

Fzr1 Cas9-CKO Strategy

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Reviewer:Lingyan Wu

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Project Overview



Project Name Fzr1

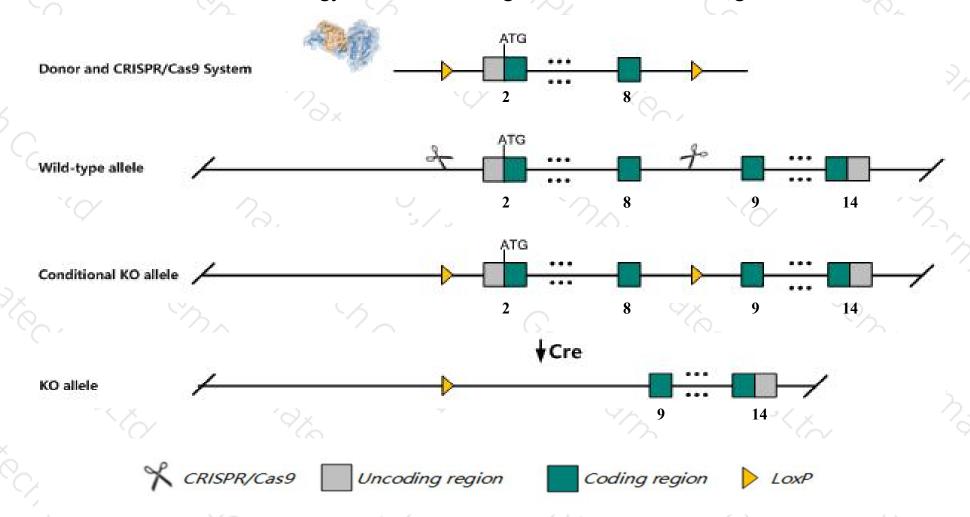
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



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



Technical routes



- ➤ 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 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, 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.
- \gt 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological 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.

Gene information (NCBI)



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

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

Summary

☆ ?

Official Symbol Fzr1 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:ENSMUSG00000020235

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 <u>human all</u>

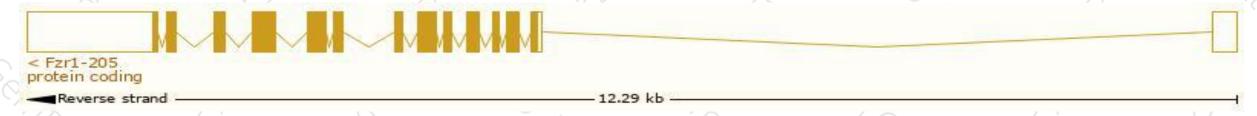
Transcript information (Ensembl)



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

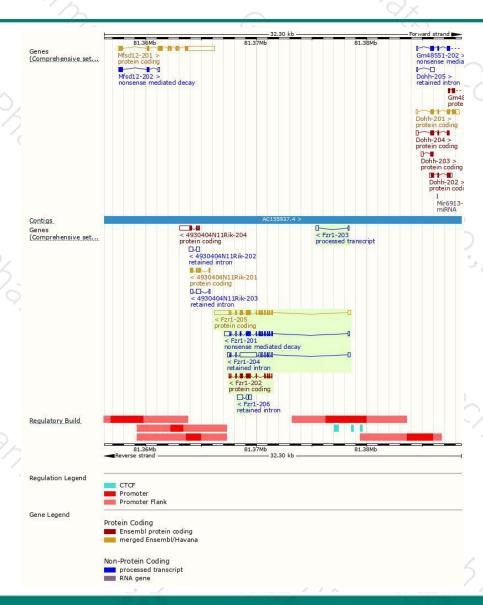
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fzr1-205	ENSMUST00000140901.7	3057	493aa	Protein coding	CCDS24057	Q3U3D4 Q9R1K5	TSL:1 GENCODE basic APPRIS P1
Fzr1-202	ENSMUST00000118812.1	1215	404aa	Protein coding	9-3	D3YTV2	TSL:5 GENCODE basic
Fzr1-201	ENSMUST00000020457.13	1956	361aa	Nonsense mediated decay	828	F8WJ80	TSL:1
Fzr1-203	ENSMUST00000127203.1	369	No protein	Processed transcript	1-11		TSL:3
Fzr1-204	ENSMUST00000138343.1	2780	No protein	Retained intron	1-11	(44)	TSL:1
Fzr1-206	ENSMUST00000150824.1	870	No protein	Retained intron	: :23	0E3	TSL:2

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



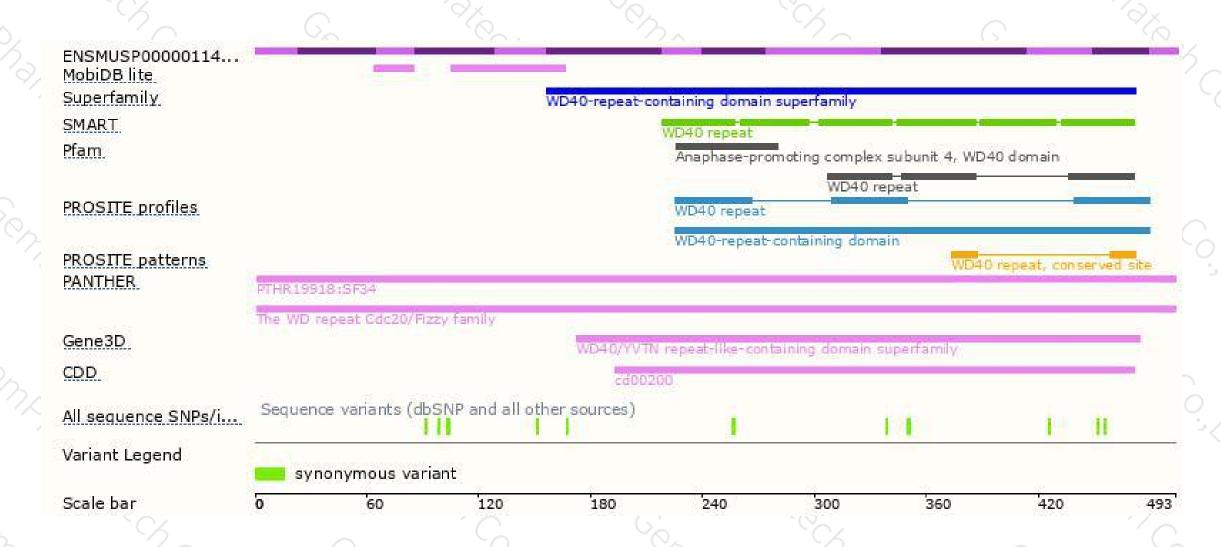
Genomic location distribution





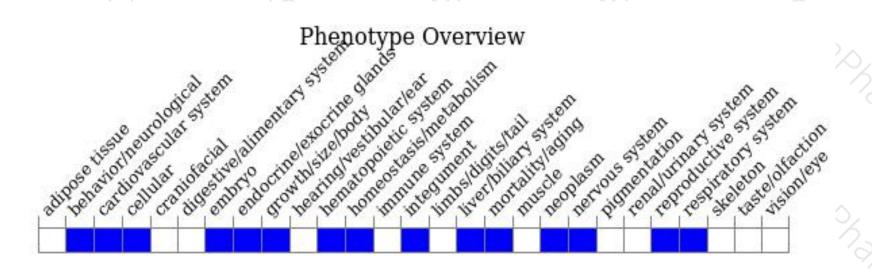
Protein domain





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 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.



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





