

Fzr1 Cas9-KO Strategy

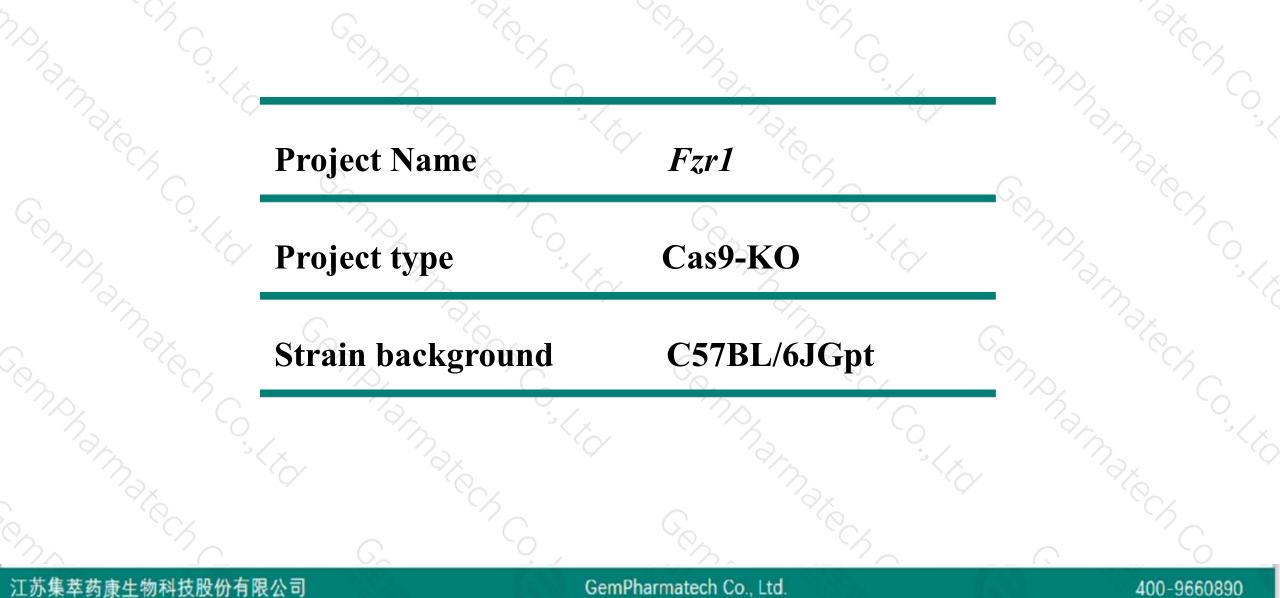
Designer:Shilei Zhu

Reviewer:Lingyan Wu

Design Date: 2020-12-8

Project Overview

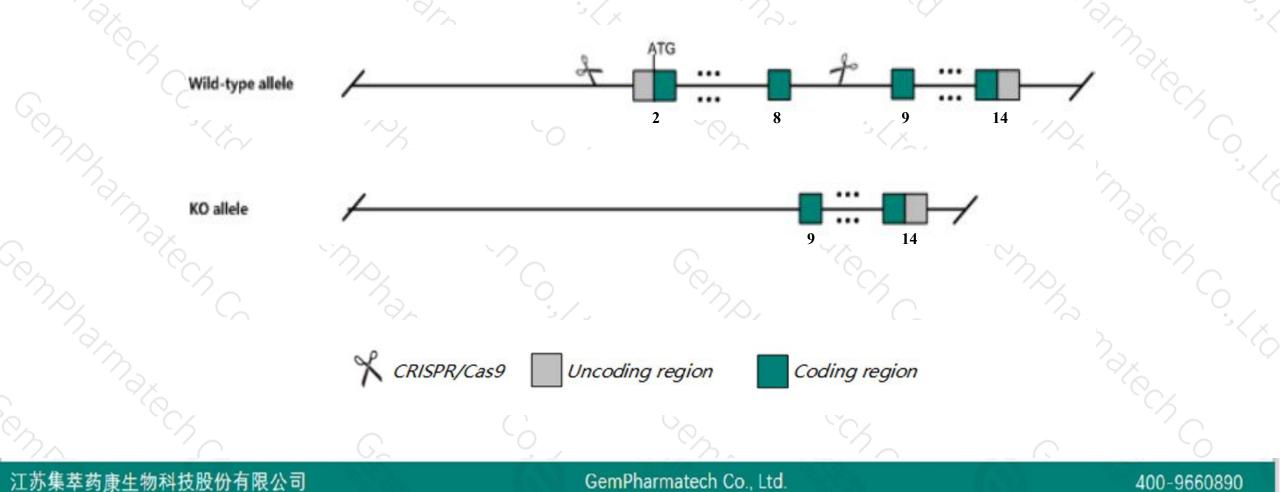




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Fzr1 gene. The schematic diagram is as follows:





> The *Fzr1* gene has 6 transcripts. According to the structure of *Fzr1* gene, exon2-exon8 of *Fzr1*-205(ENSMUST00000140901.7) 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 Fzr1 gene. The brief process is as follows: CRISPR/Cas9 system 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.



- According to the existing MGI data, homozygous null mutants die embryonically at around E9.5-E12.5 with poorly developed placentae, no placental giant cells and/or erythroblast deficiency. Homozygous MEFs undergo premature senescence. Heterozygotes exhibit learning/memory defects and/or elevatedspontaneous epithelial tumor incidence.
- ➤ The Intron8 is only 530bp,loxp insertion may affect mRNA splicing.
- > The *Fzr1* gene is located on the Chr10. 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.
- ≻The deletion region in this strategy is less than 5K from the 5 end of gene 4930404N11Rik.
- ➤ The intron8-9 is only 530bp,loxp insertion may affect mRNA splicing.
- The 3 'loxp will be placed on exon 8 of transcript *Fzr1*-204.
- > The 3 'loxp may be placed on exon 1 of transcript *Fzr1*-206.

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Gene information (NCBI)



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Fzr1 fizzy and cell division cycle 20 related 1 [Mus musculus (house mouse)]

Gene ID: 56371, updated on 13-Mar-2020

Summary

Official Symbol	Fzrl provided by MGI
Official Full Name	fizzy and cell division cycle 20 related 1 provided by MGI
Primary source	MGI:MGI:1926790
See related	Ensembl:ENSMUSG0000020235
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW108046, Cdh1, FZR, FZR2, Fyr, HCDH, HCDH1
Expression	Ubiquitous expression in ovary adult (RPKM 80.2), testis adult (RPKM 77.0) and 28 other tissuesSee more
Orthologs	human all

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Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fzr1-205	ENSMUST00000140901.7	3057	<u>493aa</u>	Protein coding	CCDS24057	Q3U3D4 Q9R1K5	TSL:1 GENCODE basic APPRIS P1
Fzr1-202	ENSMUST00000118812.1	1215	<u>404aa</u>	Protein coding	-	D3YTV2	TSL:5 GENCODE basic
Fzr1-201	ENSMUST0000020457.13	1956	<u>361aa</u>	Nonsense mediated decay	828	<u>F8WJ80</u>	TSL:1
Fzr1-203	ENSMUST00000127203.1	369	No protein	Processed transcript	1.71	0.70	TSL:3
Fzr1-204	ENSMUST00000138343.1	2780	No protein	Retained intron	79 4 55	1	TSL:1
Fzr1-206	ENSMUST00000150824.1	870	No protein	Retained intron	572) 1973	450	TSL:2

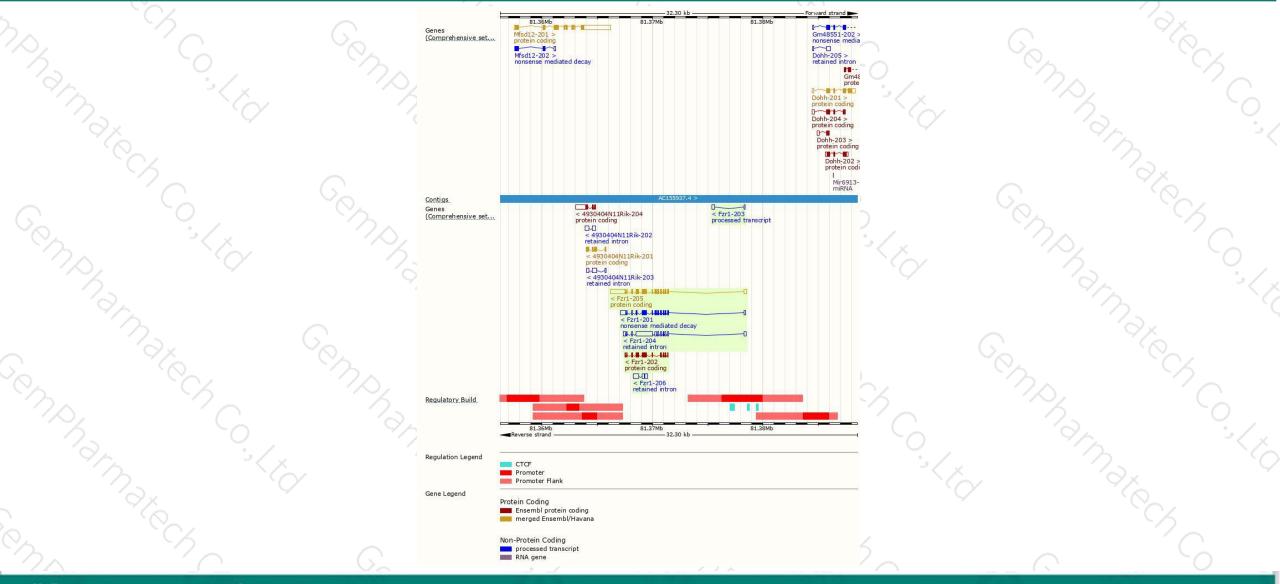
The strategy is based on the design of *Fzr1-205* transcript, the transcription is shown below:



- 12.29 kb -

Genomic location distribution





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Protein domain



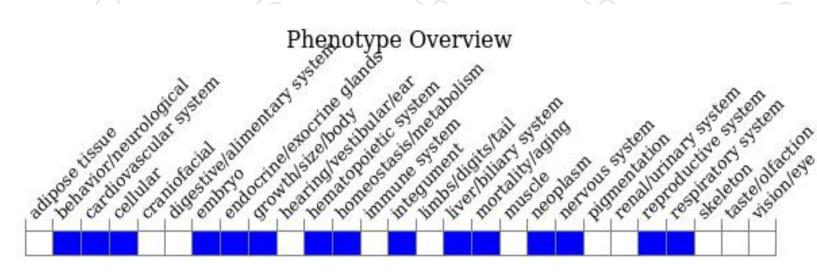


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Mouse phenotype description(MGI)





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, homozygous null mutants die embryonically at around E9.5-E12.5 with poorly developed placentae, no placental giant cells and/or erythroblast deficiency. Homozygous MEFs undergo premature senescence. Heterozygotes exhibit learning/memory defects and/or elevatedspontaneous epithelial tumor incidence.

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



