

***Zp3* Cas9-CKO Strategy**

Designer: Xueting Zhang

Project Overview

Project Name

Zp3

Project type

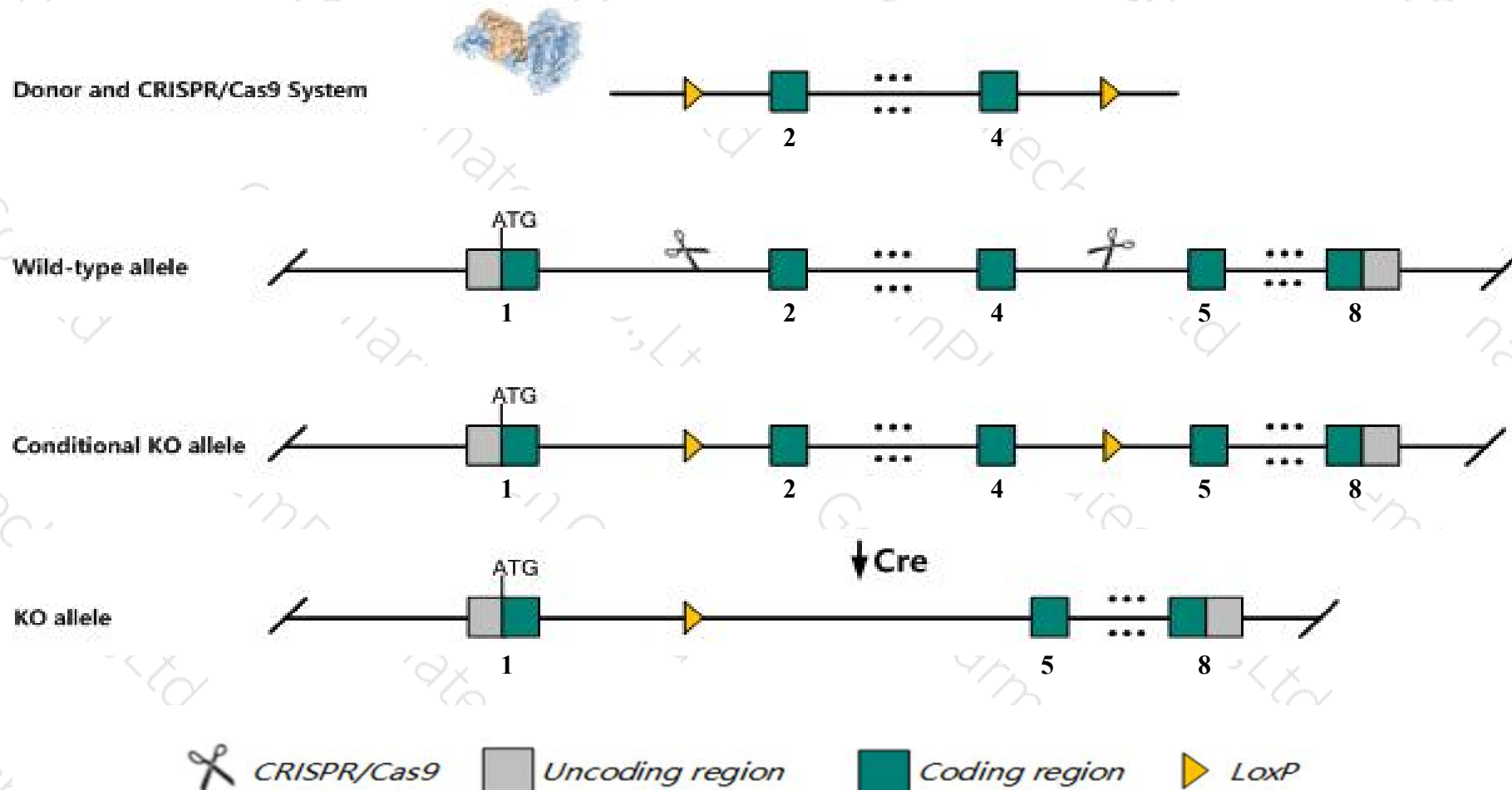
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Zp3* gene. The schematic diagram is as follows:



- The *Zp3* gene has 2 transcripts. According to the structure of *Zp3* gene, exon2-exon4 of *Zp3-201* (ENSMUST00000005073.12) transcript is recommended as the knockout region. The region contains 407bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Zp3* 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, Homozygous female mutants are infertile. In these females oocytes lack a zona pellucida and cumulus-oocyte complexes are disrupted. Oocytes of heterozygous females have a thin zona, but females are fertile.
- The N-terminal of *Zp3* gene will remain 83aa, it may remain the partial function of *Zp3* gene.
- The insertion of 5' Loxp may influence the regulatory function of the N-terminal of *Ssc4d* gene.
- The *Zp3* gene is located on the Chr5. 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)

Zp3 zona pellucida glycoprotein 3 [Mus musculus (house mouse)]

Gene ID: 22788, updated on 31-Jan-2019

Summary



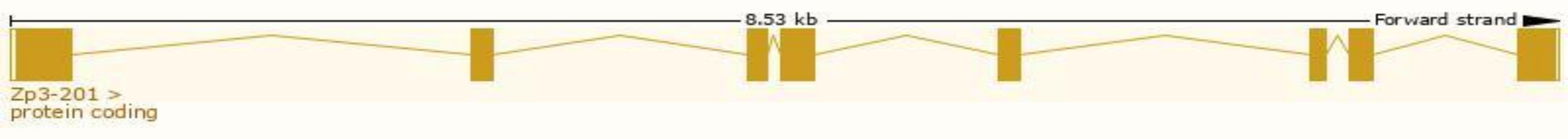
Official Symbol	Zp3 provided by MGI
Official Full Name	zona pellucida glycoprotein 3 provided by MGI
Primary source	MGI:MGI:99215
See related	Ensembl:ENSMUSG000000004948
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Zp-3
Expression	Restricted expression toward ovary adult (RPKM 68.2) See more
Orthologs	human all

Transcript information (Ensembl)

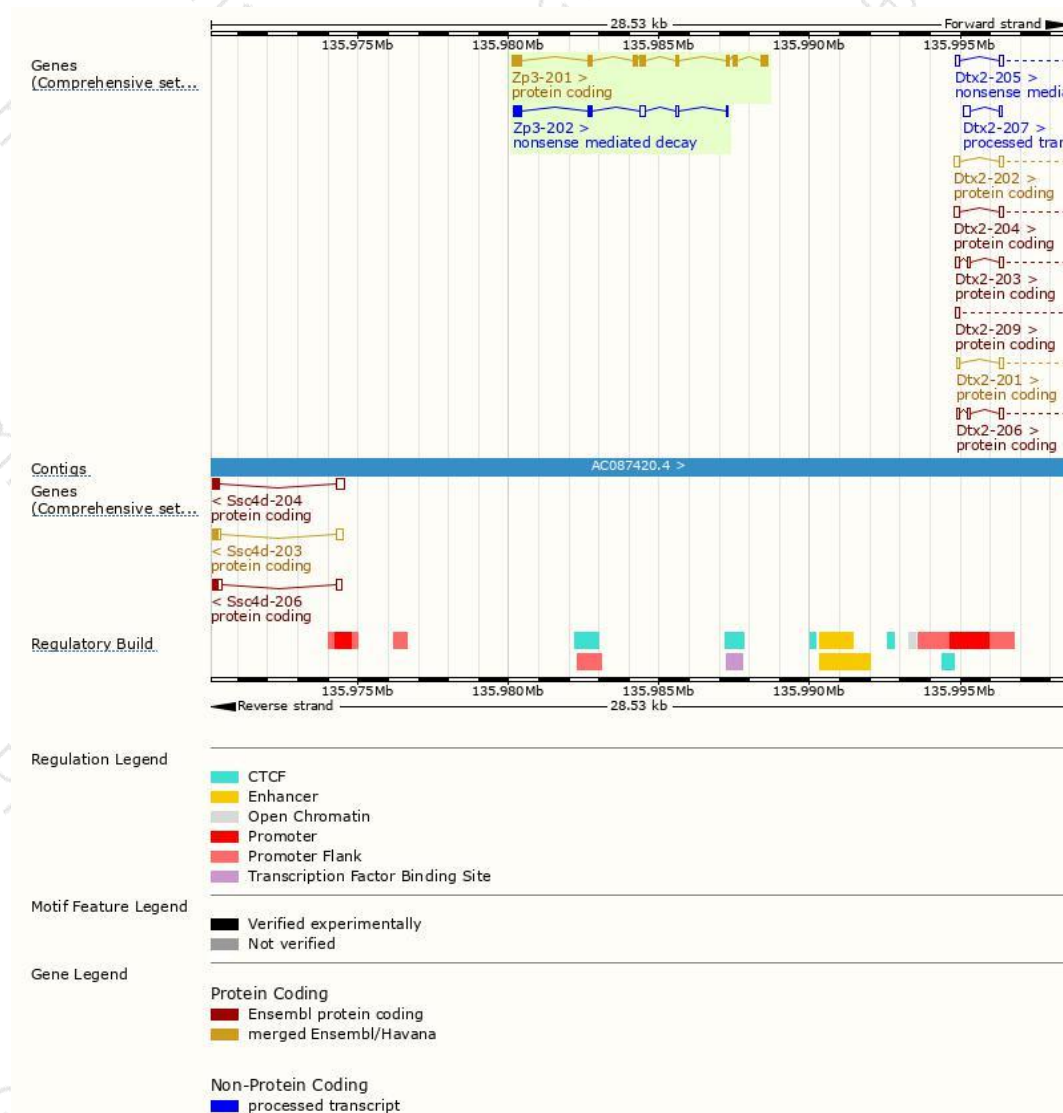
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zp3-201	ENSMUST00000005073.12	1323	424aa	Protein coding	CCDS19749	P10761	TSL:1 GENCODE basic APPRIS P1
Zp3-202	ENSMUST00000131563.1	734	136aa	Nonsense mediated decay	-	F6VD35	CDS 5' incomplete TSL:5

The strategy is based on the design of *Zp3-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain

ENSMUSP000000005...

Transmembrane heli...

Low complexity (Seq)

Cleavage site (Sign...

hmmpanther

PTHR11576

PTHR11576:SF2

SMART domains

Zona pellucida domain

Prints domain

Zona pellucida domain

Pfam domain

Zona pellucida domain

PROSITE profiles

Zona pellucida domain

PROSITE patterns

Zona pellucida domain, conserved site

Gene3D

2.60.40.3210

2.60.40.4100

All sequence SNPs/i...

Sequence variants (dbSNP and all other sources)

Variant Legend

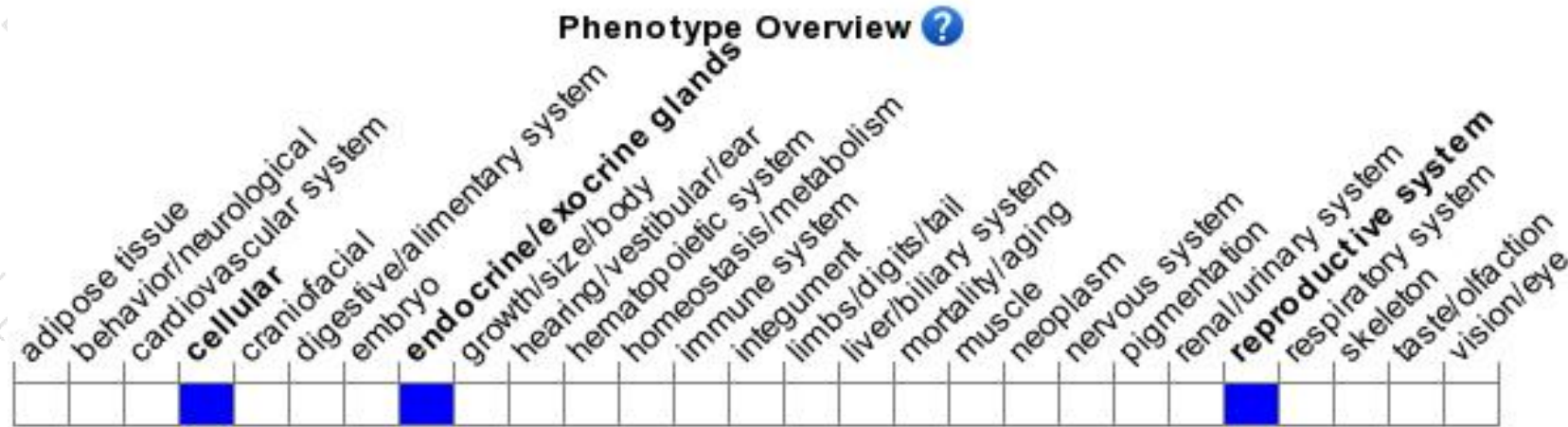
missense variant

synonymous variant

Scale bar

0 40 80 120 160 200 240 280 320 360 424

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 female mutants are infertile. In these females oocytes lack a zona pellucida and cumulus-oocyte complexes are disrupted. Oocytes of heterozygous females have a thin zona, but females are fertile.

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

Tel: 400-9660890

