

Zar1 Cas9-CKO Strategy

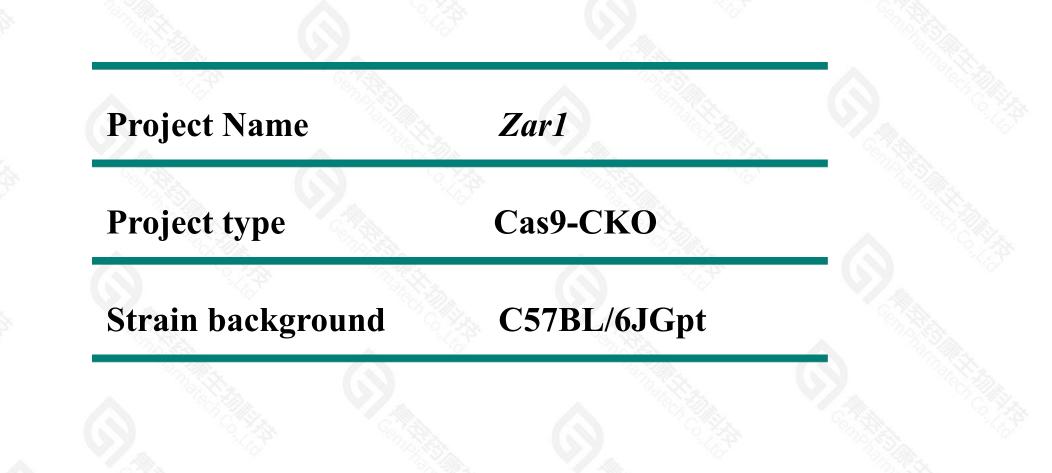
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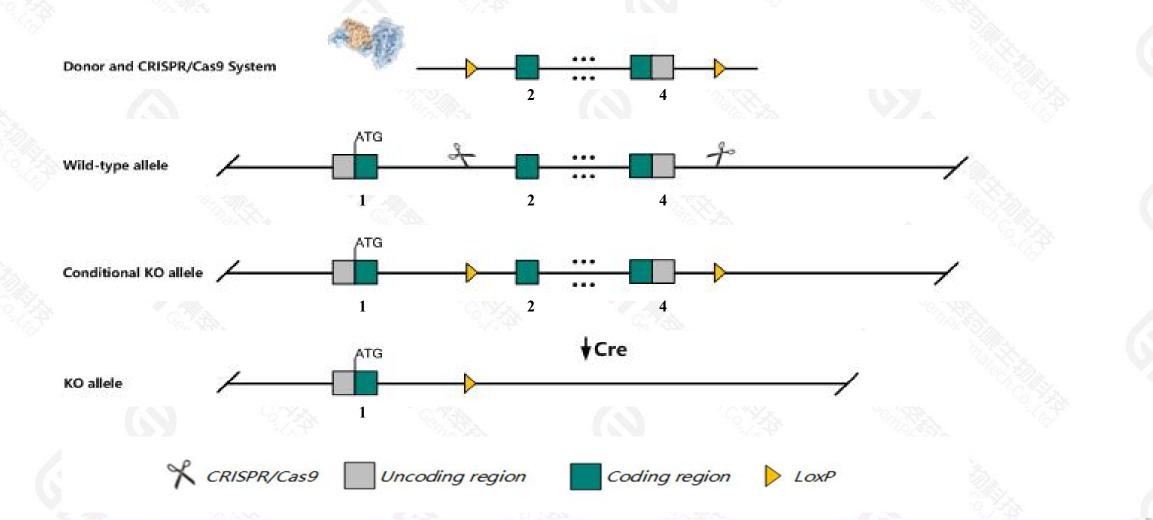
Project Overview





Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the Zar1 gene. The schematic diagram is as follows:



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Technical routes



The Zar1 gene has 3 transcripts. According to the structure of Zar1 gene, exon2-exon4 of Zar1-201(ENSMUST00000073528.4) transcript is recommended as the knockout region. The region contains 312bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify Zar1 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, ovarian development and oogenesis are normal in homozygous null females, however they are infertile due to a failure at the oocyte to embryo transition.
- > The Zar1 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)



Zar1 zygote arrest 1 [Mus musculus (house mouse)]

Gene ID: 317755, updated on 17-Dec-2020

Summary

Official Symbol	Zar1 provided by MGI
Official Full Name	zygote arrest 1 provided by <u>MGI</u>
Primary source	MGI:MGI:2180337
See related	Ensembl:ENSMUSG0000063935
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
Expression	Restricted expression toward ovary adult (RPKM 33.1)See more
Orthologs	human all

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Transcript information (Ensembl)

The gene has 3 transcripts, all transcripts are shown below:

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Zar1-201	ENSMUST0000073528.4	1429	<u>361aa</u>	Protein coding	CCDS19331		TSL:1, GENCODE basic, APPRIS P1,
Zar1-203	ENSMUST00000202174.2	758	<u>204aa</u>	Protein coding	-		CDS 5' incomplete , TSL:3 ,
Zar1-202	ENSMUST00000200883.2	2494	No protein	Retained intron	-		TSL:NA ,

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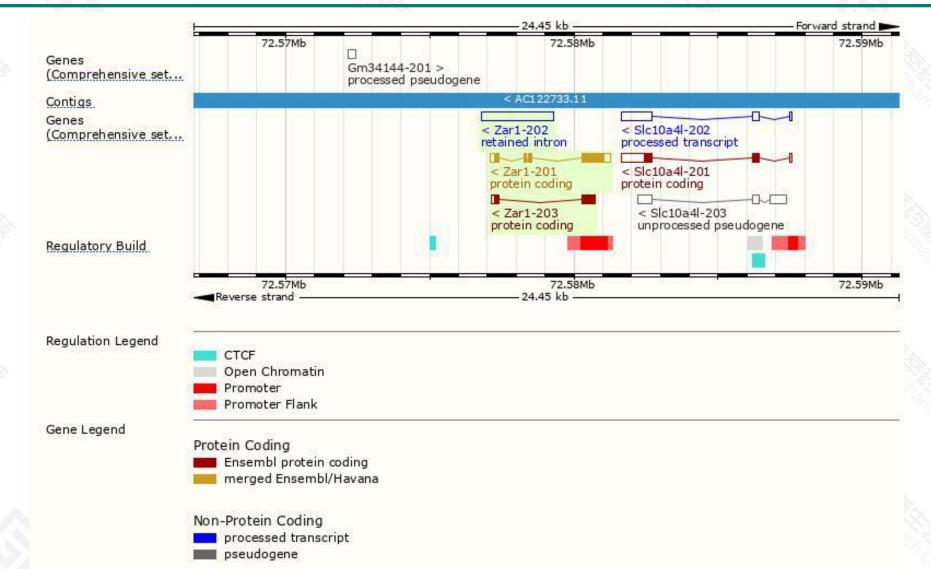
The strategy is based on the design of Zar1-201 transcript, the transcription is shown below:

Zar1-201 stein coding Reverse strand				

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Genomic location distribution





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Protein domain



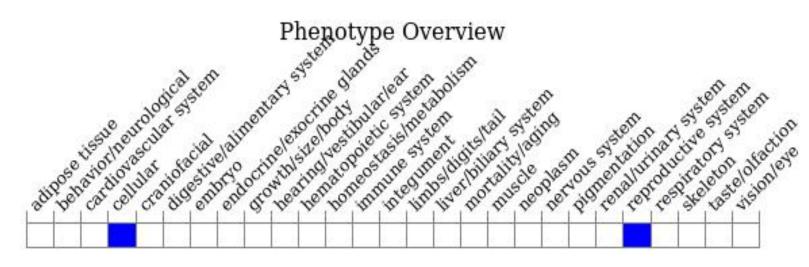


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Mouse phenotype description(MGI)



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, ovarian development and oogenesis are normal in homozygous null females, however they are infertile due to a failure at the oocyte to embryo transition.



If you have any questions, you are welcome to inquire. Tel: 400-9660890



