

Ambp Cas9-CKO Strategy

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Project Overview

Project Name

Ambp

Project type

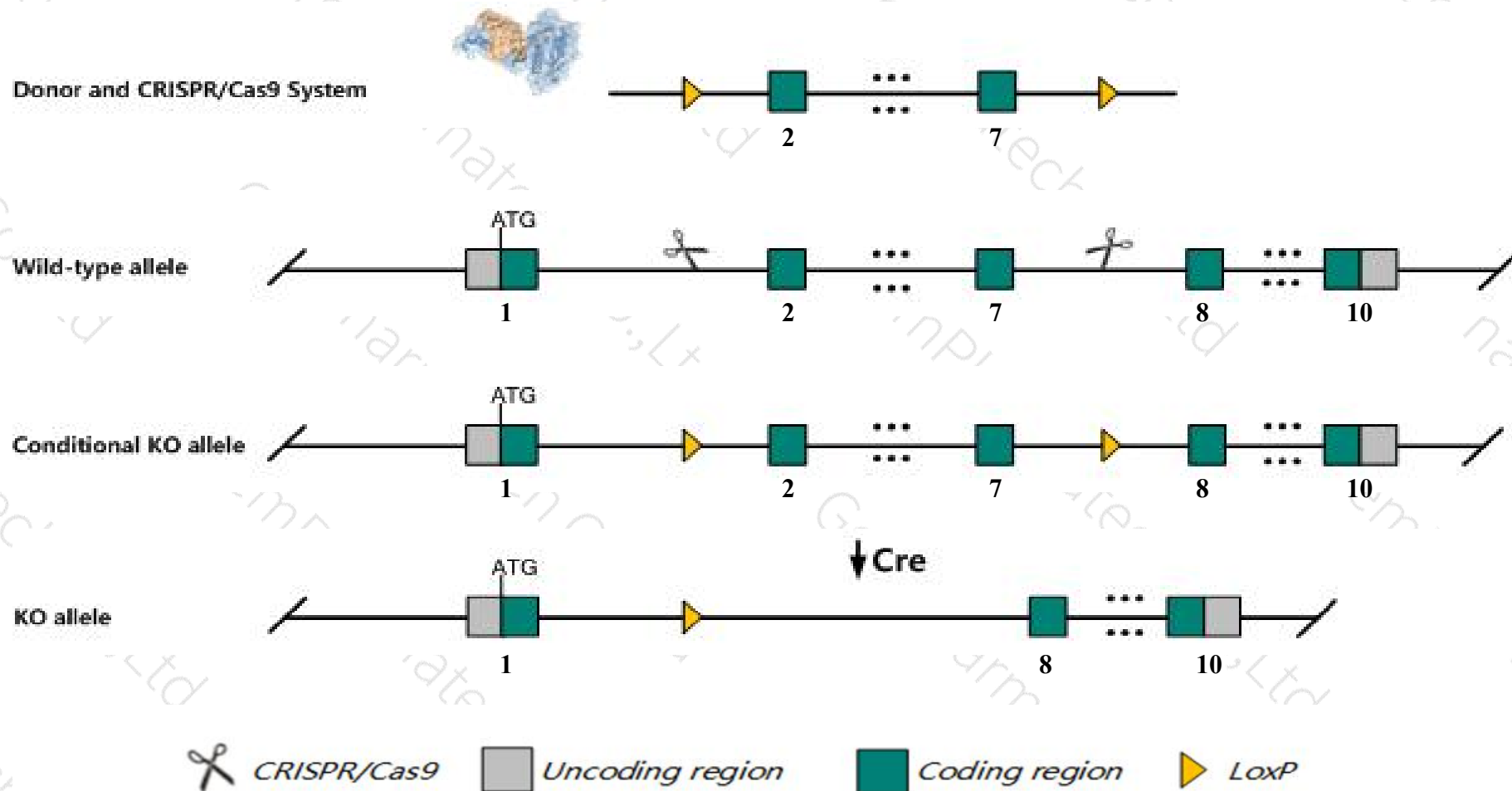
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Ambp* gene has 2 transcripts. According to the structure of *Ambp* gene, exon2-exon7 of *Ambp*-201 (ENSMUST00000030041.4) transcript is recommended as the knockout region. The region contains 568bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ambp* 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.

- According to the existing MGI data, Female homozygotes for targeted null mutations exhibit reduced ovulation rates, oocytes lacking a zona pellucida, and a very low fertilization rate.
- The *Ambp* gene is located on the Chr4. 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.

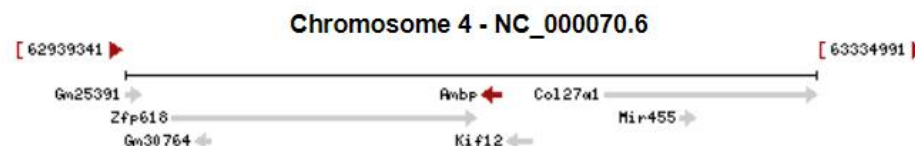
Gene information (NCBI)

Ambp alpha 1 microglobulin/bikunin precursor [*Mus musculus* (house mouse)]

Gene ID: 11699, updated on 5-Nov-2019

Summary

Official Symbol	Ambp provided by MGI
Official Full Name	alpha 1 microglobulin/bikunin precursor provided by MGI
Primary source	MGI:MGI:88002
See related	Ensembl:ENSMUSG00000028356
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	UTI; ASPI; Itil; HI-30; Intin4; A1194774
Summary	This gene encodes a fusion protein that undergoes proteolytic processing to generate two mature proteins: alpha-1-microglobulin (A1m) is a heme-binding plasma glycoprotein of the lipocalin superfamily of proteins that bind to hydrophobic molecules, whereas bikunin belongs to the superfamily of Kunitz-type protease inhibitors. The transgenic mice specifically lacking bikunin, but not A1m, exhibit female infertility and an increased sensitivity to lung metastasis. [provided by RefSeq, Oct 2015]
Expression	Biased expression in liver E18 (RPKM 1051.1), liver adult (RPKM 770.6) and 3 other tissues See more
Orthologs	human all

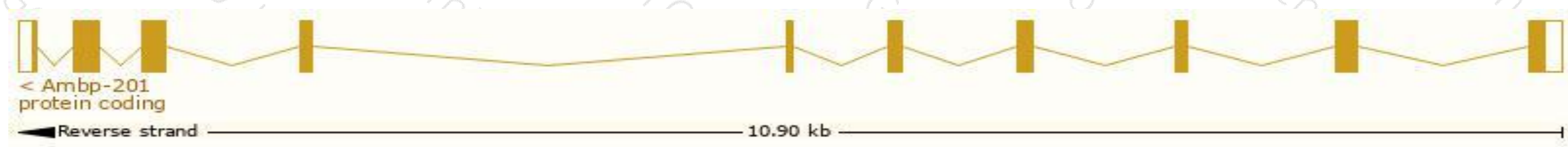


Transcript information (Ensembl)

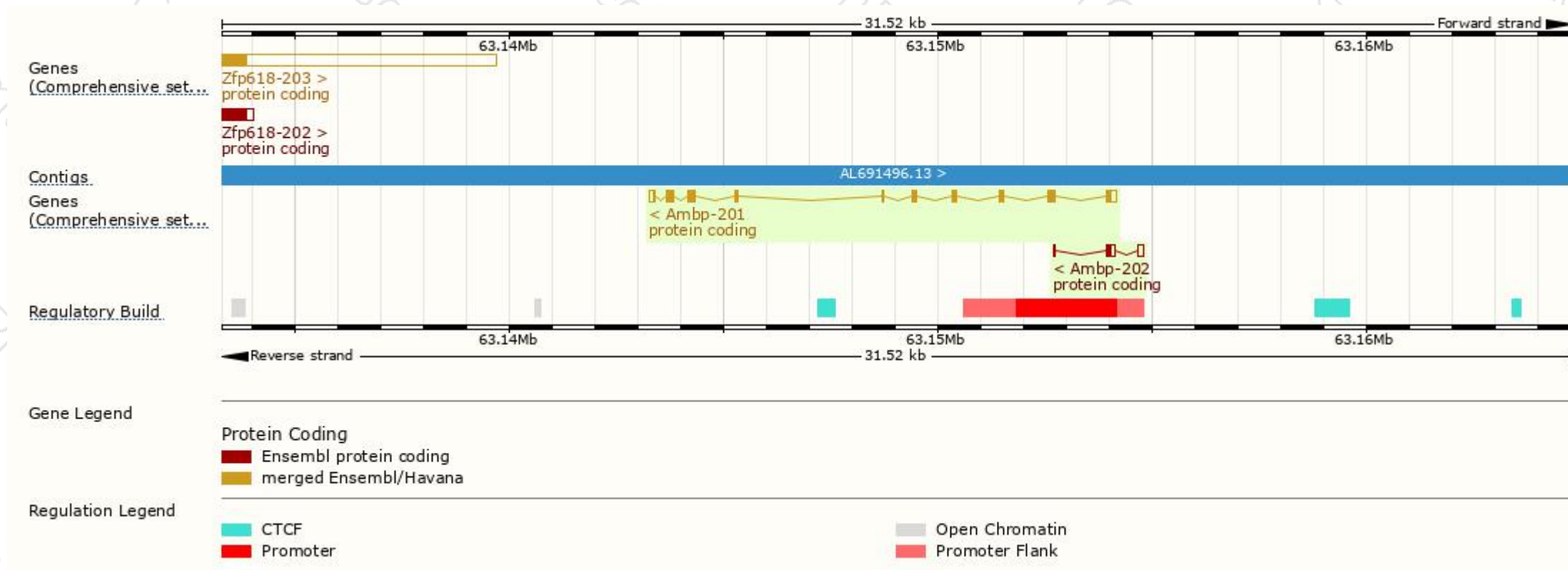
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Ambp-201	ENSMUST00000030041.4	1268	349aa	ENSMUSP00000030041.4	Protein coding	CCDS18248	Q07456	TSL:1 GENCODE basic APPRIS P1
Ambp-202	ENSMUST00000142901.1	375	47aa	ENSMUSP00000118965.1	Protein coding	-	I7HIQ9	CDS 3' incomplete TSL:3

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



Genomic location distribution

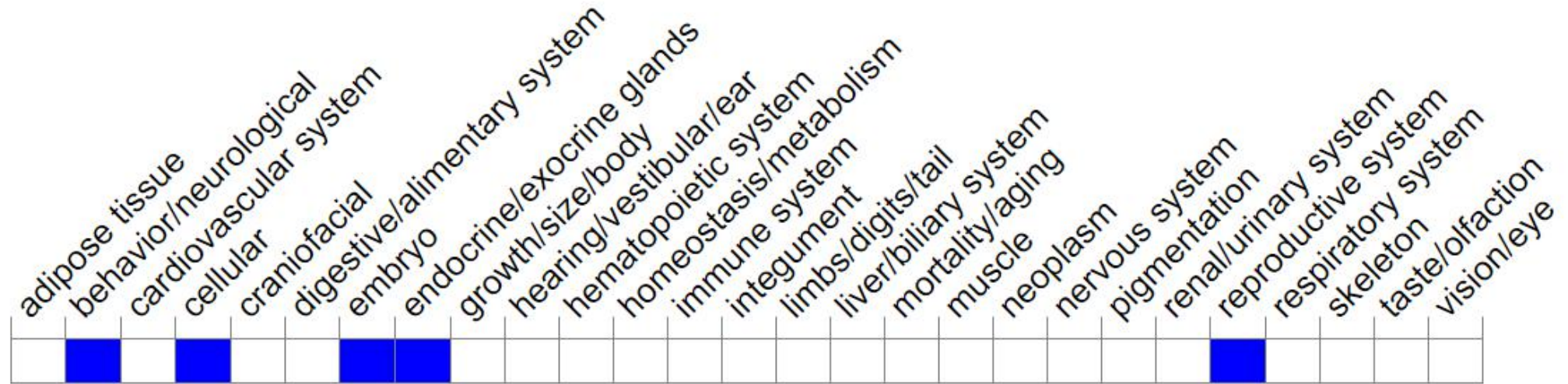


Protein domain



Mouse phenotype description(MGI)

Phenotype Overview ?



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).

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

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