GemPharmatech™

Agbl5 Cas9-KO Strategy

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Reviewer: Xingkai Xiao

Design Date: 2023-12-22

Overview

Target Gene Name

- *Agbl5*

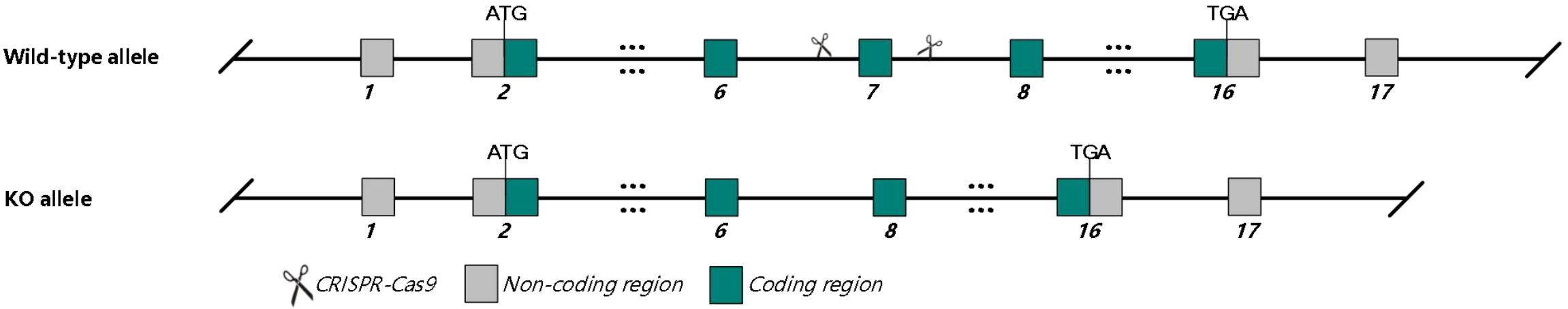
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy

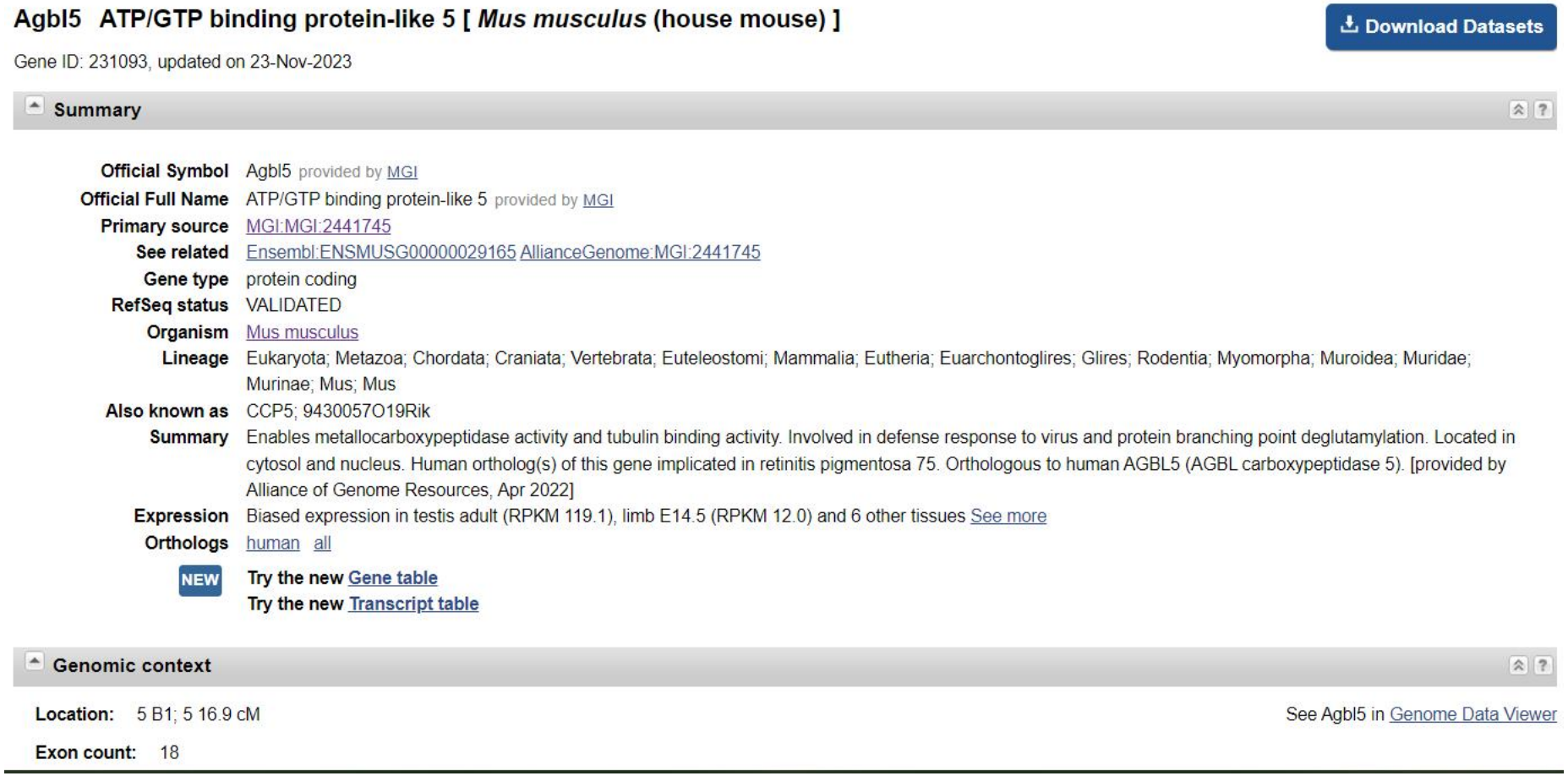


Schematic representation of CRISPR-Cas9 engineering used to edit the *Agbl5* gene.

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: 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 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.

Gene Information



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

Download Datasets

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

Summary

Official Symbol	Agbl5 provided by MGI
Official Full Name	ATP/GTP binding protein-like 5 provided by MGI
Primary source	MGI:MGI:2441745
See related	Ensembl:ENSMUSG00000029165 AllianceGenome:MGI:2441745
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	CCP5; 9430057O19Rik
Summary	Enables metallocarboxypeptidase activity and tubulin binding activity. Involved in defense response to virus and protein branching point deglutamylation. Located in cytosol and nucleus. Human ortholog(s) of this gene implicated in retinitis pigmentosa 75. Orthologous to human AGBL5 (AGBL carboxypeptidase 5). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Biased expression in testis adult (RPKM 119.1), limb E14.5 (RPKM 12.0) and 6 other tissues See more
Orthologs	human all

NEW Try the new Gene table
Try the new Transcript table

Genomic context

Location: 5 B1; 5 16.9 cM — See Agbl5 in Genome Data Viewer

Exon count: 18

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

Transcript Information

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

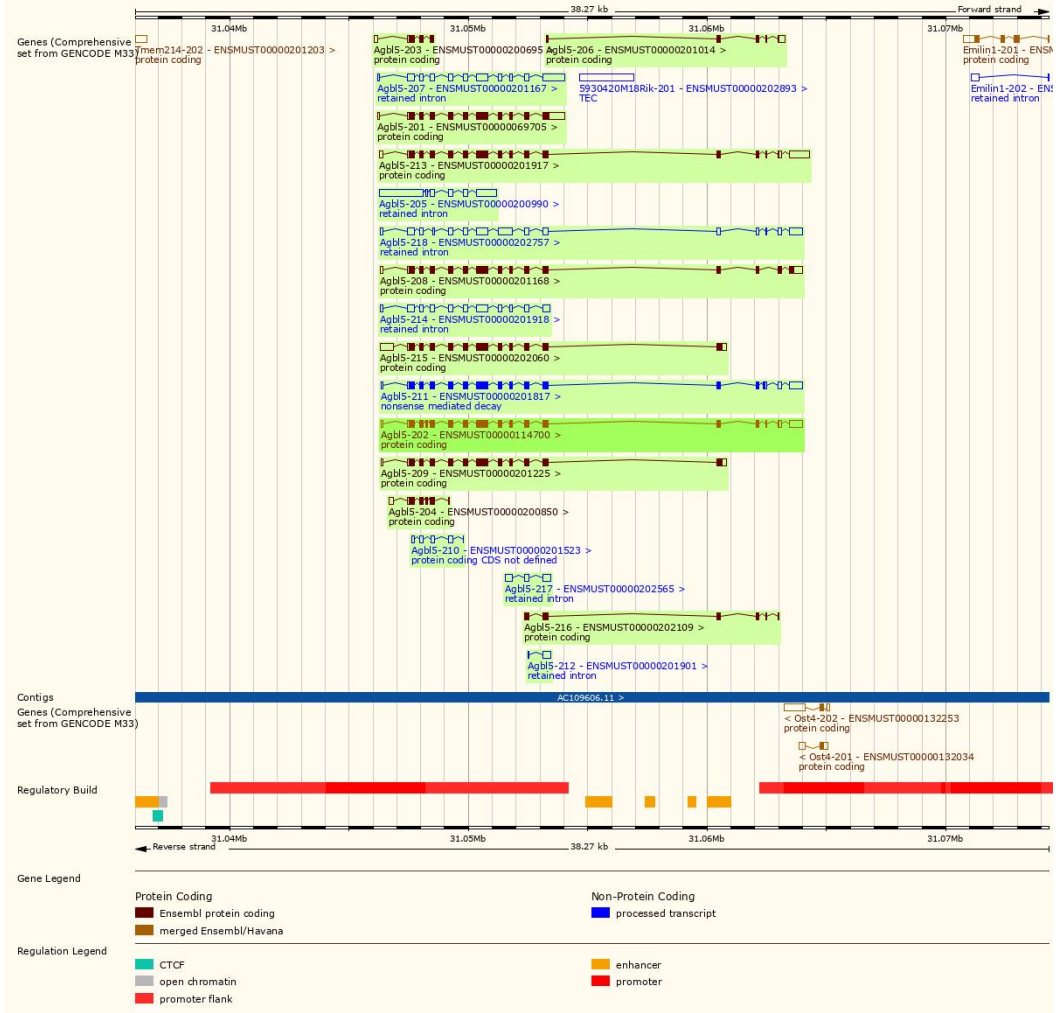
Show/hide columns (1 hidden) Filter

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000201168.4	Agbl5-208	3199	886aa	Protein coding		Q09M02-1	Ensembl Canonical GENCODE basic APPRIS P2 TSL:5
ENSMUST00000114700.9	Agbl5-202	3296	846aa	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	770aa	Protein coding	CCDS80242	Q09M02-3	GENCODE basic TSL:1
ENSMUST00000201225.4	Agbl5-209	2646	770aa	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	256aa	Protein coding		A0A0J9YV16	TSL:5 CDS 5' and 3' incomplete
ENSMUST00000200850.2	Agbl5-204	940	231aa	Protein coding		A0A0J9YUP2	TSL:5 CDS 3' incomplete
ENSMUST00000200695.4	Agbl5-203	730	178aa	Protein coding		A0A0J9YUC3	TSL:3 CDS 3' incomplete
ENSMUST00000201014.2	Agbl5-206	644	137aa	Protein coding		F6TQ98	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		-	TSL:1
ENSMUST00000201167.4	Agbl5-207	2922	No protein	Retained intron		-	TSL:1
ENSMUST00000201918.4	Agbl5-214	2352	No protein	Retained intron		-	TSL:1
ENSMUST00000202565.2	Agbl5-217	839	No protein	Retained intron		-	TSL:2
ENSMUST00000201901.2	Agbl5-212	408	No protein	Retained intron		-	TSL:2

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

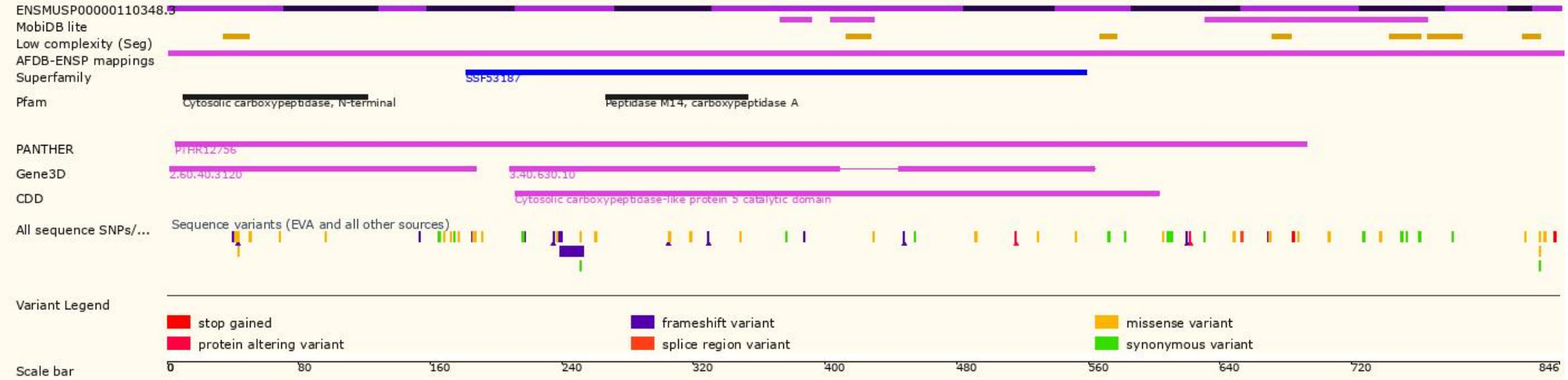
Source: http://asia.ensembl.org/

Genomic Information



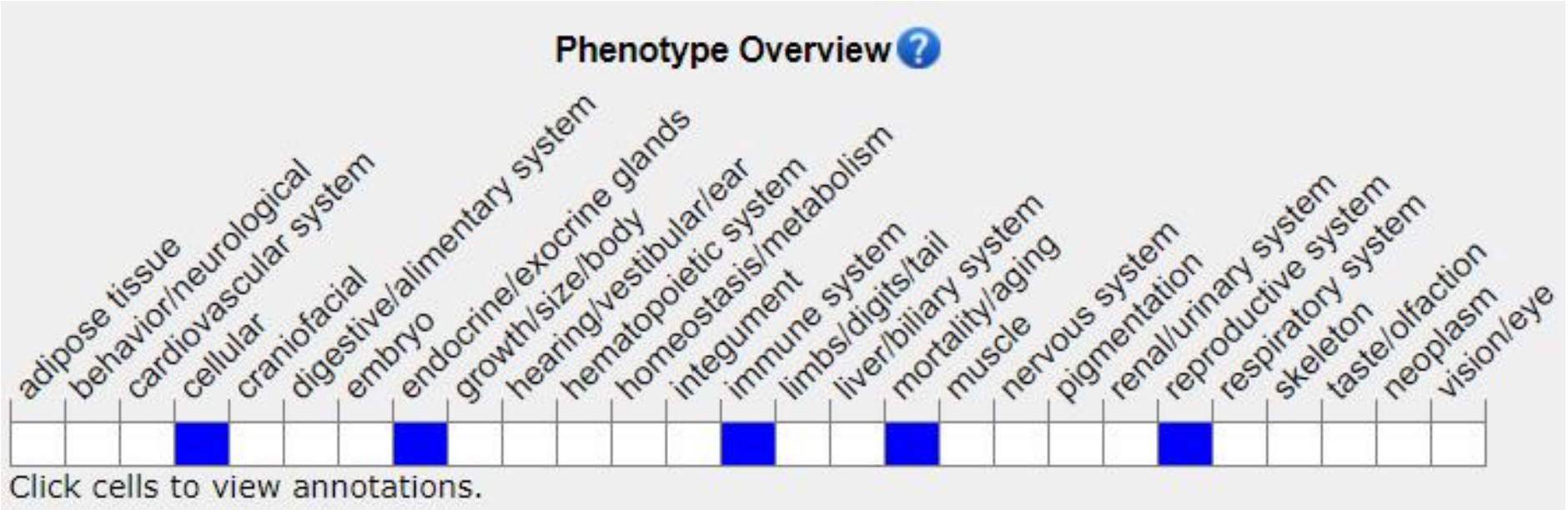
Source: http://asia.ensembl.org/

Protein Information



Source: https://www.ensembl.org

Mouse Phenotype Information (MGI)



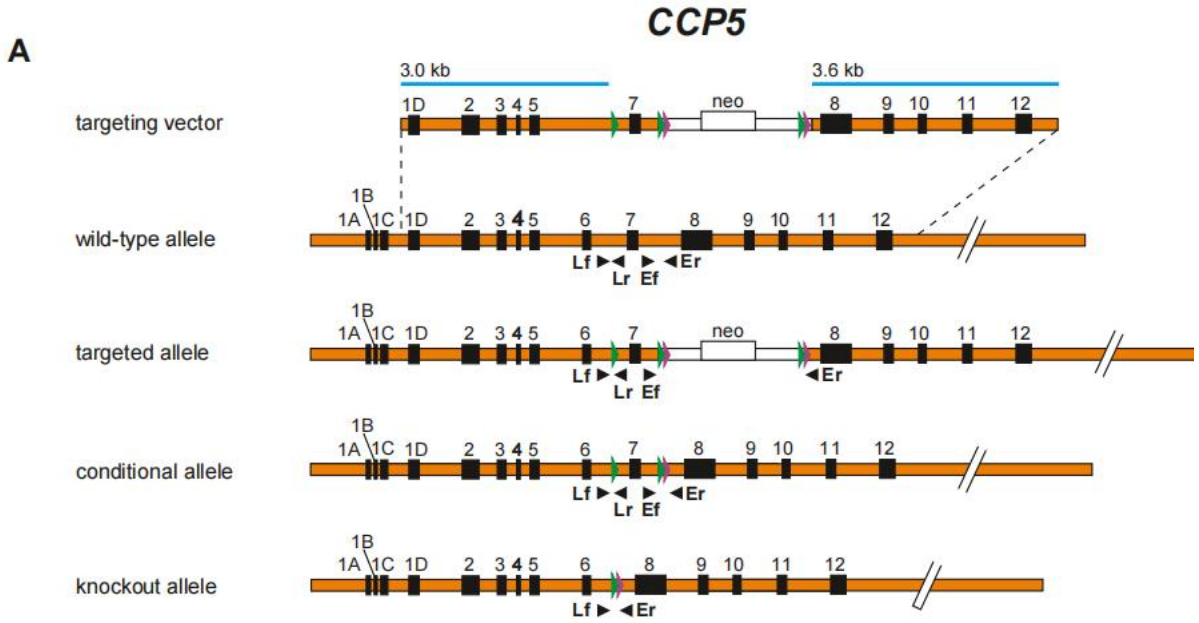
Homozygotes for a knock-out allele show increased susceptibility to infection with DNA viruses. Homozygotes for a different knock-out allele show defects in multiple steps of spermatogenesis leading to manchette malformation, supernumerary centrioles, defective sperm axonemes, and male infertility.

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.
- 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 risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Reference



Ccp5

The conditional mutant mouse line for *CCP5* (on exon 7) was established at the Mouse Clinical Institute (MCI, Illkirch, France). The targeting vector was constructed as follows. The 5' (3.0 kb), 3' (3.6 kb) and inter-loxP (0.6 kb) fragments were PCR-amplified and sequentially subcloned into an MCI proprietary vector containing the LoxP sites and a Neo

[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.