

Rtkn2 Cas9-CKO Strategy

Designer:

Zihe Cui

Reviewer :

Jia Yu

Design Date:

2020-9-17

Project Overview



Project Name

Rrkn2

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

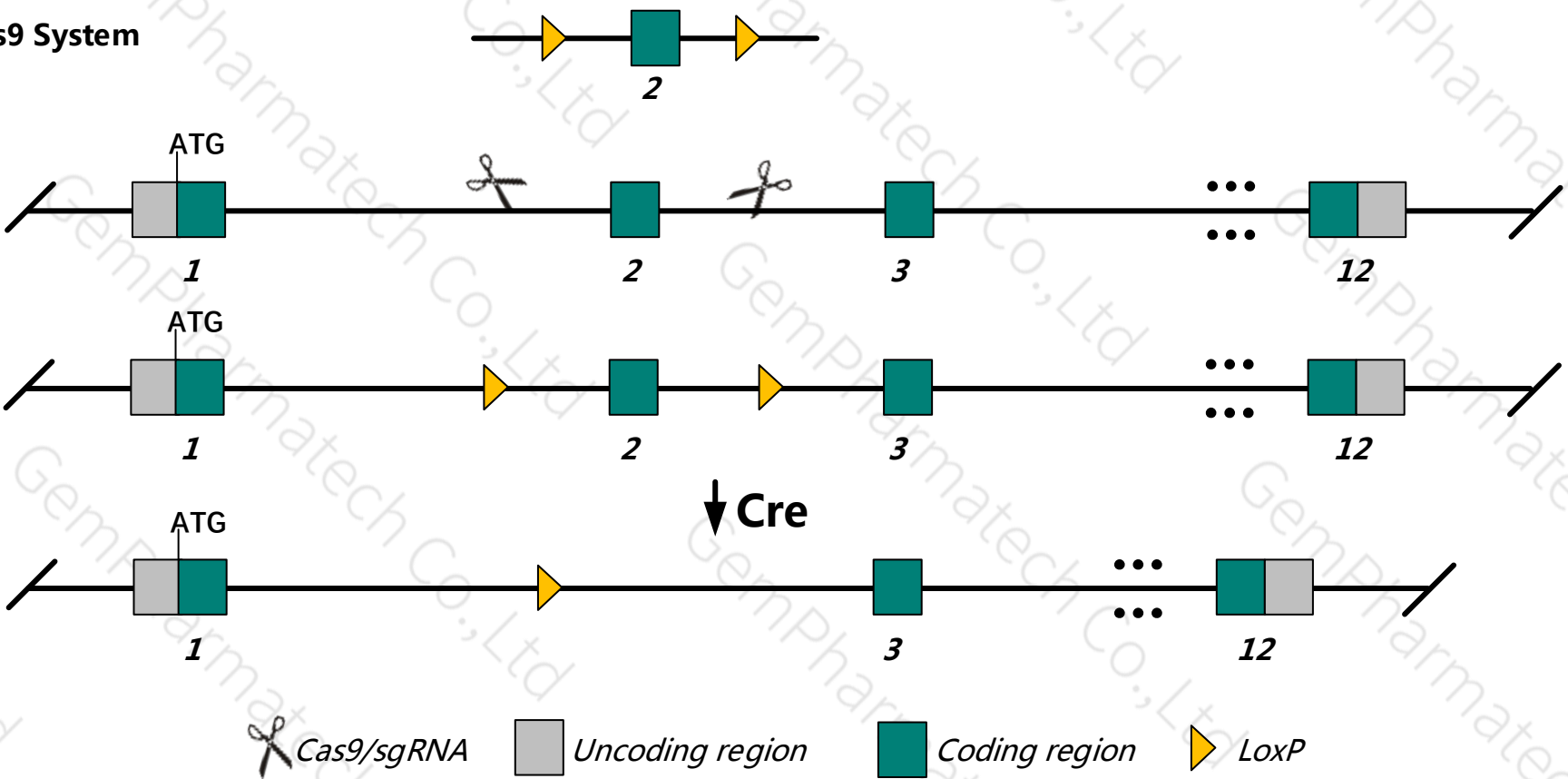
This model will use CRISPR/Cas9 technology to edit the *Rtkn2* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



- The *Rtkn2* gene has 6 transcript. According to the structure of *Rtkn2* gene, exon2 of *Rtkn2*-204 (ENSMUST00000118160.7) transcript is recommended as the knockout region. The region contains 206bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rtkn2* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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/6J Gpt mice.
- The flox mice was 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.

- The *Rtkn2* gene is located on the Chr10. 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)

Rtkn2 rhotekin 2 [*Mus musculus* (house mouse)]

Gene ID: 170799, updated on 25-Aug-2020

Summary



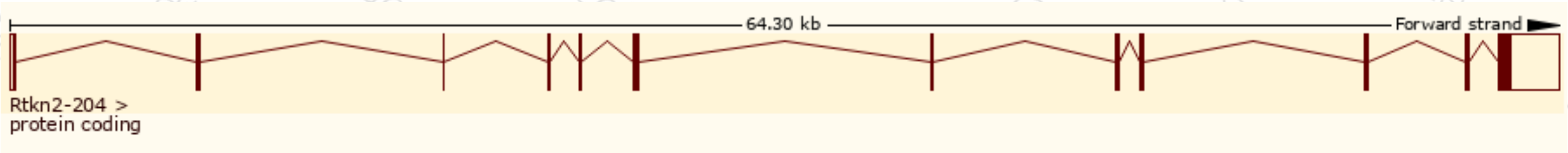
Official Symbol	Rtkn2 provided by MGI
Official Full Name	rhotekin 2 provided by MGI
Primary source	MGI:MGI:2158417
See related	Ensembl:ENSMUSG000000037846
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Mbf; RTK; Plekh; Plekhk1; B130039D23Rik
Expression	Biased expression in lung adult (RPKM 8.1), CNS E11.5 (RPKM 1.4) and 4 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

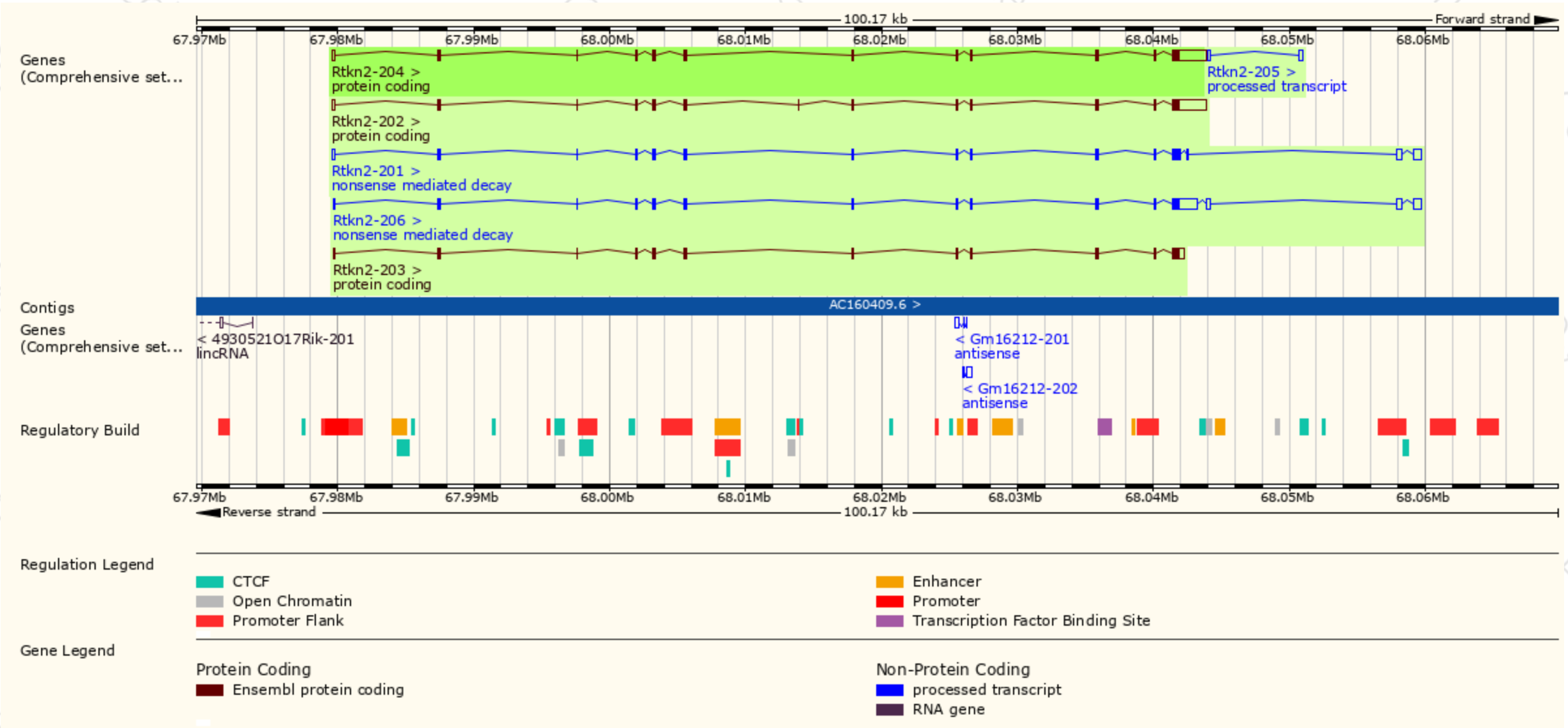
The gene has 6 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rtkn2-206	ENSMUST00000147556.7	4432	601aa	Nonsense mediated decay	-	Q14B46	TSL:1
Rtkn2-201	ENSMUST00000068994.13	3110	604aa	Nonsense mediated decay	-	Q14B46	TSL:1
Rtkn2-205	ENSMUST00000123147.1	546	No protein	Processed transcript	-	-	TSL:3
Rtkn2-204	ENSMUST00000118160.7	3970	604aa	Protein coding	-	Q14B46	TSL:1 Gencode basic APPRIS P5
Rtkn2-202	ENSMUST00000105437.7	3932	602aa	Protein coding	-	E9QP76	TSL:5 Gencode basic APPRIS ALT2
Rtkn2-203	ENSMUST00000117086.1	2246	601aa	Protein coding	-	Q14B46	TSL:1 Gencode basic APPRIS ALT2

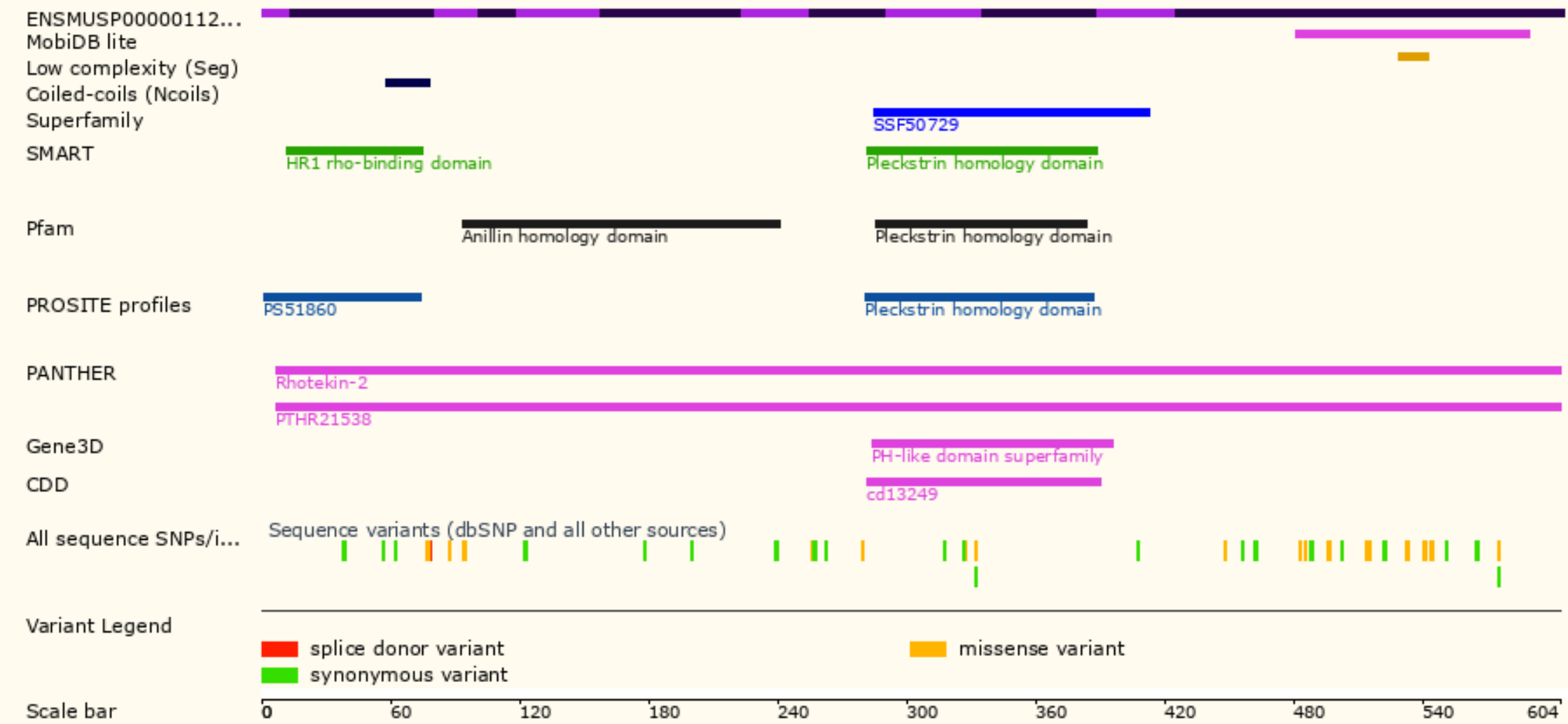
The strategy is based on the design of *Rtkn2*-204 transcript,The transcription is shown below:



Genomic location distribution



Protein domain



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



集萃药康生物科技
GemPharmatech Co.,Ltd

