Abcb5 Cas9-CKO Strategy

Designer: Jinling Wang

Reviewer: Jiayuan Yao

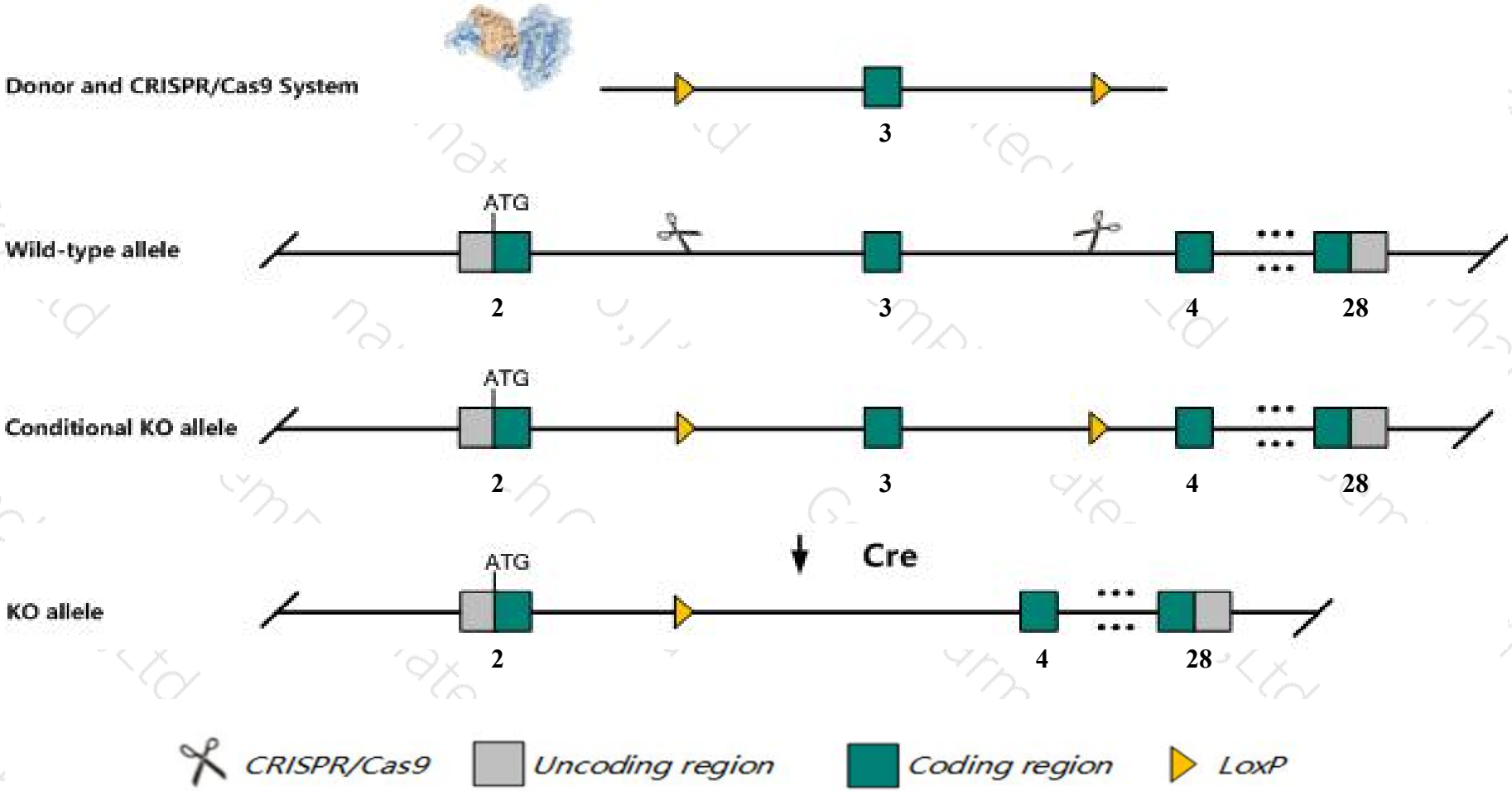
Design Date: 2018-12-4

Project Overview

Project Name	***Abcb5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Abcb5* gene. The schematic diagram is as follows:



Technical routes

➢ The *Abcb5* gene has 3 transcripts. According to the structure of *Abcb5* gene, exon3 of *Abcb5-201*(ENSMUST00000035515.4) transcript is recommended as the knockout region.The region contains 58bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Abcb5* 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,mice homozygous for a null allele display limbal stem cell abnormalities, impaired cornea development and repair, and retinal abnormalities.

➢ The *Abcb5* gene is located on the Chr12. 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

Abcb5 ATP-binding cassette, sub-family B (MDR/TAP), member 5 [Mus musculus (house mouse)]

Gene ID: 77706, updated on 3-Feb-2019

Summary

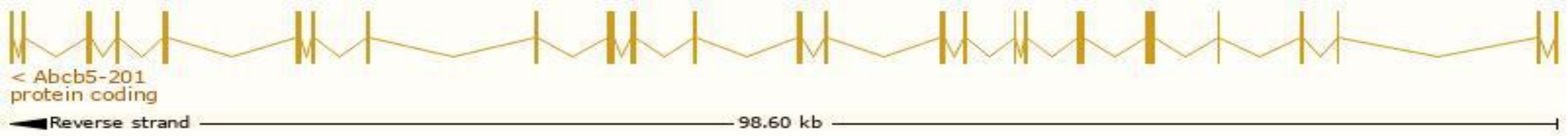
Official Symbol	Abcb5 provided by MGI
Official Full Name	ATP-binding cassette, sub-family B (MDR/TAP), member 5 provided by MGI
Primary source	MGI:MGI:1924956
See related	Ensembl:ENSMUSG00000072791
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	9230106F14Rik
Expression	Restricted expression toward genital fat pad adult (RPKM 9.9) See more
Orthologs	human all

Transcript information（Ensembl）

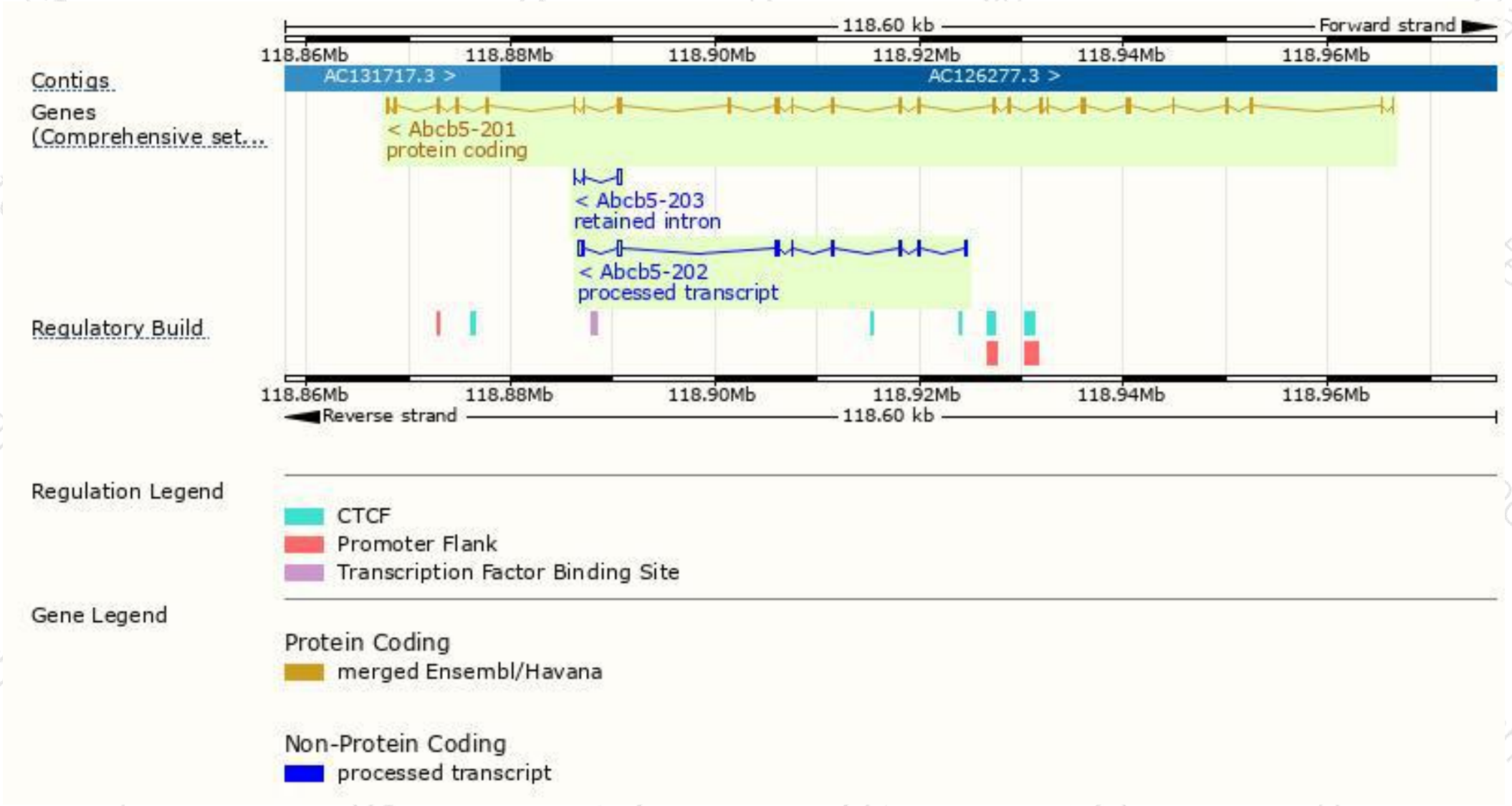
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Abcb5-201	ENSMUST00000035515.4	3876	1255aa	Protein coding	CCDS49198	B5X0E4	TSL:1 GENCODE basic APPRIS P1
Abcb5-202	ENSMUST00000100982.4	1591	No protein	Processed transcript	-	-	TSL:1
Abcb5-203	ENSMUST00000177311.7	463	No protein	Retained intron	-	-	TSL:1

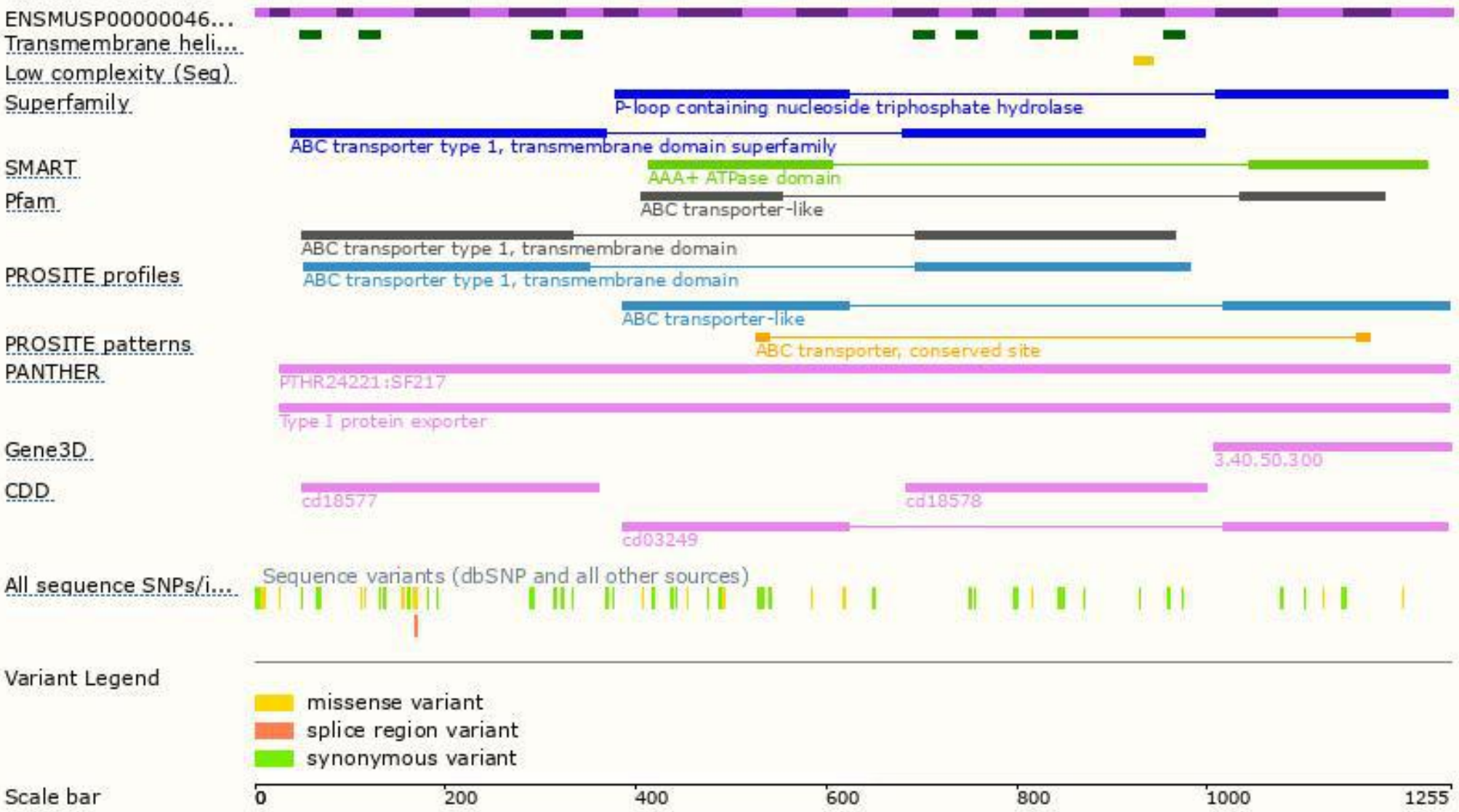
The strategy is based on the design of *Abcb5-201* transcript,the transcription is shown below:



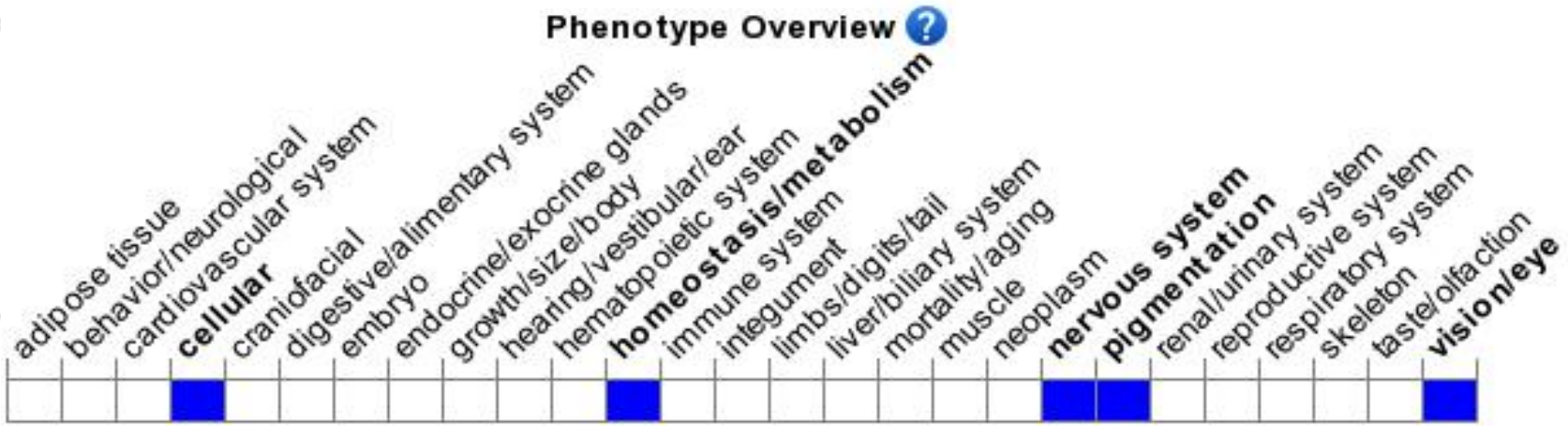
Genomic location distribution



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,mice homozygous for a null allele display limbal stem cell abnormalities, impaired cornea development and repair, and retinal abnormalities.

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd