

# Neurod4 Cas9-KO Strategy

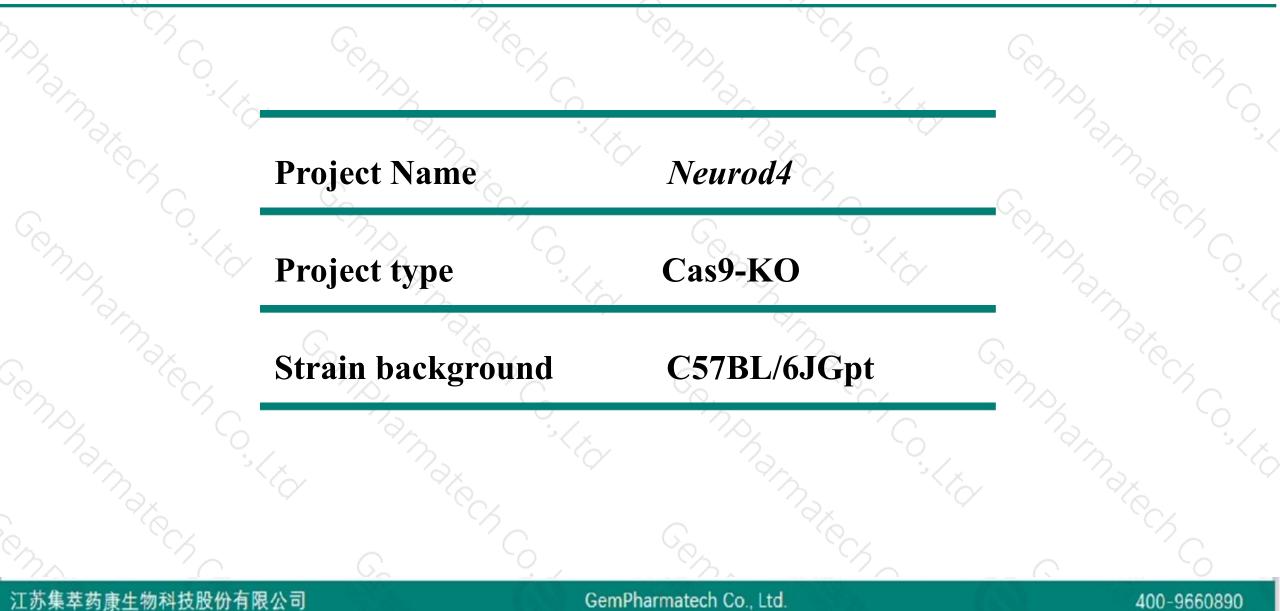
**Designer: Huimin Su** 

**Reviewer: Ruiuri Zhang** 

**Design Date: 2020-6-28** 

### **Project Overview**

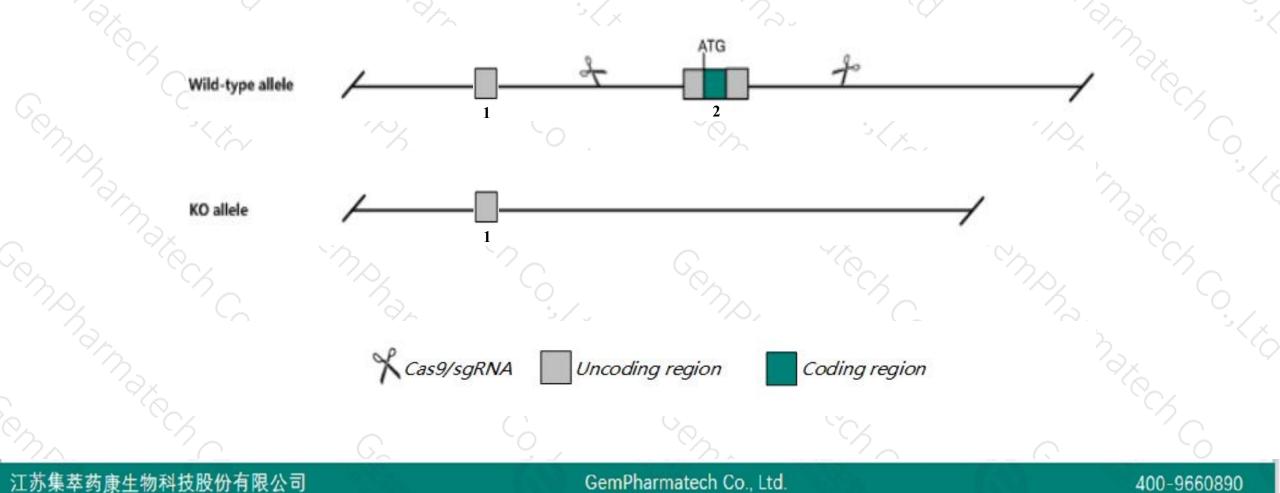




## **Knockout** strategy



This model will use CRISPR/Cas9 technology to edit the Neurod4 gene. The schematic diagram is as follows:





➤ The Neurod4 gene has 1 transcript. According to the structure of Neurod4 gene, exon2 of Neurod4-201(ENSMUST00000061571.4) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Neurod4* gene. The brief process is as follows: CRISPR/Cas9 system 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.



> According to the existing MGI data, homozygotes for a targeted null mutation exhibit reduced growth, ataxia, and high postnatal mortality. Mutants show impaired postnatal cerebellar development, with thinner inner granular cell and molecular layers.

The *Neurod4* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

# Gene information (NCBI)



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#### Neurod4 neurogenic differentiation 4 [ Mus musculus (house mouse) ]

Gene ID: 11923, updated on 26-Jun-2020

#### Summary

Official Symbol Neurod4 provided by MGI Official Full Name neurogenic differentiation 4 provided by MGI Primary source MGI:MGI:108055 See related Ensembl:ENSMUSG00000048015 Gene type protein coding RefSeg status REVIEWED Organism Mus musculus Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Lineage Mus: Mus Also known as ATH-3; Atoh3; Math3; MATH-3; bHLHa4; Al846749 This gene belongs to the neurogenic differentiation factor family and encodes a basic helix-loop-helix (bHLH) transcription factor which is expressed in the developing Summary nervous system with high levels of expression in the brain, retina and cranial ganglions. Expression gradually becomes restricted to the neural retina. It is a key gene in the Ngn2-regulated neuronal differentiation pathway, coordinating the onset of cortical gene transcription. This gene also regulates amacrine cell fate determination in the retina. [provided by RefSeq, Jul 2016] Biased expression in CNS E11.5 (RPKM 1.8), CNS E14 (RPKM 0.6) and 4 other tissues See more Expression Orthologs human all

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# **Transcript information (Ensembl)**



The gene has 1 transcript, and the transcript is shown below:

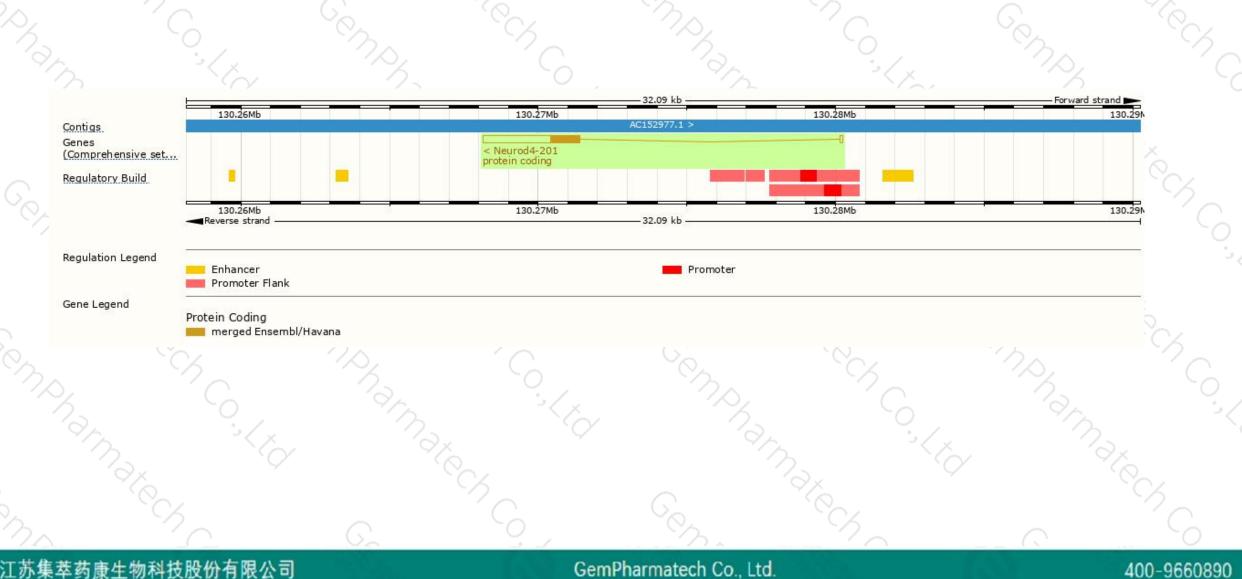
Name 🖕	Transcript ID	bp 🖕	Protein A	Biotype 💧	CCDS 🖕	UniProt 🖕		Flags	(
Neurod4-201	ENSMUST0000061571.4	3345	<u>330aa</u>	Protein coding	<u>CCDS24353</u> @	<u>009105@Q545C0</u> @	TSL:1	GENCODE basic	APPRIS P1

The strategy is based on the design of *Neurod4-201* transcript, the transcription is shown below:

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# **Genomic location distribution**





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# **Protein domain**



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	PROSITE profiles PIRSF PANTHER	Neurogenic differentiation factor NeuroD PTHR 19290 Neurogenic differentiation factor 4	Myc-type, basic helix-loop-hel		domain of unknown function		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7/2	Gene3D CDD All sequence SNPs/i		Helix-loop-helix DNA-binding o Myc-type, basic helix-loop-he other sources)	elix (bHLH) domain			5. 34
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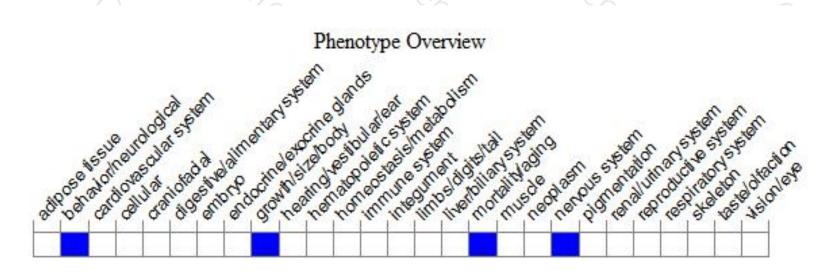
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# Mouse phenotype description(MGI)





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, homozygotes for a targeted null mutation exhibit reduced growth, ataxia, and high postnatal mortality. Mutants show impaired postnatal cerebellar development, with thinner inner granular cell and molecular layers.

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



