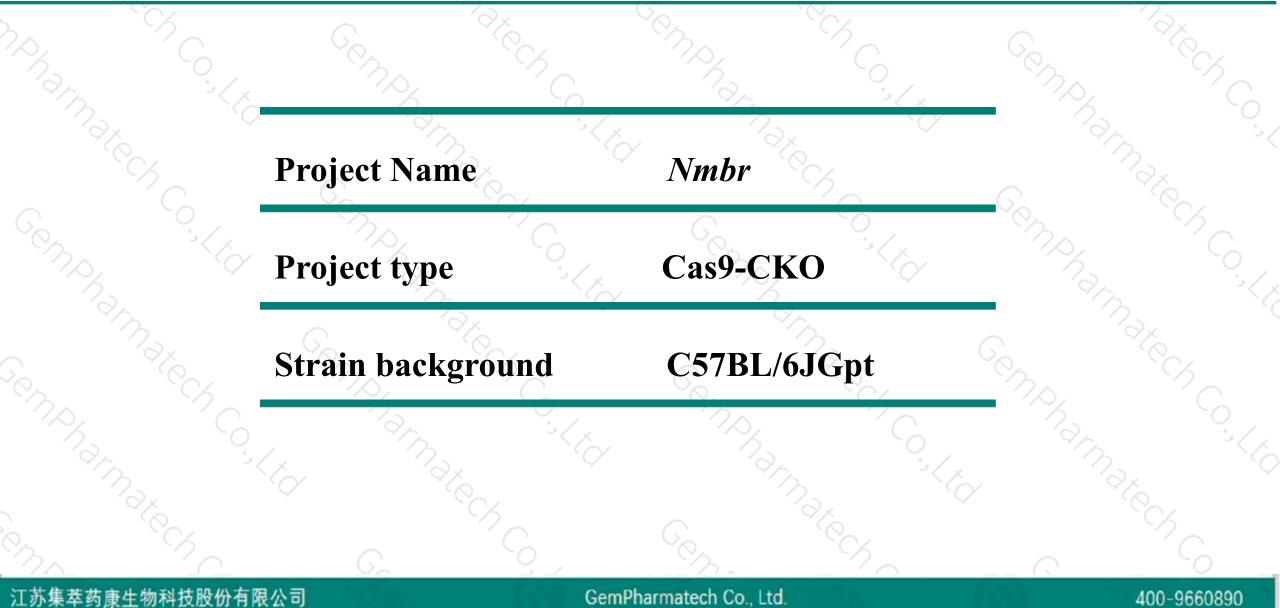


Nmbr Cas9-CKO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2020-1-14

Project Overview

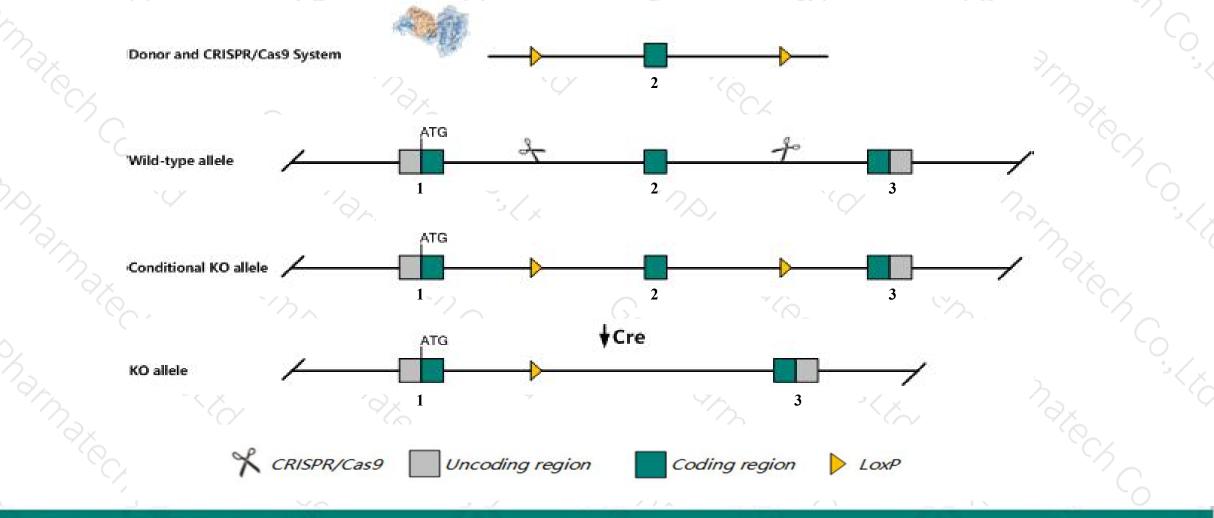




Conditional Knockout strategy



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



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The Nmbr gene has 8 transcripts. According to the structure of Nmbr gene, exon2 of Nmbr-201 (ENSMUST0000020015.9) transcript is recommended as the knockout region. The region contains 349bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Nmbr* 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-out allele show a 50% reduction in the thermoregulatory response to neuromedin B as well as impaired maternal behavior in response to restraint-induced stress.
- The N-terminal of *Nmbr* gene will remain several amino acids ,it may remain the partial function of *Nmbr* gene.
 Transcript *Nmbr*-202&204&205 may not be affected.
- ➤ The effect on transcript *Nmbr*-206&207&208 is unknown.
- The floxed region is near to the N-terminal of *Gm47698* and *B230364G03Rik* gene, this strategy may influence the regulatory function of the N-terminal of these genes.
- The Nmbr gene is located on the Chr10. 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)



\$?



See related	Ensembl:ENSMUSG0000019865
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	BB182387
Expression	Biased expression in testis adult (RPKM 4.4), frontal lobe adult (RPKM 2.7) and 5 other tissues See more
Orthologs	human all
nomic context	

See Nmbr in Genome Data Viewer

Annotation release	Status	Assembly	Chr	Location	2
108	current	GRCm38.p6 (GCF_000001635.26)	10	NC_000076.6 (1475935614771458)	
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	10	NC_000076.5 (1448009514490362)	

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The gene has 8 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nmbr-201	ENSMUST00000020015.9	1599	<u>390aa</u>	Protein coding	CCDS23707	054799 Q0VEH1	TSL:1 GENCODE basic APPRIS P1
mbr-207	ENSMUST00000190751.1	921	<u>179aa</u>	Protein coding	, e	A0A087WQJ7	TSL:1 GENCODE basic
mbr-206	ENSMUST00000190114.1	662	<u>141aa</u>	Protein coding	<u>.</u>	A0A087WRS7	TSL:3 GENCODE basic
mbr-208	ENSMUST00000191238.6	640	<u>24aa</u>	Protein coding	<u>_</u>	A0A087WPA3	CDS 3' incomplete TSL:3
Nmbr-203	ENSMUST00000186382.6	1354	<u>257aa</u>	Nonsense mediated decay	2	A0A087WP36	TSL:1
mbr-202	ENSMUST00000186175.1	672	No protein	Retained intron			TSL:3
mbr-205	ENSMUST00000188021.6	910	No protein	IncRNA	2	4	TSL:1
mbr-204	ENSMUST00000187449.6	447	No protein	IncRNA	2 2	2	TSL:3

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

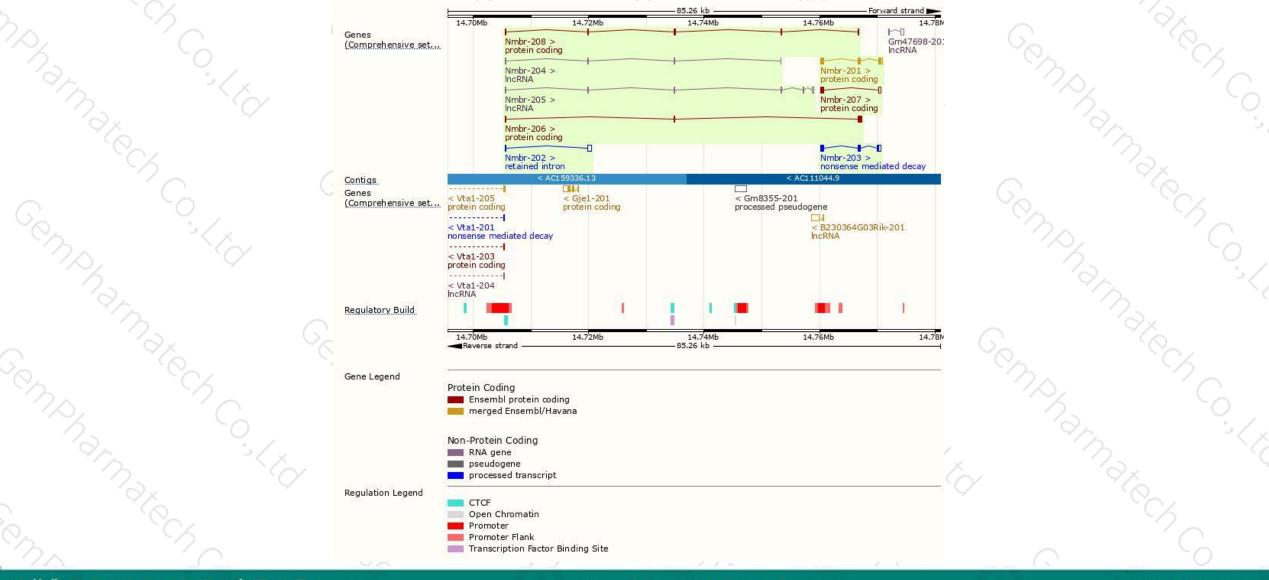


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Genomic location distribution



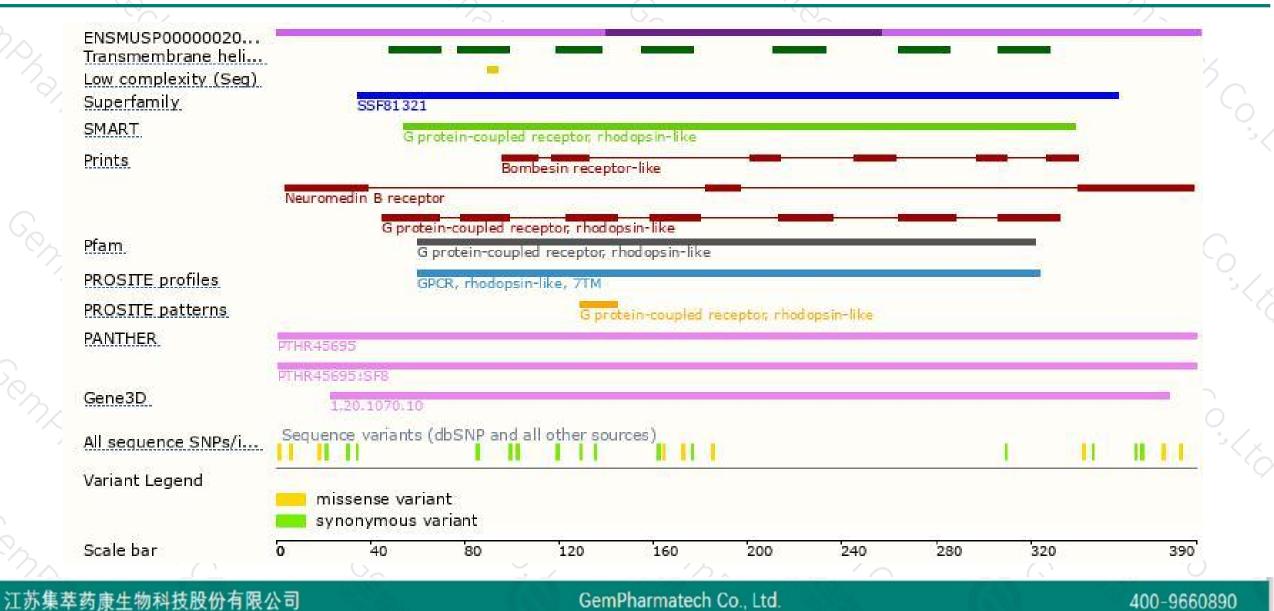


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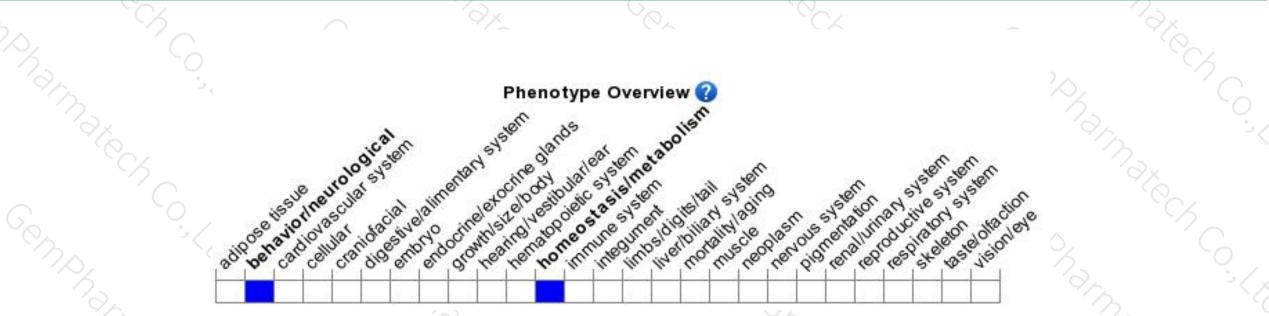
Protein domain





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, Mice homozygous for a knock-out allele show a 50% reduction in the thermoregulatory response to neuromedin B as well as impaired maternal behavior in response to restraint-induced stress.



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



