Braf Cas9-KO Strategy

Designer: Xiaojing Li

Design Date: 2019-9-16

Reviewer: JiaYu

Project Overview



Project Name

Braf

Project type

Cas9-KO

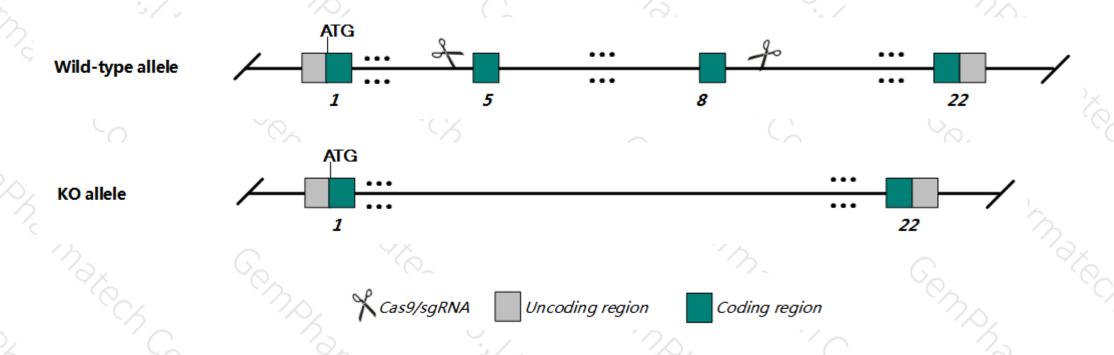
Strain background

C57BL/6JGpt

Knockout strategy



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



Technical routes



- The *Braf* gene has 5 transcripts. According to the structure of *Braf* gene, exon 5-8 of *Braf*-201 transcript (ENSMUST00000002487.14) is recommended as the Knockout region. The region contains 473bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Braf* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9, 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.

Notice



- According to the existing MGI data, Homozygous null embryos die during organogenesis, are smaller, have enlarged blood vessels, hemorrhaging, poor circulation, slow heartbeat and abnormal endothelial cell development. Mice homozygous for a targeted allele activated in neurons exhibit impaired neuronal differentiation.
- ➤ The *Braf* 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)



Braf Braf transforming gene [Mus musculus (house mouse)]

Gene ID: 109880, updated on 21-May-2019

Summary

Official Symbol Braf provided by MGI

Official Full Name Braf transforming gene provided by MGI

Primary source MGI:MGI:88190

See related Ensembl: ENSMUSG00000002413

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 B-raf; Braf2; Braf-2; C87398; AA120551; AA387315; AA473386; C230098H17; D6Ertd631e; 9930012E13Rik

Expression Ubiquitous expression in frontal lobe adult (RPKM 6.7), bladder adult (RPKM 6.5) and 25 other tissues See more

Orthologs human all

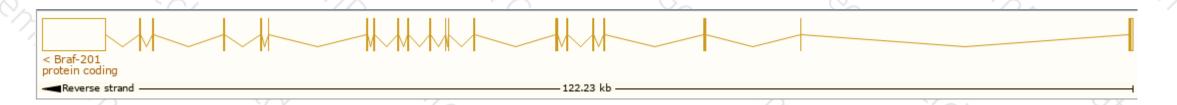
Transcript information (Ensembl)



The gene has 5 transcripts, and all transcripts are shown below:

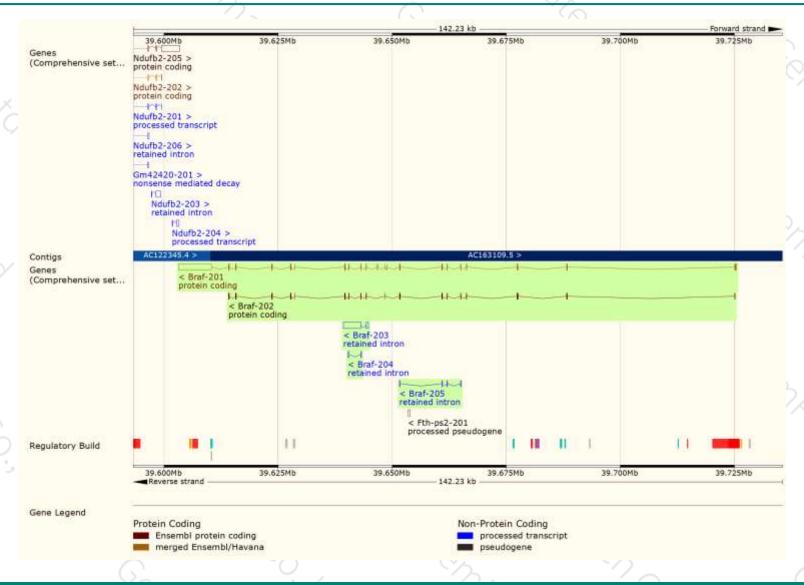
Name 🍦	Transcript ID A	bp 🌲	Protein 🍦	Biotype 🍦	CCDS 🍦	UniProt 🍦	Flags
Braf-201	ENSMUST00000002487.14	9728	<u>804aa</u>	Protein coding	CCDS39463 ₽	<u>P28028</u> ₽	TSL:1 GENCODE basic APPRIS P1
Braf-202	ENSMUST00000101497.3	2253	<u>750aa</u>	Protein coding	-	<u>F6SZ47</u> ₽	CDS 5' incomplete TSL:5
Braf-203	ENSMUST00000167073.1	4475	No protein	Retained intron	-	-	TSL:1
Braf-204	ENSMUST00000167169.1	384	No protein	Retained intron	-	-	TSL:3
Braf-205	ENSMUST00000169647.1	528	No protein	Retained intron	-	-	TSL:2

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



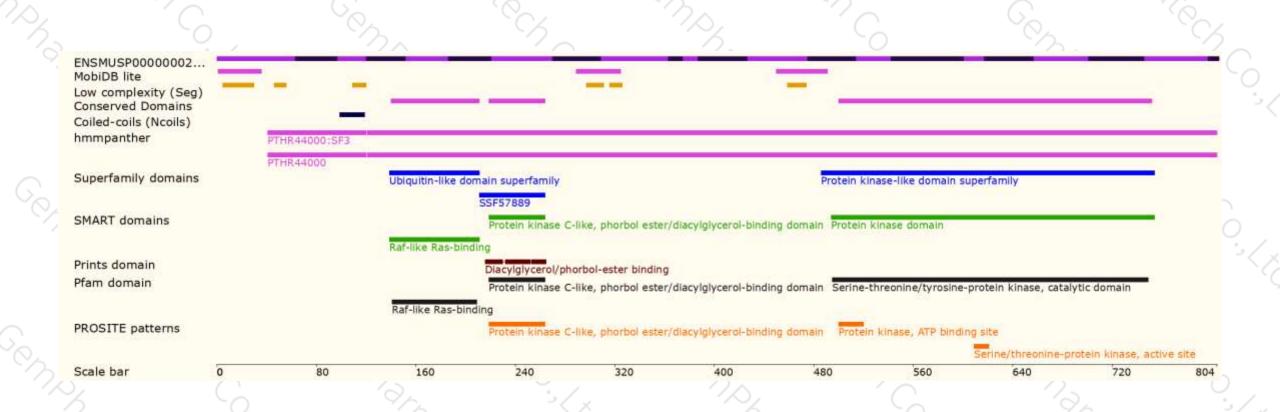
Genomic location distribution





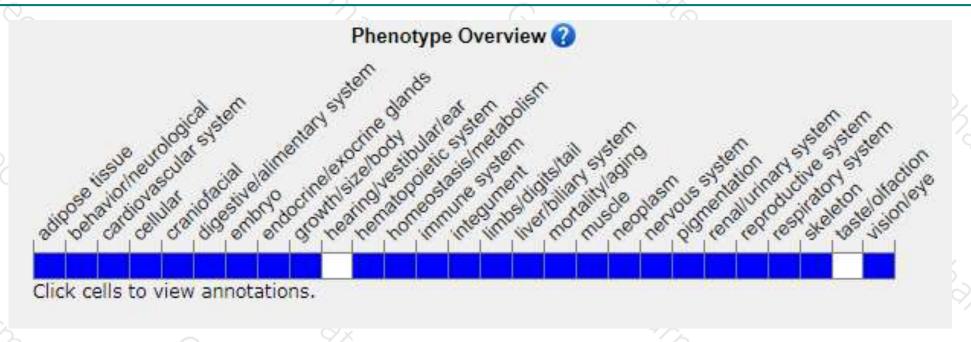
Protein domain





Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(http://www.informatics.jax.org/).

Homozygous null embryos die during organogenesis, are smaller, have enlarged blood vessels, hemorrhaging, poor circulation, slow heartbeat and abnormal endothelial cell development. Mice homozygous for a targeted allele activated in neurons exhibit impaired neuronal differentiation.

If you have any questions, you are welcome to inquire. Tel: 025-5864 1534





