Raf1 Cas9-KO Strategy

Designer: Huan Fan

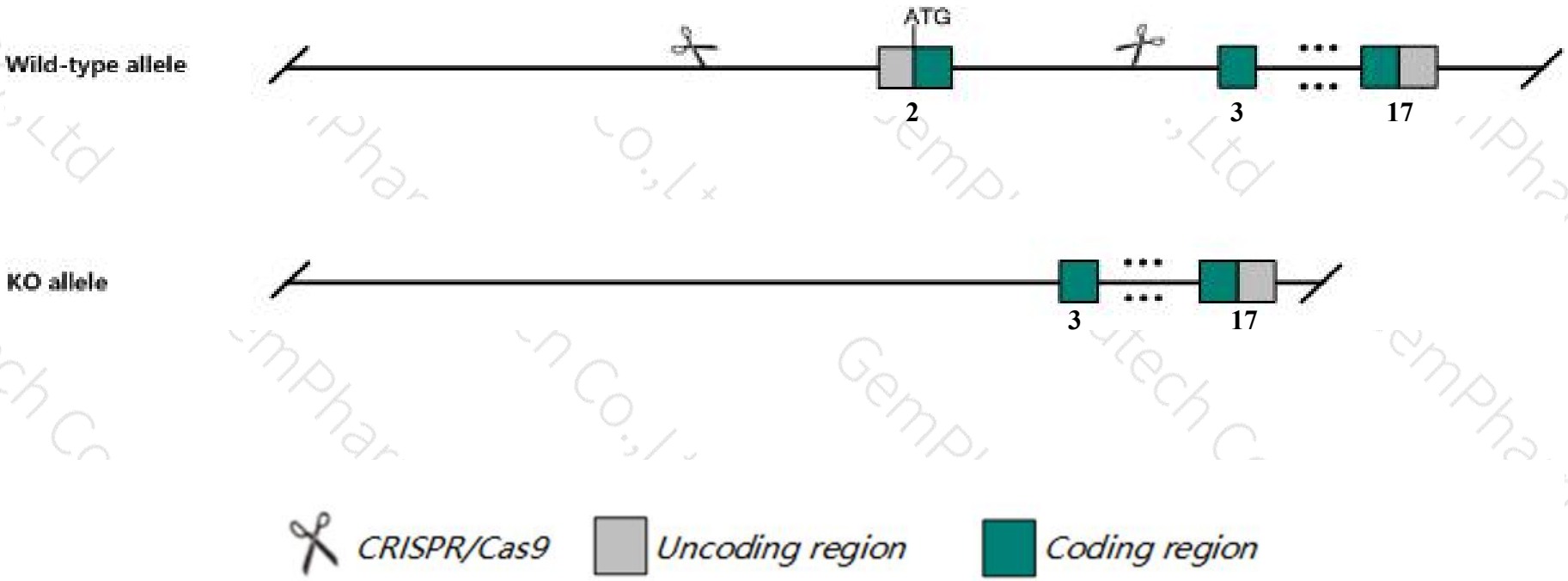
Design Date: 2019-7-25

Project Overview

Project Name	***Raf1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Raf1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Raf1* gene has 11 transcripts. According to the structure of *Raf1* gene, exon2 of *Raf1-201* (ENSMUST00000000451.13) 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 *Raf1* gene. The brief process is as follows: CRISPR/Cas9 system v

Notice

➢ According to the existing MGI data,Homozygotes for targeted null mutations are growth retarded, with hypocellular fetal livers, placental anomalies, and defects of skin and lungs, resulting in lethality around mid-gestation. Mice heterozygous for a knock-in allele exhibit hypertrophic cardiomyopathy.

➢Transcript *Raf1-206,209* may not be affected.

➢ The *Raf1* 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）

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Raf1 v-raf-leukemia viral oncogene 1 [Mus musculus (house mouse)]

Gene ID: 110157, updated on 31-Jan-2019

Summary

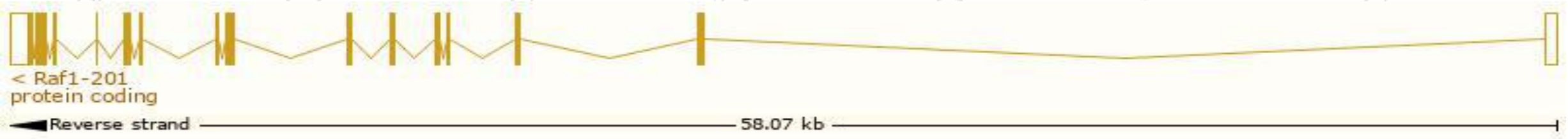
Official Symbol	Raf1 provided by MGI
Official Full Name	v-raf-leukemia viral oncogene 1 provided byMGI
Primary source	MGI:MGI:97847
See related	Ensembl:ENSMUSG00000000441
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6430402F14Rik, AA990557, BB129353, Craf1, D830050J10Rik, Raf-1, c-Raf, cRaf, v-Raf
Expression	Ubiquitous expression in CNS E14 (RPKM 50.6), whole brain E14.5 (RPKM 48.5) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

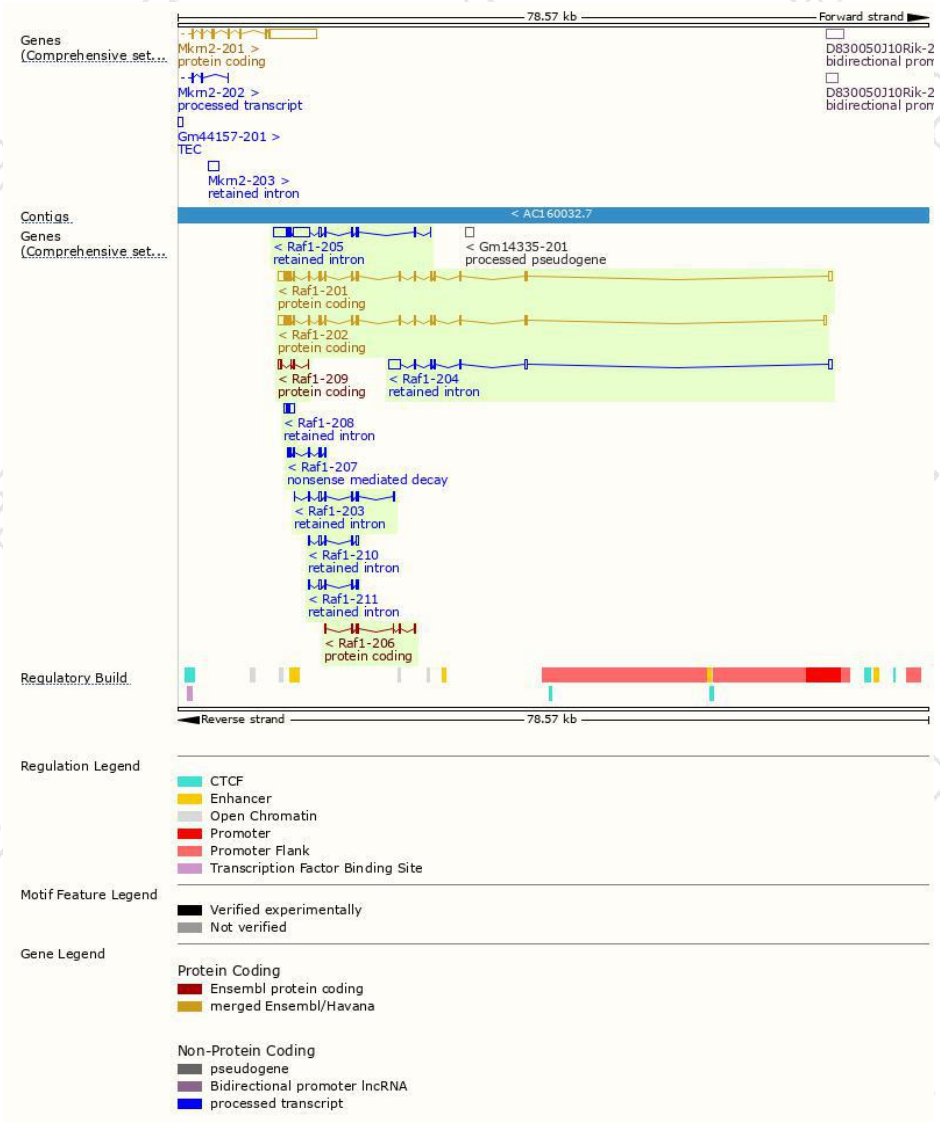
The gene has 11 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Raf1-201	ENSMUST00000000451.13	3070	648aa	Protein coding	CCDS20441	Q99N57	TSL:1 GENCODE basic APPRIS P1
Raf1-202	ENSMUST00000112949.7	2946	648aa	Protein coding	CCDS20441	Q99N57	TSL:1 GENCODE basic APPRIS P1
Raf1-206	ENSMUST00000147979.2	613	205aa	Protein coding	-	F6TUC4	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Raf1-209	ENSMUST00000203759.1	538	96aa	Protein coding	-	A0A0N4SWH5	CDS 5' incomplete TSL:5
Raf1-207	ENSMUST00000203142.1	676	118aa	Nonsense mediated decay	-	A0A0N4SV36	CDS 5' incomplete TSL:3
Raf1-205	ENSMUST00000130528.7	3968	No protein	Retained intron	-	-	TSL:1
Raf1-204	ENSMUST00000127503.1	2277	No protein	Retained intron	-	-	TSL:1
Raf1-208	ENSMUST00000203276.1	838	No protein	Retained intron	-	-	TSL:1
Raf1-210	ENSMUST00000203826.2	745	No protein	Retained intron	-	-	TSL:5
Raf1-203	ENSMUST00000124553.3	732	No protein	Retained intron	-	-	TSL:5
Raf1-211	ENSMUST00000204512.1	674	No protein	Retained intron	-	-	TSL:3

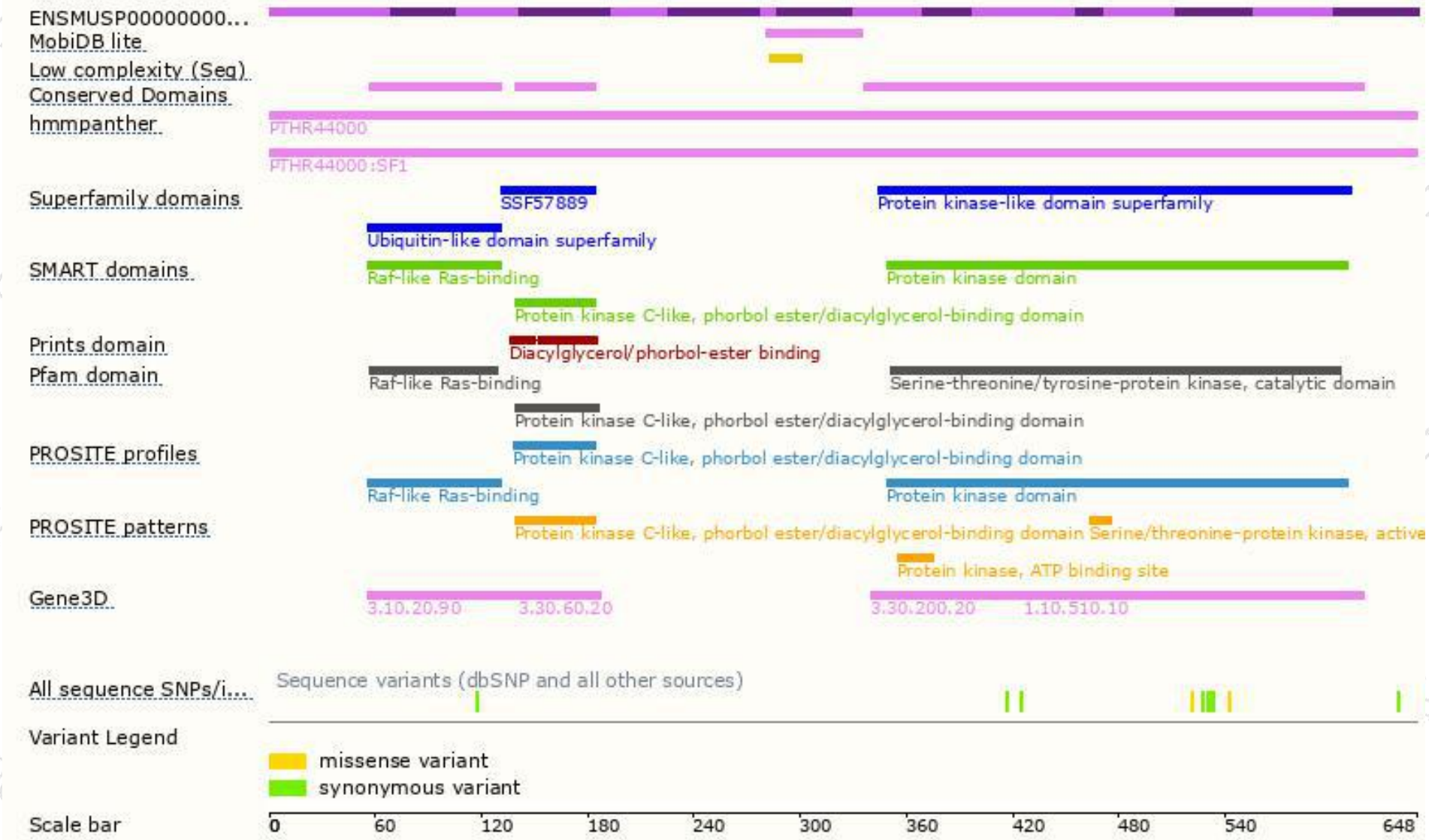
The strategy is based on the design of *Raf1-201* transcript,The transcription is shown below



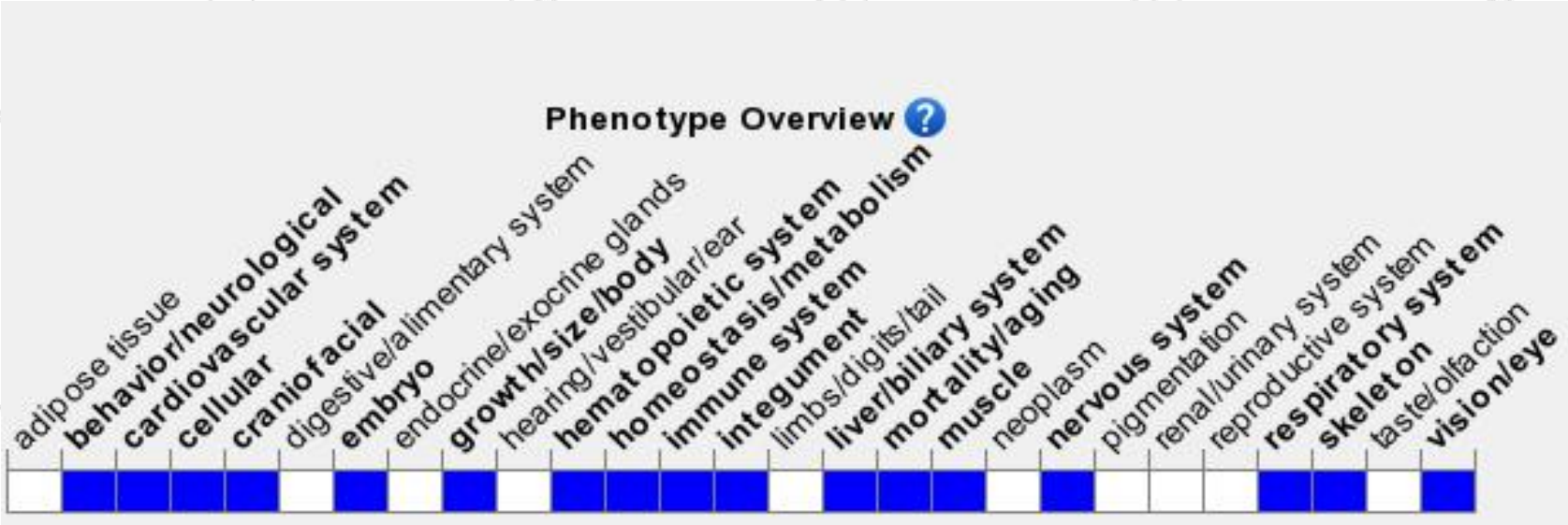
Genomic location distribution



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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