Kras Cas9-KO Strategy

Designer: Jinling Wang

Design Date: 2019-7-26

Project Overview



Project Name

Project type Cas9-KO

Strain background

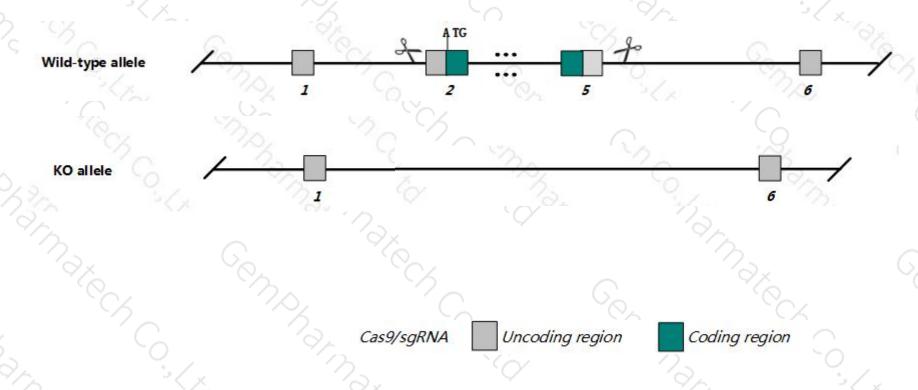
C57BL/6JGpt

Kras

Knockout strategy



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



Technical routes



- The *Kras* gene has 7 transcripts, According to the structure of *Kras* gene, exon2-5 of *Kras*-202 transcript is recommended as the knockout region. The region contains the all coding sequence. Knock out the region, result in destruction of protein.
- In this project we use CRISPR/Cas9 technology to modify *Kras* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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: Mice homozygous for a null allele exhibit embryonic lethality, decreased fetal growth, pericardial edema, anemia, and liver hypoplasia. Mice heterozygous for various knock-in alleles exhibit increased tumorigenesis.
- The *Kras* 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)



Kras Kirsten rat sarcoma viral oncogene homolog [Mus musculus (house mouse)]

Gene ID: 16653, updated on 4-Dec-2018

- Summary

2

Official Symbol Kras provided by MGI

Official Full Name Kirsten rat sarcoma viral oncogene homolog provided by MGI

Primary source MGI:MGI:96680

See related Ensembl:ENSMUSG00000030265 Vega:OTTMUSG00000022179

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 ras; p21B; K-Ras; K-ras; Kras2; Ki-ras; Kras-2; K-Ras 2; c-K-ras; Al929937; c-Ki-ras

Expression Ubiquitous expression in CNS E18 (RPKM 17.0), whole brain E14.5 (RPKM 16.2) and 26 other tissues See more

Orthologs human all

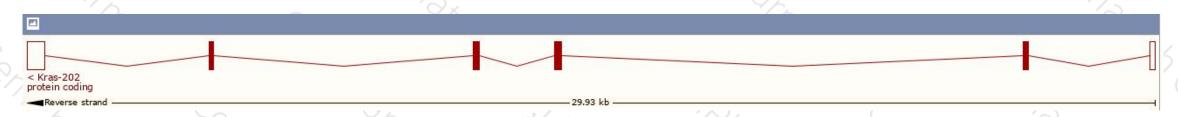
Transcript information (Ensembl)



The gene has 7 transcripts, and all transcripts are shown below:

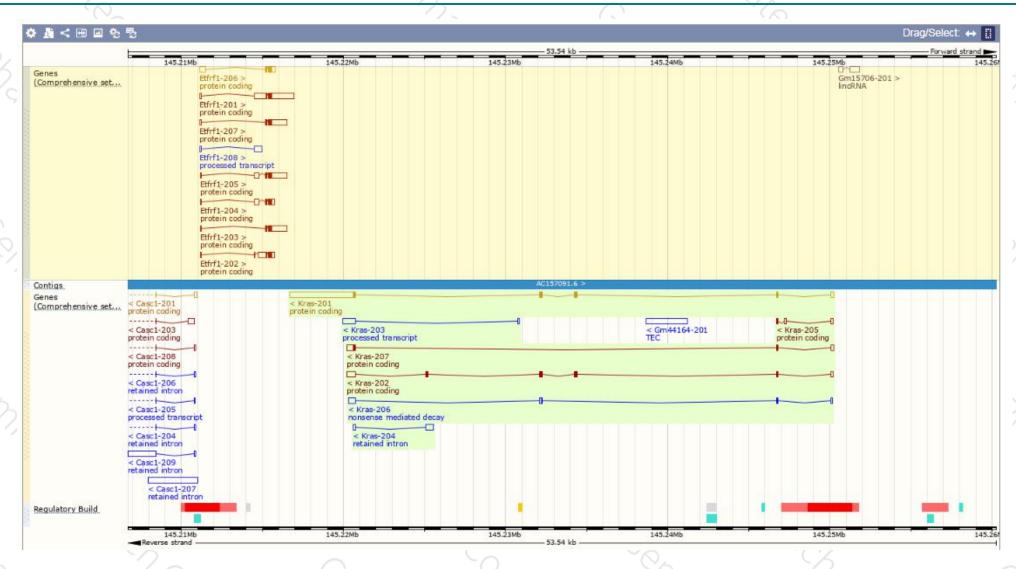
| Name ▲ | Transcript ID | bp 🌲 | Protein | Biotype | CCDS 🍦 | UniProt | Flags |
|----------|-----------------------|------|--------------|-------------------------|-------------|----------------|---------------------------------|
| Kras-201 | ENSMUST00000032399.11 | 4678 | <u>188aa</u> | Protein coding | CCDS20693 ₽ | P32883@Q5J7N1@ | TSL:1 GENCODE basic APPRIS P2 |
| Kras-202 | ENSMUST00000111710.7 | 1194 | <u>189aa</u> | Protein coding | - | P32883@Q0VDV7@ | TSL:5 GENCODE basic APPRIS ALT1 |
| Kras-203 | ENSMUST00000123972.1 | 816 | No protein | IncRNA | - | 121 | TSL:3 |
| Kras-204 | ENSMUST00000149314.1 | 599 | No protein | Retained intron | 3.53 | 15. | TSL:2 |
| Kras-205 | ENSMUST00000155145.1 | 381 | <u>34aa</u> | Protein coding | - | B2KGV5₽ | CDS 3" incomplete TSL:3 |
| Kras-206 | ENSMUST00000156486.1 | 832 | <u>40aa</u> | Nonsense mediated decay | - | E9Q8V2₽ | TSL:5 |
| Kras-207 | ENSMUST00000203147.2 | 817 | <u>75aa</u> | Protein coding | | A0A0N4SVY1@ | TSL:5 GENCODE basic |

The strategy is based on the design of *Kras-*202 transcript, The transcription is shown below



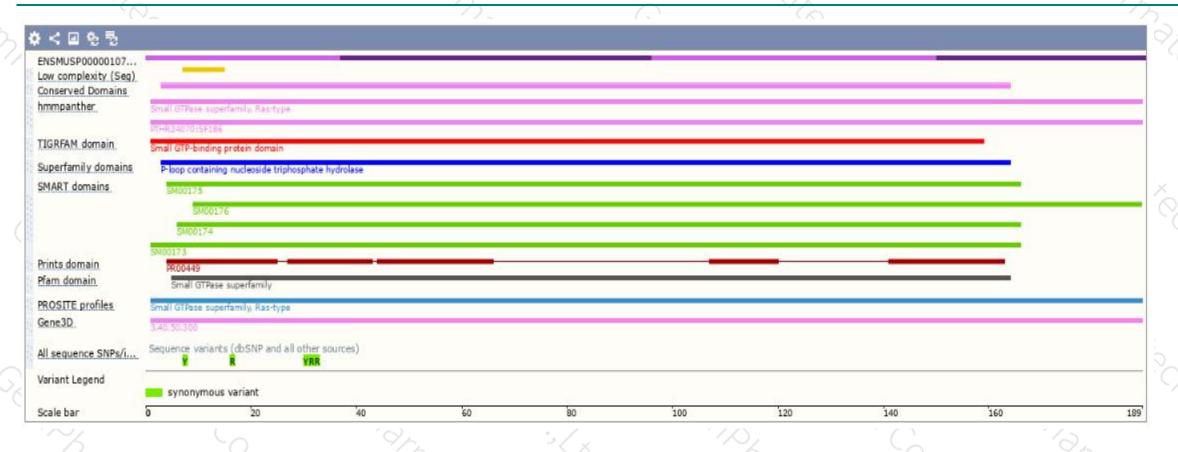
Genomic location distribution





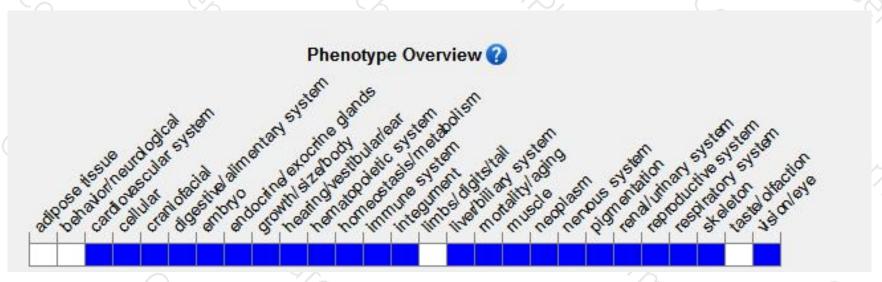
Protein domain





Mouse phenotype description(MGI)





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

Mice homozygous for a null allele exhibit embryonic lethality, decreased fetal growth, pericardial edema, anemia, and liver hypoplasia. Mice heterozygous for various knock-in alleles exhibit increased tumorigenesis.

If you have any questions, you are welcome to inquire. Tel: 400-9660890





