

***Rassf8* Cas9-KO Strategy**

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Design Date: 2020-8-24

Project Overview

Project Name

Rassf8

Project type

Cas9-KO

Strain background

C57BL/6JGpt

- The *Rassf8* gene has 4 transcripts. According to the structure of *Rassf8* gene, exon3-exon5 of *Rassf8*-203(ENSMUST00000111704.7) transcript is recommended as the knockout region. The region contains 1157bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rassf8* 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.

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rassf8* gene. The schematic diagram is as follows:



- The *Rassf8* gene is located on the Chr6. 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)

Rassf8 Ras association (RalGDS/AF-6) domain family (N-terminal) member 8 [Mus musculus (house mouse)]

Gene ID: 71323, updated on 13-Mar-2020

Summary



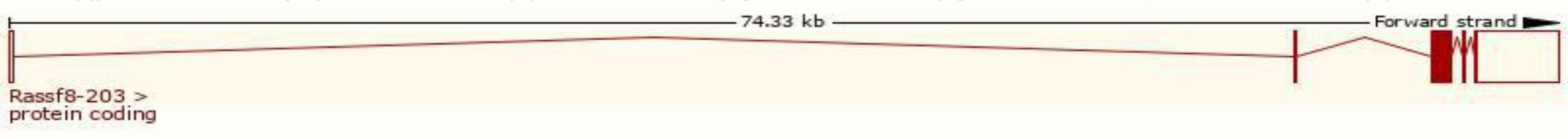
Official Symbol	Rassf8 provided by MGI
Official Full Name	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8 provided by MGI
Primary source	MGI:MGI:1918573
See related	Ensembl:ENSMUSG00000030259
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5133400D11Rik, AA815821, AW123240, Hoj1, mHoj-1
Expression	Ubiquitous expression in placenta adult (RPKM 11.3), limb E14.5 (RPKM 7.6) and 26 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

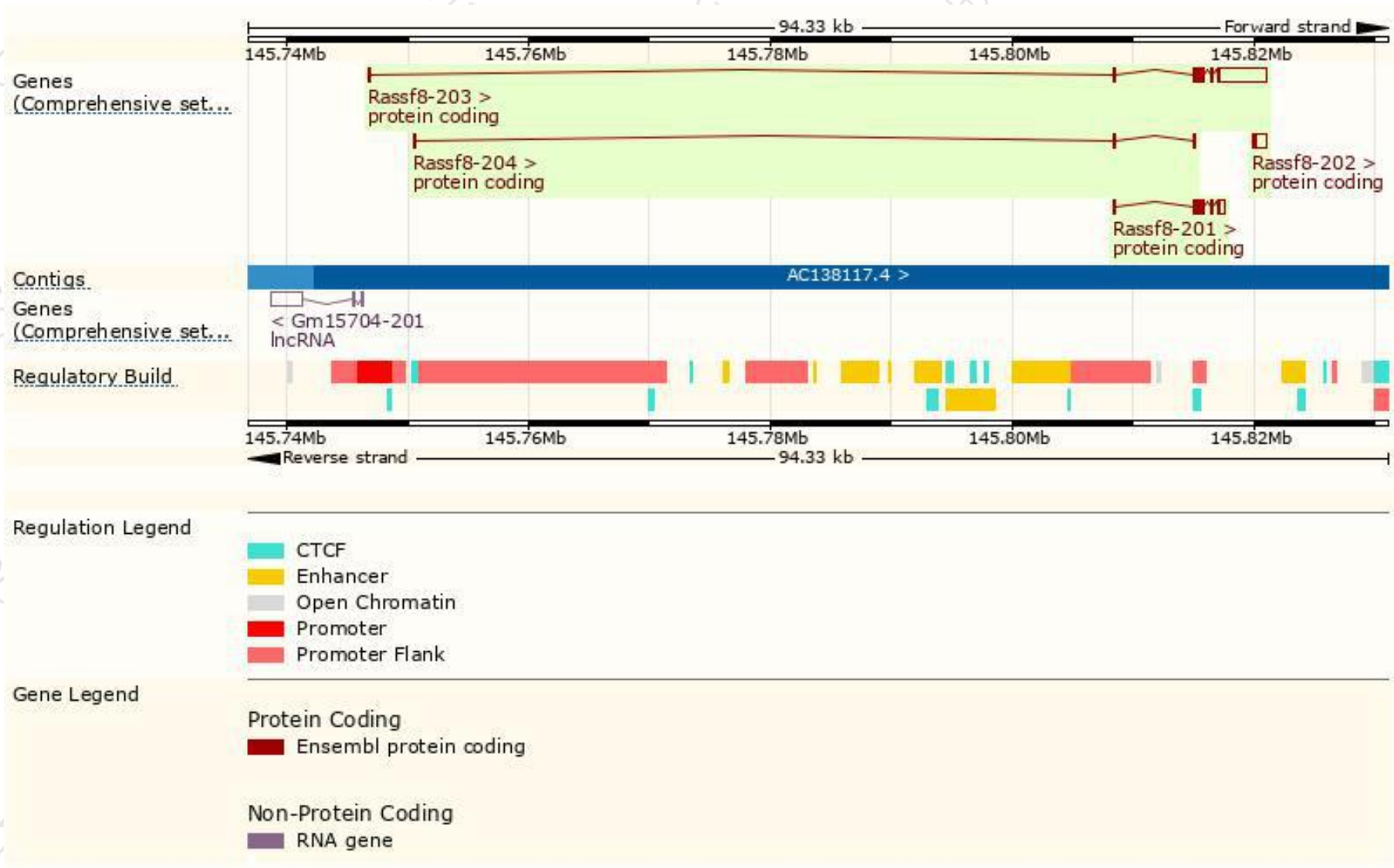
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rassf8-203	ENSMUST00000111704.7	5446	419aa	Protein coding	CCDS20696	Q8CJ96	TSL:5 GENCODE basic APPRIS P1
Rassf8-201	ENSMUST00000032388.4	1733	419aa	Protein coding	CCDS20696	Q8CJ96	TSL:1 GENCODE basic APPRIS P1
Rassf8-202	ENSMUST00000058538.6	1131	102aa	Protein coding	-	F7BB71	TSL:NA GENCODE basic
Rassf8-204	ENSMUST00000140966.1	427	79aa	Protein coding	-	E9QA00	CDS 3' incomplete TSL:2

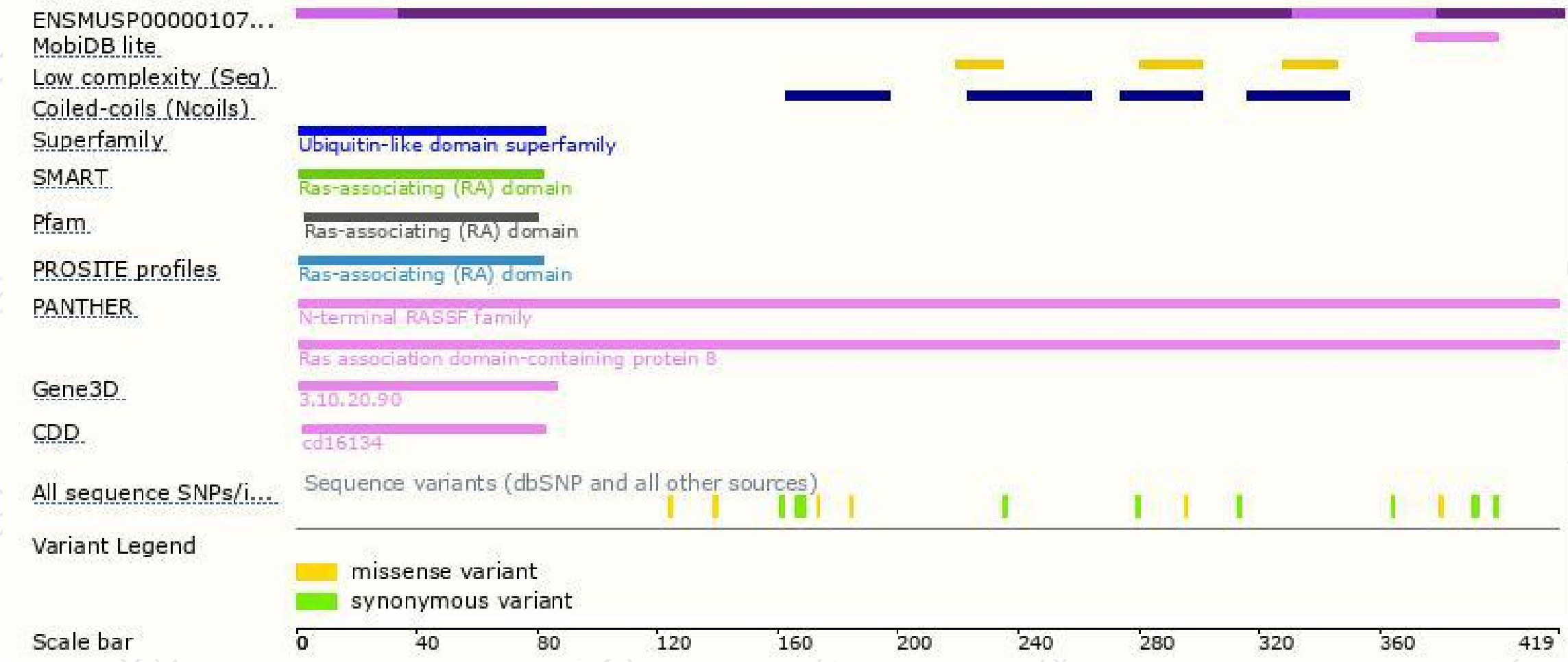
The strategy is based on the design of *Rassf8-203* transcript,the transcription is shown below:



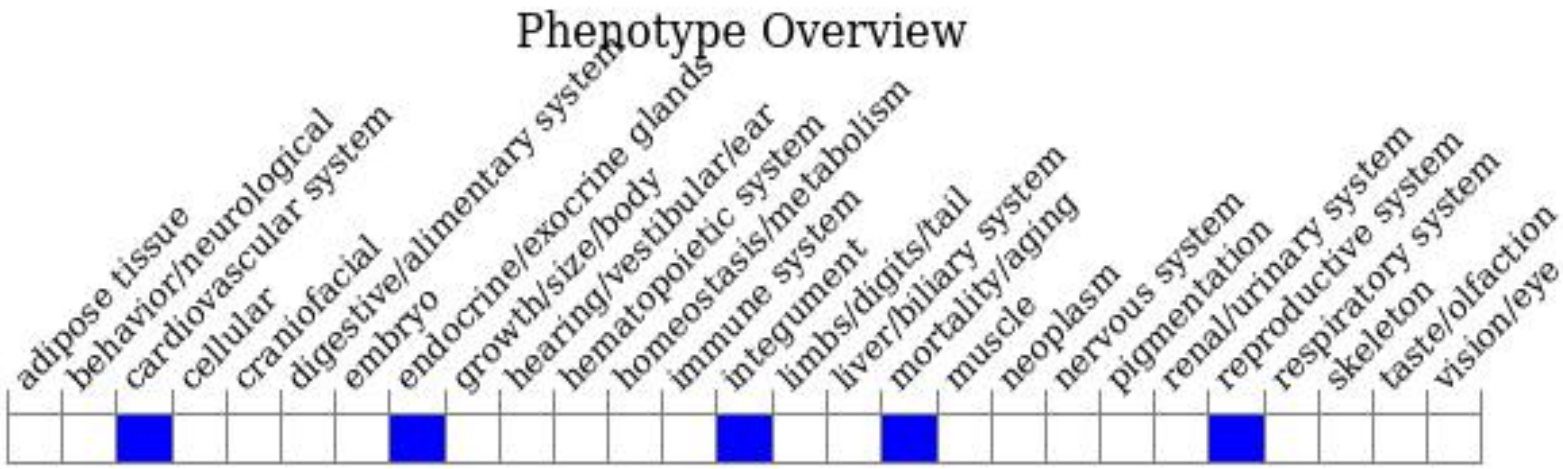
Genomic location distribution



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/>).

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

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