

Pde10a Cas9-CKO Strategy

Designer: Reviewer:

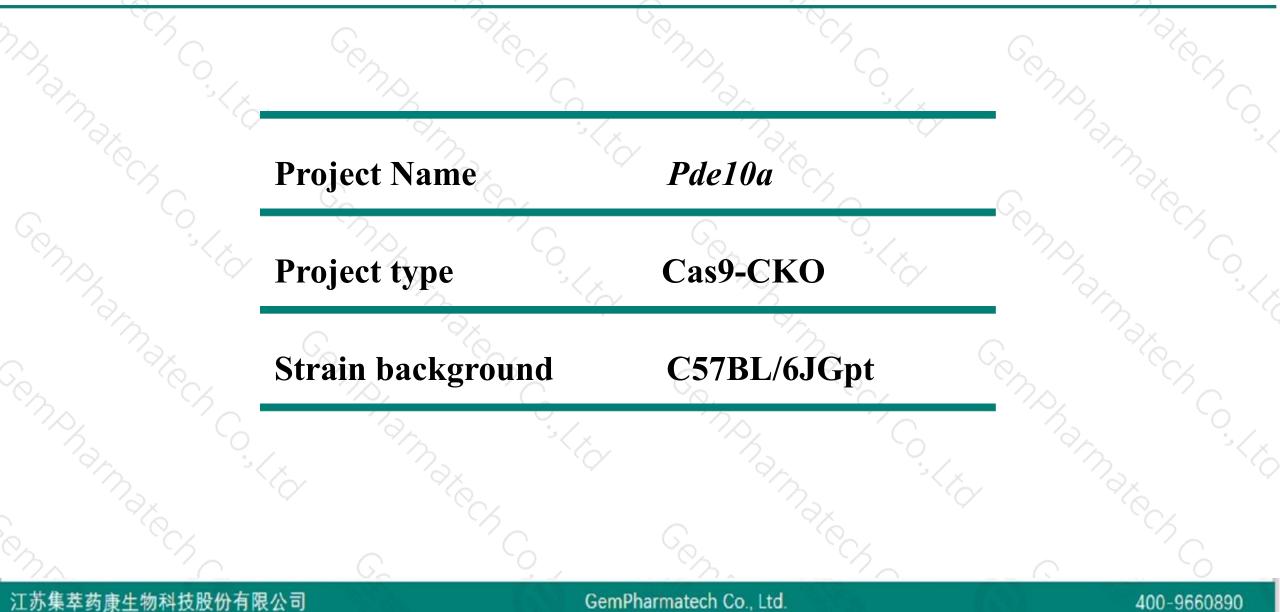
Design Date:

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2020-1-7

Project Overview

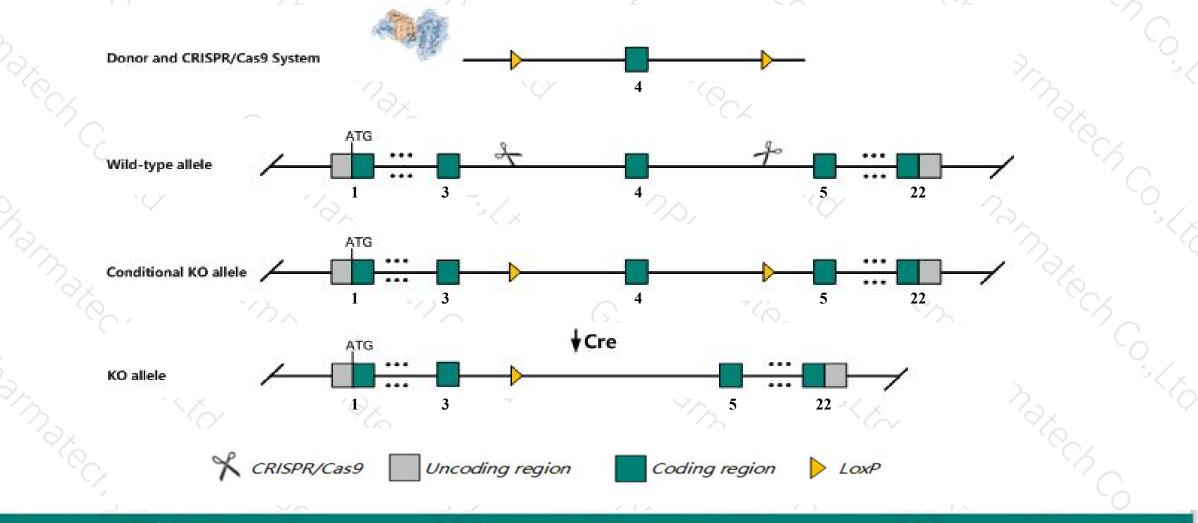




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Pde10a* gene. The schematic diagram is as follows:



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The Pde10a gene has 14 transcripts. According to the structure of Pde10a gene, exon4 of Pde10a-202 (ENSMUST00000089085.9) transcript is recommended as the knockout region. The region contains 121bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Pde10a* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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.



- According to the existing MGI data, Homozygous mutation of this gene results in decreased exploratory behavior, hypoactivity, and a delay in the acquisition of conditioned avoidance behavior. A hypomorphic allele results in increased social behavior. Mice homozygous for a knock-out allele exhibit resistance to diet-induced obesity.
- The Pde10a gene is located on the Chr17. 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)



Pde10a phosphodiesterase 10A [Mus musculus (house mouse)]

Gene ID: 23984, updated on 7-Apr-2019

Summary

Official Symbol	Pde10a provided by MGI
Official Full Name	phosphodiesterase 10A provided by MGI
Primary source	MGI:MGI:1345143
See related	Ensembl:ENSMUSG0000023868
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
Expression	Biased expression in CNS E18 (RPKM 7.5), cortex adult (RPKM 4.9) and 13 other tissues See more
Orthologs	human all

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Transcript information (Ensembl)



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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pde10a-202	ENSMUST0000089085.9	7769	<u>796aa</u>	Protein coding	CCDS28384	Q8CA95	TSL:1 GENCODE basic APPRIS P3
Pde10a-206	ENSMUST00000115720.7	3328	<u>779aa</u>	Protein coding	CCDS84274	<u>Q8CA95</u>	TSL:1 GENCODE basic APPRIS ALT2
Pde10a-211	ENSMUST00000149440.7	4897	<u>727aa</u>	Protein coding	23	Q8CA95	TSL:1 GENCODE basic
Pde10a-208	ENSMUST00000115724.8	3611	<u>850aa</u>	Protein coding	29	A0A384DV92	TSL:5 GENCODE basic
Pde10a-201	ENSMUST00000024647.12	3089	<u>716aa</u>	Protein coding	-	F8WHK3	TSL:1 GENCODE basic APPRIS ALT:
Pde10a-204	ENSMUST00000115715.7	2783	<u>716aa</u>	Protein coding	-8	F8WHK3	TSL:5 GENCODE basic APPRIS ALT:
Pde10a-213	ENSMUST00000233052.1	748	<u>238aa</u>	Protein coding	23	A0A3B2W3N3	CDS 3' incomplete
Pde10a-209	ENSMUST00000136160.1	459	<u>123aa</u>	Protein coding	20	A0A3B2WCR8	CDS 3' incomplete TSL:5
Pde10a-205	ENSMUST00000115717.8	7630	<u>151aa</u>	Nonsense mediated decay	-	<u>S4R197</u>	TSL:5
Pde10a-207	ENSMUST00000115722.7	3673	No protein	Retained intron	-8	()	TSL:1
Pde10a-210	ENSMUST00000141877.1	782	No protein	Retained intron	23	144	TSL:1
Pde10a-214	ENSMUST00000233652.1	409	No protein	Retained intron	10	123	
Pde10a-203	ENSMUST00000115708.1	2471	No protein	IncRNA	50	1781	TSL:1
Pde10a-212	ENSMUST00000233016.1	498	No protein	IncRNA	-	689	

The strategy is based on the design of Pde10a-202 transcript, The transcription is shown below

— 184.96 kb –

Pde10a-202 > protein coding

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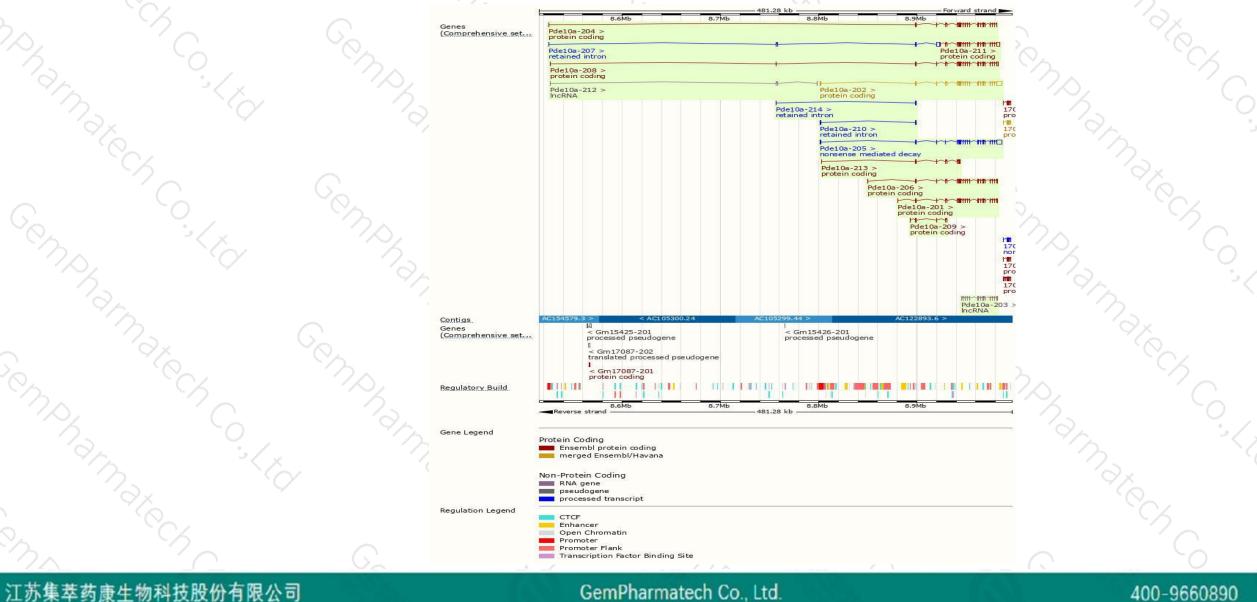
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Forward strand

Genomic location distribution

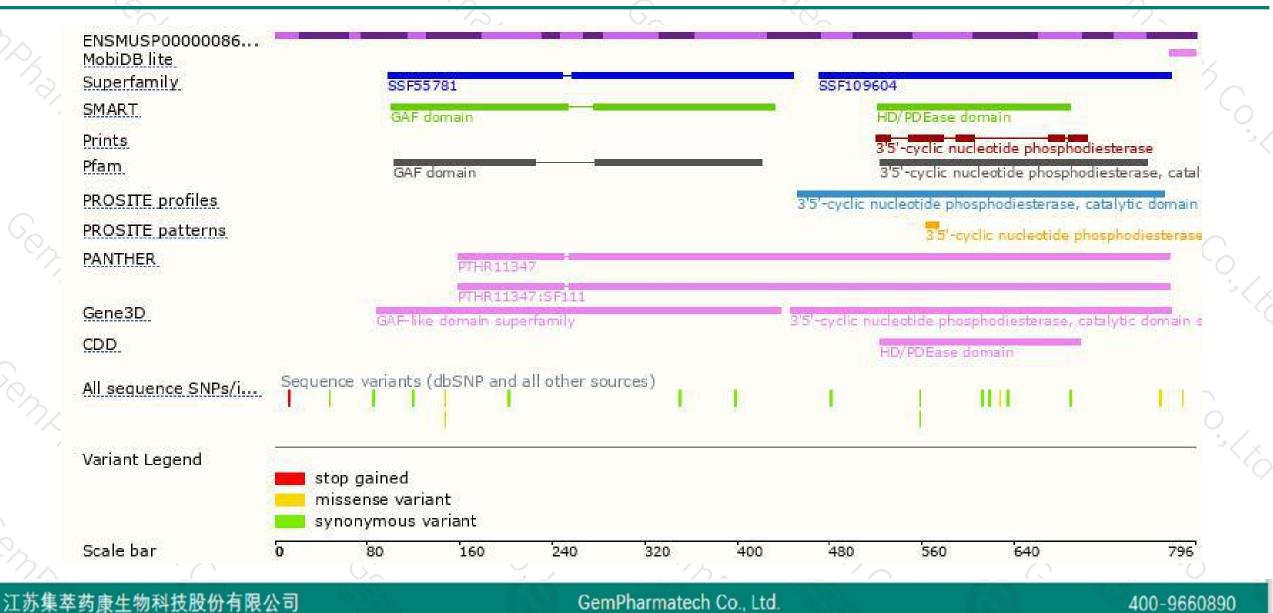




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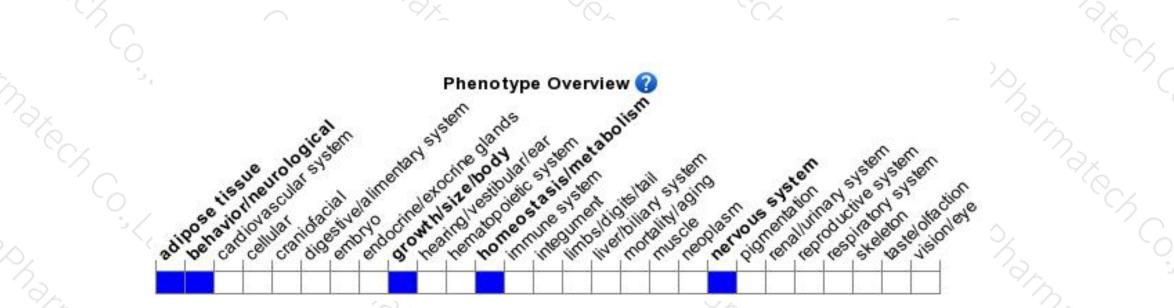
Protein domain





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, Homozygous mutation of this gene results in decreased exploratory behavior, hypoactivity, and a delay in the acquisition of conditioned avoidance behavior. A hypomorphic allele results in increased social behavior. Mice homozygous for a knock-out allele exhibit resistance to diet-induced obesity.

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



