

# Abcb5 Cas9-KO Strategy

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#### Overview

#### Target Gene Name

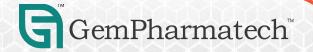
• Abcb5

#### Project Type

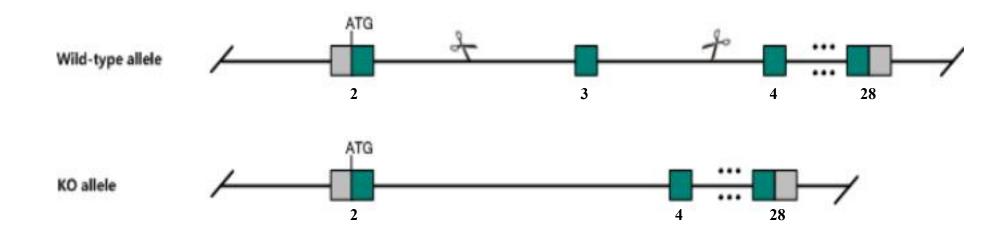
• Cas9-KO

#### Genetic Background

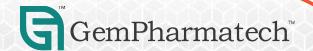
• C57BL/6JGpt



## Strain Strategy







#### **Technical Information**

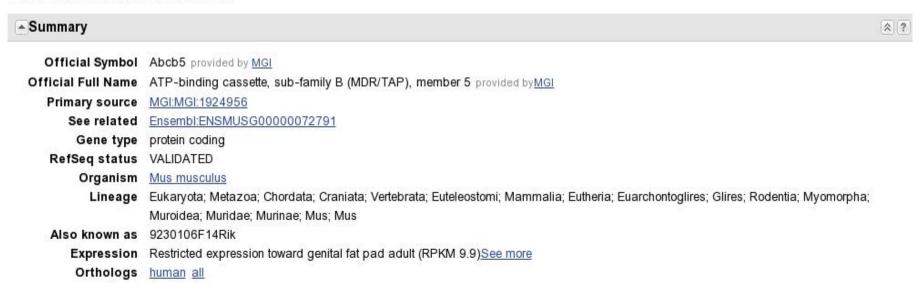
- The *Abcb5* gene has 3 transcripts. According to the structure of *Abcb5* gene, exon3 of *Abcb5*-201 (ENSMUST00000035515.5) transcript is recommended as the knockout region. The region contains 58bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Abcb5* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 ontarget amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.



#### Gene Information

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

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



Source: https://www.ncbi.nlm.nih.gov/

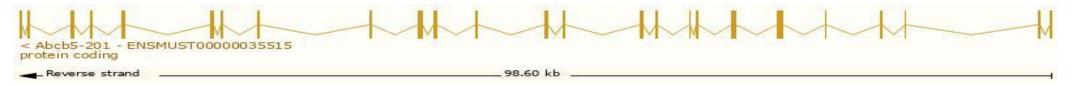


### Transcript Information

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Abcb5-201	ENSMUST00000035515.4	3876	<u>1255aa</u>	Protein coding	CCDS49198	B5X0E4	TSL:1 GENCODE basic APPRIS P1
Abcb5-202	ENSMUST00000100982.4	1591	No protein	Processed transcript	, <del>8</del>		TSL:1
Abcb5-203	ENSMUST00000177311.7	463	No protein	Retained intron	18	2	TSL:1

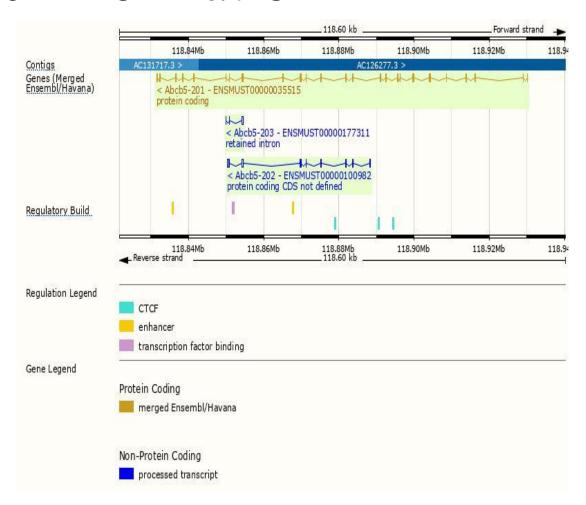
The strategy is based on the design of *Abcb5*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org



### Genomic Information





Source: : https://www.ensembl.org

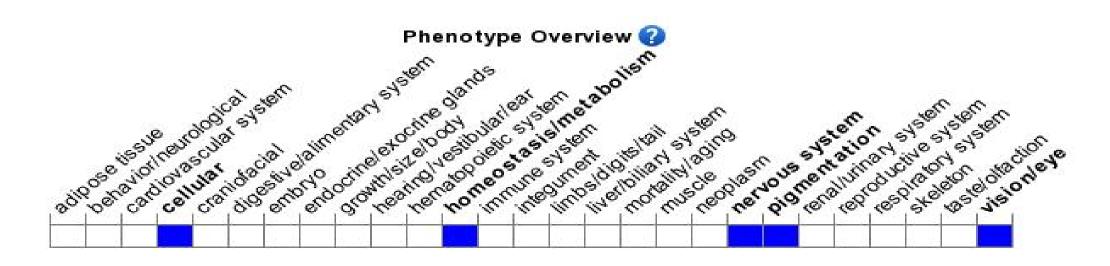
#### Protein Information





Source: : https://www.ensembl.org

### Mouse Phenotype Information (MGI)



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



Source: https://www.informatics.jax.org

### Important Information

- *Abcb5* is located on Chr12. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

