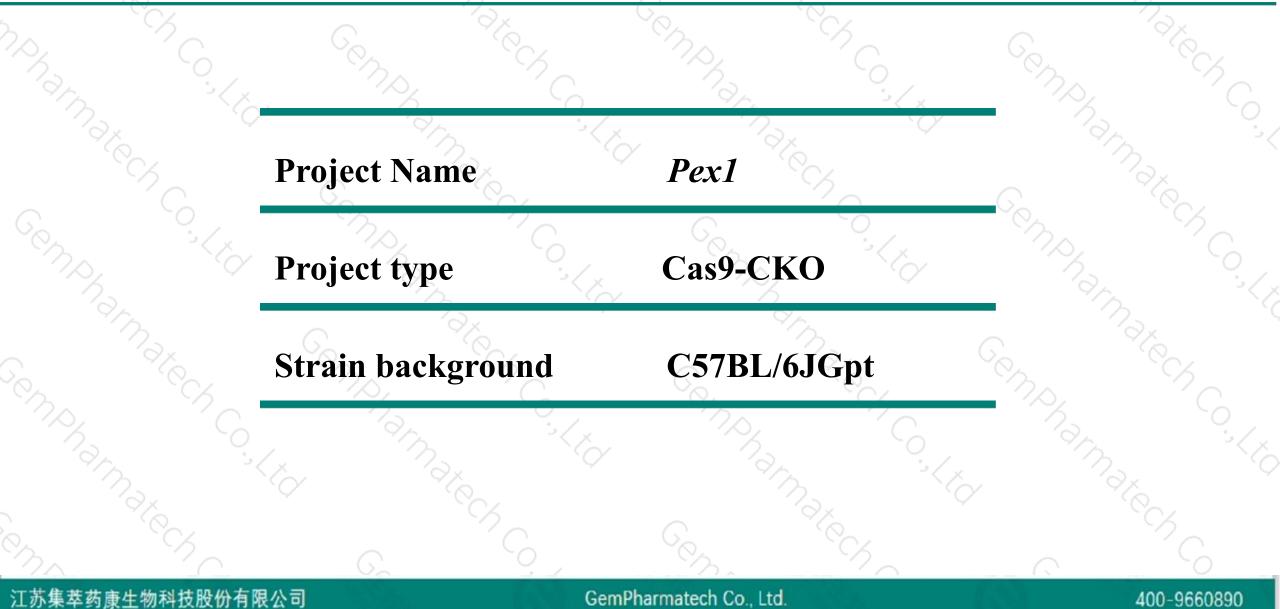


Pex1 Cas9-CKO Strategy

Designer: Xueting Zhang Reviewer:Yanhua Shen Date:2020-03-10

Project Overview

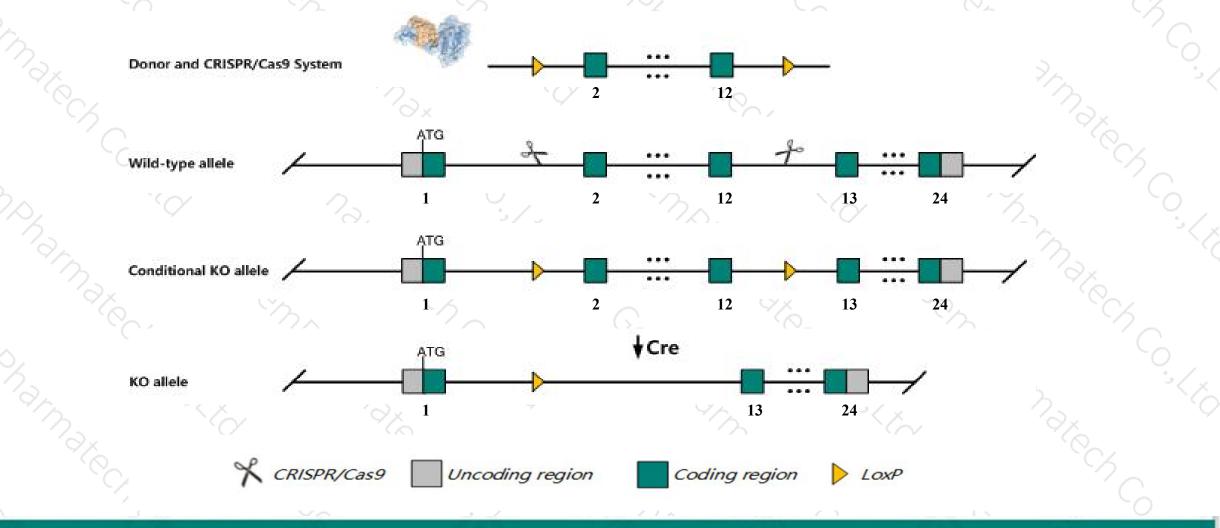




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Pex1* gene. The schematic diagram is as follows:



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The Pex1 gene has 14 transcripts. According to the structure of Pex1 gene, exon2-exon12 of Pex1-202 (ENSMUST00000121291.7) transcript is recommended as the knockout region. The region contains 1945bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Pex1* 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, Mice homozygous for a knock-in allele display premature death, postnatal growth retardation, fatty livers, a bile acid defect associated with intestinal lipid malabsorption and cholestasis, and a retinopathy associated with retinal cone cell degenerationand abnormal cone and rod electrophysiology.
- The floxed region is near to the N-terminal of *Rbm48* gene, this strategy may influence the regulatory function of the N-terminal of *Rbm48* gene.
- ➤ Transcript *Pex1*-2029&210&211&212&214 may not be affected.
- The *Pex1* 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.

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Gene information (NCBI)



Pex1 peroxisomal biogenesis factor 1 [Mus musculus (house mouse)]

Gene ID: 71382, updated on 25-Feb-2020

Summary

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Official Symbol	Pex1 provided by MGI
Official Full Name	peroxisomal biogenesis factor 1 provided by MGI
Primary source	MGI:MGI:1918632
See related	Ensembl:ENSMUSG0000005907
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; Mus; Mus
Also known as	ZWS1; 5430414H02Rik; E330005K07Rik
Expression	Ubiquitous expression in placenta adult (RPKM 4.3), CNS E14 (RPKM 3.6) and 28 other tissues See more
Orthologs	human all

Genomic context

Location: 5; 5 A1 See Pex1 in Genome Data Viewer Exon count: 25 Annotation release Status Assembly Chr Location GRCm38.p6 (GCF_000001635.26) NC_000071.6 (3596066..3637230) 108 current 5 NC_000071.5 (3596066..3637101) Build 37.2 previous assembly MGSCv37 (GCF_000001635.18) 5

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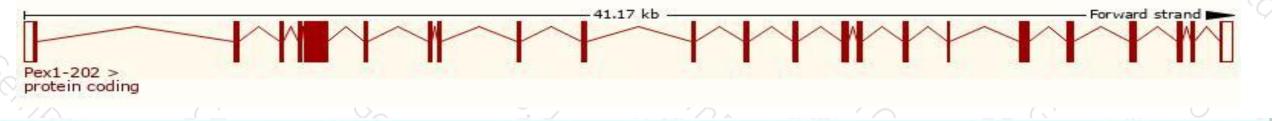
Transcript information (Ensembl)



The gene has 14 transcripts, all transcripts are shown below:

1 Mar	No. of Contract of					and and a	
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pex1-202	ENSMUST00000121291.7	4555	<u>1284aa</u>	Protein coding	CCDS80201	Q5BL07	TSL:5 GENCODE basic APPRIS ALT2
Pex1-201	ENSMUST0000006061.12	4433	<u>1244aa</u>	Protein coding	CCDS19065	Q5BL07	TSL:1 GENCODE basic APPRIS P3
ex1-205	ENSMUST00000142516.1	727	<u>69aa</u>	Protein coding	1920	D3Z5A7	CDS 3' incomplete TSL:3
ex1-204	ENSMUST00000126545.1	639	<u>213aa</u>	Protein coding	8 <u>8</u> 8	F6RUH9	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
ex1-208	ENSMUST00000195894.1	528	<u>143aa</u>	Protein coding	(5)	A0A0G2JE39	TSL:3 GENCODE basic
ex1-206	ENSMUST00000143132.1	664	<u>76aa</u>	Nonsense mediated decay		F7CF88	CDS 5' incomplete TSL:5
ex1-207	ENSMUST00000143959.1	3491	No protein	Retained intron	1920		TSL:1
ex1-213	ENSMUST00000199035.1	987	No protein	Retained intron	100	21	TSL:NA
ex1-203	ENSMUST00000123268.1	746	No protein	Retained intron	151	-	TSL:3
ex1-210	ENSMUST00000196432.1	668	No protein	Retained intron			TSL:NA
ex1-211	ENSMUST00000196692.1	638	No protein	Retained intron	120	2	TSL:NA
ex1-214	ENSMUST00000199213.1	487	No protein	Retained intron	10 <u>1</u> 1	20 20	TSL:2
Pex1-212	ENSMUST00000197167.1	474	No protein	Retained intron	(5)	5	TSL:2
ex1-209	ENSMUST00000196124.1	403	No protein	Retained intron	690	-	TSL:NA
						7 5	

The strategy is based on the design of *Pex1-202* transcript, The transcription is shown below



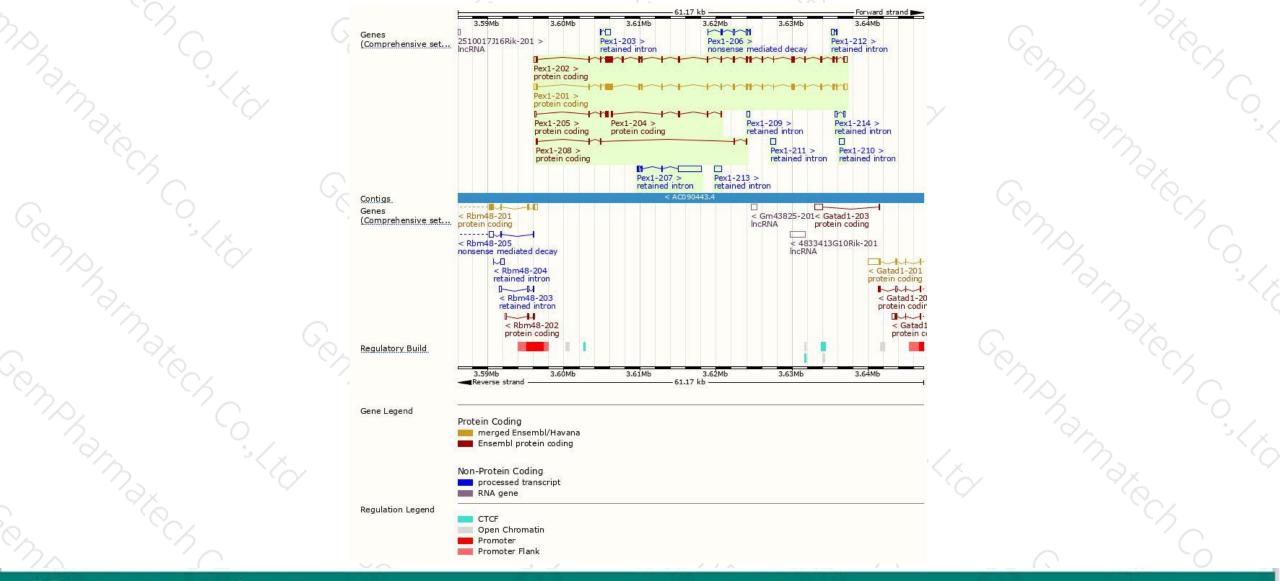
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Genomic location distribution



400-9660890

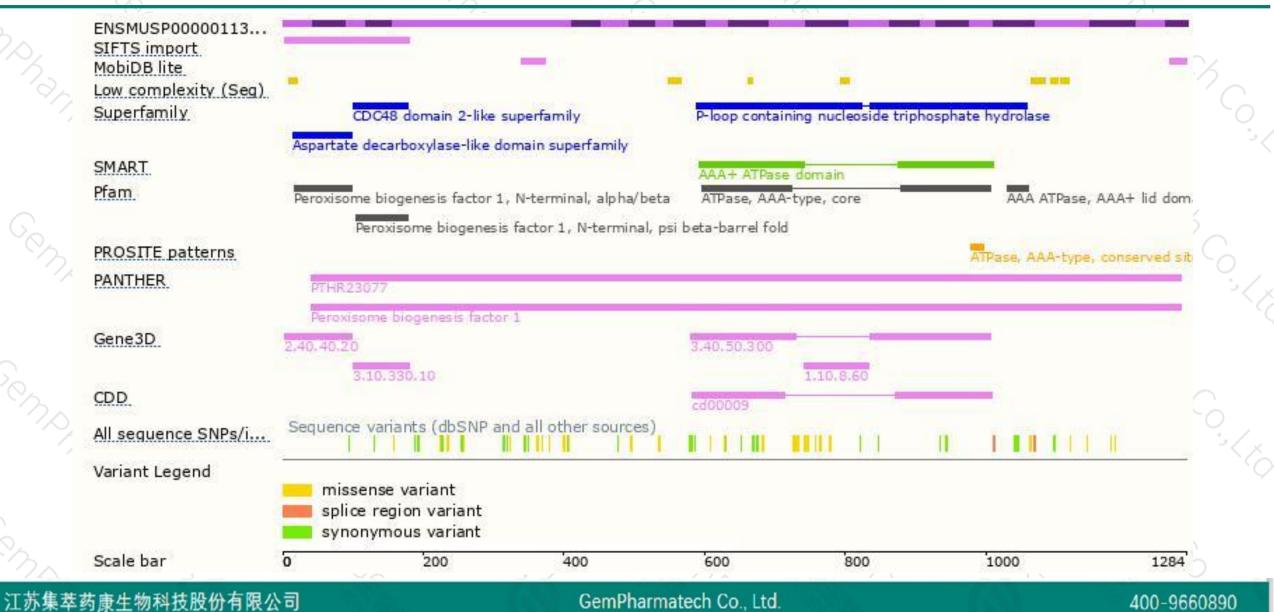


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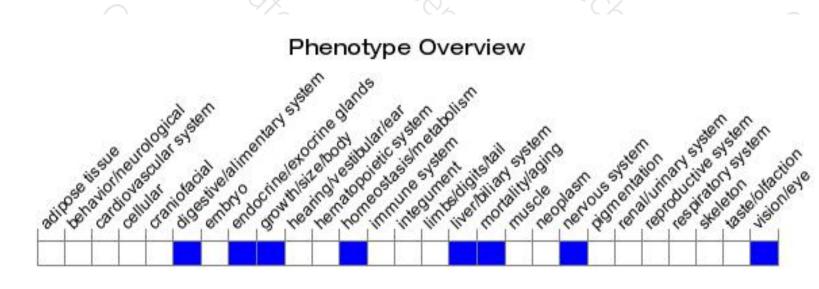
Protein domain





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, Mice homozygous for a knock-in allele display premature death, postnatal growth retardation, fatty livers, a bile acid defect associated with intestinal lipid malabsorption and cholestasis, and a retinopathy associated with retinal cone cell degeneration abnormal cone and rod electrophysiology.

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



