

# Aqp8 Cas9-CKO Strategy

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## Overview

#### Target Gene Name

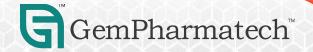
• Aqp8

#### Project Type

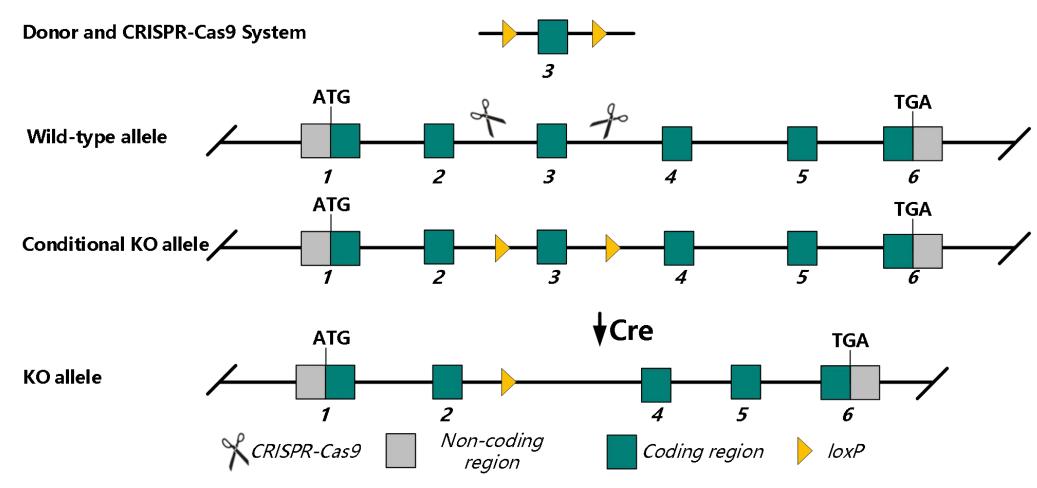
• Cas9-CKO

#### Genetic Background

• C57BL/6JGpt



## Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Aqp8 gene.



#### Technical Information

- The Aqp8 gene has 3 transcripts. According to the structure of Aqp8 gene, exon 3 of Aqp8-202 (ENSMUST00000098056.6) transcript is recommended as the knockout region. The region contains 127 bp of coding sequences. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Aqp8* 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 on-target 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.
- 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.



#### Gene Information





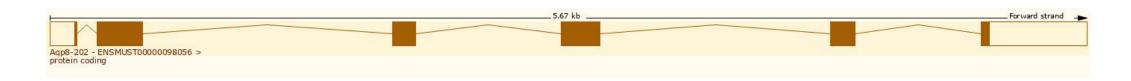
Source: https://www.ncbi.nlm.nih.gov/

# Transcript Information

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

Transcript ID ▼	Name 🍦	bp 🛊	Protein 🍦	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000206106.2	Aqp8-203	1159	No protein	Protein coding CDS not defined		-	TSL:1
ENSMUST00000098056.6	Aqp8-202	1451	<u>261aa</u>	Protein coding	CCDS72038 €	P56404 @	Ensembl Canonical GENCODE basic APPRIS P4 TSL:1
ENSMUST00000033023.10	Aqp8-201	1487	260aa	Protein coding	CCDS85406 &	A0A0X1KG59₺	GENCODE basic   APPRIS ALT1   TSL:1

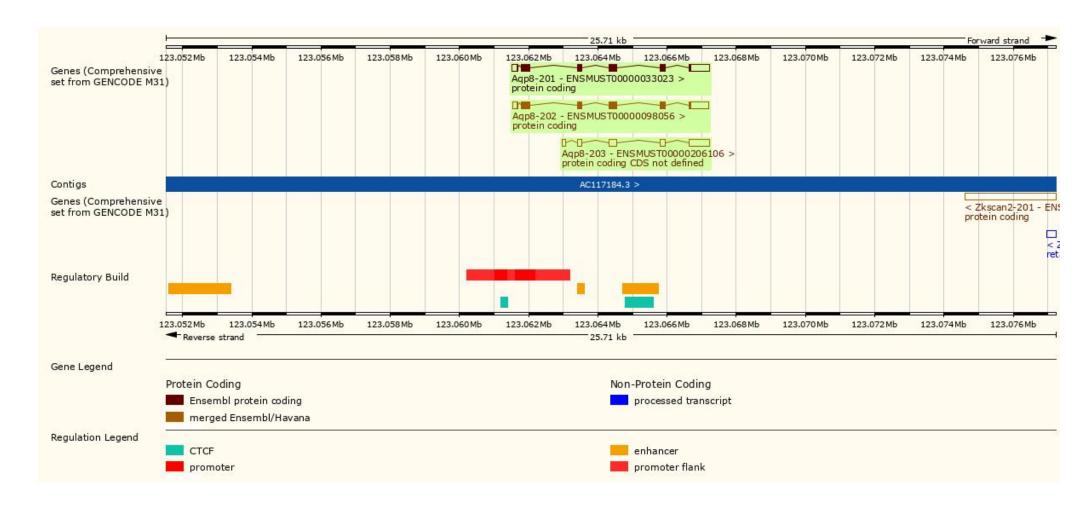
The strategy is based on the design of Aqp8-202 transcript, the transcription is shown below:



Source: https://www.ensembl.org



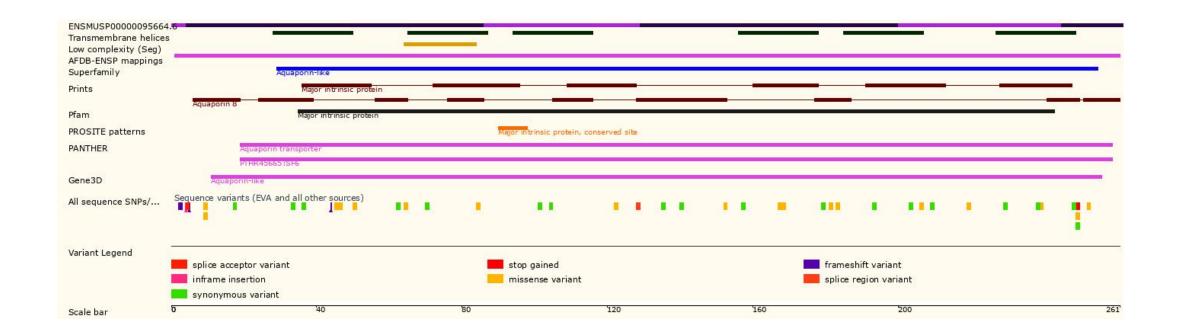
## Genomic Information





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

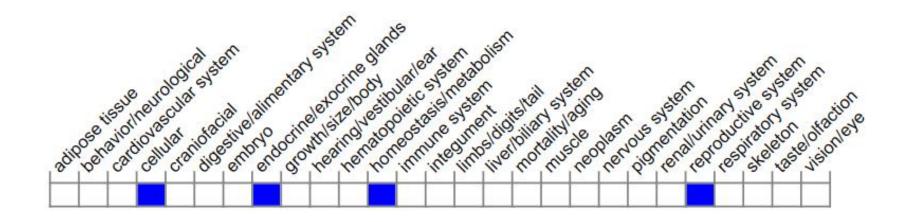
## Protein Information





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

## Mouse Phenotype Information (MGI)



Males homozygous for a null allele are fertile but show enlarged testes and reduced water permeability in testis plasma membranes. Females show increased ovulation, corpora lutea number and fertility, and a decrease in the plasma membrane water permeability and apoptosis rate of granulosa cells.



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

## Important Information

- Males homozygous for a null allele are fertile but show enlarged testes and reduced water permeability in testis plasma membranes. Females show increased ovulation, corpora lutea number and fertility, and a decrease in the plasma membrane water permeability and apoptosis rate of granulosa cells.
- Intron 3-4 (793 bp) of Aqp8 are small and loxp insertion may interfere with normal splicing.
- Aqp8 is located on Chr 7. 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 risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

