Adam17 Cas9-CKO Strategy

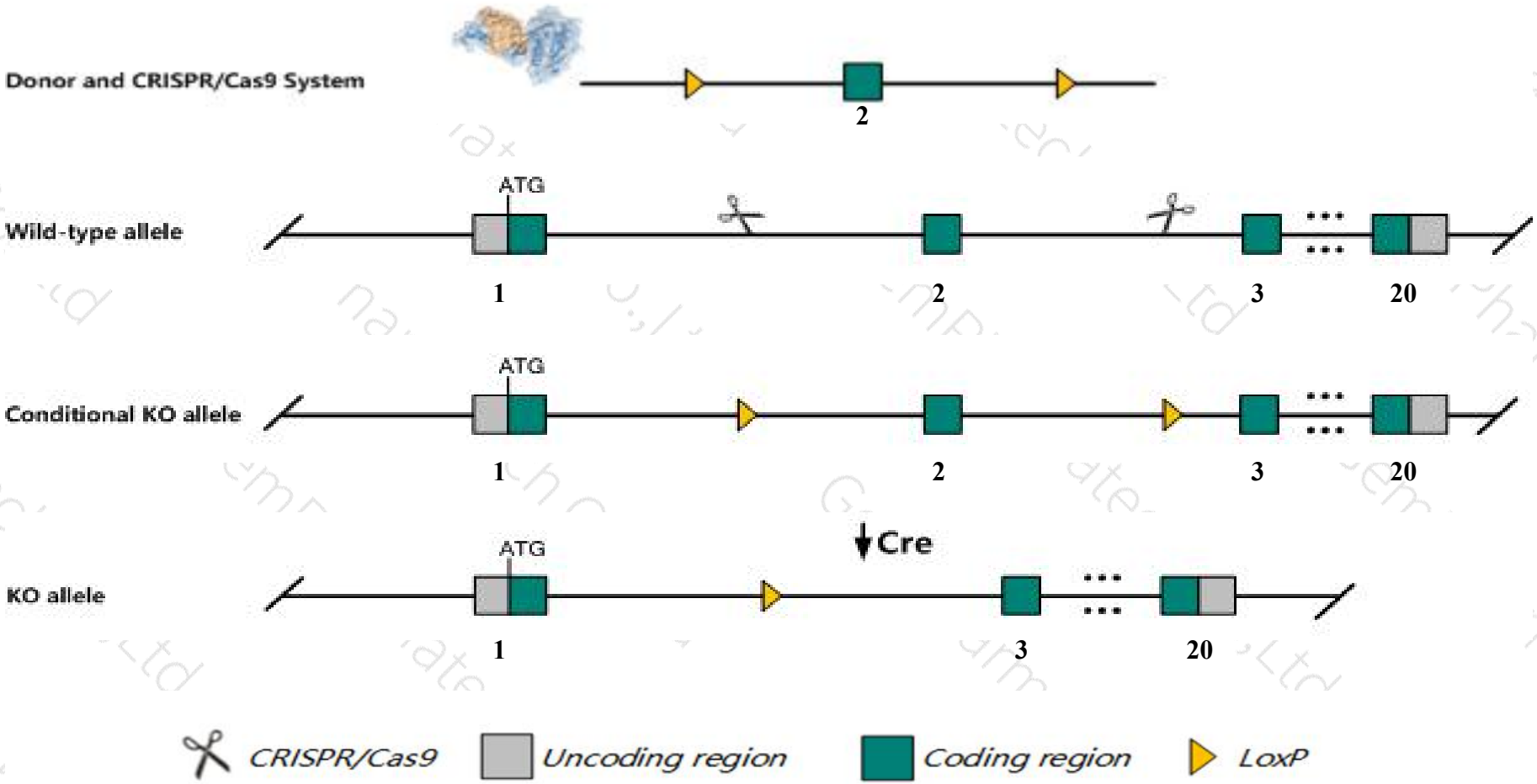
Designer: Huimin Su

Project Overview

Project Name	***Adam17***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Adam17* gene. The schematic diagram is as follows:



Technical routes

集萃药康 GemPharmatech

- The *Adam17* gene has 10 transcripts. According to the structure of *Adam17* gene, exon2 of *Adam17-202* (ENSMUST00000101551.9) transcript is recommended as the knockout region. The region contains 133bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Adam17* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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.

Notice

➢ According to the existing MGI data,Most mice homozygous for targeted mutations that inactivate the gene die perinatally with stunted vibrissae and open eyelids. Survivors display various degrees of eye degeneration, perturbed hair coats, curly vibrissae, and irregular pigmentation patterns. Histological analysis of fetuses reveal defects in epithelial cell maturation and organization in multiple organs.

➢ The *Adam17* gene is located on the Chr12. 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information （NCBI）

集萃药康 GemPharmatech

Adam17 a disintegrin and metallopeptidase domain 17 [Mus musculus (house mouse)]

Gene ID: 11491, updated on 16-Feb-2019

Summary

Official Symbol	Adam17 provided by MGI
Official Full Name	a disintegrin and metallopeptidase domain 17 provided by MGI
Primary source	MGI:MGI:1096335
See related	Ensembl:ENSMUSG00000052593
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	CD156b, Tace
Summary	This gene encodes a member of a disintegrin and metalloprotease (ADAM) family of endoproteases that play important roles in various biological processes including cell signaling, adhesion and migration. The encoded preproprotein undergoes proteolytic processing to generate a mature enzyme that is involved in the proteolytic release of membrane-bound proteins in a process called ectodomain shedding. Mice lacking the encoded protein die in utero or fail to survive beyond one week of age. Alternative splicing results in multiple transcript variants encoding different isoforms, some of which may undergo similar processing. [provided by RefSeq, May 2016]
Expression	Ubiquitous expression in placenta adult (RPKM 12.8), CNS E11.5 (RPKM 9.9) and 28 other tissues See more
Orthologs	human all

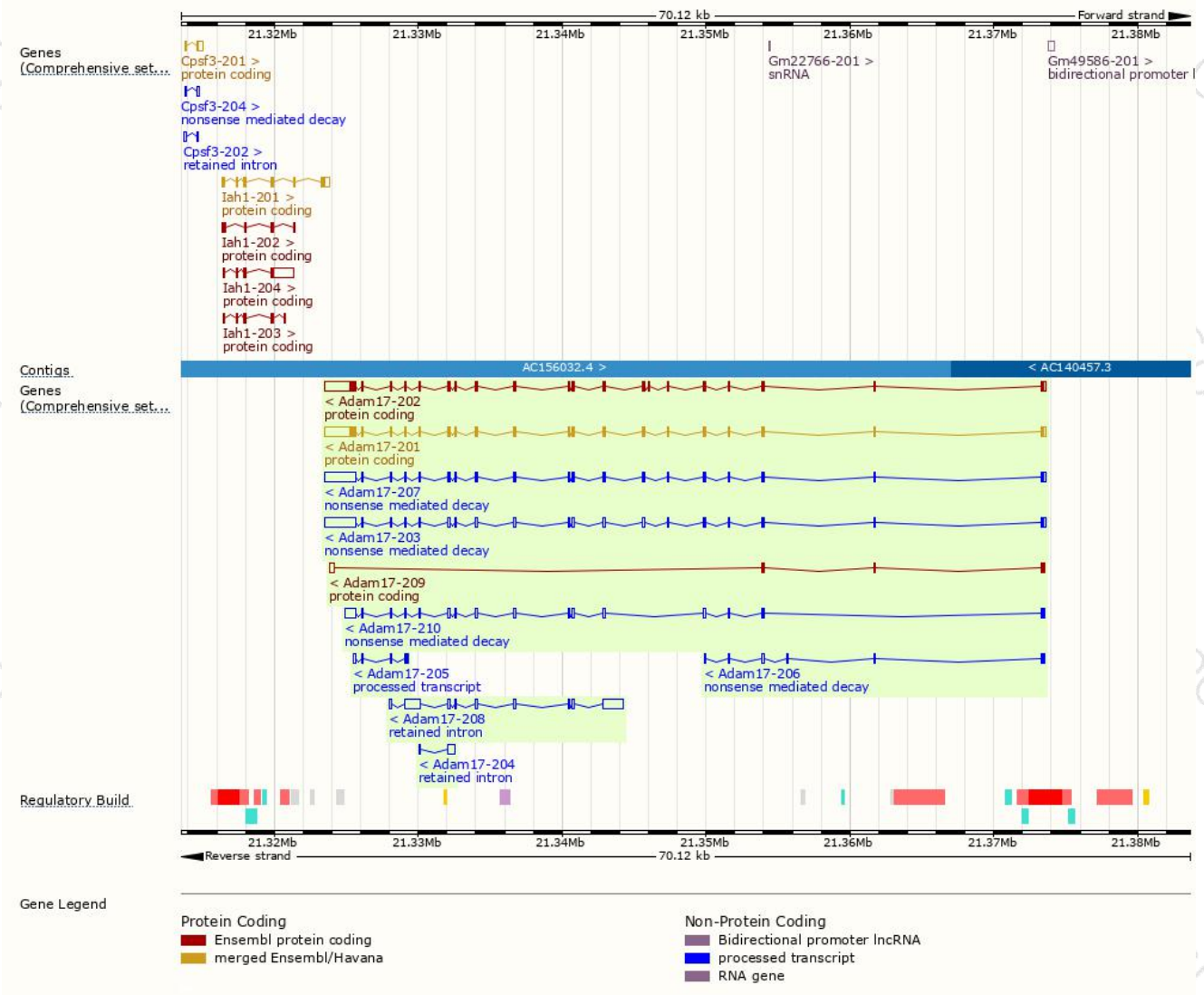
Transcript information（Ensembl）

The gene has 10 transcripts,all transcripts are shown below:

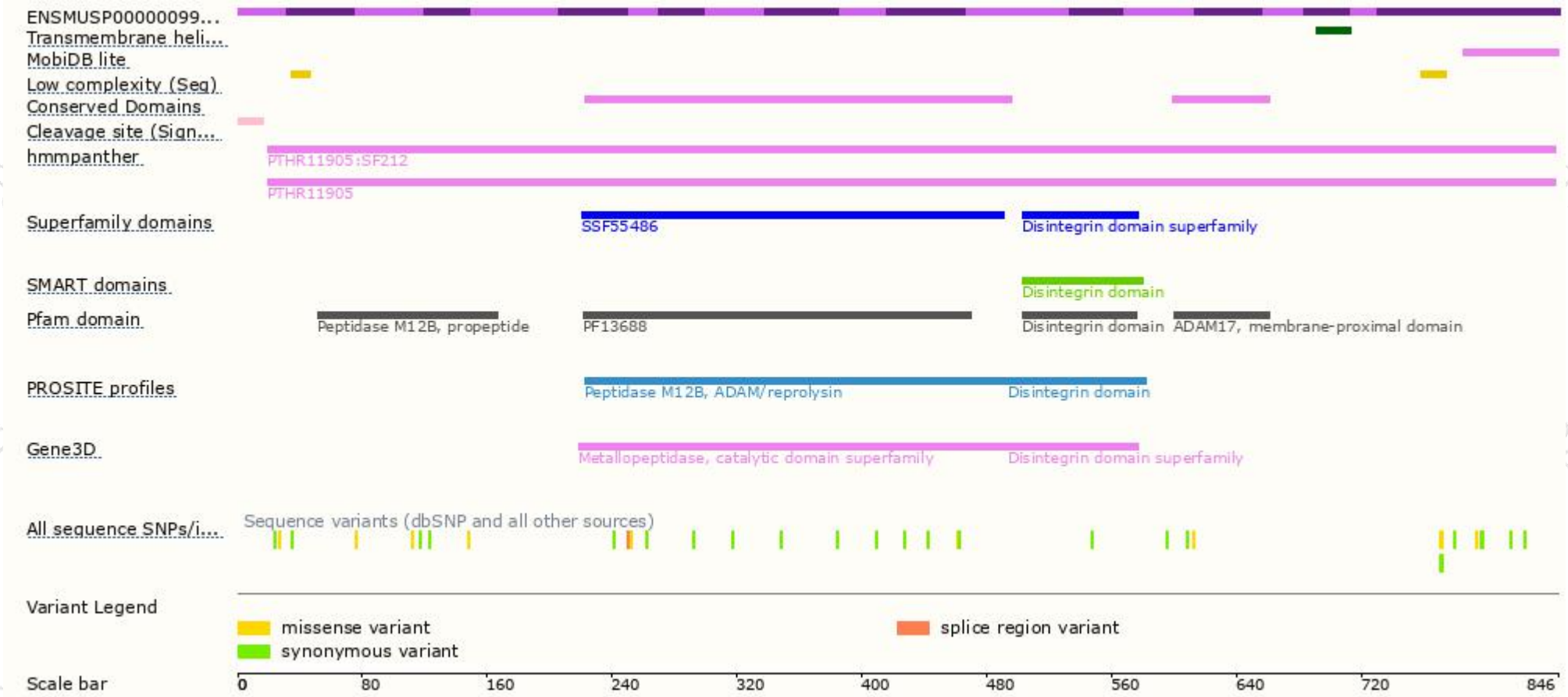
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Adam17-202	ENSMUST00000101551.9	4469	846aa	Protein coding	CCDS70376	E9PXU2	TSL:1 GENCODE basic
Adam17-201	ENSMUST00000064536.12	4443	827aa	Protein coding	CCDS25836	Q9Z0F8	TSL:1 GENCODE basic APPRIS P1
Adam17-209	ENSMUST00000232107.1	834	123aa	Protein coding	-	A0A338P6I8	GENCODE basic
Adam17-207	ENSMUST00000145118.7	4427	655aa	Nonsense mediated decay	-	Q9Z0F8	TSL:1
Adam17-203	ENSMUST00000127974.7	4423	222aa	Nonsense mediated decay	-	J3QNB3	TSL:1
Adam17-210	ENSMUST00000232526.1	2540	38aa	Nonsense mediated decay	-	A0A338P6F3	
Adam17-206	ENSMUST00000142092.1	702	94aa	Nonsense mediated decay	-	J3QMF2	TSL:3
Adam17-205	ENSMUST00000141799.1	375	No protein	Processed transcript	-	-	TSL:2
Adam17-208	ENSMUST00000155115.1	3456	No protein	Retained intron	-	-	TSL:1
Adam17-204	ENSMUST00000132339.1	537	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Adam17-202* transcript,The transcription is shown below

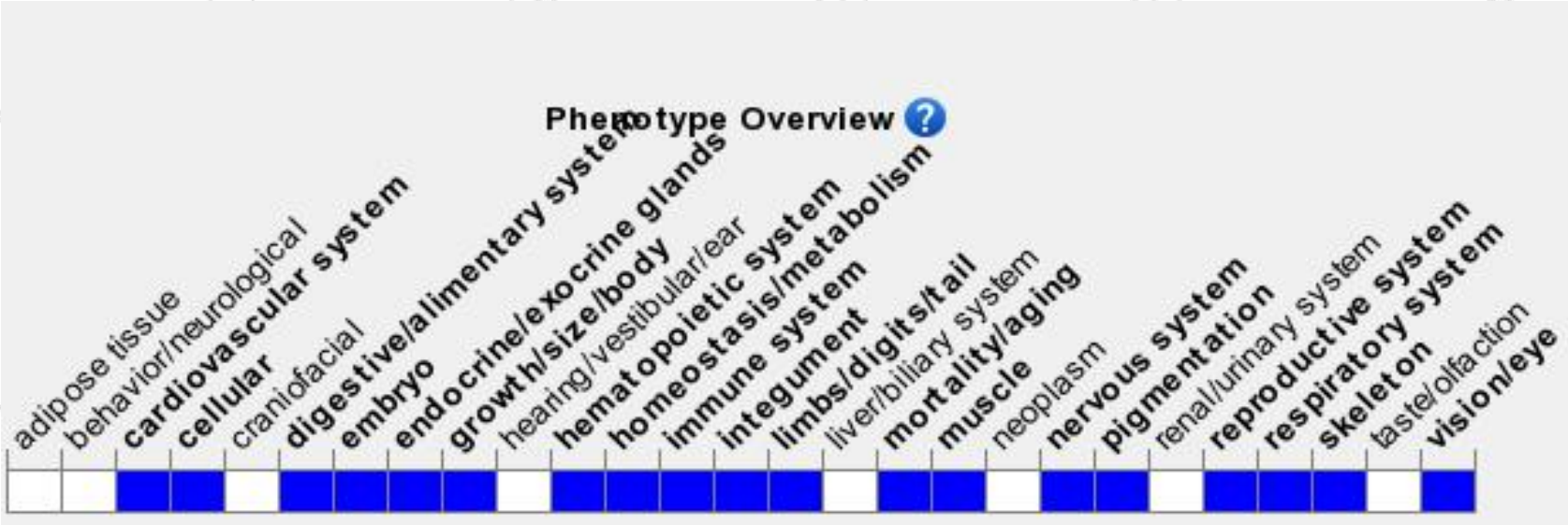
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



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

According to the existing MGI data,Most mice homozygous for targeted mutations that inactivate the gene die perinatally with stunted vibrissae and open eyelids. Survivors display various degrees of eye degeneration, perturbed hair coats, curly vibrissae, and irregular pigmentation patterns. Histological analysis of fetuses reveal defects in epithelial cell maturation and organization in multiple organs.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd