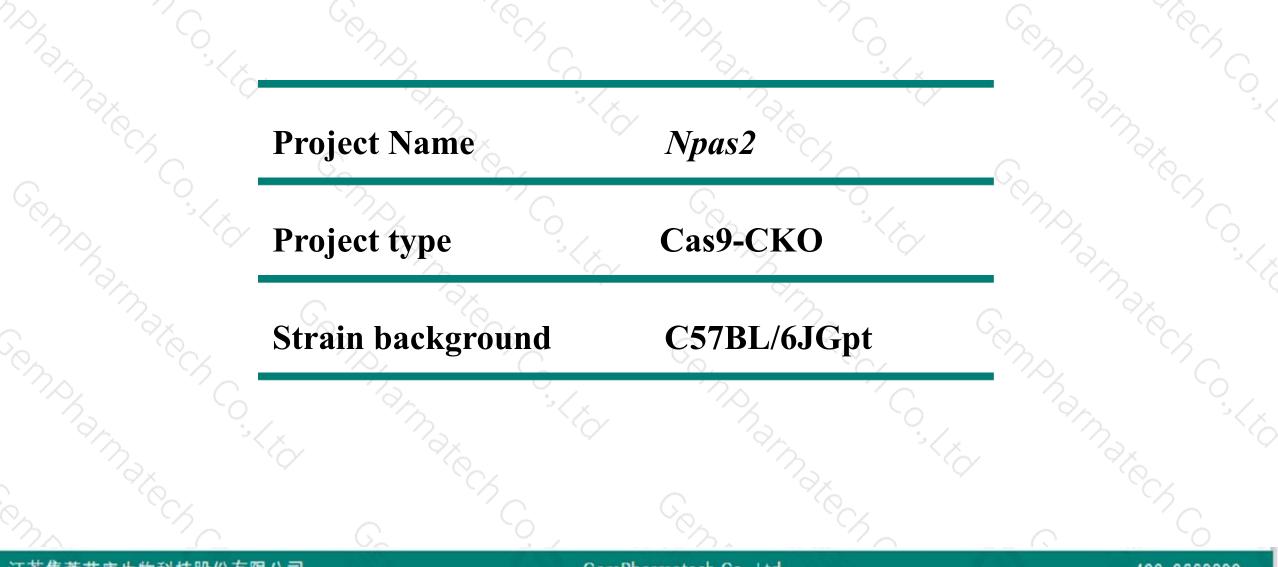


# Npas2 Cas9-CKO Strategy

Designer: Reviewer: Design Date: Yang Zeng Jia Yu 2020-2-10

# **Project Overview**





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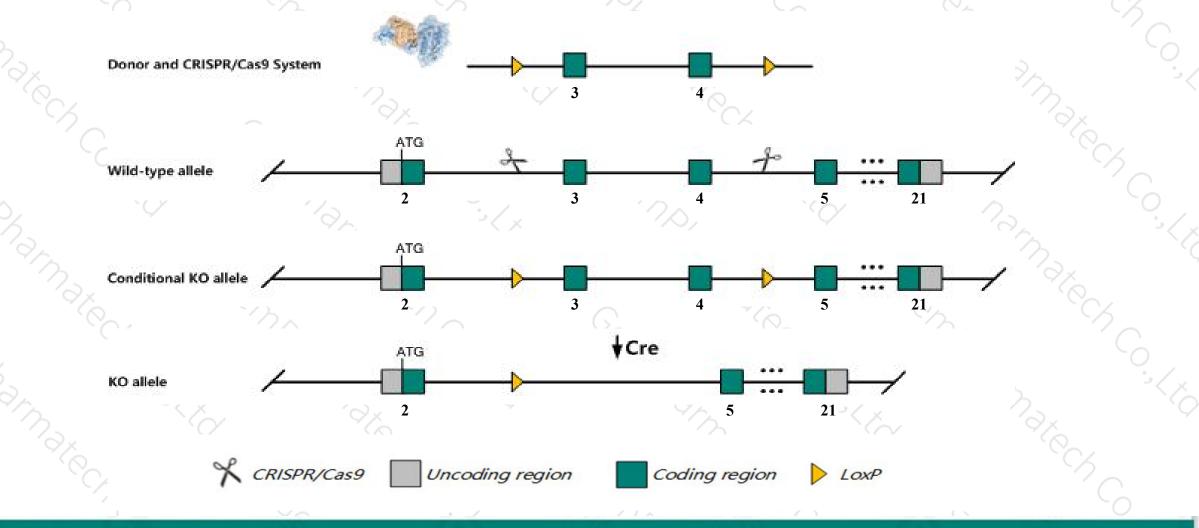
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## **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the Npas2 gene. The schematic diagram is as follows:



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The Npas2 gene has 3 transcripts. According to the structure of Npas2 gene, exon3-exon4 of Npas2-201 (ENSMUST00000056815.8) transcript is recommended as the knockout region. The region contains 241bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Npas2* 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.



- According to the existing MGI data, Targeted mutation of this gene results in deficits in complex emotional long-term memory tasks.
- Transcript Npas2-202 lncRNA may not be affected.
- The Npas2 gene is located on the Chr1. 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)



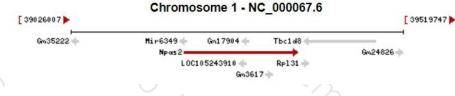
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#### Npas2 neuronal PAS domain protein 2 [ Mus musculus (house mouse) ]

Gene ID: 18143, updated on 12-Aug-2019

#### Summary

Official Symbol	Npas2 provided by MGI						
Official Full Name	neuronal PAS domain protein 2 provided by MGI						
Primary source	MGI:MGI:109232						
See related	Ensembl:ENSMUSG0000026077						
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	MOP4; bHLHe9						
Summary	The protein encoded by this gene is a member of the basic helix-loop-helix (bHLH)-PAS family of transcription factors. The encoded						
	protein may play a regulatory role in the acquisition of specific types of memory. It also may function as a part of a molecular clock						
	operative in the mammalian forebrain. [provided by RefSeq, Dec 2014]						
Expression	Broad expression in small intestine adult (RPKM 16.1), colon adult (RPKM 13.4) and 17 other tissues See more						
Orthologs	human all						
	Chromosome 1 - NC 000067.6						



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# **Transcript information (Ensembl)**



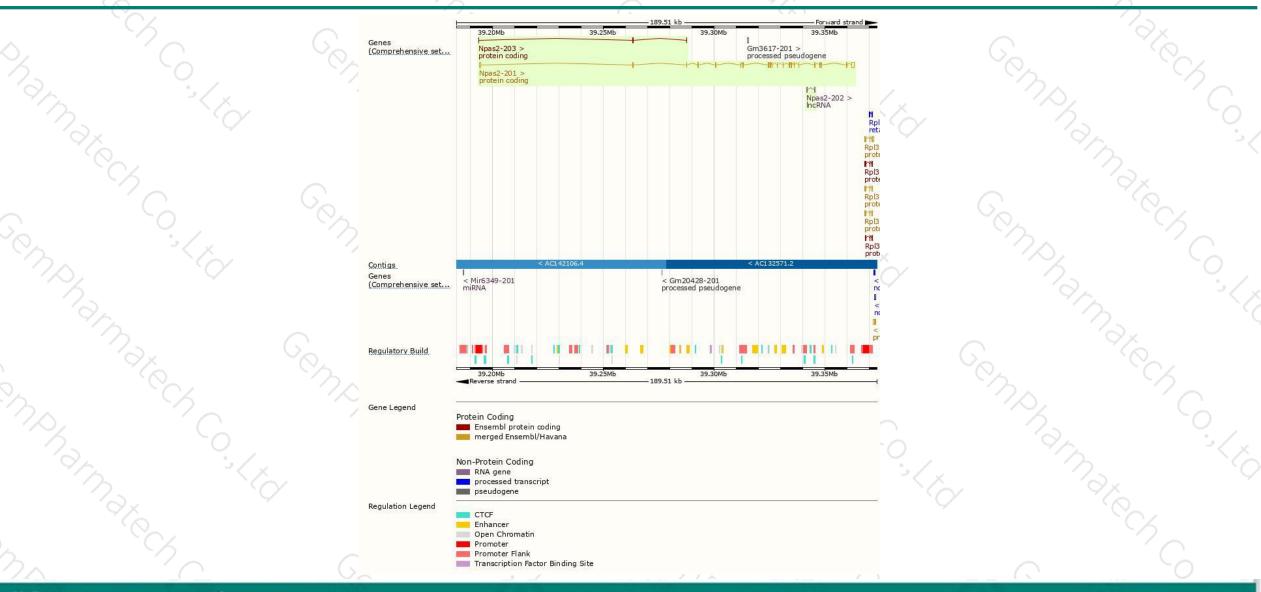
#### The gene has 3 transcripts, all transcripts are shown below:

Name 🖕	Transcript ID	bp 🖕	Protein 🖕	Translation ID	Biotype 🍦	CCDS 🖕	UniProt 🖕	Flags
Npas2-201	ENSMUST0000056815.8	4236	<u>816aa</u>	ENSMUSP0000054719.7	Protein coding	CCDS48244@	<u>G3X9B7</u> ₽	TSL:1 GENCODE basic APPRIS P1
Npas2-203	ENSMUST00000173050.7	484	<u>60aa</u>	ENSMUSP00000134241.1	Protein coding	1.5	<u>G3UYV8</u> ₽	CDS 3' incomplete TSL:2
Npas2-202	ENSMUST00000172825.1	474	No protein	∭	IncRNA	120	5	TSL:2

The strategy is based on the design of Npas2-201 transcript, The transcription is shown below



### **Genomic location distribution**



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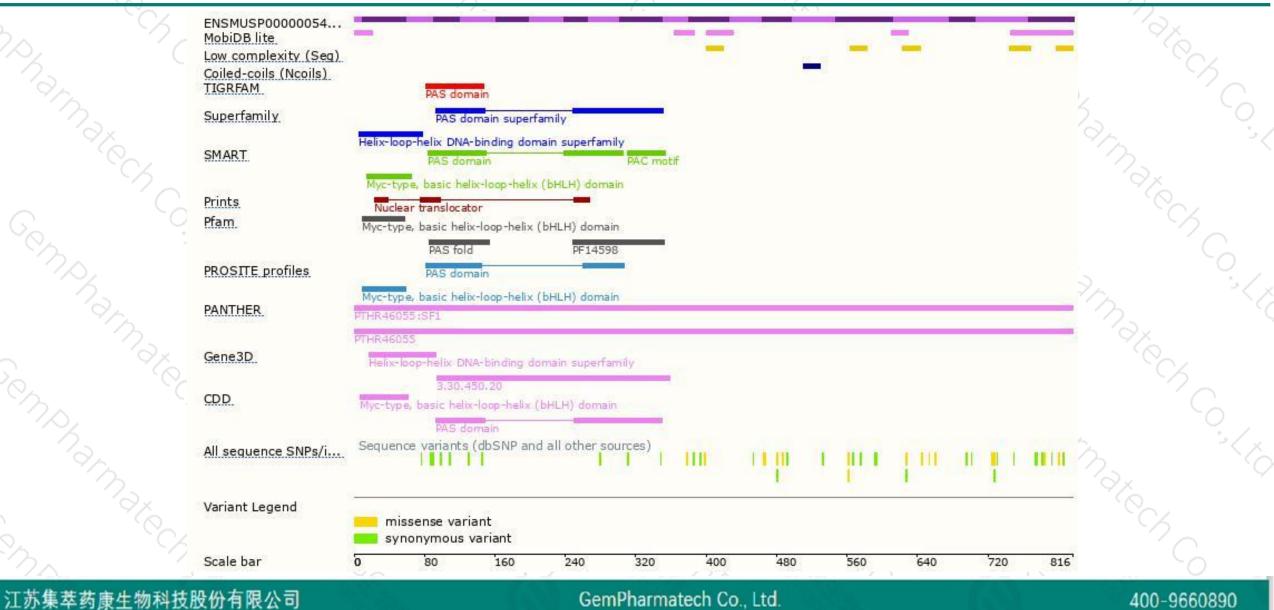
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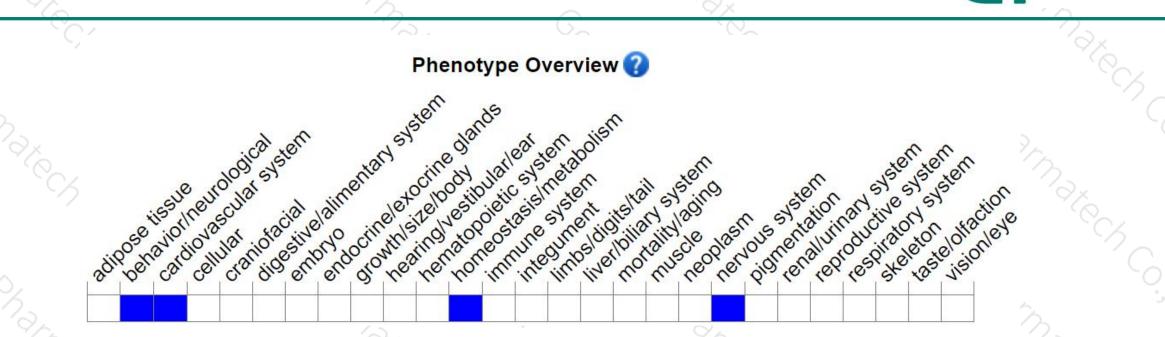
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### **Protein domain**





### 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, Targeted mutation of this gene results in deficits in complex emotional long-term memory tasks



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



