Psap Cas9-CKO Strategy

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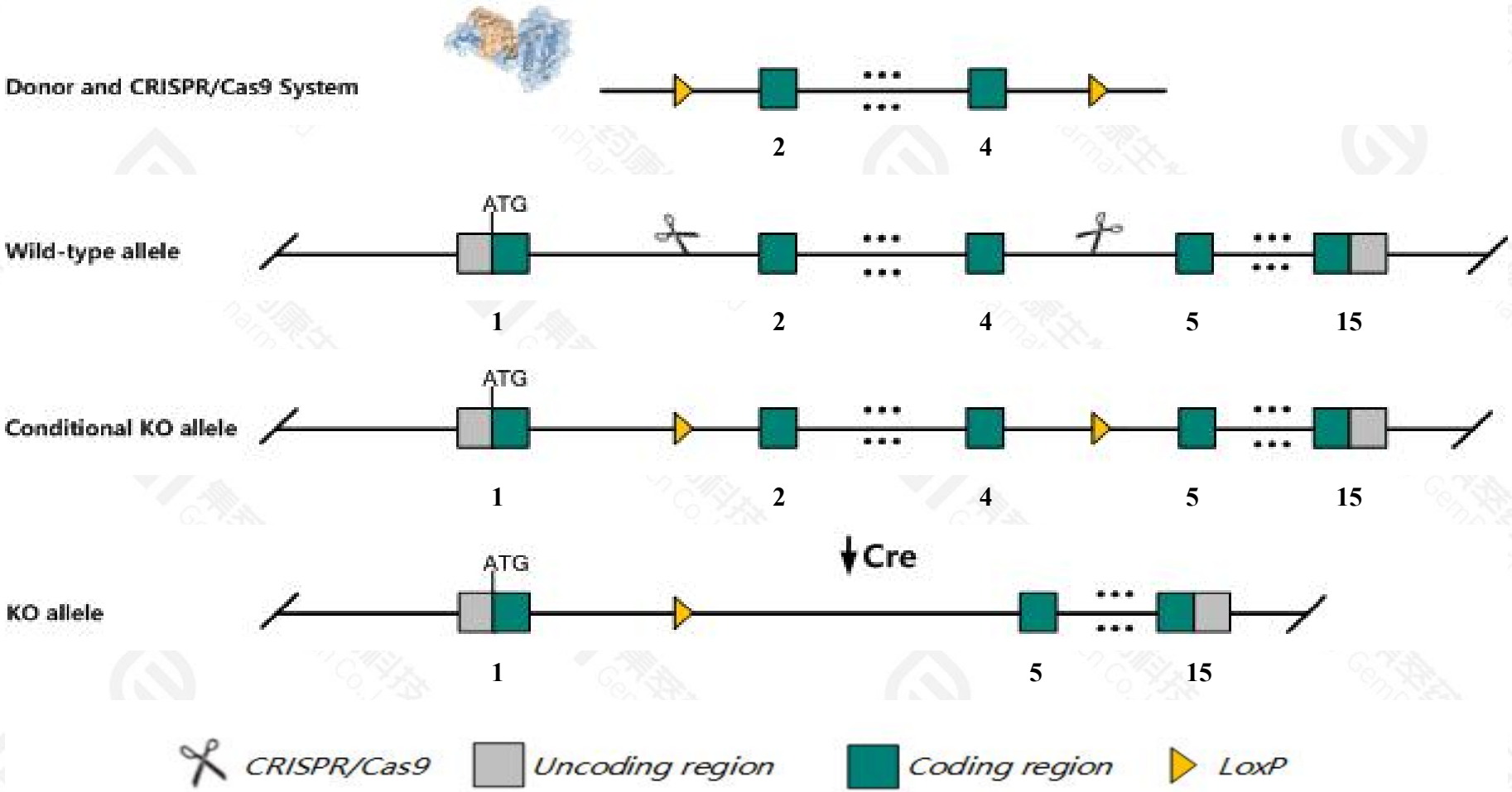
Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Psap* gene has 5 transcripts. According to the structure of *Psap* gene, exon2-exon4 of *Psap-205*(ENSMUST00000179238.8) transcript is recommended as the knockout region.The region contains 335bp coding sequence. Knock out the region will result in disruption of protein function.

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

➢ According to the existing MGI data,homozygotes for a targeted null mutation die either neonatally or around 7 weeks. At 30 days, mutants show hypomyelination, PAS-positive material in the nervous system, and accumulation of ceramides in brain, liver, and kidney.

➢ The *Psap* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information（NCBI）

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Psap prosaposin [Mus musculus (house mouse)]

Gene ID: 19156, updated on 17-Dec-2020

Summary

Official Symbol	Psap provided by MGI
Official Full Name	prosaposin provided byMGI
Primary source	MGI:MGI:97783
See related	Ensembl:ENSMUSG00000004207
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	AI037048, SGP, SGP-1
Summary	This gene encodes a multifunctional glycoprotein that plays a role in the intracellular metabolism of various sphingolipids or secreted into the plasma, milk or cerebrospinal fluid. The encoded protein undergoes proteolytic processing to generate four different polypeptides known as saposin A, B, C or D, that are required for the hydrolysis of certain sphingolipids by lysosomal hydrolases. Alternately, the encoded protein is secreted into body fluids where it exhibits neurotrophic and myelinotrophic activities. A complete lack of the encoded protein is fatal to mice either at the neonatal stage or within the first month due to severe leukodystrophy and sphingolipid accumulation. Alternative splicing results in multiple transcript variants encoding different isoforms that may undergo similar processing to generate the mature saposins. [provided by RefSeq, Sep 2015]
Expression	Ubiquitous expression in subcutaneous fat pad adult (RPKM 636.6), mammary gland adult (RPKM 598.6) and 28 other tissuesSee more
Orthologs	human all

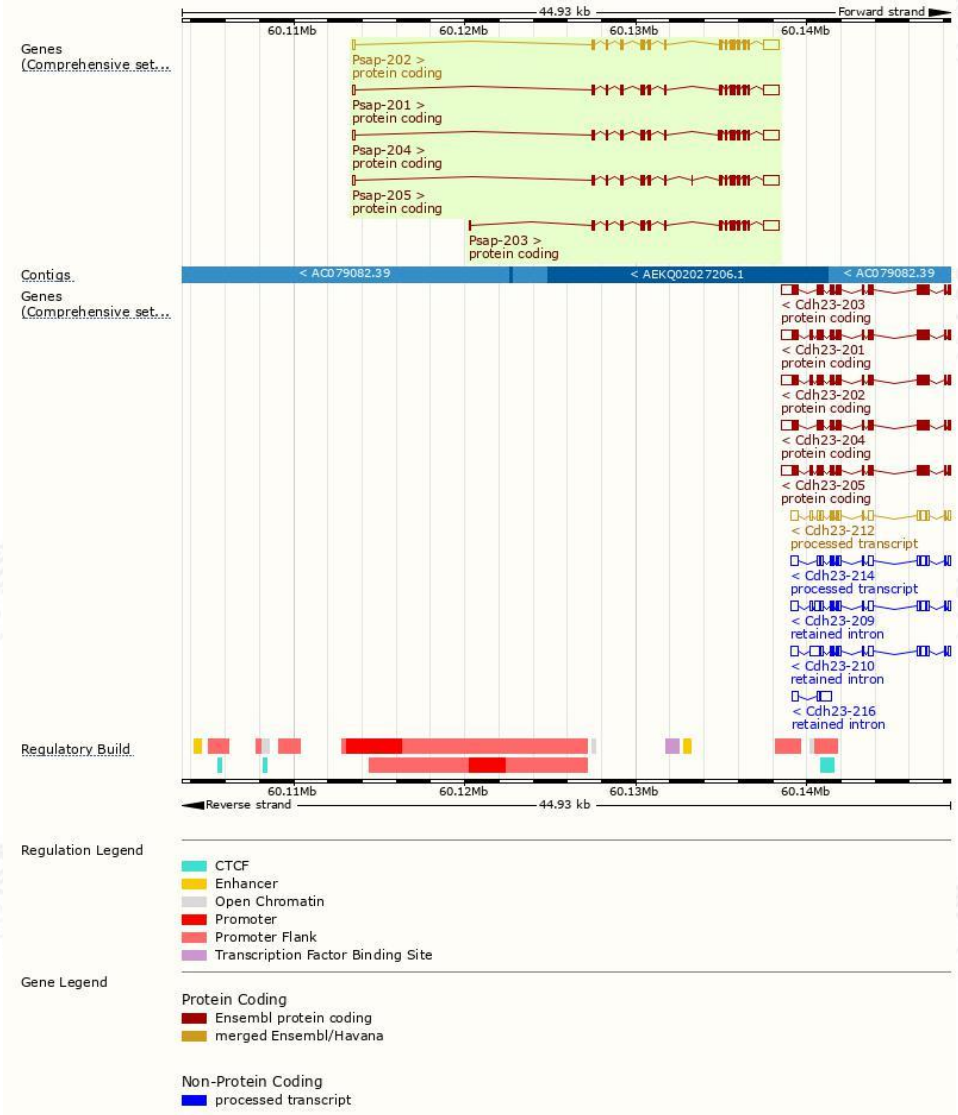
Transcript information（Ensembl）

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

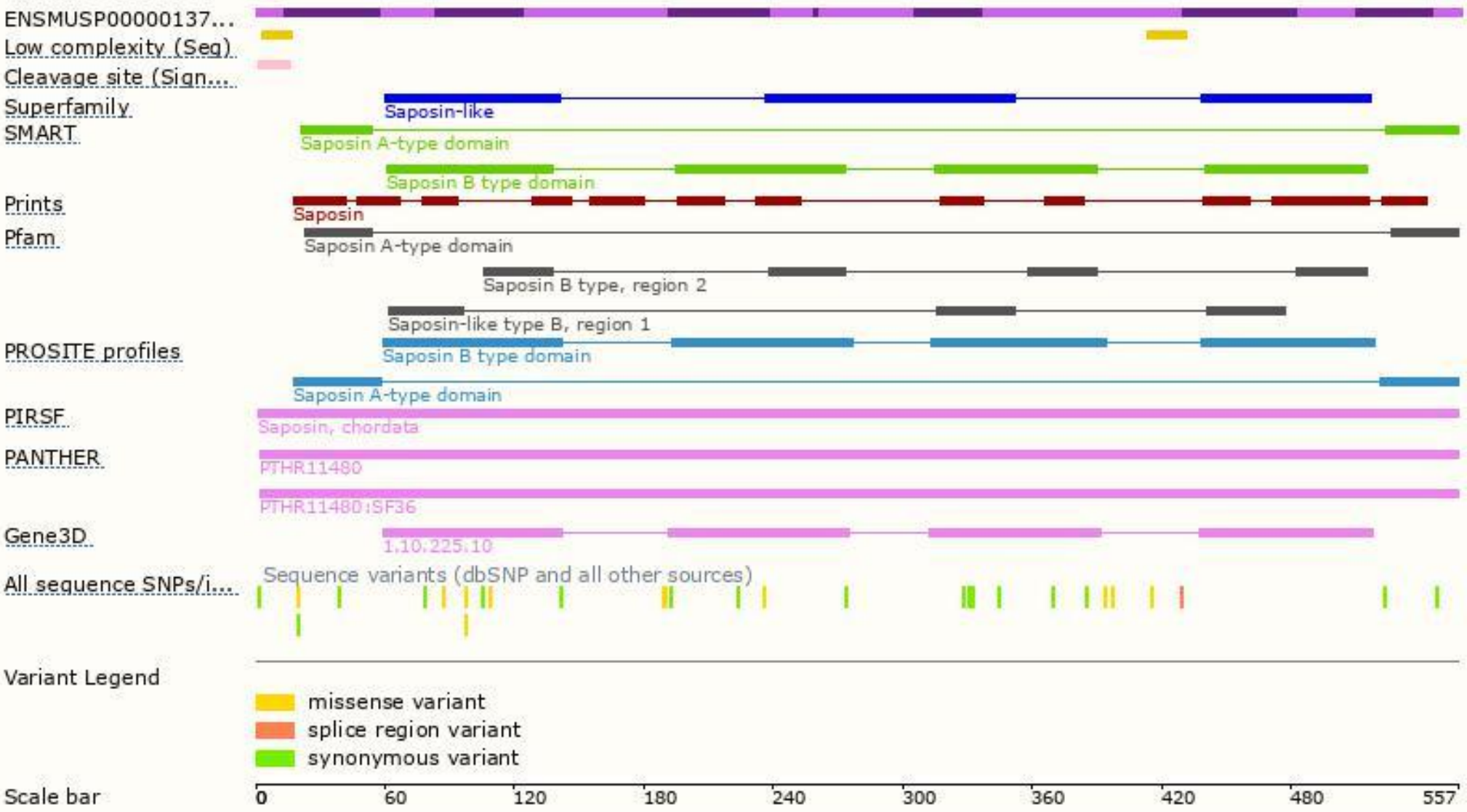
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Psap-205	ENSMUST00000179238.8	2657	557aa	Protein coding	CCDS35911		TSL:5 , GENCODE basic , APPRIS P3 ,
Psap-202	ENSMUST00000105465.8	2646	554aa	Protein coding	CCDS48567		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Psap-203	ENSMUST00000165878.2	2564	551aa	Protein coding	CCDS48568		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Psap-204	ENSMUST00000177779.8	2657	557aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Psap-201	ENSMUST00000004316.15	2654	556aa	Protein coding	-		TSL:1 , GENCODE basic , APPRIS ALT2 ,

The strategy is based on the design of *Psap-205* transcript,the transcription is shown below:

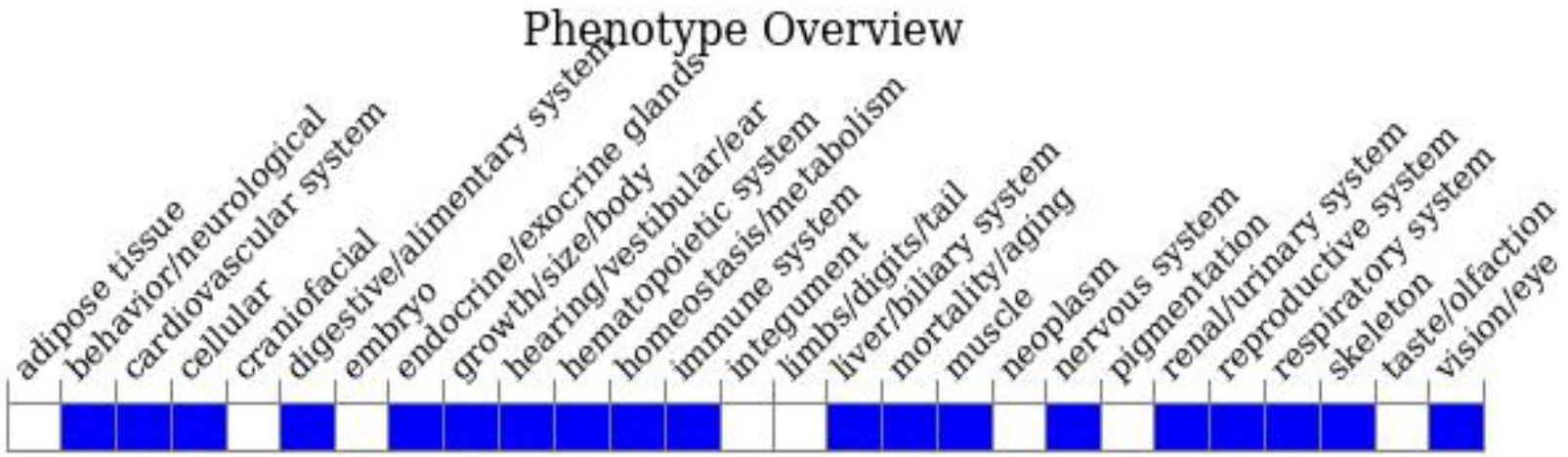
Genomic location distribution



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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