

Agbl5 Cas9-CKO Strategy

Designer: Xiangli Bian

Reviewer: Xingkai Xiao

Design Date: 2023-12-22

Overview

Target Gene Name

• *Agbl5*

Project Type

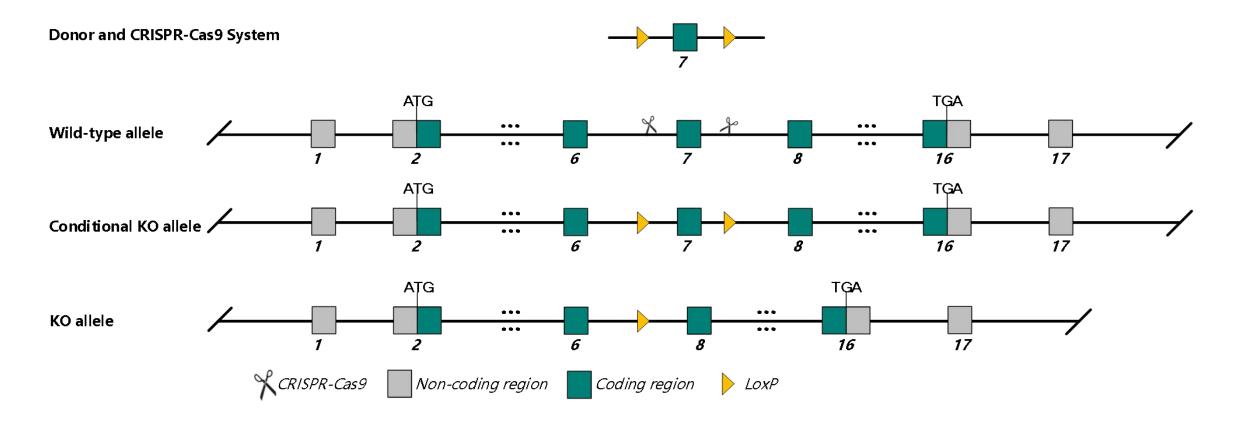
• Cas9-CKO

Genetic Background

• C57BL/6JGpt



Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the Agbl5 gene.



Technical Information

- The *Agbl5* gene has 18 transcripts. According to the structure of *Agbl5* gene, exon 7 of *Agbl5*-202 (ENSMUST00000114700.9) is recommended as the knockout region. The region contains 179 bp of coding sequence. Knocking out the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Agbl5* 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

Agbl5 ATP/GTP binding protein-like 5 [Mus musculus (house mouse)]

≛ Download Datasets

Gene ID: 231093, updated on 23-Nov-2023



https://www.ncbi.nlm.nih.gov/gene/231093



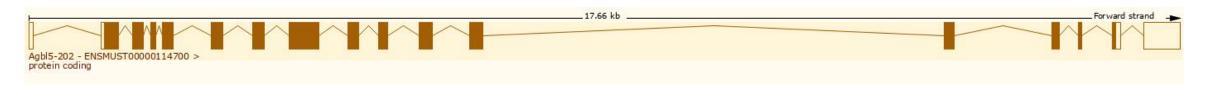
Exon count: 18

Transcript Information

The gene has 18 transcripts, all transcripts are shown below:

Show/hide columns (1 hidden)							Filter	
Transcript ID	Name 🍦	bp 👙	Protein ▼	Biotype	CCDS 🍦	UniProt Match	Flags	
ENSMUST00000201168.4	Agbl5-208	3199	<u>886aa</u>	Protein coding		Q09M02-1 ₽	Ensembl Canonical GENCODE basic APPRIS P2 TSL:	
ENSMUST00000114700.9	Agbl5-202	3296	<u>846aa</u>	Protein coding	CCDS39048₺	Q09M02-7₽	GENCODE basic TSL:1	
ENSMUST00000201917.4	Agbl5-213	3579	817aa	Protein coding		Q09M02-2₽	GENCODE basic APPRIS ALT1 TSL:1	
ENSMUST00000201817.4	Agbl5-211	3305	808aa	Nonsense mediated decay		Q09M02-4₽	TSL:1	
ENSMUST00000202060.4	Agbl5-215	3121	<u>770aa</u>	Protein coding	CCDS80242₺	Q09M02-3 ₽	GENCODE basic TSL:1	
ENSMUST00000201225.4	Agbl5-209	2646	<u>770aa</u>	Protein coding	CCDS80242₺	Q09M02-3 ₽	GENCODE basic TSL:1	
ENSMUST00000069705.11	Agbl5-201	2922	719aa	Protein coding	CCDS19165₺	Q09M02-6 ₽	GENCODE basic TSL:1	
ENSMUST00000202109.5	Agbl5-216	768	<u>256aa</u>	Protein coding		A0A0J9YV16₺	TSL:5 CDS 5' and 3' incomplete	
ENSMUST00000200850.2	Agbl5-204	940	<u>231aa</u>	Protein coding		A0A0J9YUP2₺	TSL:5 CDS 3' incomplete	
ENSMUST00000200695.4	Agbl5-203	730	<u>178aa</u>	Protein coding		A0A0J9YUC3₺	TSL:3 CDS 3' incomplete	
ENSMUST00000201014.2	Agbl5-206	644	<u>137aa</u>	Protein coding		<u>F6TQ98</u> ₽	TSL:5 CDS 5' incomplete	
ENSMUST00000201523.2	Agbl5-210	577	No protein	Protein coding CDS not defined		-	TSL:3	
ENSMUST00000202757.4	Agbl5-218	3466	No protein	Retained intron			TSL:2	
ENSMUST00000200990.4	Agbl5-205	3267	No protein	Retained intron		829	TSL:1	
ENSMUST00000201167.4	Agbl5-207	2922	No protein	Retained intron		050	TSL:1	
ENSMUST00000201918.4	Agbl5-214	2352	No protein	Retained intron		-	TSL:1	
ENSMUST00000202565.2	Agbl5-217	839	No protein	Retained intron		1-1	TSL:2	
ENSMUST00000201901.2	Agbl5-212	408	No protein	Retained intron		100	TSL:2	

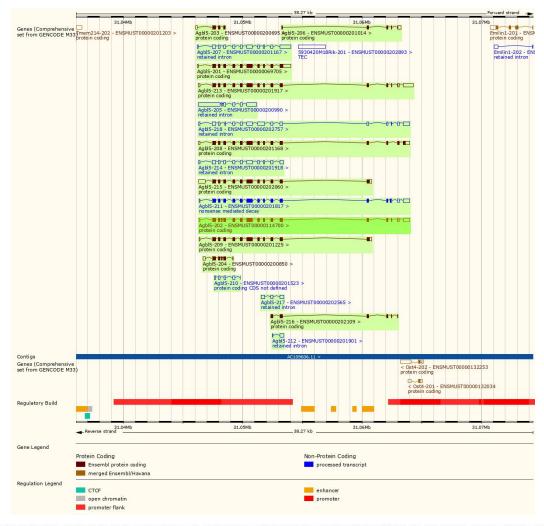
The strategy is based on the design of *Agbl5*-202 transcript, the transcription is shown below:





Source: http://asia.ensembl.org/

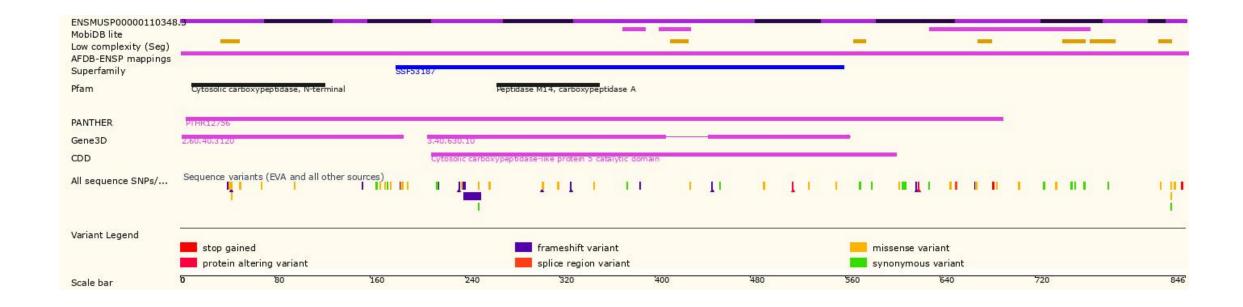
Genomic Information





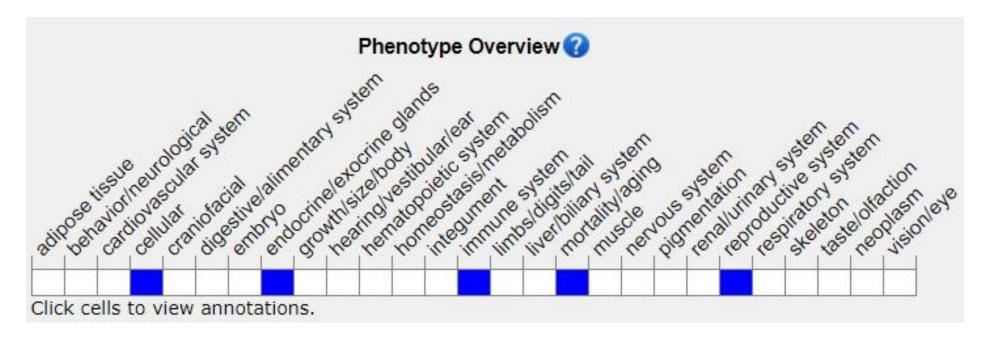
Source: http://asia.ensembl.org/

Protein Information





Mouse Phenotype Information (MGI)



Homozygotes for a knock-out allele show increased susceptibility to infection with DNA viruses. Homozygotes for a different knock-out allele show defects in multiple steps of spermatogenesis leading to manchette malformation, supernumerary centrioles, defective sperm axonemes, and male infertility.



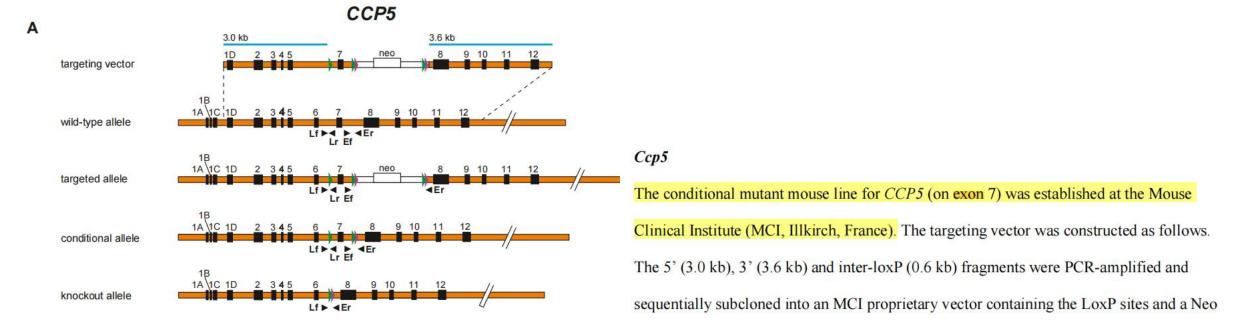
Source: https://www.informatics.jax.org

Important Information

- The knockout region is about 4.5 kb away from the 5' of the 5930420M18Rik gene, which may affect the regulation of this gene.
- The intron 6-7 of *Agbl5* is 447 bp, the loxp insertion may affect the regulation of this gene.
- The intron 7-8 of Agbl5 is 388 bp, the loxp insertion may affect the regulation of this gene.
- This stratergy may not affect *Agbl5*-203, *Agbl5*-204, *Agbl5*-206, *Agbl5*-212, *Agbl5*-216 and *Agbl5*-217 transcript.
- *Agbl5* is located on Chr 5. 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.



Reference



[1] Giordano T, Gadadhar S, Bodakuntla S, Straub J, Leboucher S, Martinez G, Chemlali W, Bosc C, Andrieux A, Bieche I, Arnoult C, Geimer S, Janke C. Loss of the deglutamylase CCP5 perturbs multiple steps of spermatogenesis and leads to male infertility. J Cell Sci. 2019 Feb 7;132(3):jcs226951. doi: 10.1242/jcs.226951. PMID: 30635446.

