

Prkaca Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Prkaca

Project type

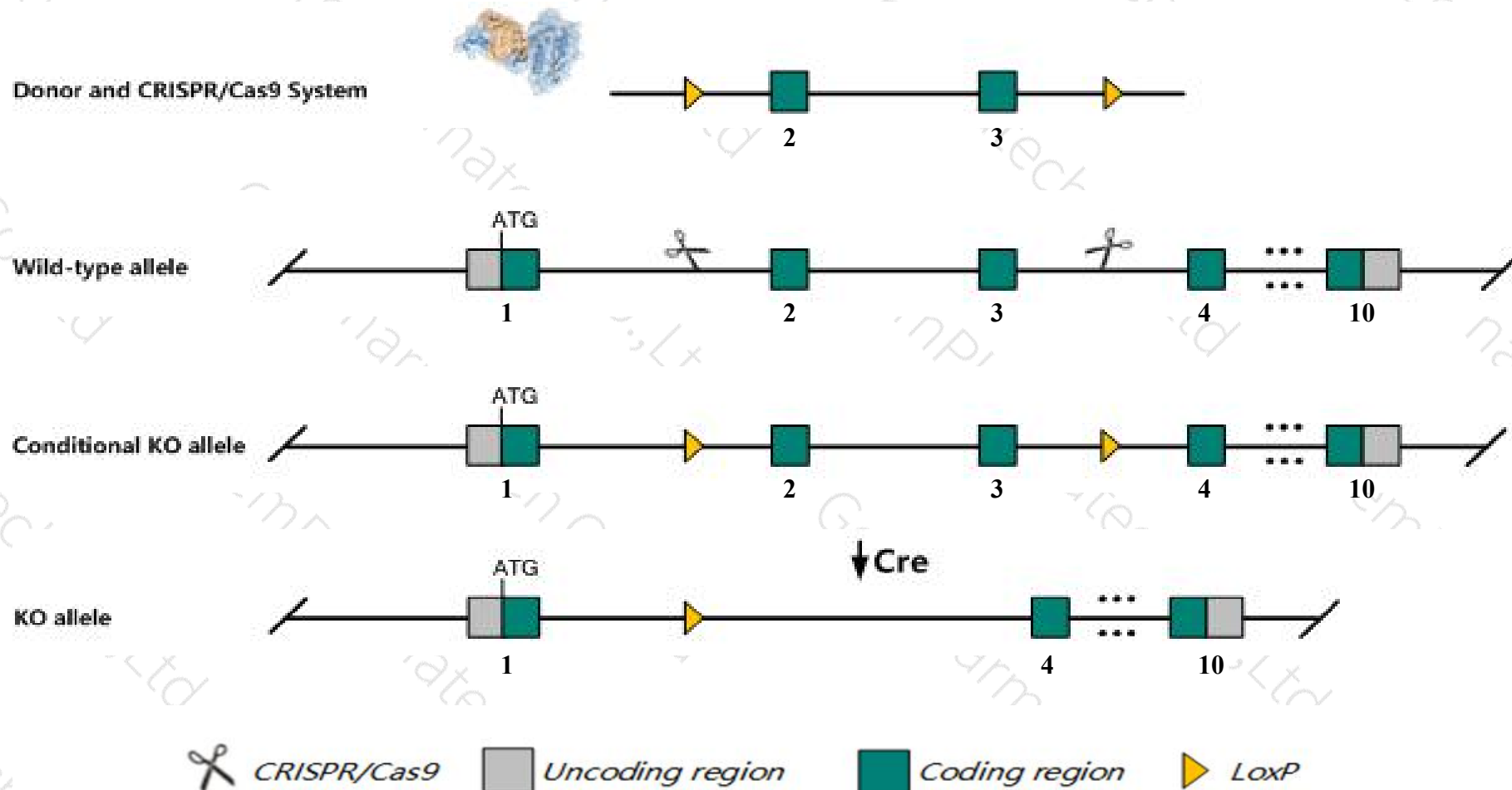
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Prkaca* gene has 2 transcripts. According to the structure of *Prkaca* gene, exon2-exon3 of *Prkaca-201* (ENSMUST00000005606.7) transcript is recommended as the knockout region. The region contains 191bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Prkaca* gene. The brief process is as follows: CRISPR/Cas9 system 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 and cell types.

- According to the existing MGI data, Homozygous mutant mice are highly susceptible to perinatal lethality. Surviving mice are runted and while spermatogenesis progresses normally, mature sperm shows impaired motility.
- The *Prkaca* gene is located on the Chr8. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

Prkaca protein kinase, cAMP dependent, catalytic, alpha [*Mus musculus* (house mouse)]

Gene ID: 18747, updated on 12-Aug-2019

Summary

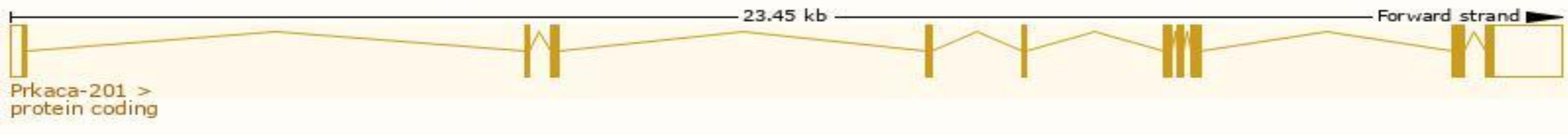
Official Symbol	Prkaca provided by MGI
Official Full Name	protein kinase, cAMP dependent, catalytic, alpha provided by MGI
Primary source	MGI:MGI:97592
See related	Ensembl:ENSMUSG000000005469
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	PKCD; Pkaca
Summary	This gene encodes a member of the serine/threonine protein kinase family. The holoenzyme, protein kinase A (also known as cyclic-AMP dependent protein kinase), mediates cellular response to changes in cyclic-AMP levels. This gene encodes the alpha catalytic subunit of protein kinase A. Protein kinase A-mediated signaling is transduced via phosphorylation of target proteins, and is important for many cellular functions, including mammalian sperm maturation and motility. Alternative splicing results in multiple transcript variants. A pseudogene of this gene has been defined on the X chromosome. [provided by RefSeq, Apr 2013]
Expression	Ubiquitous expression in heart adult (RPKM 86.7), subcutaneous fat pad adult (RPKM 59.9) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

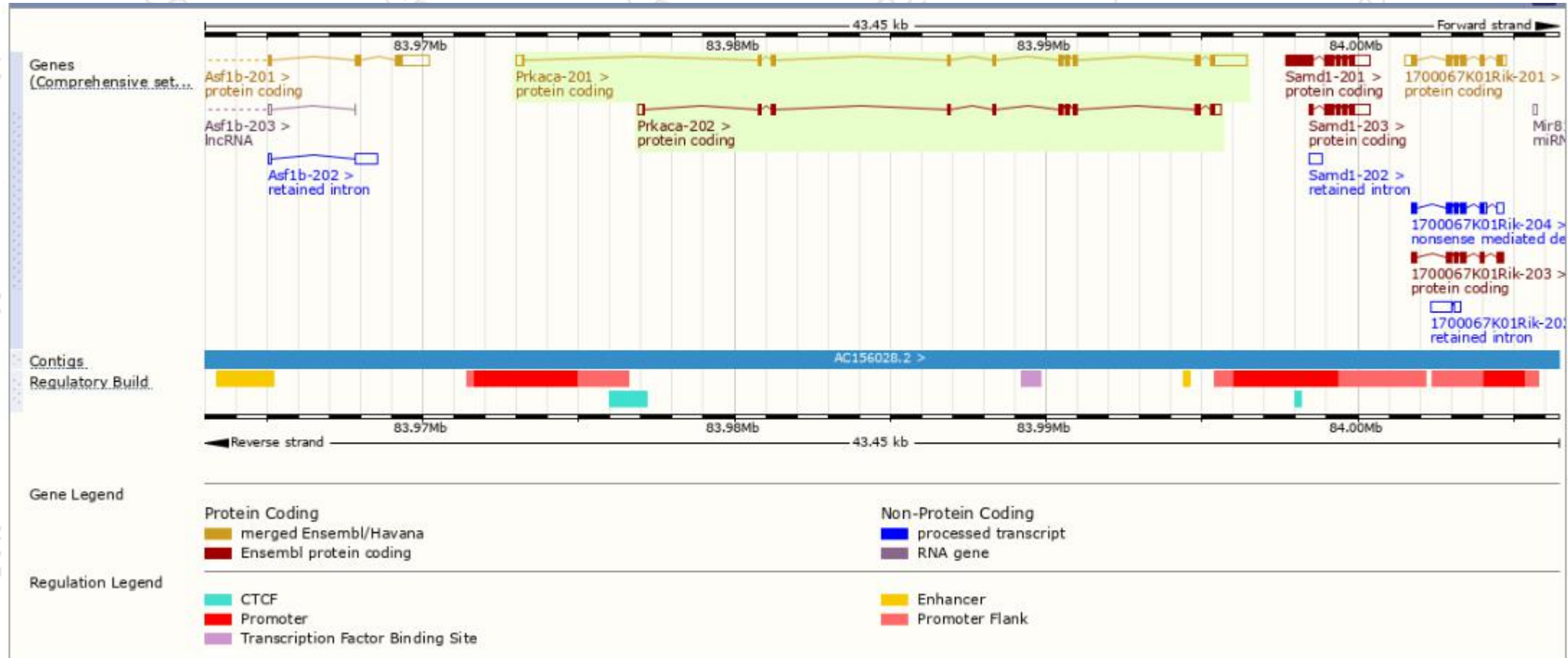
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Prkaca-201	ENSMUST00000005606.7	2276	351aa	ENSMUSP00000005606.6	Protein coding	CCDS22463	P05132	TSL:1 GENCODE basic APPRIS P3
Prkaca-202	ENSMUST00000211558.1	1399	343aa	ENSMUSP00000147256.1	Protein coding	CCDS85574	P05132	TSL:5 GENCODE basic APPRIS ALT1

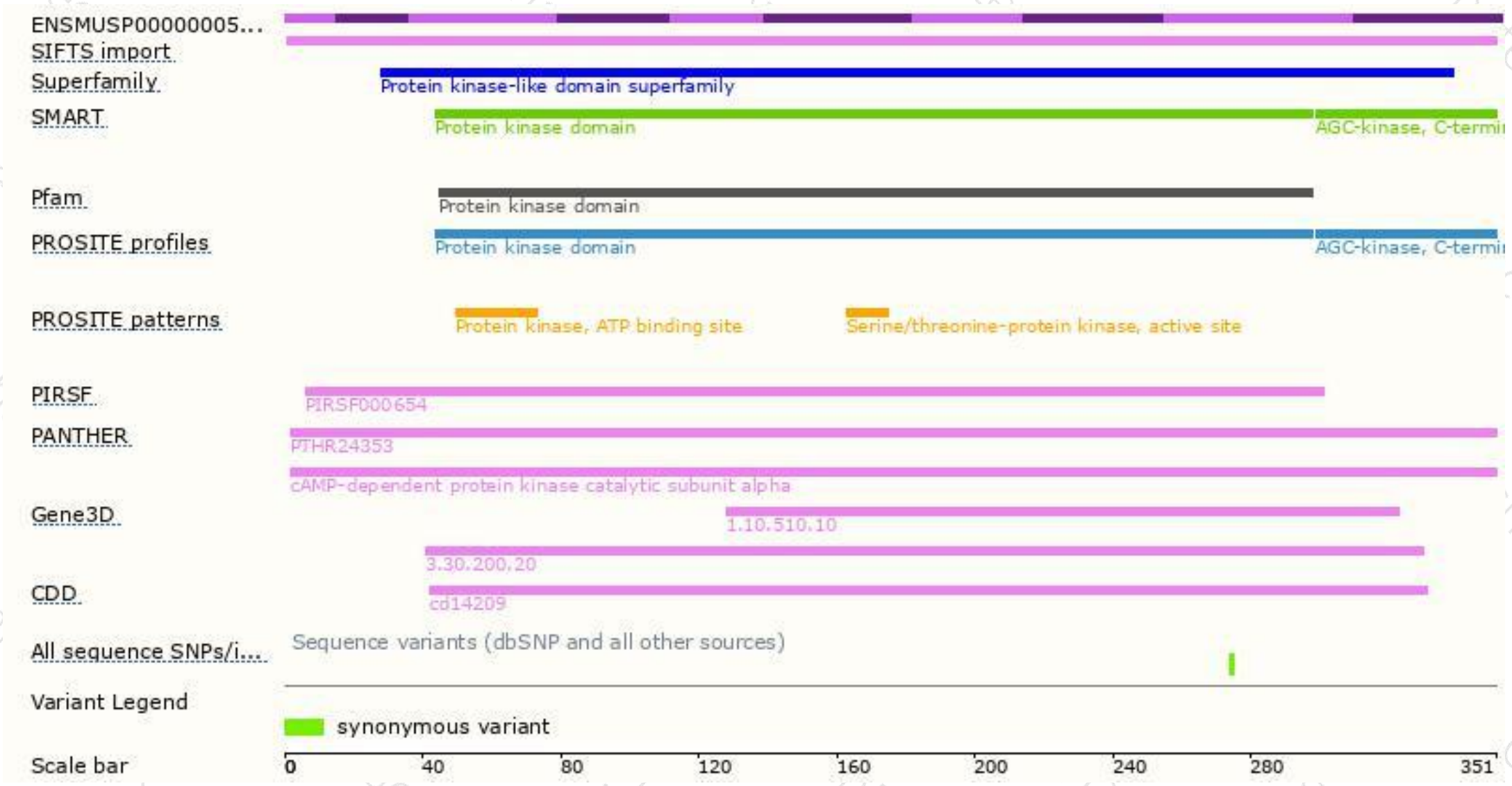
The strategy is based on the design of *Prkaca-201* transcript,The transcription is shown below



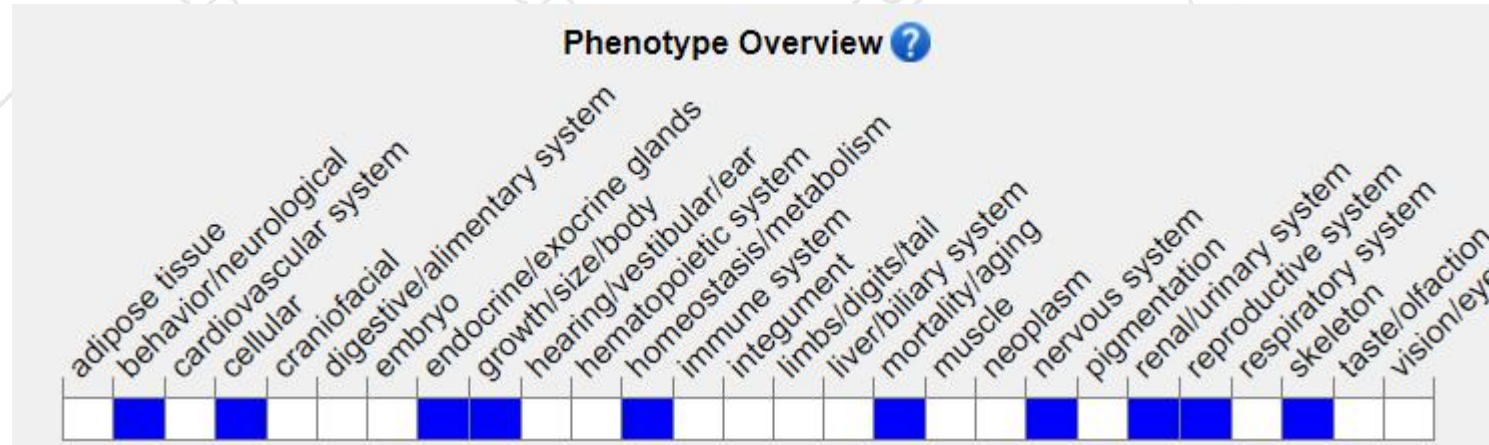
Genomic location distribution



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/>).

According to the existing MGI data, Homozygous mutant mice are highly susceptible to perinatal lethality. Surviving mice are runted and while spermatogenesis progresses normally, mature sperm shows impaired motility.

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

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