

Kras Cas9-CKO Strategy

Designer:

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Design Date:

2019-7-27

Project Overview

Project Name

Kras

Project type

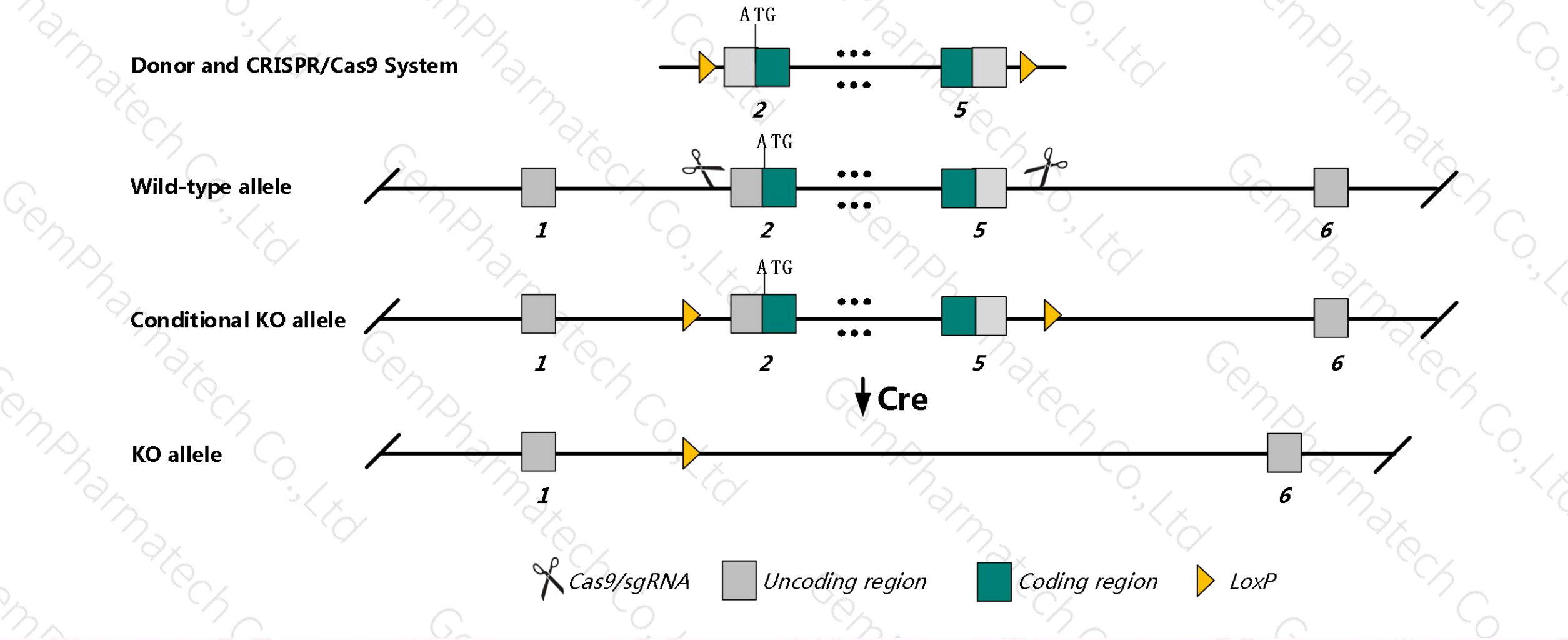
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- 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, donor was constructed. Cas9, gRNA 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 or cell types.

- 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 loxp insertion 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

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; A1929937; 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](#)

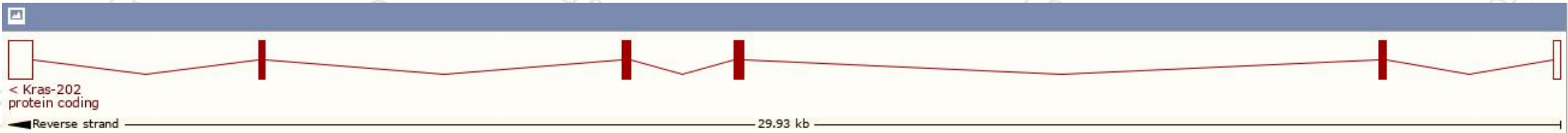
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

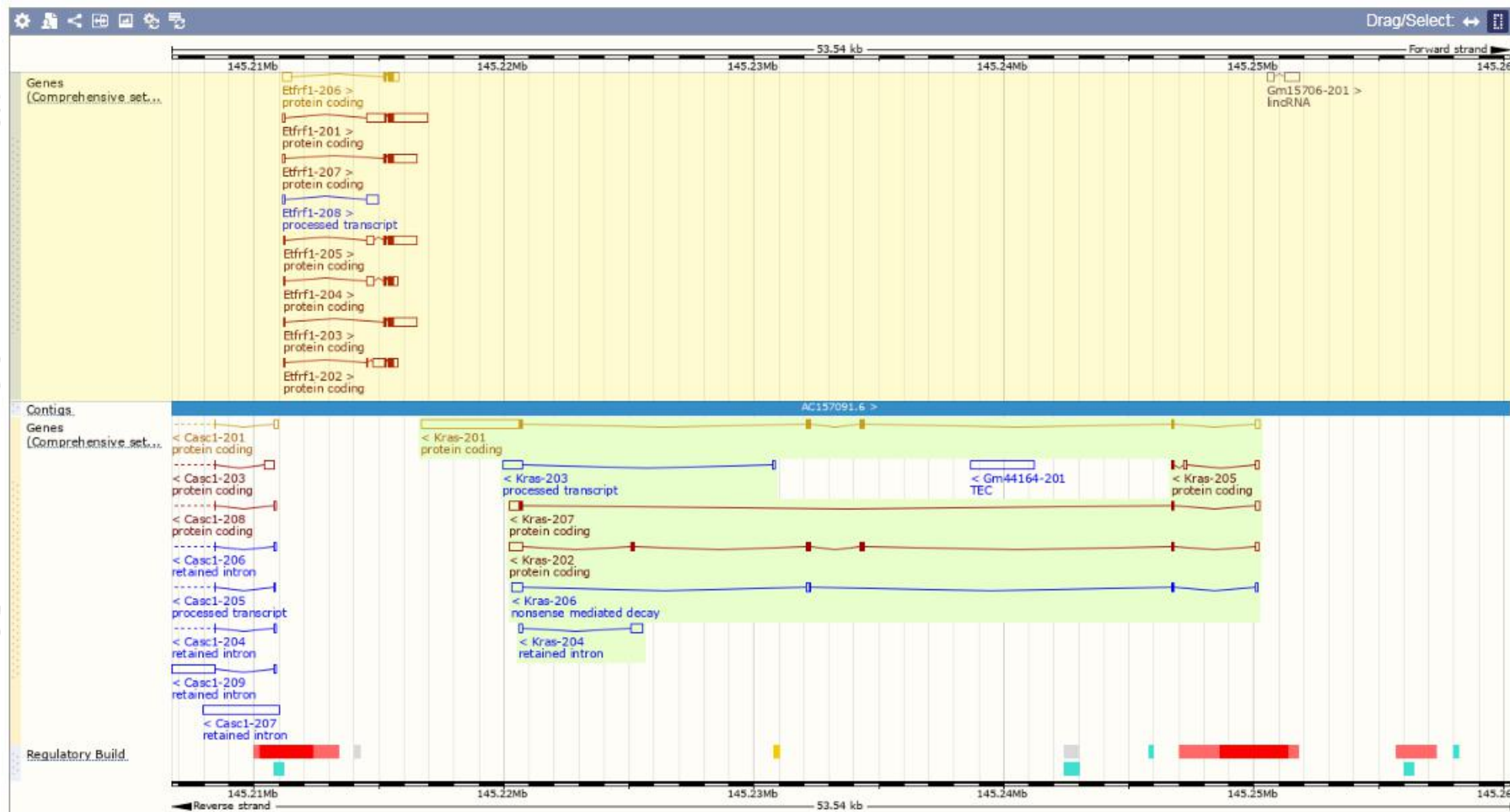
The gene has 7 transcripts, and all transcripts are shown below:

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

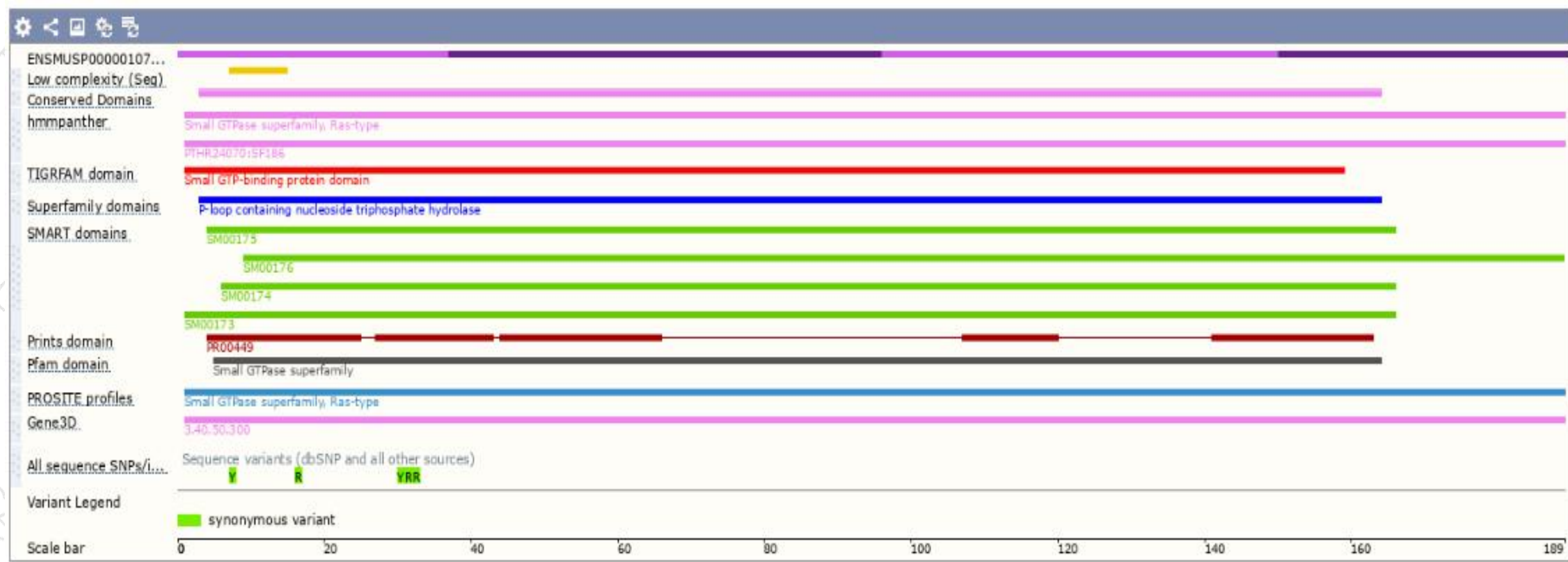
The strategy is based on the design of *Kras*-202 transcript, The transcription is shown below



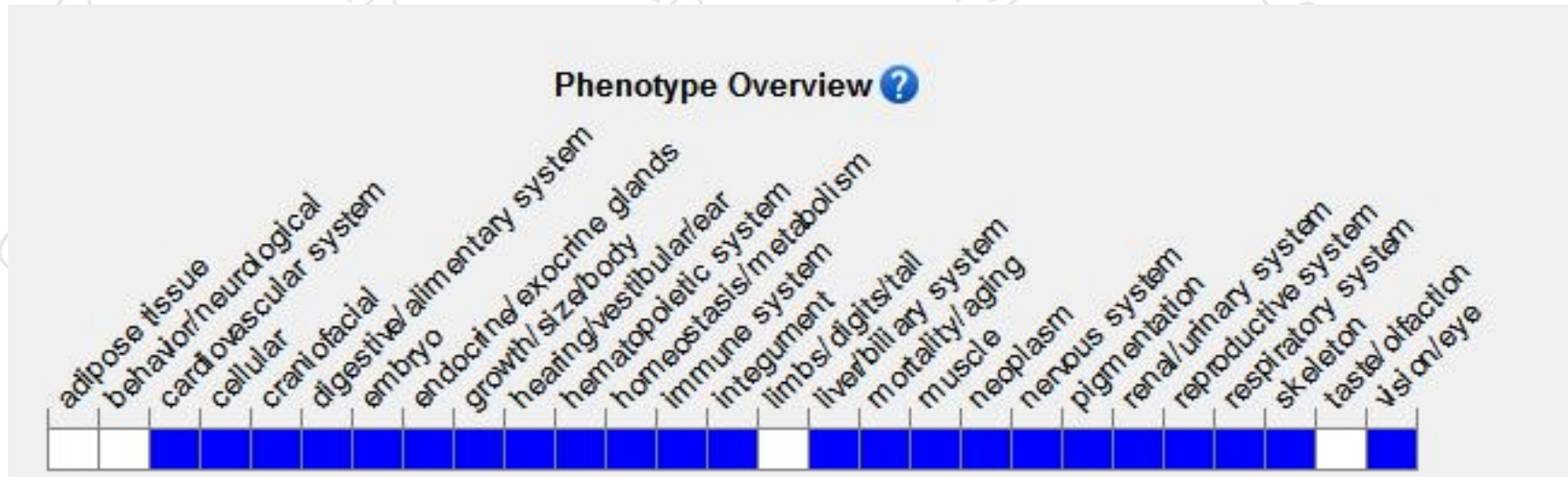
Genomic location distribution



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.
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