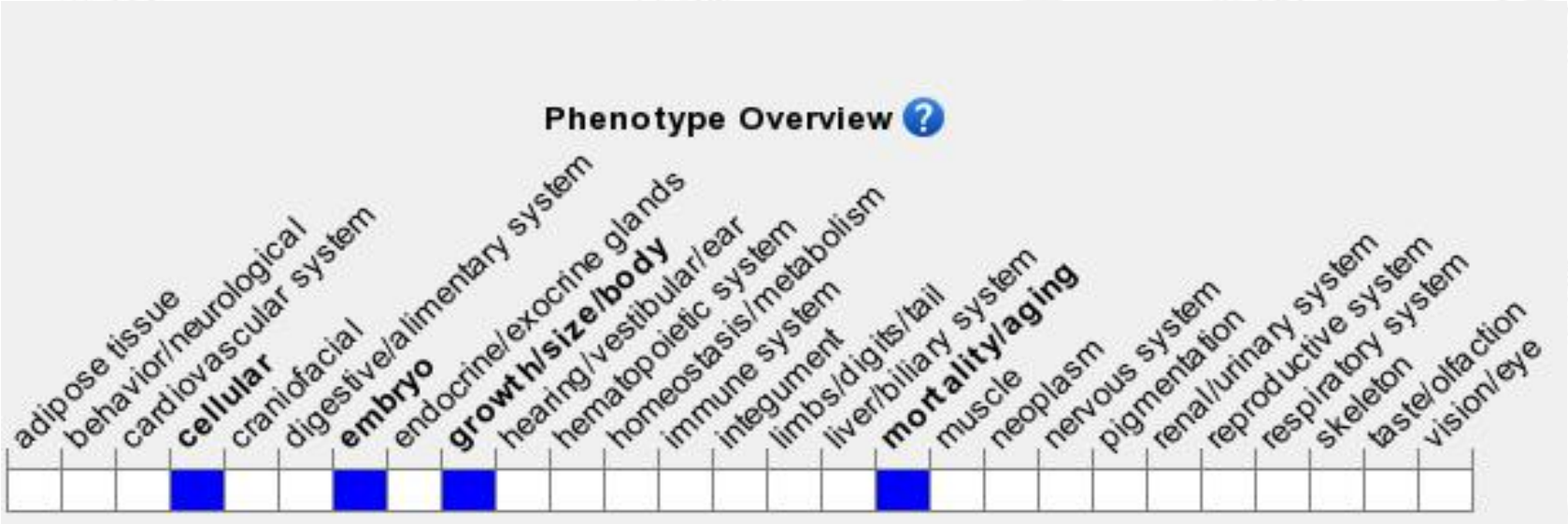
Genomic location distribution



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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