# Pea15a Cas9-CKO Strategy

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**Reviewer: Xueting Zhang** 

**Design Date: 2021-6-2** 

## **Project Overview**



Project Name Pea15a

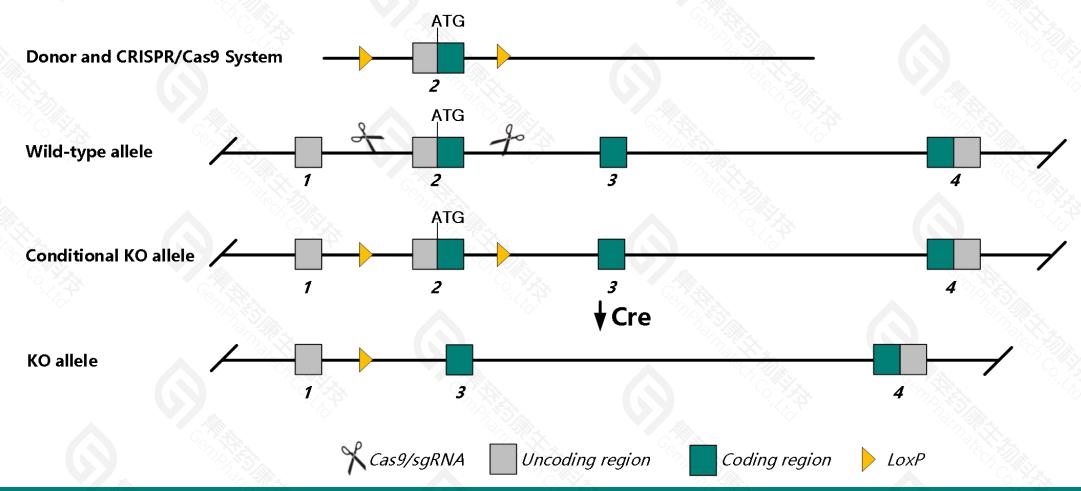
Project type Cas9-CKO

Strain background C57BL/6JGpt

### Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Pea15a* gene. The schematic diagram is as follows:



### **Technical routes**



- The *Peal5a* gene has 5 transcripts. According to the structure of *Peal5a* gene, exon2 of *Peal5a*-201 (
  ENSMUST00000013842.12) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Pea15a* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

### **Notice**



- According to the existing MGI data, homozygous null mice exhibit reduced body weight. Although Tnfa-induced apoptosis was increased in astrocytes in vitro, glial cell and brain morphology is normal.
- The *Peal5a* gene is located on the Chr1. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

## Gene information (NCBI)



☆ ?

#### Pea15a proliferation and apoptosis adaptor protein 15A [ Mus musculus (house mouse) ]

**≛** Download Datasets

Gene ID: 18611, updated on 18-May-2021

Summary

Official Symbol Pea15a provided by MGI

Official Full Name proliferation and apoptosis adaptor protein 15A provided by MGI

Primary source MGI:MGI:104799

See related Ensembl: ENSMUSG00000013698

Gene type protein coding
RefSeq status REVIEWED
Organism Mus musculus

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Murinae; Mus; Mus

Also known as Mat; Pea; Mat1; PEA-; Pkcs; Pea15; PEA-15; Pkcs15

Summary This gene encodes an adaptor protein that functions as a negative regulator of apoptosis induced by tumor necrosis factor-alpha, tumor necrosis factor-related apoptosis-inducing ligand,

and Fas, through its interaction with fas-associated protein with death domain and caspase-8. It also regulates proliferation signaling by relocating the extracellular signal-regulated protein kinases 1 and 2 to the cytosol. The protein encoded by this gene contains an N-terminal death effector domain and a long, flexible C-terminal tail. In humans, the encoded protein is an endogenous substrate for protein kinase C. This protein is overexpressed in type 2 diabetes mellitus, where it may contribute to insulin resistance in glucose uptake. Multiple pseudogenes

of this gene have been identified. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Aug 2016]

Expression Broad expression in cerebellum adult (RPKM 121.2), frontal lobe adult (RPKM 117.6) and 24 other tissues See more

Orthologs <u>human</u> all

Try the new Gene table

Try the new Transcript table

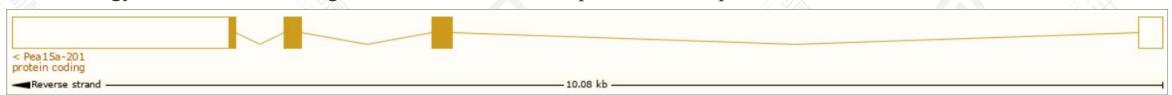
## Transcript information (Ensembl)



The gene has 5 transcripts, and all transcripts are shown below:

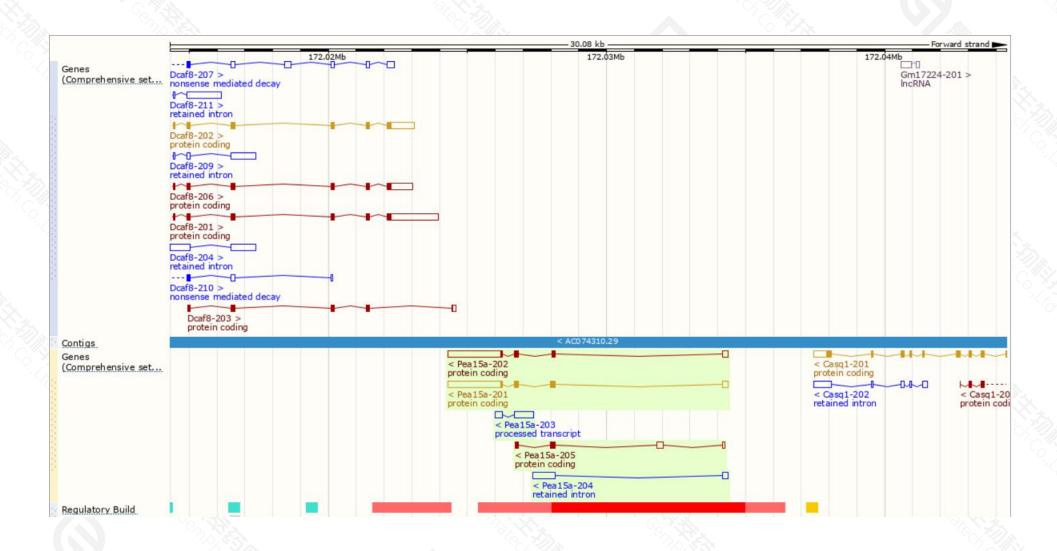
Name 🍦	Transcript ID	bp 🛊	Protein	Biotype	CCDS	UniProt Match	Flags
Pea15a-201	ENSMUST00000013842.12	2501	<u>130aa</u>	Protein coding	CCDS15510₽	<u>Q62048-1</u> ₽	GENCODE basic APPRIS P1 TSL:1
Pea15a-202	ENSMUST00000111247.8	2435	<u>108aa</u>	Protein coding	-	Q62048-2₽	GENCODE basic TSL:3
Pea15a-205	ENSMUST00000155109.2	600	<u>92aa</u>	Protein coding	020	D3Z375 ₽	TSL:3 CDS 3' incomplete
Pea15a-203	ENSMUST00000125361.2	980	No protein	Processed transcript	1000	-	TSL:2
Pea15a-204	ENSMUST00000152432.2	1015	No protein	Retained intron		2	TSL:2

The strategy is based on the design of *Pea15a*-201 transcript, The transcription is shown below:



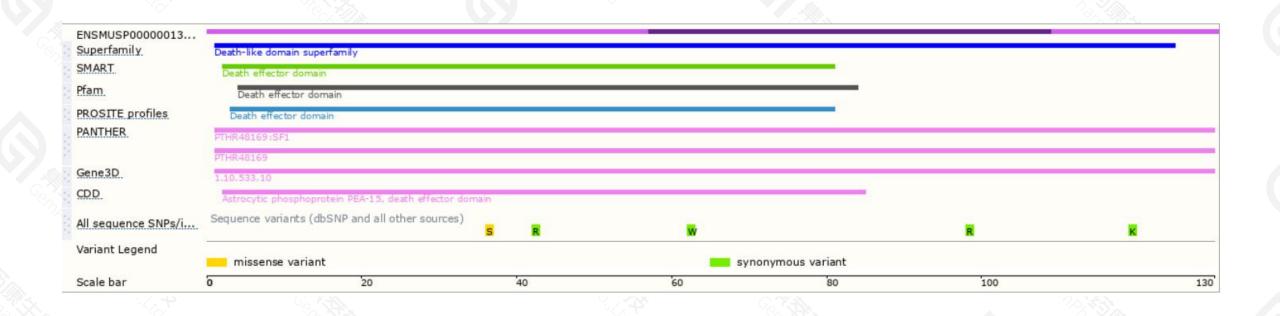
### Genomic location distribution





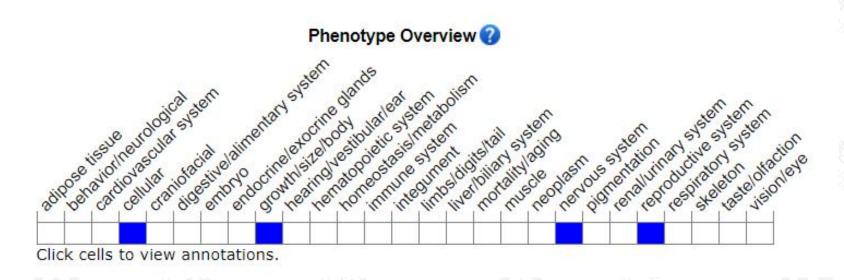
### Protein domain





## 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, homozygous null mice exhibit reduced body weight. Although Tnfa-induced apoptosis was increased in astrocytes in vitro, glial cell and brain morphology is normal.

If you have any questions, you are welcome to inquire. Tel: 025-5864 1534





