Rnase4 Cas9-CKO Strategy

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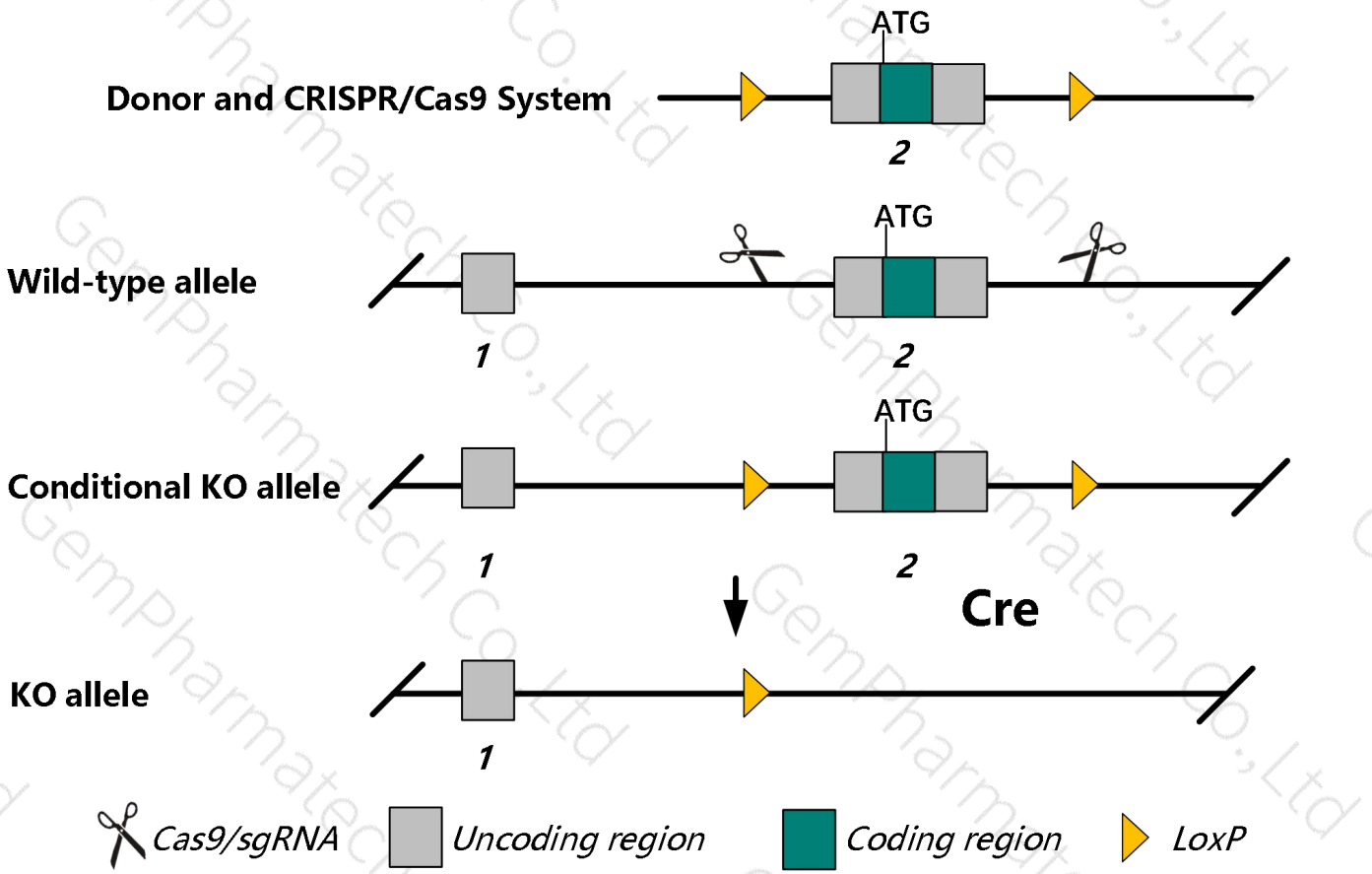
Project Overview

Project Name	***Rnase4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rnase4* gene. The schematic diagram is as follows:



Technical routes

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- The *Rnase4* gene has 2 transcripts. According to the structure of *Rnase4* gene, exon2 of *Rnase4-201* (ENSMUST00000022428.12) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rnase4* 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 *Rnase4* gene is located on the Chr14. 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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Rnase4 ribonuclease, RNase A family 4 [*Mus musculus* (house mouse)]

Gene ID: 58809, updated on 12-Aug-2019

Summary

Official Symbol	Rnase4 provided by MGI
Official Full Name	ribonuclease, RNase A family 4 provided by MGI
Primary source	MGI:MGI:1926217
See related	Ensembl:ENSMUSG00000021876
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Rab1; C730049F20Rik
Summary	This gene encodes a member of the pancreatic ribonuclease A superfamily. The encoded enzyme is sereted and has unique uridine specificity. This gene resides in a cluster of highly related genes. It shares dual promoters and 5' exons with the angiogenin, ribonuclease, RNase A family, 5 gene. Each gene splices to a unique downstream exon that contains its complete coding region. Two alternatively spliced variants, with different 5' exons but the same coding exon, have been identified. [provided by RefSeq, Jun 2009]
Expression	Biased expression in liver adult (RPKM 541.8), lung adult (RPKM 184.6) and 10 other tissues See more
Orthologs	human all

Genomic context

Location: 14; 14 C1

See Rnase4 in Genome Data Viewer

Exon count: 3

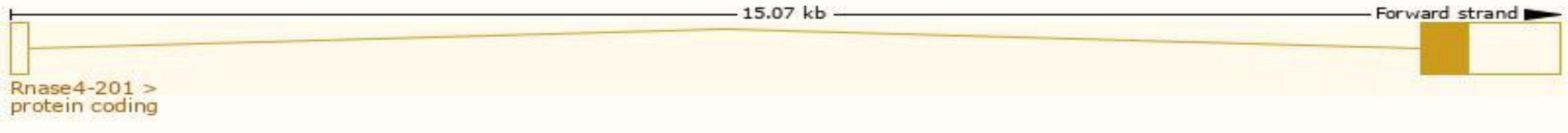
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	14	NC_000080.6 (51091077..51106151)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	14	NC_000080.5 (51710752..51725826)

Transcript information（Ensembl）

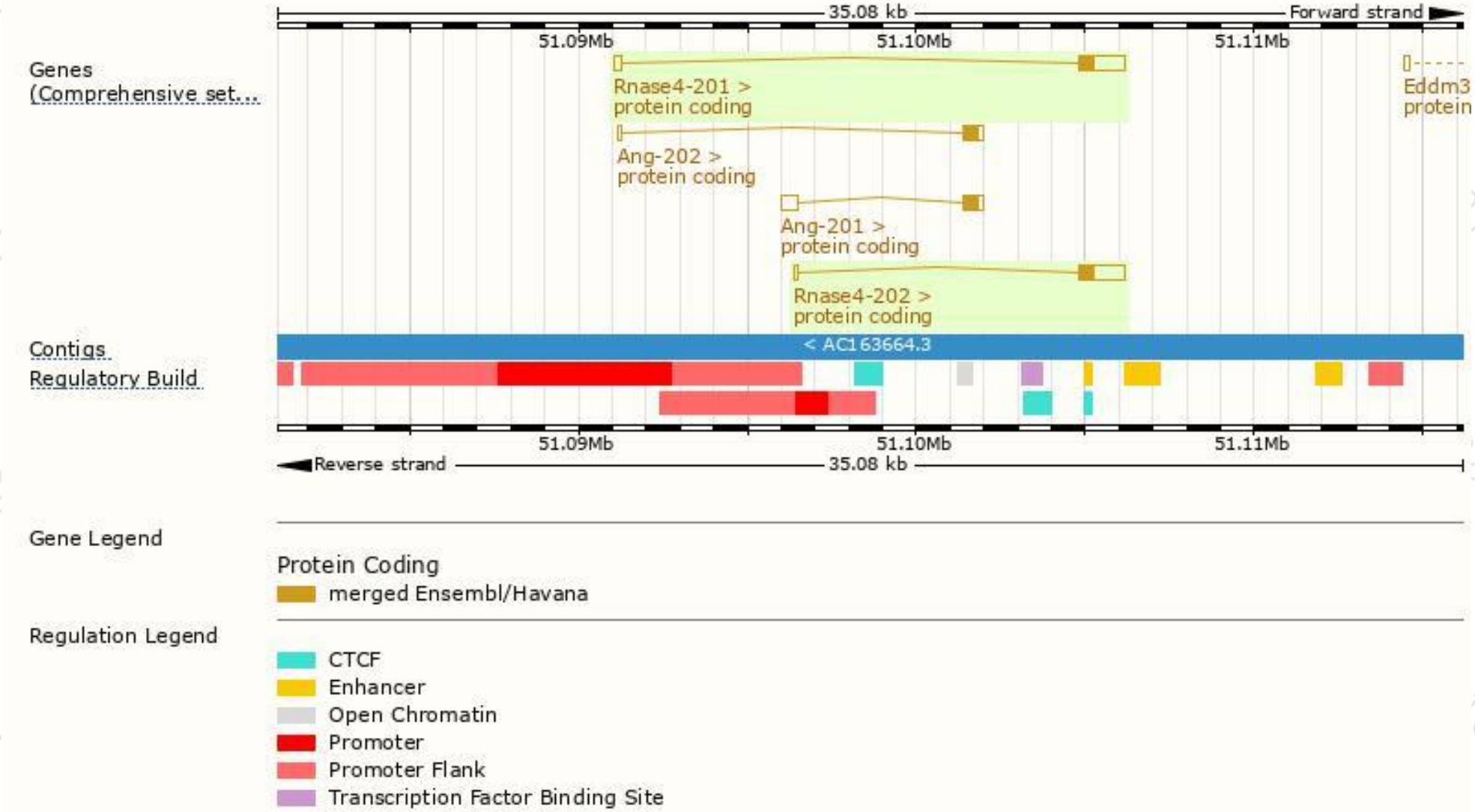
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rnase4-201	ENSMUST00000022428.12	1518	148aa	Protein coding	CCDS27035	Q8C7E4	TSL:1 GENCODE basic APPRIS P1
Rnase4-202	ENSMUST00000169895.2	1460	148aa	Protein coding	CCDS27035	Q8C7E4	TSL:1 GENCODE basic APPRIS P1

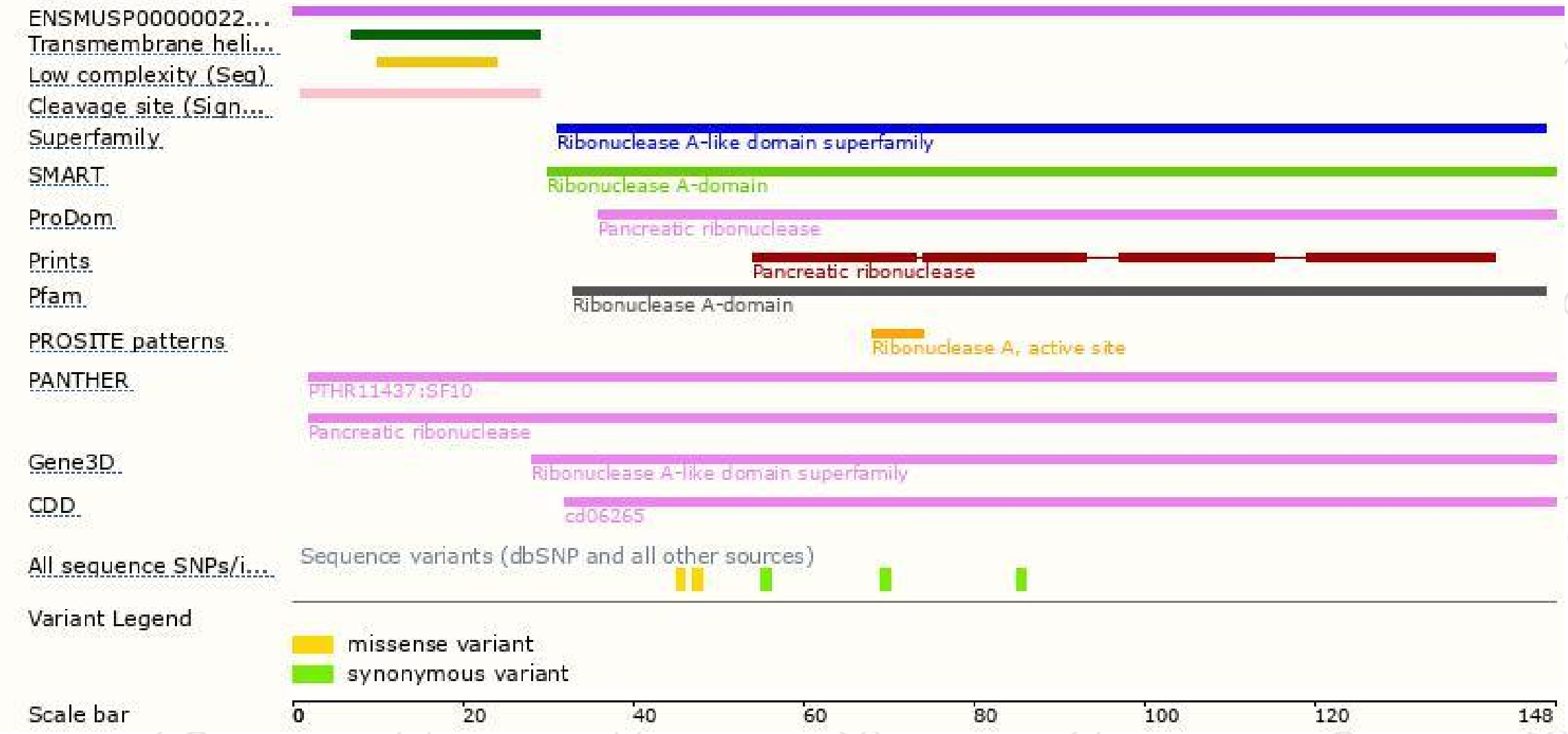
The strategy is based on the design of *Rnase4-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



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

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