

Abcd3 Cas9-KO Strategy

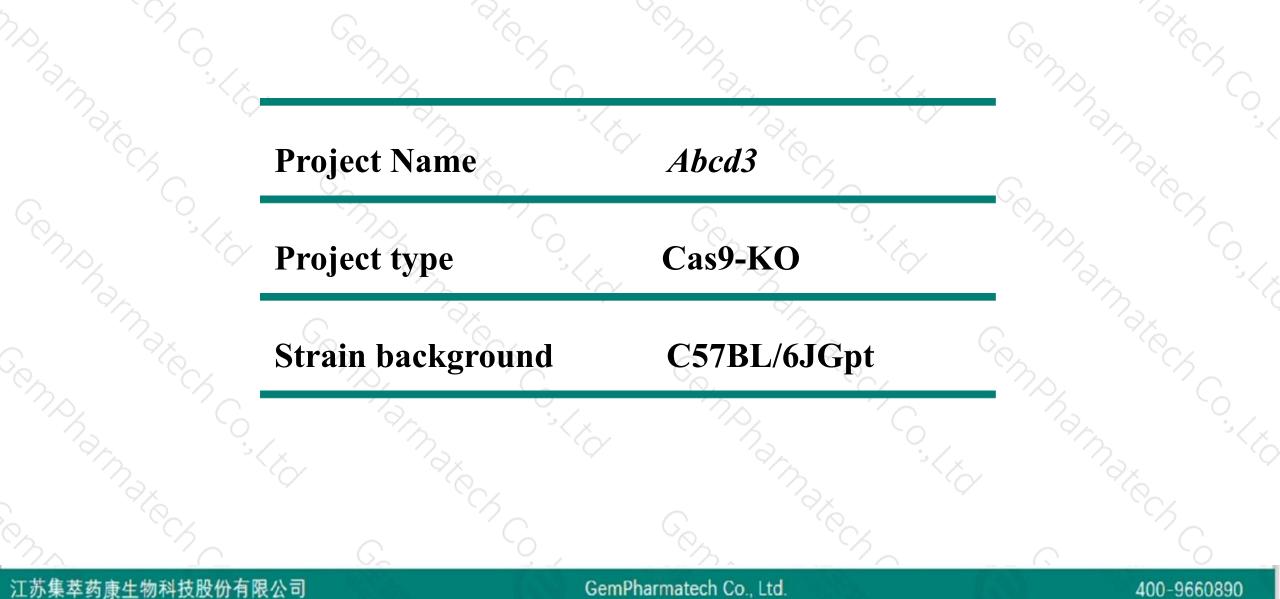
Designer: Reviewer: Design Date:

Bingxuan Li Ruirui Zhang

2020-3-25

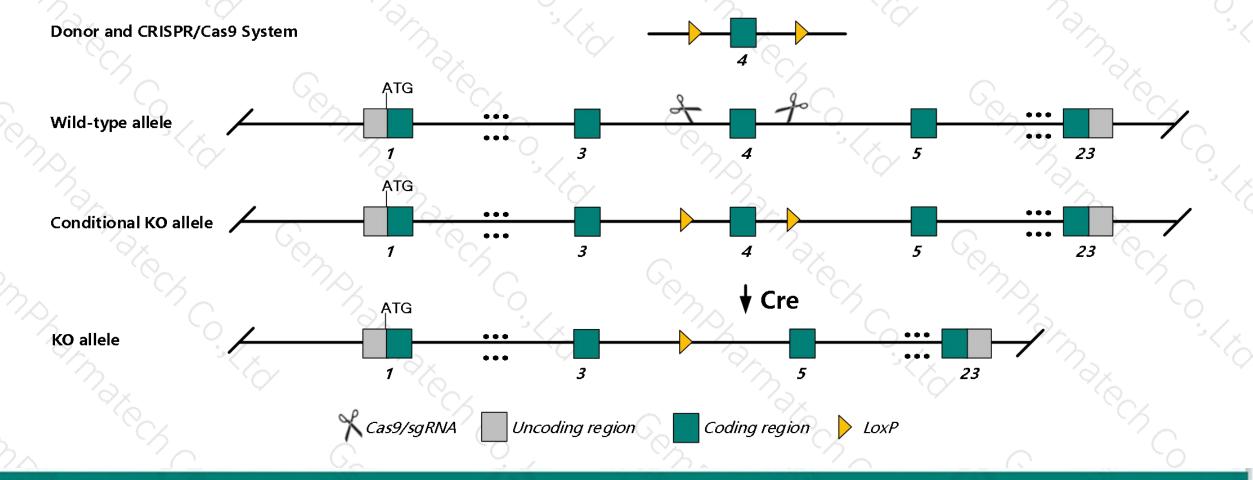
Project Overview







This model will use CRISPR/Cas9 technology to edit the Abcd3 gene. The schematic diagram is as follows:



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- The Abcd3 gene has 6 transcripts. According to the structure of Abcd3 gene, exon4 of Abcd3-201 (ENSMUST00000029770.7) transcript is recommended as the knockout region. The region contains 89bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Abcd3* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.



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- According to the existing MGI data, Mice homozygous for a null mutation show enlarged livers, abnormal bile composition and peroxisome abnormalities.
- The Abcd3 gene is located on the Chr3. 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.

Notice

Gene information (NCBI)



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Abcd3 ATP-binding cassette, sub-family D (ALD), member 3 [Mus musculus (house mouse)]

Gene ID: 19299, updated on 13-Mar-2020

Summary

Official Symbol Abcd3 provided by MGI Official Full Name ATP-binding cassette, sub-family D (ALD), member 3 provided by MGI Primary source MGI:MGI:1349216 See related Ensembl:ENSMUSG0000028127 Gene type protein coding RefSeg status REVIEWED Organism Mus musculus Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Murinae: Mus: Mus Also known as PMP68; PMP70; Pxmp1; Al313901; AU018866; AW146054 The membrane-associated protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport Summary various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the ALD subfamily, which is involved in peroxisomal import of fatty acids and/or fatty acyl-CoAs in the organelle. All known peroxisomal ABC transporters are half transporters which require a partner half transporter molecule to form a functional homodimeric or heterodimeric transporter. This peroxisomal membrane protein likely plays an important role in peroxisome biogenesis. Mutations have been associated with some forms of Zellweger syndrome, a heterogeneous group of peroxisome assembly disorders. [provided by RefSeq, Jul 2008] Expression Ubiquitous expression in liver adult (RPKM 16.9), bladder adult (RPKM 15.9) and 27 other tissues See more Orthologs human all

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Transcript information (Ensembl)



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The gene	has 6 transcripts,al	l transcripts are	shown below:	

Name 🍦	Transcript ID	bp 🖕	Protein 🖕	Biotype 🔺	CCDS 🖕	UniProt 🖕	Flags 👙
Abcd3-203	ENSMUST00000196340.1	2489	No protein	Retained intron	-		TSL:NA
Abcd3-206	ENSMUST00000199593.1	638	No protein	Retained intron	-	-	TSL:1
Abcd3-201	ENSMUST0000029770.7	3489	<u>659aa</u>	Protein coding	CCDS17806 @	P55096 @	TSL:1 GENCODE basic APPRIS P1
Abcd3-204	ENSMUST00000197383.4	3156	<u>549aa</u>	Protein coding	-	<u>A0A0G2JDI9</u> &	TSL:5 GENCODE basic
Abcd3-202	ENSMUST00000195965.1	478	No protein	Processed transcript	=		TSL:5
Abcd3-205	ENSMUST00000197662.4	2467	<u>58aa</u>	Nonsense mediated decay	-	A0A0G2JGA4	TSL:5

56.40 kt

The strategy is based on the design of *Abcd3-201* transcript, the transcription is shown below:

< Abcd3-201 protein coding

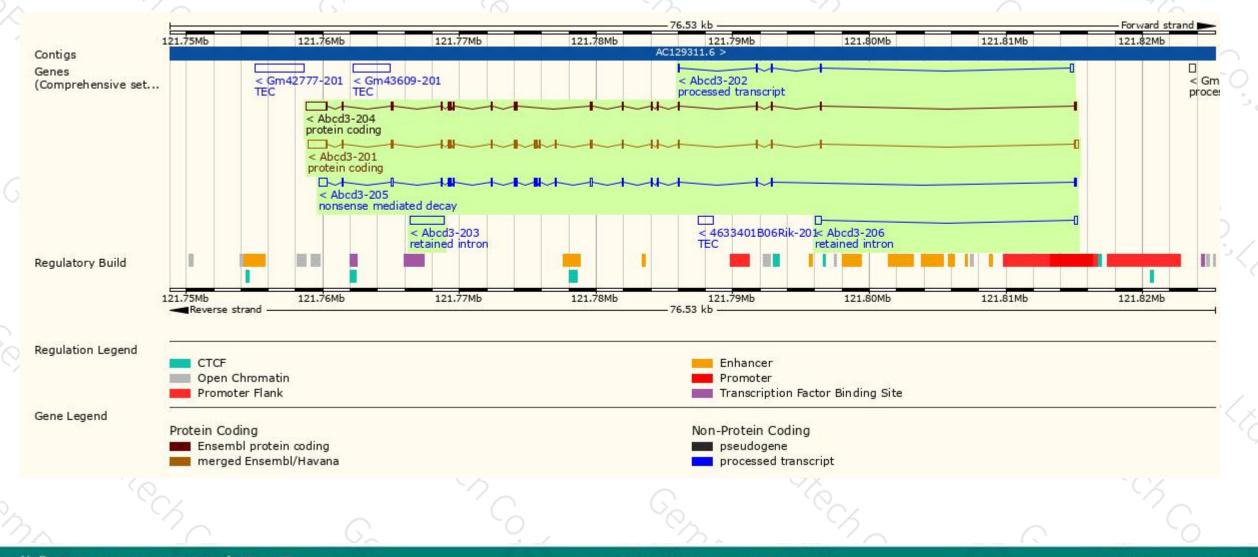
Reverse strand

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Genomic location distribution





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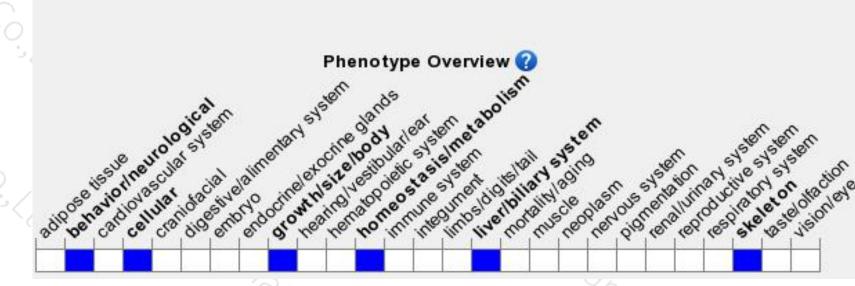
Protein domain



25												
1	ENSMUSP00000029 Transmembrane heli Low complexity (Seg) TIGRFAM	Peroxysomal long cha	in fatty acyl transporter		_							- 2
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	SMART								AAA+ ATPase do	main		-
	Pfam	Ā	BC transporter type 1, tran		ABC transporter-like							
R	PROSITE profiles		ABC transporter		ABC transporter-like							
	PROSITE patterns										ABC transporter, cons	served si,
	PANTHER	ATP-binding cassette su	b-family D member 3									
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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, Mice homozygous for a null mutation show enlarged livers, abnormal bile composition and peroxisome abnormalities.



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



