Pitpnm3 Cas9-CKO Strategy

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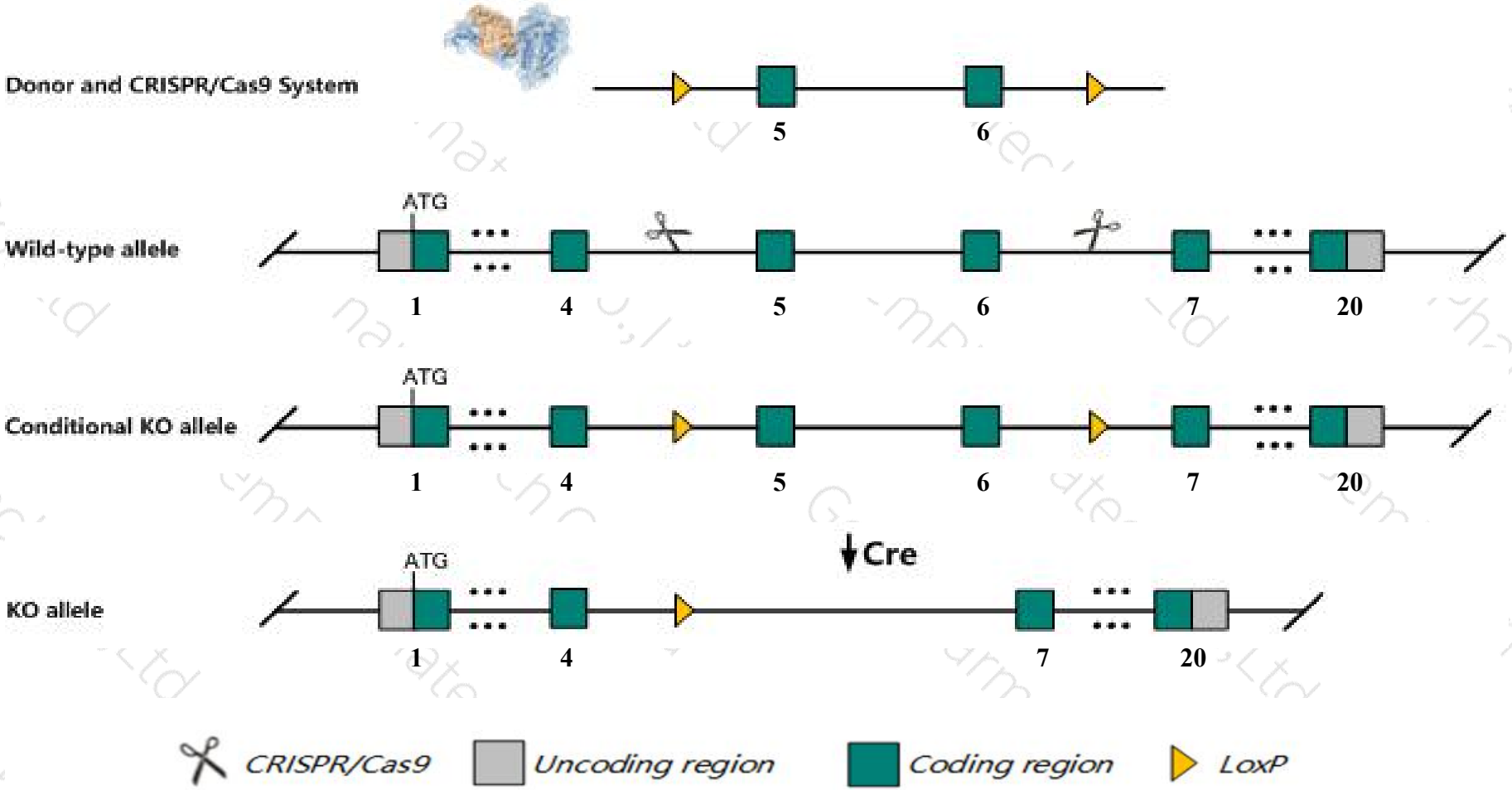
Project Overview

Project Name	***Pitpnm3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pitpnm3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Pitpnm3* gene has 5 transcripts. According to the structure of *Pitpnm3* gene, exon5-exon6 of *Pitpnm3-201*(ENSMUST00000075258.12) transcript is recommended as the knockout region.The region contains 313bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Pitpnm3* 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.

Notice

➢ The *Pitpnm3* gene is located on the Chr11. 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）

集萃药康 GemPharmatech

Pitpnm3 PITPNM family member 3 [Mus musculus (house mouse)]

Gene ID: 327958, updated on 13-Mar-2020

Summary

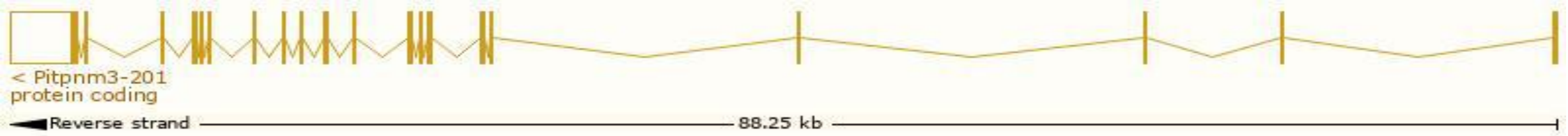
Official Symbol	Pitpnm3 provided by MGI
Official Full Name	PITPNM family member 3 provided by MGI
Primary source	MGI:MGI:2685726
See related	Ensembl:ENSMUSG00000040543
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A330068P14Rik, AI848332, Ackr6, Gm880
Expression	Broad expression in frontal lobe adult (RPKM 23.6), cortex adult (RPKM 15.6) and 17 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

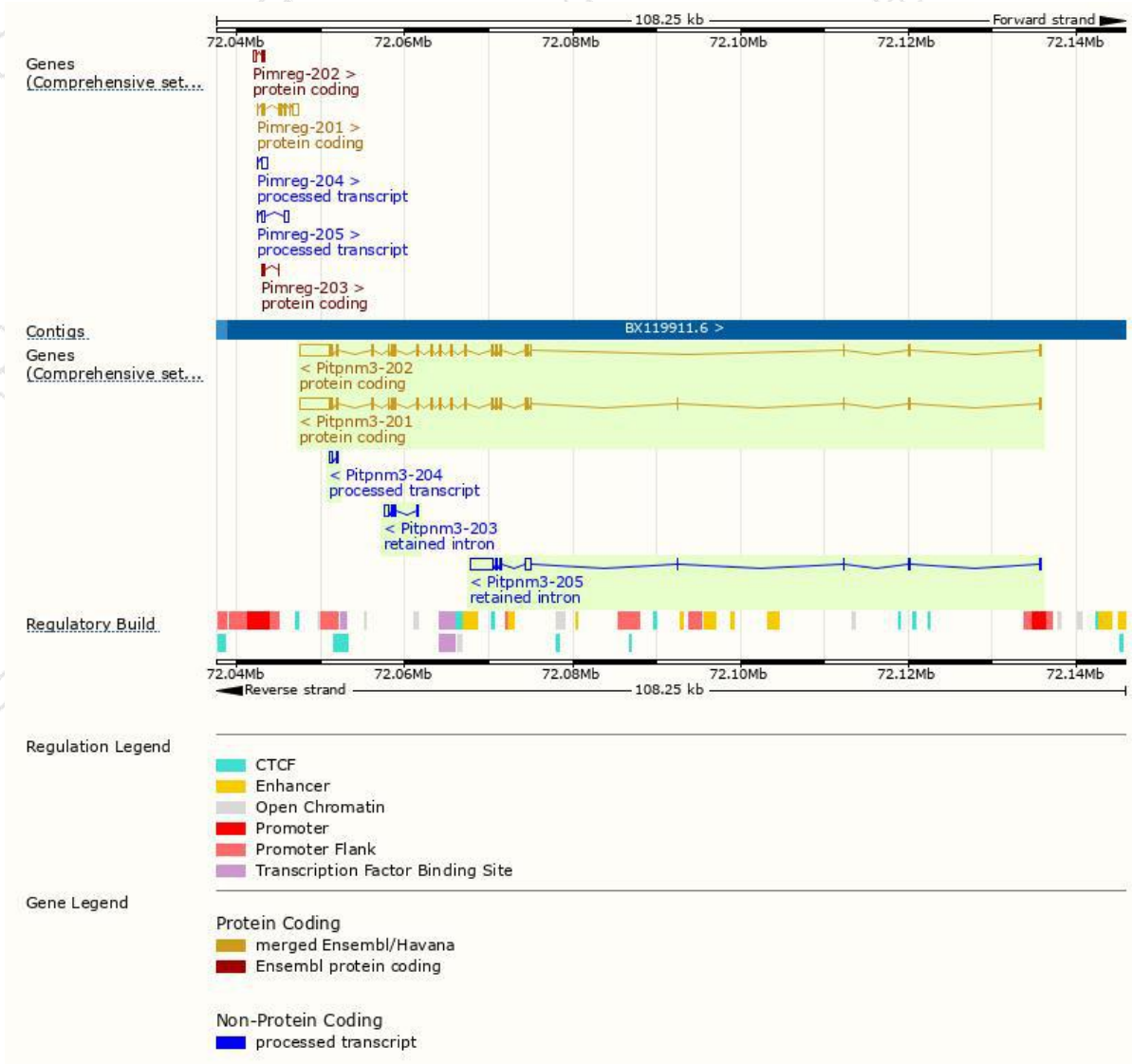
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pitpnm3-201	ENSMUST00000075258.12	6555	974aa	Protein coding	CCDS24978	Q3UHE1	TSL:1 GENCODE basic APPRIS P3
Pitpnm3-202	ENSMUST00000108508.2	6450	958aa	Protein coding	CCDS36213	Q3UHE1	TSL:1 GENCODE basic APPRIS ALT2
Pitpnm3-204	ENSMUST00000134210.1	461	No protein	Processed transcript	-	-	TSL:2
Pitpnm3-205	ENSMUST00000142471.1	3993	No protein	Retained intron	-	-	TSL:2
Pitpnm3-203	ENSMUST00000132781.1	879	No protein	Retained intron	-	-	TSL:2

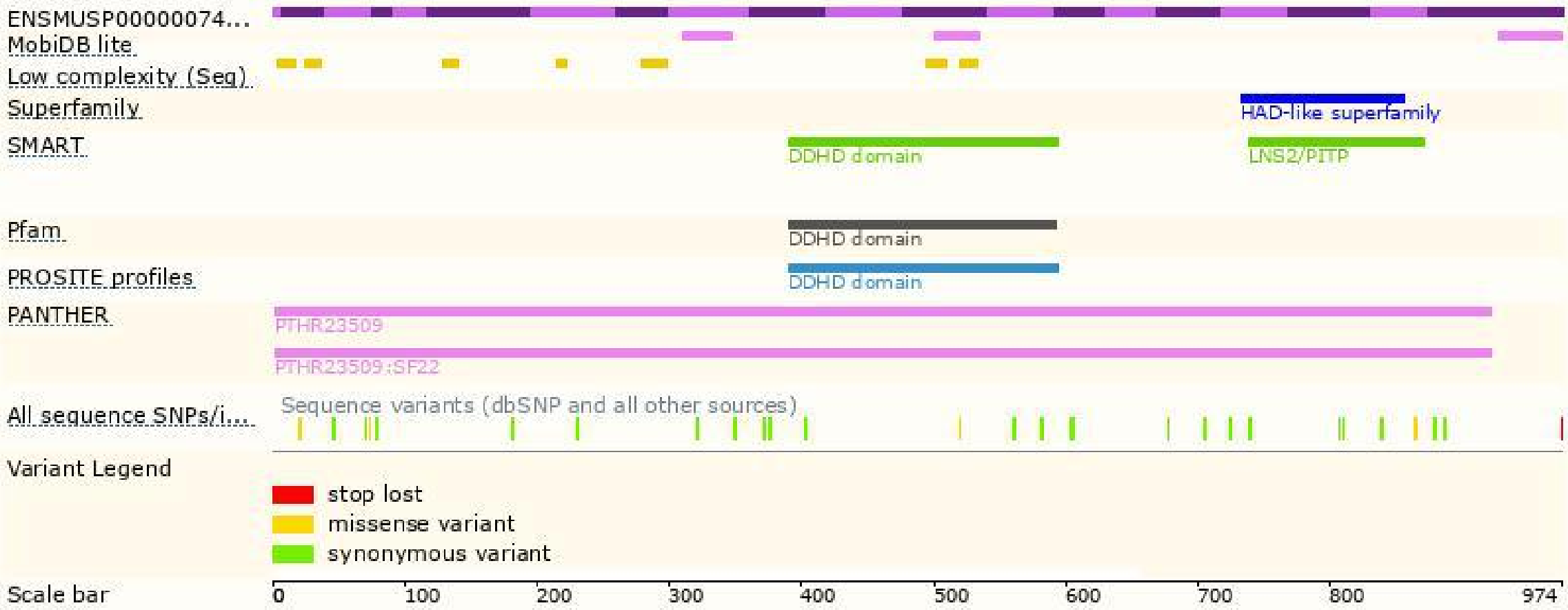
The strategy is based on the design of *Pitpnm3-201* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



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

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