

Pex3 Cas9-CKO Strategy

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Reviewer: Rui Xiong

Design Date: 2021-3-2

Project Overview



Project Name Pex3

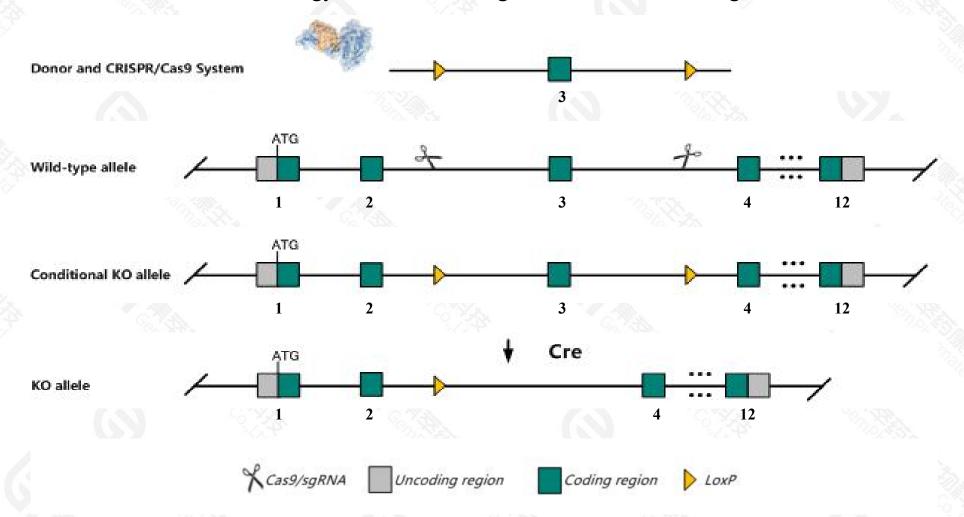
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Pex3* gene. The schematic diagram is as follows:



Technical routes



- > The *Pex3* gene has 7 transcripts. According to the structure of *Pex3* gene, exon3 of *Pex3-201*(ENSMUST00000019945.14) transcript is recommended as the knockout region. The region contains 82bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Pex3* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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 mutants exhibit abnormal sebaceous gland, hair follicle bulge, and cornea morphology. An increase in B and T cell numbers and mean platelet volume, and vertebral transformation are also seen.
- > The *Pex3* 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.

Gene information (NCBI)



Pex3 peroxisomal biogenesis factor 3 [Mus musculus (house mouse)]

Gene ID: 56535, updated on 13-Mar-2020

Summary

☆ ?

Official Symbol Pex3 provided by MGI

Official Full Name peroxisomal biogenesis factor 3 provided by MGI

Primary source MGI:MGI:1929646

See related Ensembl:ENSMUSG00000019809

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 1700014F15Rik, 2810027F19Rik, 2900010N04Rik

Expression Ubiquitous expression in testis adult (RPKM 24.6), bladder adult (RPKM 9.4) and 24 other tissuesSee more

Orthologs <u>human all</u>

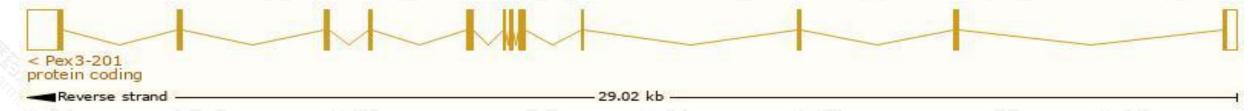
Transcript information (Ensembl)



The gene has 7 transcripts, all transcripts are shown below:

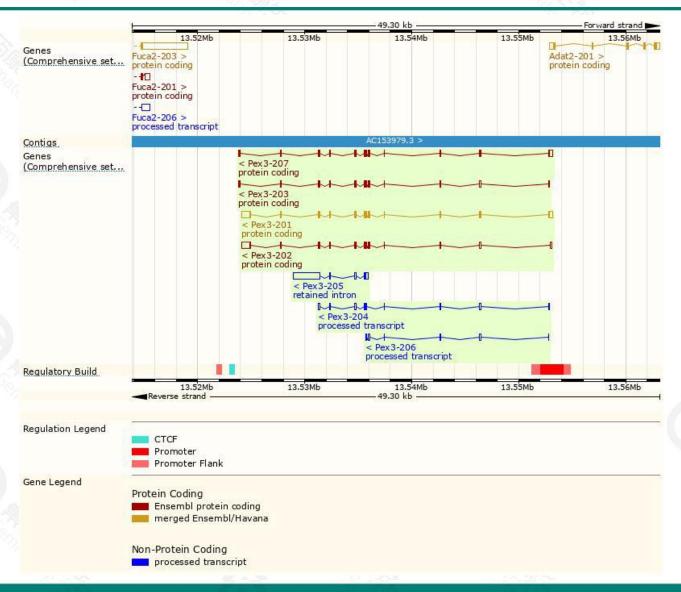
Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST00000019945.14	2129	372aa	Protein coding	CCD523702	Q3UIG2 Q9QXY9	TSL:1 GENCODE basic APPRIS P1
ENSMUST00000105539.1	1961	<u>306aa</u>	Protein coding	CCDS83681	D3Z6X4	TSL:1 GENCODE basic
ENSMUST00000170376.7	1381	359aa	Protein coding	CCDS48503	E9QA46	TSL:1 GENCODE basic
ENSMUST00000105541.7	1072	293aa	Protein coding	-	D3Z6X3	TSL:1 GENCODE basic
ENSMUST00000125207.7	771	No protein	Processed transcript	2	<u> </u>	TSL:3
ENSMUST00000145337.1	480	No protein	Processed transcript	-	類	TSL:5
ENSMUST00000133332.7	2959	No protein	Retained intron	-	-	TSL:1
	ENSMUST0000019945.14 ENSMUST00000105539.1 ENSMUST00000170376.7 ENSMUST00000105541.7 ENSMUST00000125207.7 ENSMUST00000145337.1	ENSMUST0000019945.14 2129 ENSMUST00000105539.1 1961 ENSMUST00000170376.7 1381 ENSMUST00000105541.7 1072 ENSMUST00000125207.7 771 ENSMUST00000145337.1 480	ENSMUST0000019945.14 2129 372aa ENSMUST00000105539.1 1961 306aa ENSMUST00000170376.7 1381 359aa ENSMUST00000105541.7 1072 293aa ENSMUST00000125207.7 771 No protein ENSMUST00000145337.1 480 No protein	ENSMUST00000019945.14 2129 372aa Protein coding ENSMUST00000105539.1 1961 306aa Protein coding ENSMUST00000170376.7 1381 359aa Protein coding ENSMUST00000105541.7 1072 293aa Protein coding ENSMUST00000125207.7 771 No protein Processed transcript ENSMUST00000145337.1 480 No protein Processed transcript	ENSMUST00000019945.14 2129 372aa Protein coding CCDS23702 ENSMUST00000105539.1 1961 306aa Protein coding CCDS83681 ENSMUST00000170376.7 1381 359aa Protein coding CCDS48503 ENSMUST00000105541.7 1072 293aa Protein coding - ENSMUST00000125207.7 771 No protein Processed transcript - ENSMUST00000145337.1 480 No protein Processed transcript -	ENSMUST00000019945.14 2129 372aa Protein coding CCDS23702 Q3UIG2 Q9QXY9 ENSMUST00000105539.1 1961 306aa Protein coding CCDS83681 D3Z6X4 ENSMUST00000170376.7 1381 359aa Protein coding CCDS48503 E9QA46 ENSMUST00000105541.7 1072 293aa Protein coding - D3Z6X3 ENSMUST00000125207.7 771 No protein Processed transcript - - ENSMUST00000145337.1 480 No protein Processed transcript - -

The strategy is based on the design of *Pex3-201* transcript, the transcription is shown below:



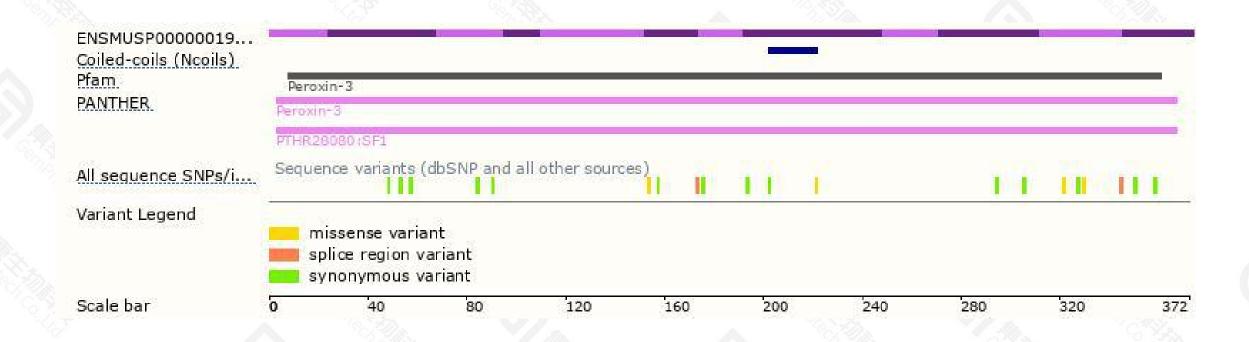
Genomic location distribution





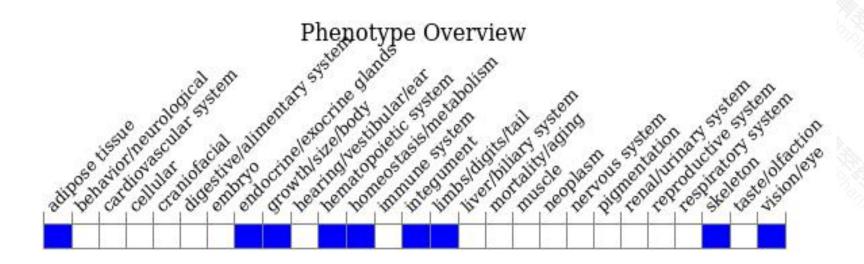
Protein domain





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,homozygous mutants exhibit abnormal sebaceous gland, hair follicle bulge, and cornea morphology. An increase in B and T cell numbers and mean platelet volume, and vertebral transformation are also seen.



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

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