Rab33b Cas9-CKO Strategy

Designer:

Reviewer:

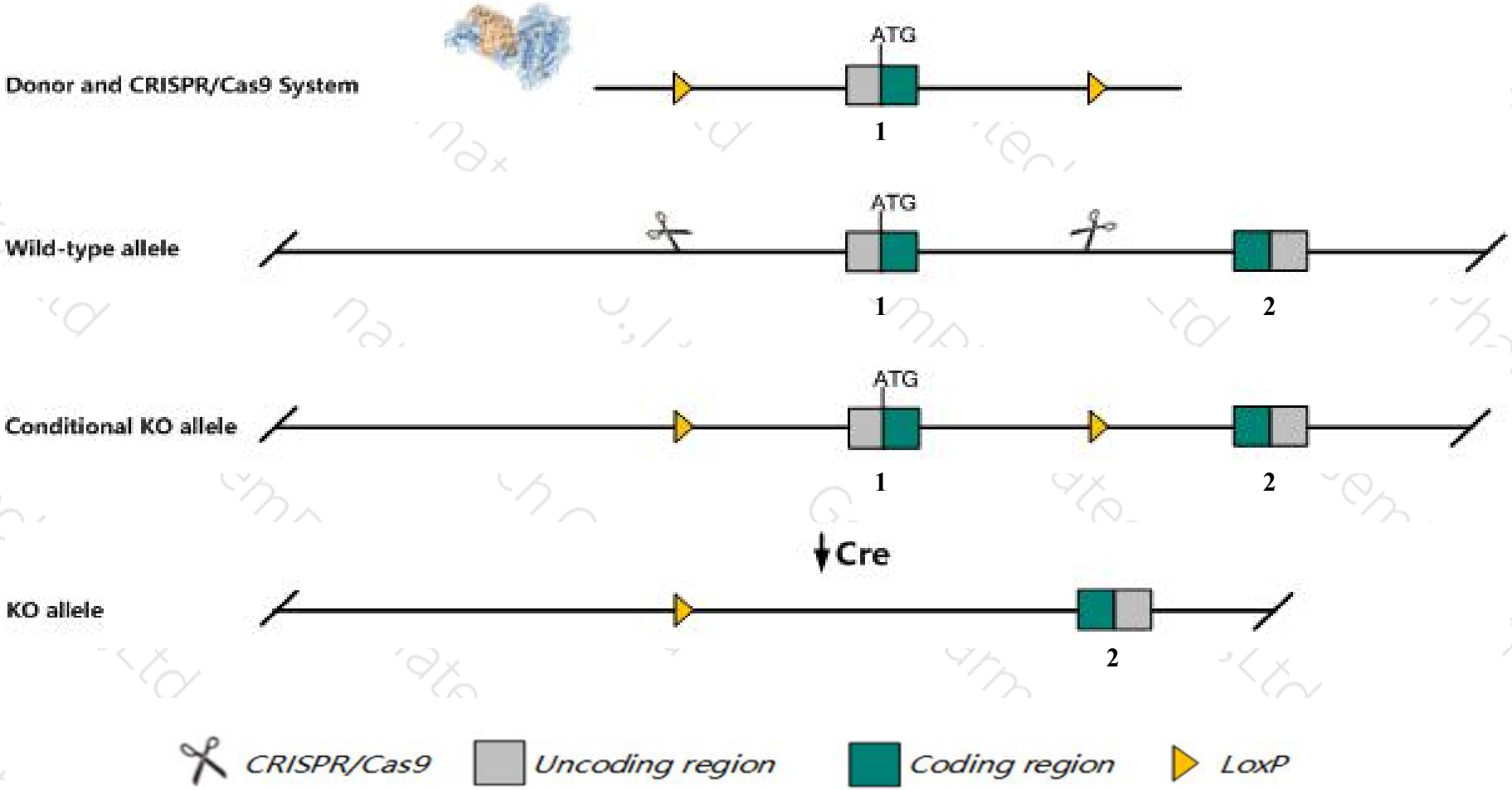
Design Date:

Project Overview

Project Name	***Rab33b***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rab33b* gene. The schematic diagram is as follows:



Technical routes

- The *Rab33b* gene has 3 transcripts. According to the structure of *Rab33b* gene, exon1 of *Rab33b-201* (ENSMUST00000054387.7) transcript is recommended as the knockout region.The region contains start codon ATG. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rab33b* 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.

Notice

➢The KO region contains functional region of the *Gm38160* gene.Knockout the region may affect the function of *Gm38160* gene.

➢ The *Rab33b* gene is located on the Chr3. 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）

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Rab33b RAB33B, member RAS oncogene family [Mus musculus (house mouse)]

Gene ID: 19338, updated on 13-Mar-2020

Summary

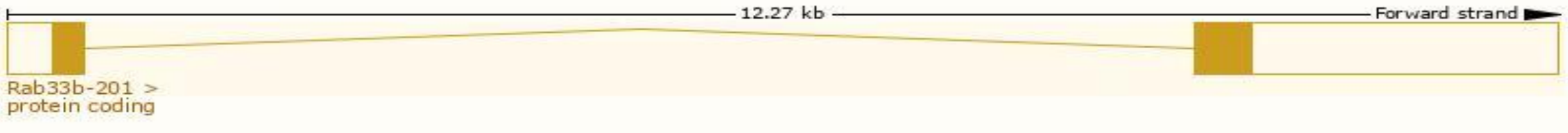
Official Symbol	Rab33b provided by MGI
Official Full Name	RAB33B, member RAS oncogene family provided by MGI
Primary source	MGI:MGI:1330805
See related	Ensembl:ENSMUSG00000027739
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Ubiquitous expression in bladder adult (RPKM 9.3), CNS E18 (RPKM 5.9) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

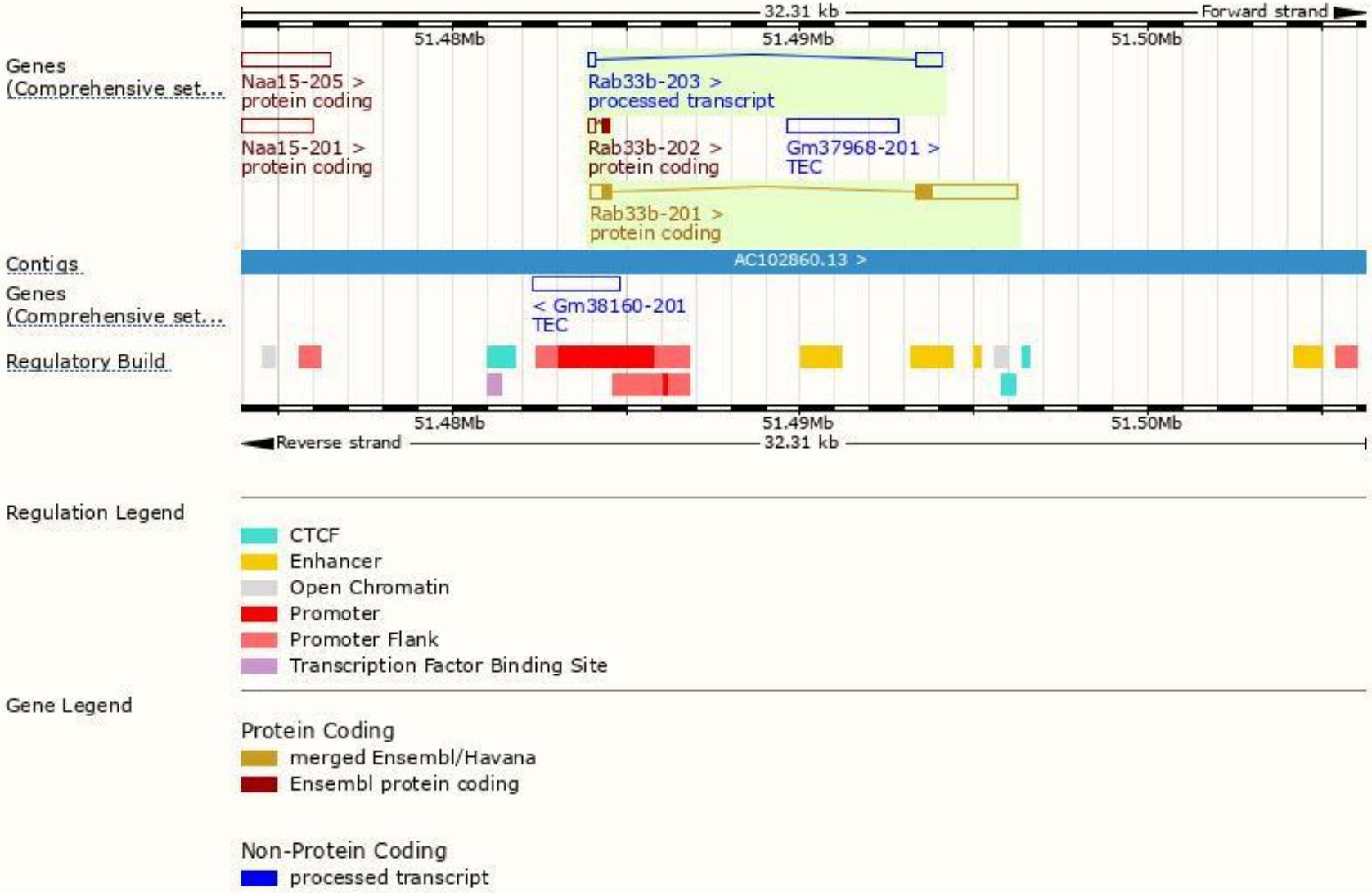
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rab33b-201	ENSMUST00000054387.7	3492	229aa	Protein coding	CCDS38427	O35963 Q0PD21	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 P1
Rab33b-202	ENSMUST00000192172.1	397	65aa	Protein coding	-	A0A0A6YY00	CDS 3' incomplete TSL:5
Rab33b-203	ENSMUST00000195715.1	933	No protein	Processed transcript	-	-	TSL:2

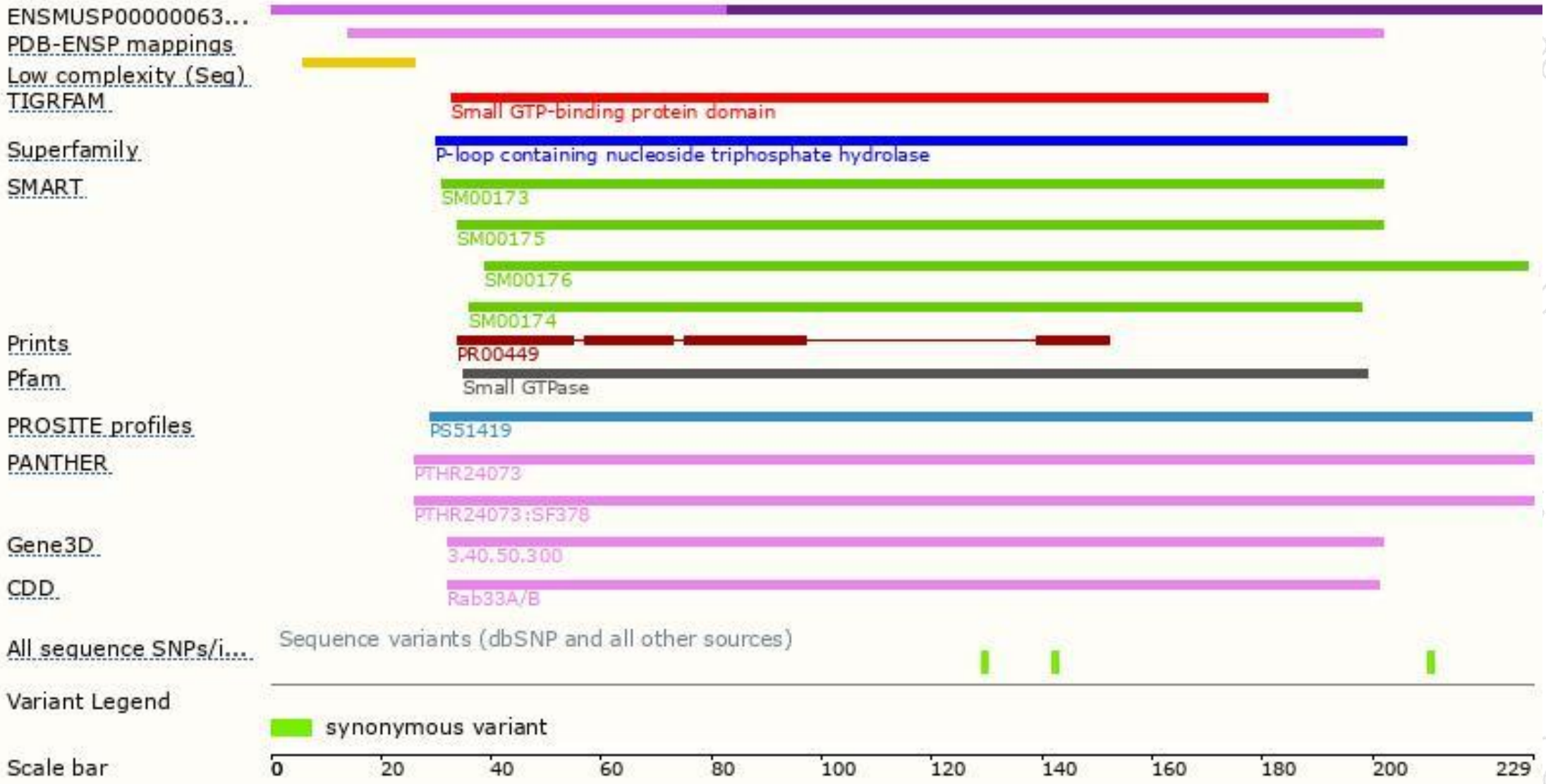
The strategy is based on the design of *Rab33b-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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