

# Neu4 Cas9-CKO Strategy

Designer: Xueting Zhang

Reviewer: Yanhua Shen

Date:2020-02-25

# **Project Overview**



**Project Name** 

Neu4

**Project type** 

Cas9-CKO

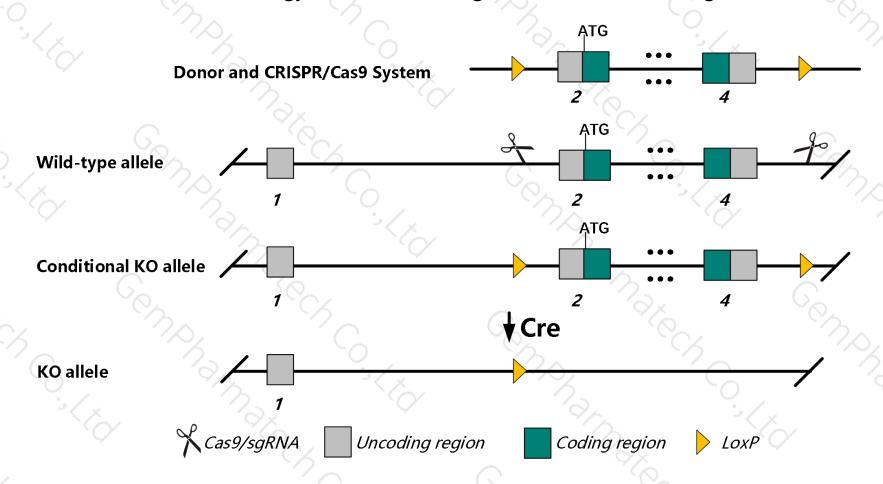
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Neu4 gene. The schematic diagram is as follows:



### Technical routes



- The *Neu4* gene has 2 transcripts. According to the structure of *Neu4* gene, exon2-exon4 of *Neu4-201* (ENSMUST0000050890.7) 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 *Neu4* 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 null allele are largely normal except increased lipid content in the lung and liver and vacuolization indicative of lysosomal storage disorder.
- > The *Neu4* 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.

### Gene information (NCBI)



#### Neu4 sialidase 4 [ Mus musculus (house mouse) ]

Gene ID: 241159, updated on 10-Oct-2019

#### Summary

↑ ?

Official Symbol Neu4 provided by MGI
Official Full Name sialidase 4 provided by MGI

Primary source MGI:MGI:2661364

See related Ensembl: ENSMUSG00000034000

Gene type protein coding
RefSeq status VALIDATED
Organism <u>Mus musculus</u>

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as 9330166104

Expression Biased expression in cortex adult (RPKM 1.5), frontal lobe adult (RPKM 1.4) and 1 other tissue See more

Orthologs human all

#### Genomic context



Location: 1; 1 D

See Neu4 in Genome Data Viewer

Exon count: 5

| Annotation release | Status            | Assembly                     | Chr | Location                       | ). |
|--------------------|-------------------|------------------------------|-----|--------------------------------|----|
| 108                | current           | GRCm38.p6 (GCF_000001635.26) | 1   | NC_000067.6 (9402049394028334) |    |
| Build 37.2         | previous assembly | MGSCv37 (GCF_000001635.18)   | 1   | NC_000067.5 (9591707095924911) |    |

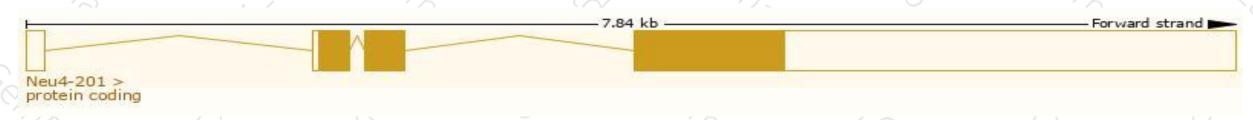
# Transcript information (Ensembl)



The gene has 2 transcripts, all transcripts are shown below:

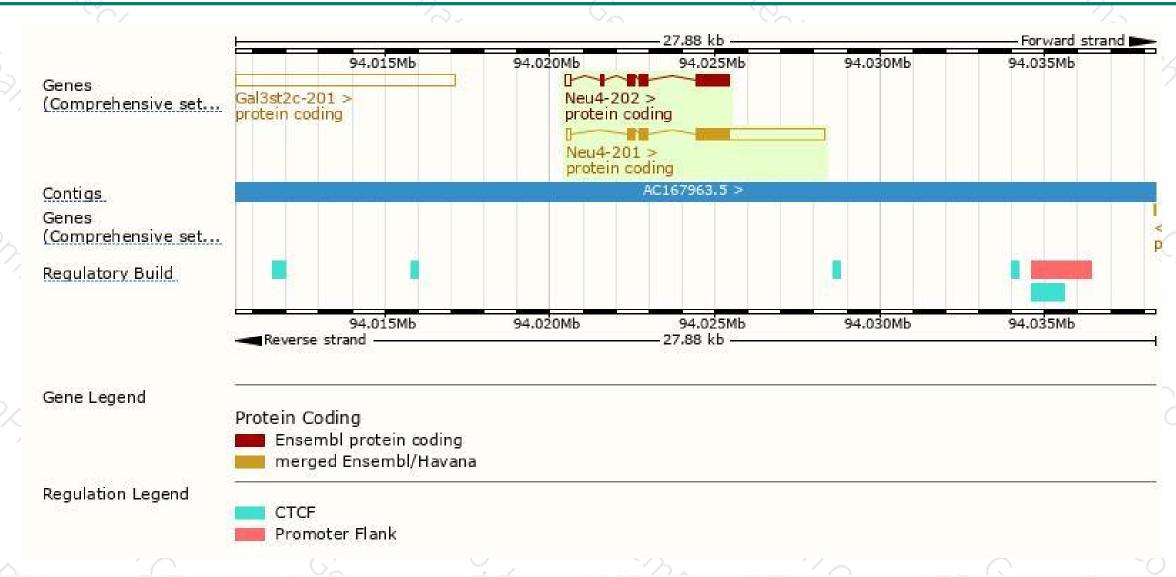
| Name     | Transcript ID        | bp   | Protein      | Biotype        | CCDS      | UniProt       | Flags                           |
|----------|----------------------|------|--------------|----------------|-----------|---------------|---------------------------------|
| Neu4-201 | ENSMUST00000050890.7 | 4511 | 478aa        | Protein coding | CCDS15199 | Q8BZL1        | TSL:1 GENCODE basic APPRIS P3   |
| Neu4-202 | ENSMUST00000190212.6 | 1719 | <u>501aa</u> | Protein coding | CCDS78660 | C5NTX9 Q8BZL1 | TSL:1 GENCODE basic APPRIS ALT2 |

The strategy is based on the design of Neu4-201 transcript, The transcription is shown below



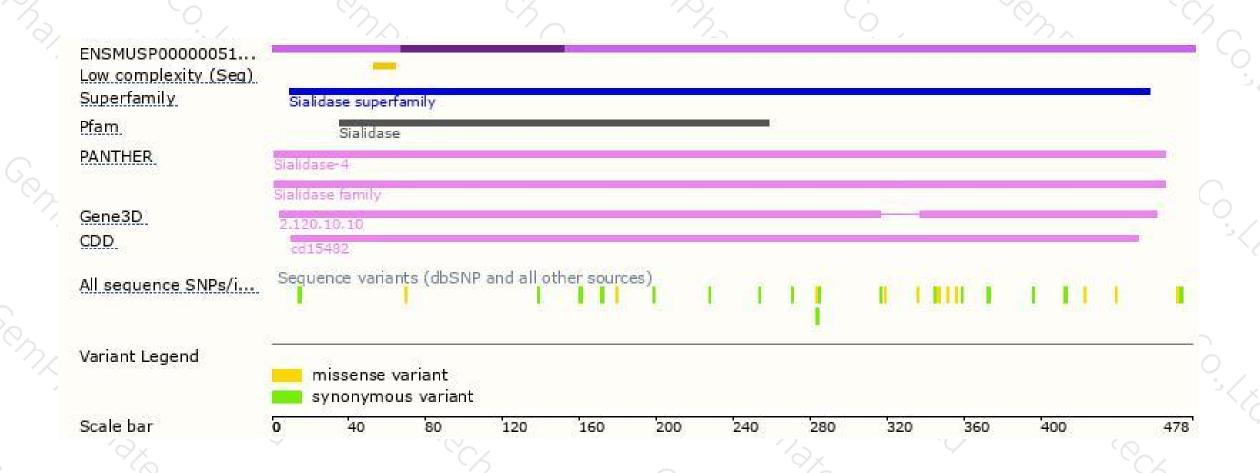
### Genomic location distribution





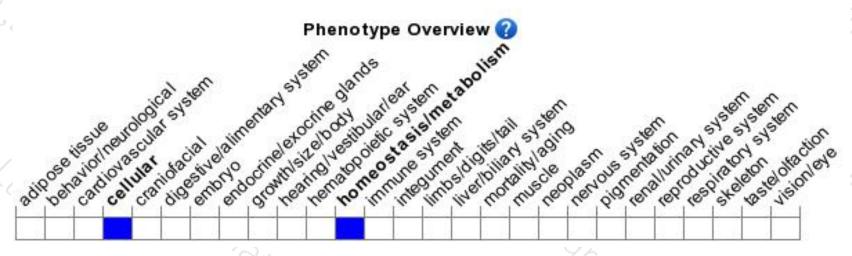
### Protein domain





# 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 null allele are largely normal except increased lipid content in the lung and liver and vacuolization indicative of lysosomal storage disorder.



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





