

Rxfp2 Cas9-CKO Strategy

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Reviewer:

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Design Date:

2019-12-26

Project Overview



Project Name

Rxfp2

Project type

Cas9-CKO

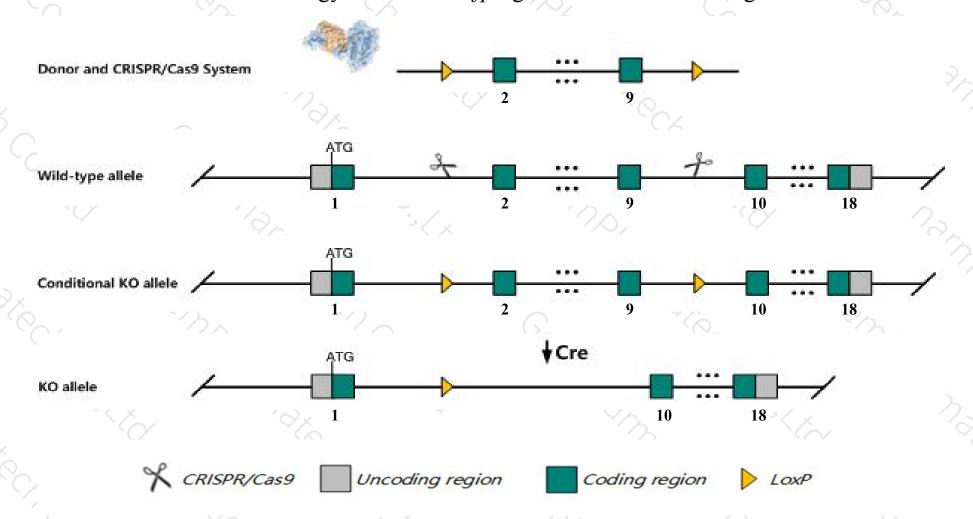
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Rxfp2* gene. The schematic diagram is as follows:



Technical routes



- The *Rxfp2* gene has 6 transcripts. According to the structure of *Rxfp2* gene, exon2-exon9 of *Rxfp2-201* (ENSMUST00000065745.9) transcript is recommended as the knockout region. The region contains 691bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Rxfp2* 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, Male homozygotes for a targeted null mutation exhibit bilateral intraabdominal cryptorchidism and sterility associated with a failure in the differentiation of the gubernaculae, ligaments that control testicular movement during development.
- > The *Rxfp2* 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)



Rxfp2 relaxin/insulin-like family peptide receptor 2 [Mus musculus (house mouse)]

Gene ID: 140498, updated on 31-Jan-2019

Summary

☆ ?

Official Symbol Rxfp2 provided by MGI

Official Full Name relaxin/insulin-like family peptide receptor 2 provided by MGI

Primary source MGI:MGI:2153463

See related Ensembl:ENSMUSG00000053368

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 Gpr106, Great, Lgr8

Expression Biased expression in testis adult (RPKM 1.6), subcutaneous fat pad adult (RPKM 1.2) and 9 other tissuesSee more

Orthologs <u>human</u> all

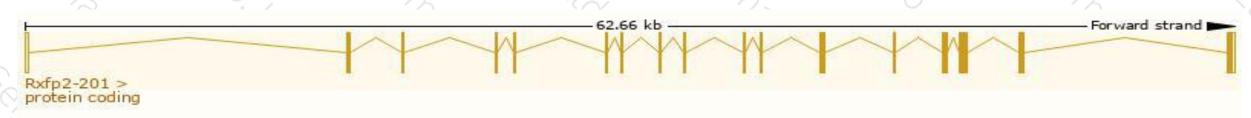
Transcript information (Ensembl)



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

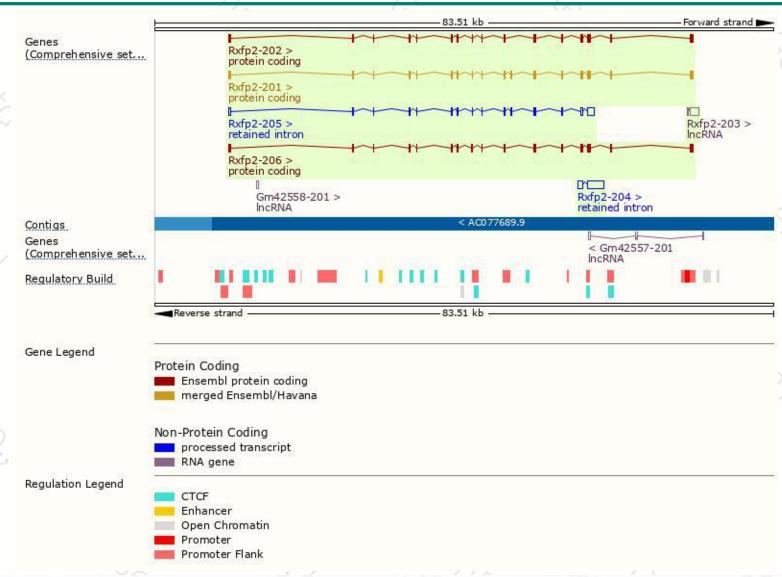
Name	Transcript ID	bp	Protein	Biotype	ccps	UniProt	Flags
Rxfp2-201	ENSMUST00000065745.9	2539	<u>737aa</u>	Protein coding	CCDS19886	Q91ZZ5	TSL:1 GENCODE basic APPRIS P1
Rxfp2-206	ENSMUST00000201612.1	2470	<u>727aa</u>	Protein coding	CCDS80479	A0A0J9YV81	TSL:1 GENCODE basic
Rxfp2-202	ENSMUST00000110496.4	2467	713aa	Protein coding	CCDS80480	E9Q0U9	TSL:1 GENCODE basic
Rxfp2-204	ENSMUST00000138901.1	2836	No protein	Retained intron	84	29	TSL:2
Rxfp2-205	ENSMUST00000143989.1	2471	No protein	Retained intron	85		TSL:2
Rxfp2-203	ENSMUST00000131212.1	1306	No protein	IncRNA	19-	-8	TSL:1

The strategy is based on the design of *Rxfp2-201* transcript, The transcription is shown below



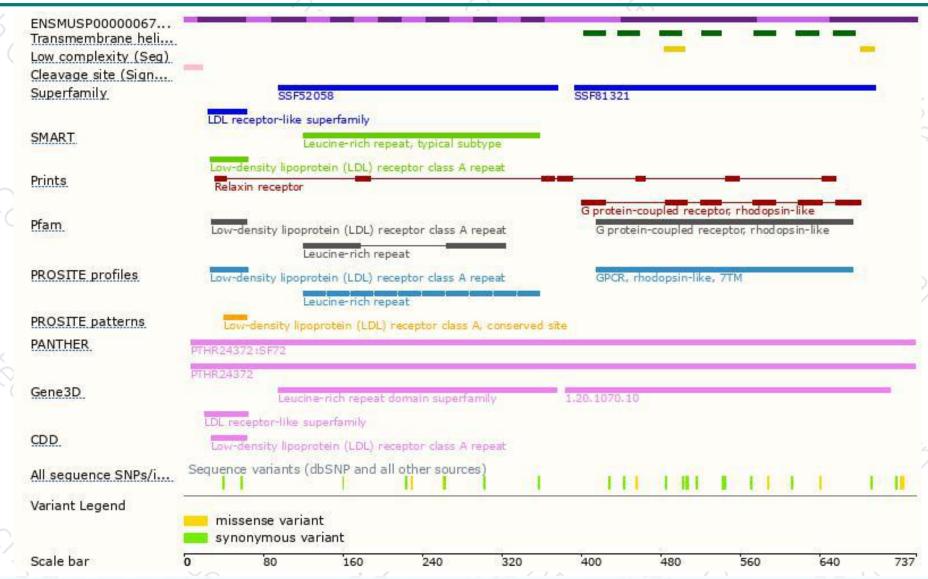
Genomic location distribution





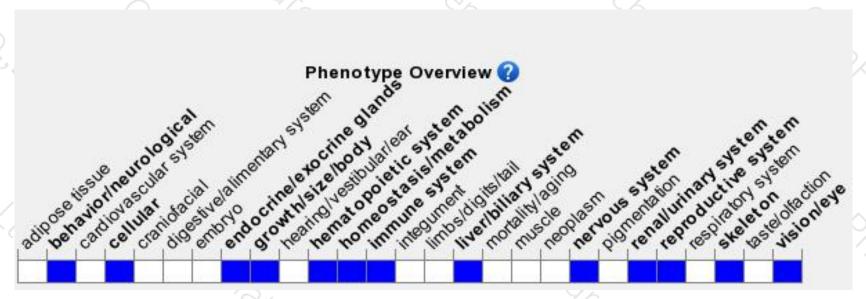
Protein domain





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, Male homozygotes for a targeted null mutation exhibit bilateral intraabdominal cryptorchidism and sterility associated with a failure in the differentiation of the gubernaculae, ligaments that control testicular movement during development.



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





