Prkcsh Cas9-CKO Strategy

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Reviewer	**Huimin Su**
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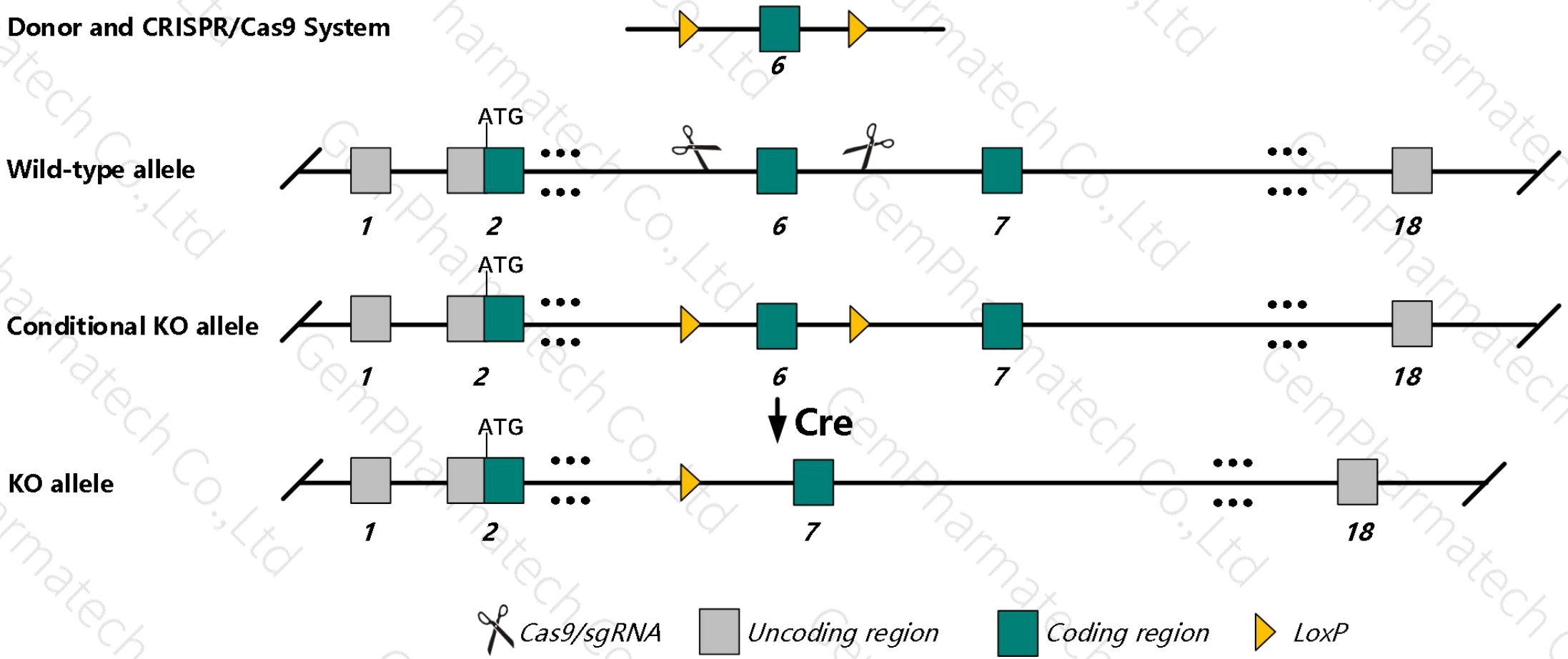
Project Overview

Project Name	***Prkcsh***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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



Technical routes

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- The *Prkcsh* gene has 5 transcripts. According to the structure of *Prkcsh* gene, exon6 of *Prkcsh-201* (ENSMUST00000003493.8) transcript is recommended as the knockout region. The region contains 118bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Prkcsh* 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 and cell types.

Notice

- According to the existing MGI data, mice homozygous for a knock-out allele exhibit embryonic lethality during organogenesis. Mice homozygous for a conditional allele activated in the kidneys or ubiquitously develop polycystic kidney and liver phenotypes, respectively.

- The distance of *Ccdc151* gene from exon6 of *Prkcsh* gene is about 3.9kb, this strategy may affect the regulatory function of the 5-terminals of Ccdc151 gene.

- The *Prkcsh* gene is located on the Chr9. 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）

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Prkcsh protein kinase C substrate 80K-H [*Mus musculus* (house mouse)]

Gene ID: 19089, updated on 12-Aug-2019

Summary

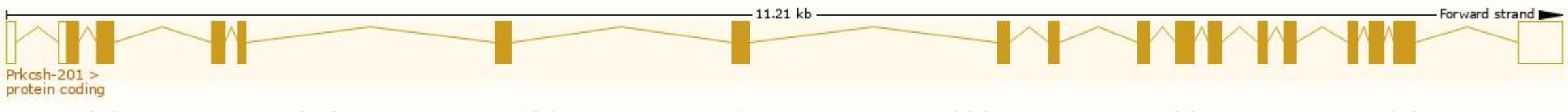
Official Symbol	Prkcsh provided by MGI
Official Full Name	protein kinase C substrate 80K-H provided by MGI
Primary source	MGI:MGI:107877
See related	Ensembl:ENSMUSG00000003402
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	80K-H; PKCSH
Expression	Ubiquitous expression in adrenal adult (RPKM 75.3), duodenum adult (RPKM 65.8) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

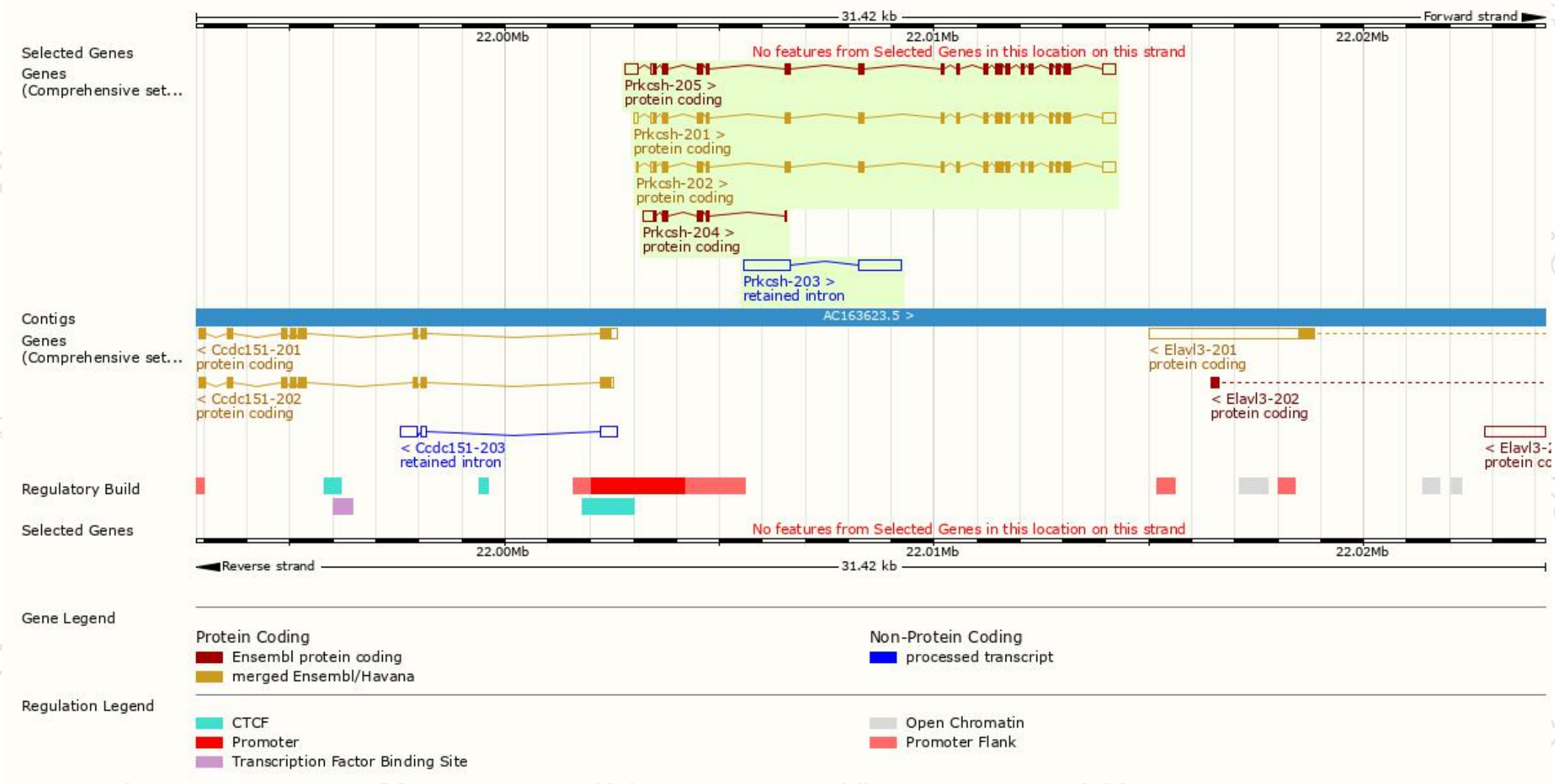
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Prkcsh-205	ENSMUST00000216344.1	2228	521aa	Protein coding	CCDS22918	O08795 Q3U518	TSL:1 GENCODE basic APPRIS P3
Prkcsh-201	ENSMUST00000003493.8	2012	521aa	Protein coding	CCDS22918	O08795 Q3U518	TSL:1 GENCODE basic APPRIS P3
Prkcsh-202	ENSMUST00000115331.9	1983	528aa	Protein coding	CCDS80967	O08795	TSL:1 GENCODE basic APPRIS ALT2
Prkcsh-204	ENSMUST00000215795.1	601	119aa	Protein coding	-	A0A1L1ST83	CDS 3' incomplete TSL:2
Prkcsh-203	ENSMUST00000214565.1	2092	No protein	Retained intron	-	-	TSL:1

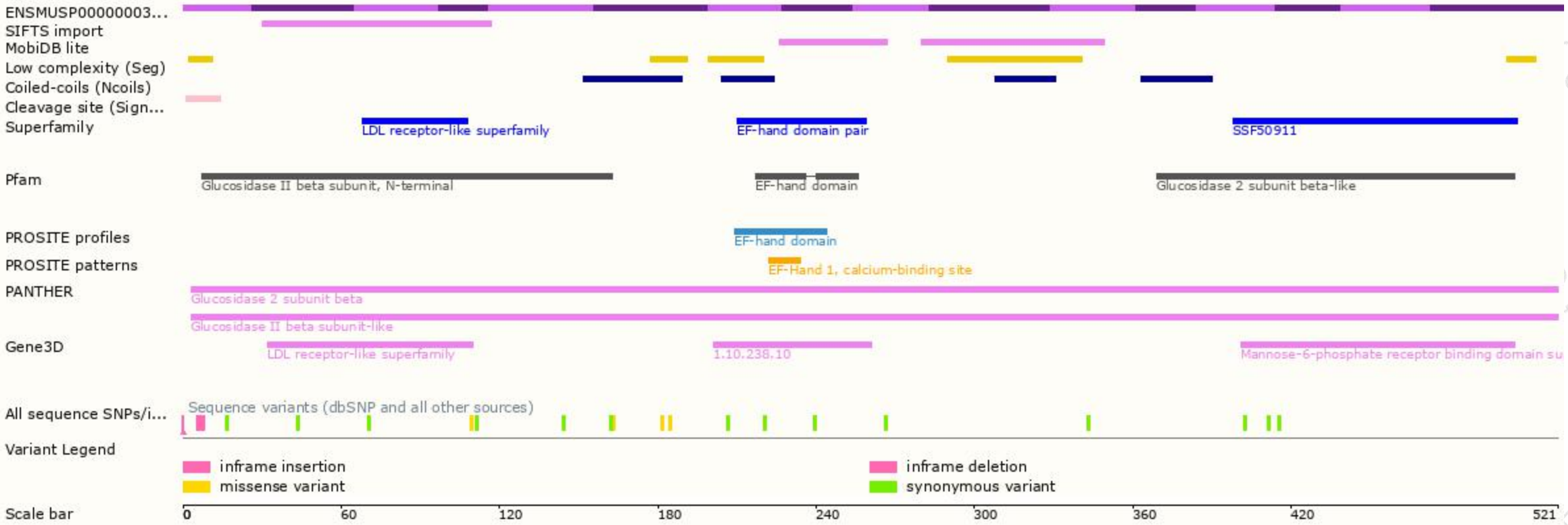
The strategy is based on the design of *Prkcsh-201* transcript,The transcription is shown below



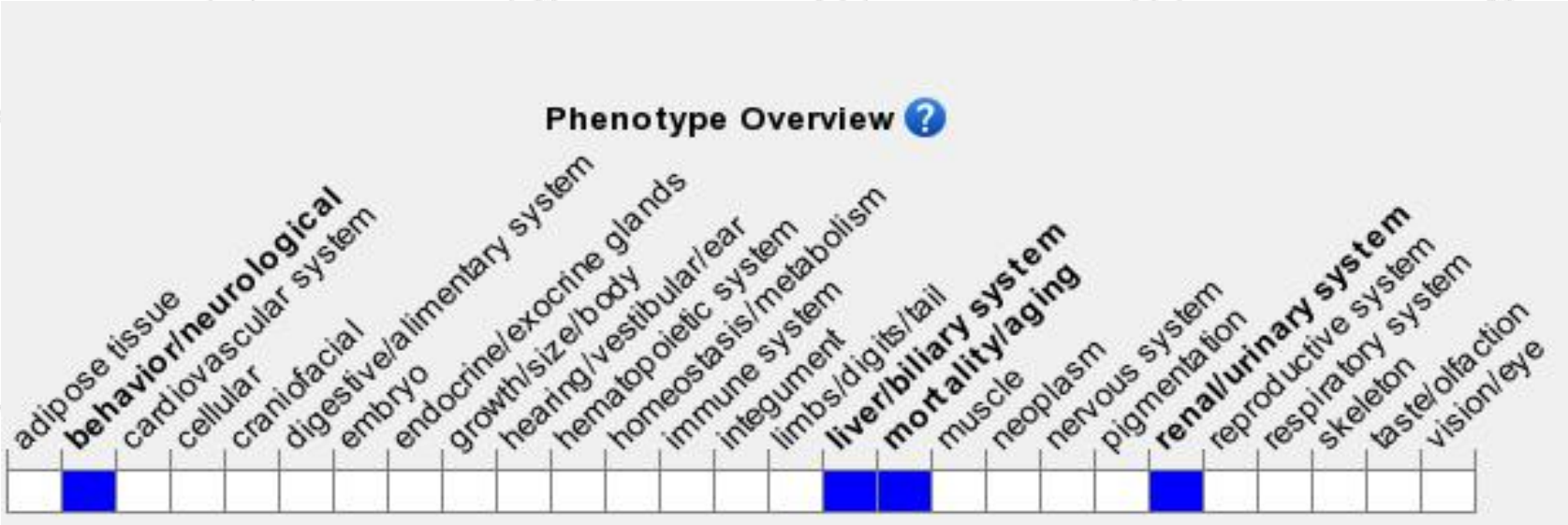
Genomic location distribution



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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