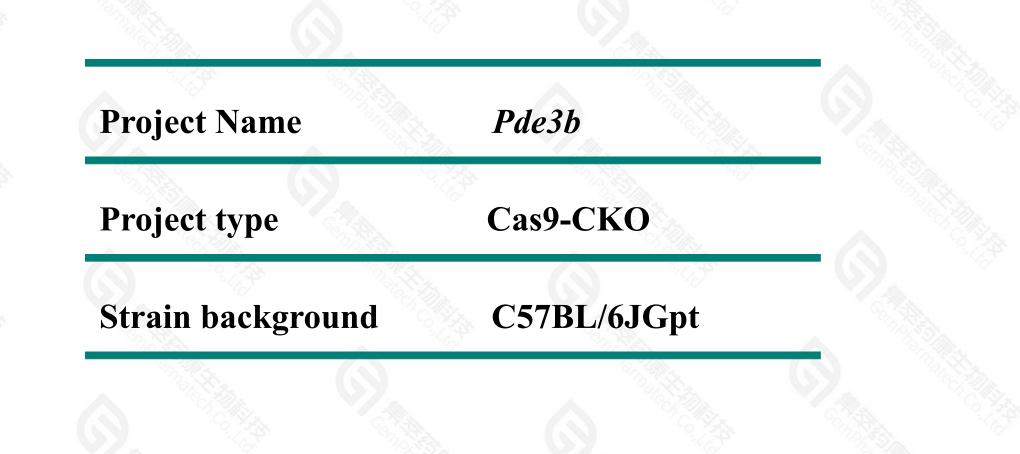


Pde3b Cas9-CKO Strategy

Designer: Qiong Zhou

Project Overview





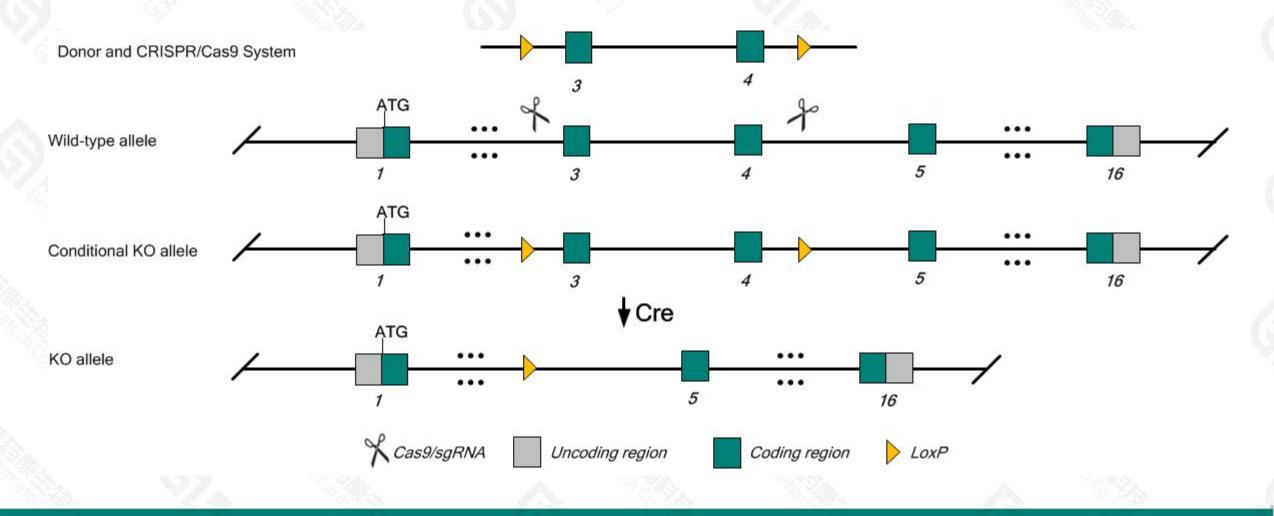
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Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the Pde3b gene. The schematic diagram is as follows:



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Technical routes



The Pde3b gene has 4 transcripts. According to the structure of Pde3b gene, exon3-exon4 of Pde3b-201(ENSMUST00000032909.9) transcript is recommended as the knockout region. The region contains 389bp coding sequence. Knock out the region will result in disruption of protein function.

> In this project we use CRISPR/Cas9 technology to modify *Pde3b* 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.

 \succ 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.



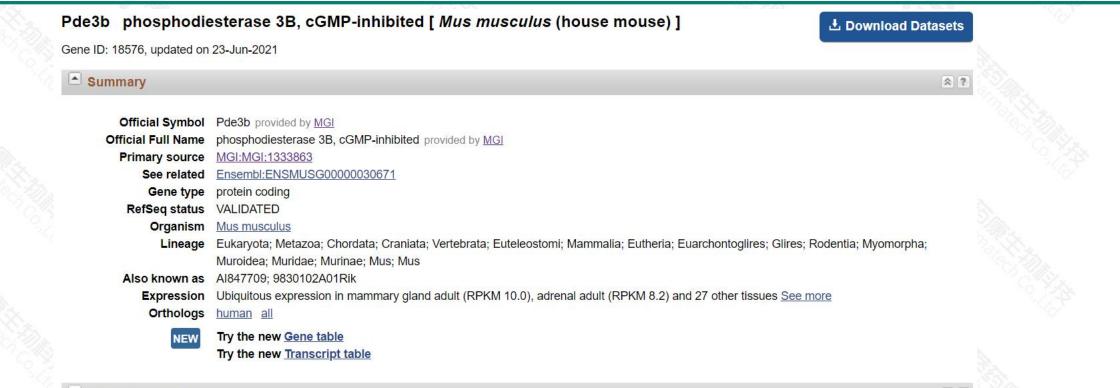
- According to the existing MGI data, Mutants show abnormalities in glycerol and fatty acid levels, along with changes in adipocyte morphology and decreased body fat percentage.
- Transcript *Pde3b-202, Pde3b-203, Pde3b-204* may not be affected.

> The *Pde3b* gene is located on the Chr7. 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)





Genomic context

2

See Pde3b in Genome Data Viewer

Location: 7 F1; 7 59.46 cM

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Exon count: 17

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Annotation release Status Assembly Chr Location GRCm39 (GCF 000001635.27) NC_000073.7 (114014388..114137173) 109 7 current GRCm38.p6 (GCF_000001635.26) NC_000073.6 (114415222..114537938) 108.20200622 previous assembly Build 37.2 previous assembly MGSCv37 (GCF_000001635.18) NC_000073.5 (121558768..121681451)

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Transcript information (Ensembl)

The gene has 4 transcripts, all transcripts are shown below:

Name 🍦	Transcript ID 🖕	bp 🖕	Protein 🖕	Biotype 🍦	CCDS 🍦	UniProt Match 🖕	Flags
Pde3b-201	ENSMUST0000032909.9	6573	<u>1099aa</u>	Protein coding	<u>CCDS21760</u> &	<u>E9QLQ3</u> &	GENCODE basic APPRIS P1 TSL:1
Pde3b-204	ENSMUST00000210411.2	2658	No protein	Retained intron		-	TSL:NA
Pde3b-203	ENSMUST00000149455.2	719	No protein	Retained intron	-	-	TSL:5
Pde3b-202	ENSMUST00000140007.2	485	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Pde3b-201* transcript, the transcription is shown below:

	- 123	9.97 kb	Forward strand
Pde3b-201 > protein coding			

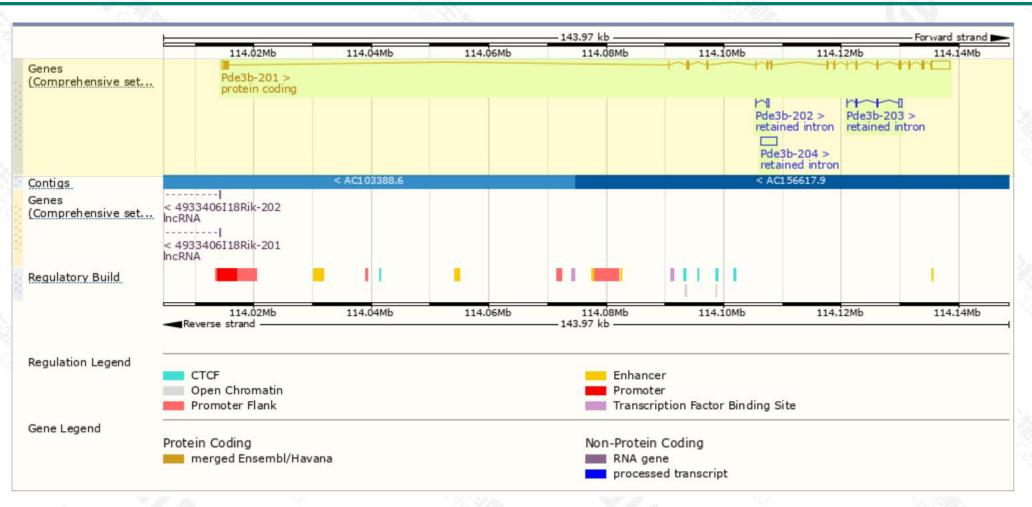
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Genomic location distribution





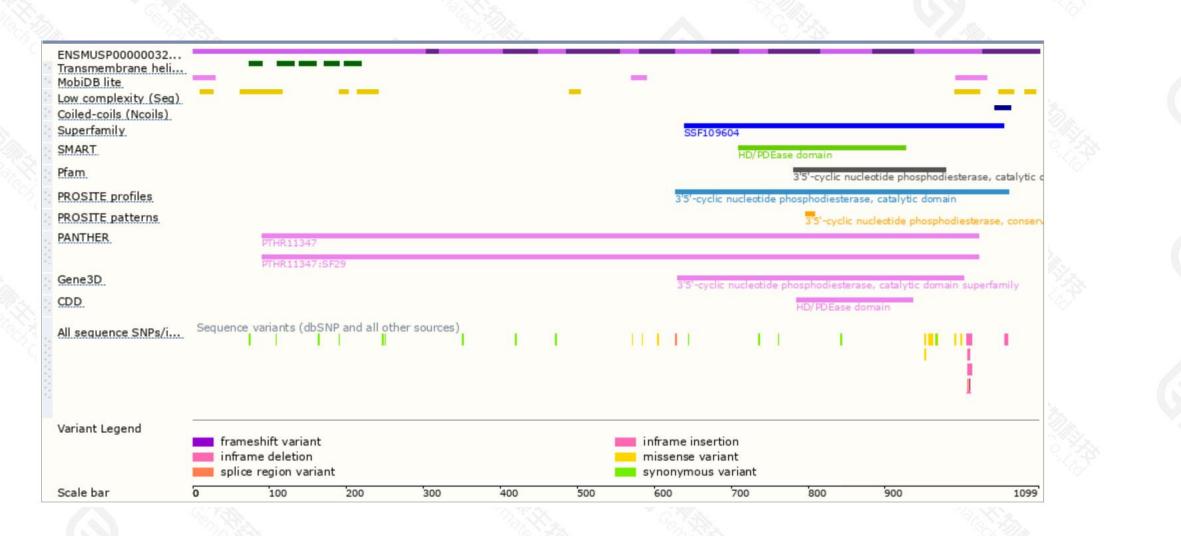
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Protein domain



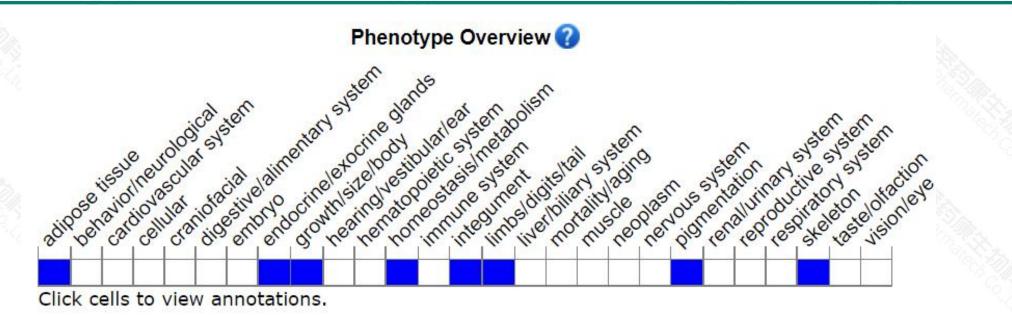


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Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(http://www.informatics.jax.org/).

Mutants show abnormalities in glycerol and fatty acid levels, along with changes in adipocyte morphology and decreased body fat percentage.

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



