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Nup35 Cas9-KO Strategy

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Reviewer: Jingling Wang

Design Date: 2024-3-11

Overview

Target Gene Name

- Nup35

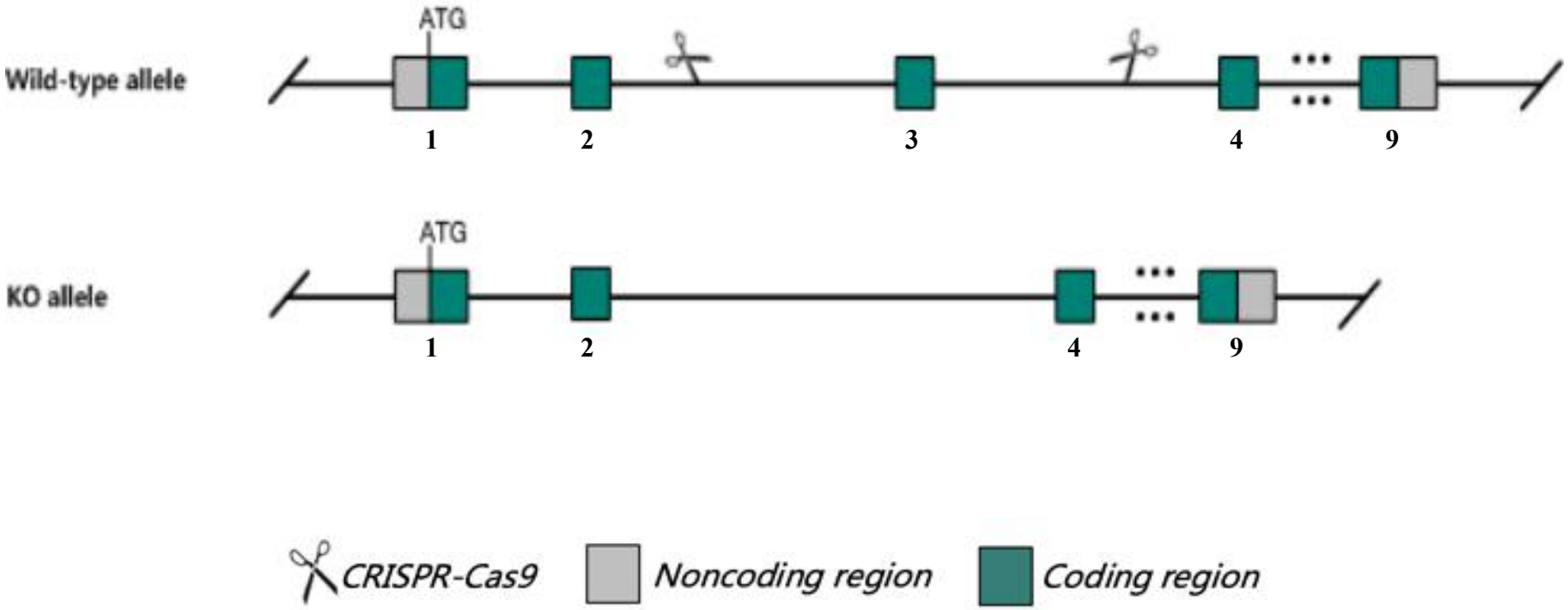
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

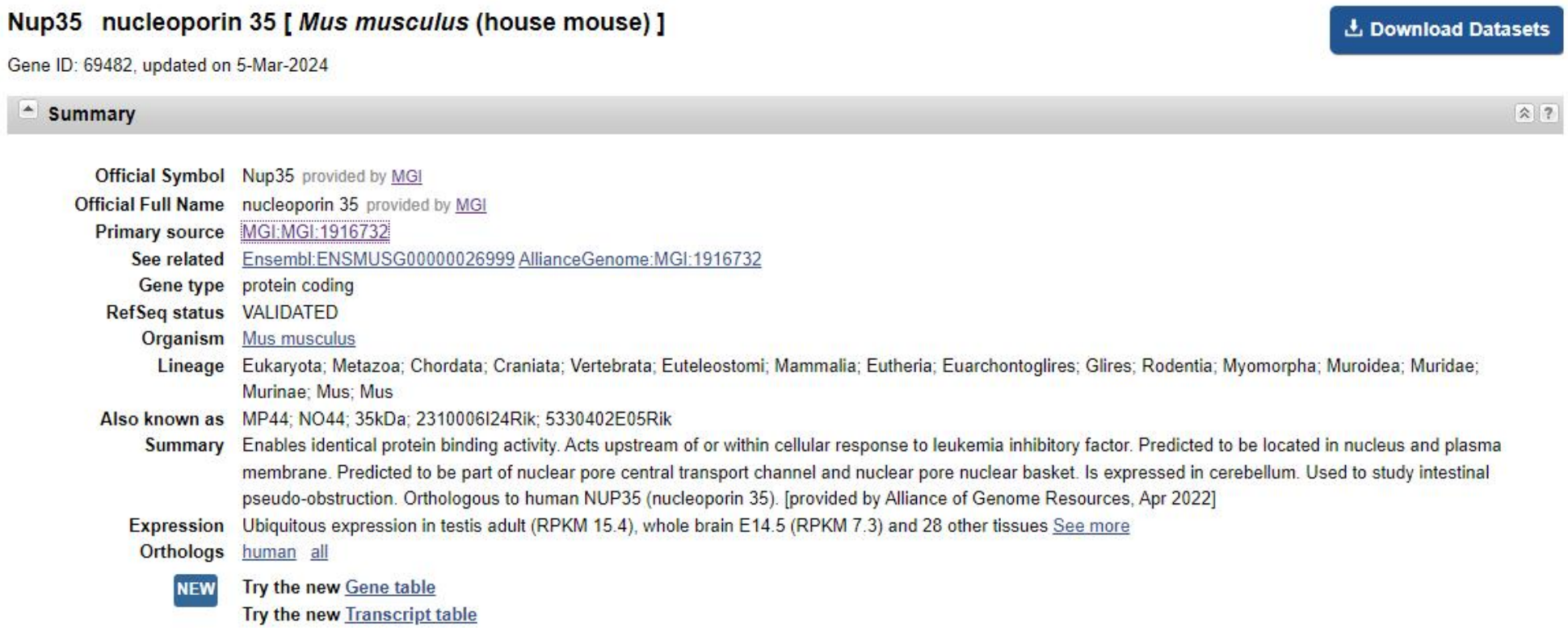
Strain Strategy



Technical Information

- The *Nup35* gene has 5 transcripts. According to the structure of *Nup35* gene, exon3 of *Nup35*-201 (ENSMUST00000028382.13) transcript is recommended as the knockout region. The region contains 128bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Nup35* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

Gene Information



Nup35 nucleoporin 35 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 69482, updated on 5-Mar-2024

Summary

Official Symbol	Nup35 provided by MGI
Official Full Name	nucleoporin 35 provided by MGI
Primary source	MGI:MGI:1916732
See related	Ensembl:ENSMUSG00000026999 AllianceGenome:MGI:1916732
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	MP44; NO44; 35kDa; 2310006I24Rik; 5330402E05Rik
Summary	Enables identical protein binding activity. Acts upstream of or within cellular response to leukemia inhibitory factor. Predicted to be located in nucleus and plasma membrane. Predicted to be part of nuclear pore central transport channel and nuclear pore nuclear basket. Is expressed in cerebellum. Used to study intestinal pseudo-obstruction. Orthologous to human NUP35 (nucleoporin 35). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in testis adult (RPKM 15.4), whole brain E14.5 (RPKM 7.3) and 28 other tissues See more
Orthologs	human all

NEW Try the new Gene table

Try the new Transcript table

Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

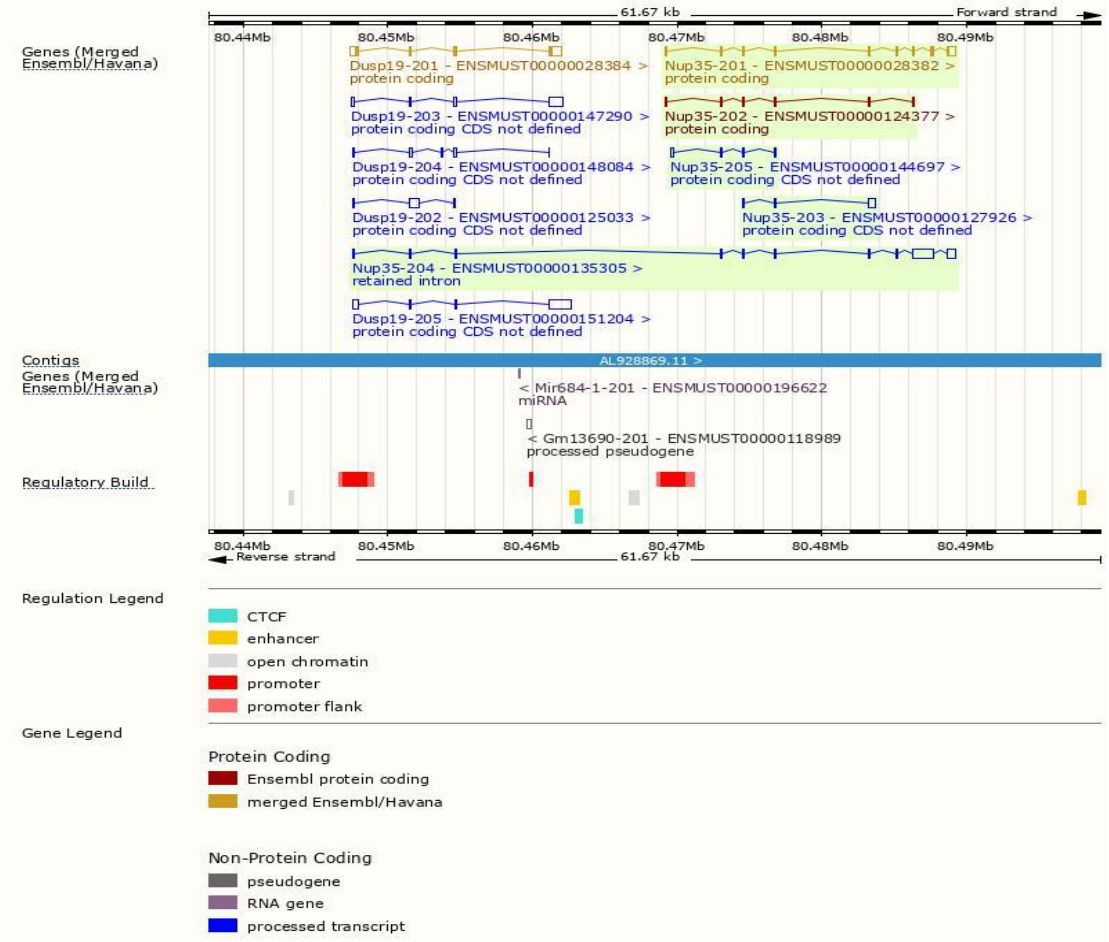
The gene has 5 transcripts,all transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000028382.13	Nup35-201	1559	325aa	Protein coding	CCDS16179	Q8R4R6	Ensembl Canonical, GENCODE basic, APPRIS P1, TSL:1
ENSMUST00000124377.2	Nup35-202	653	197aa	Protein coding		A2ATJ2	TSL:3, CDS 3' incomplete
ENSMUST00000127926.2	Nup35-203	692	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000135305.8	Nup35-204	2688	No protein	Retained intron		-	TSL:2
ENSMUST00000144697.2	Nup35-205	496	No protein	Protein coding CDS not defined		-	TSL:3

The strategy is based on the design of *Nup35*-201 transcript, the transcription is shown below:

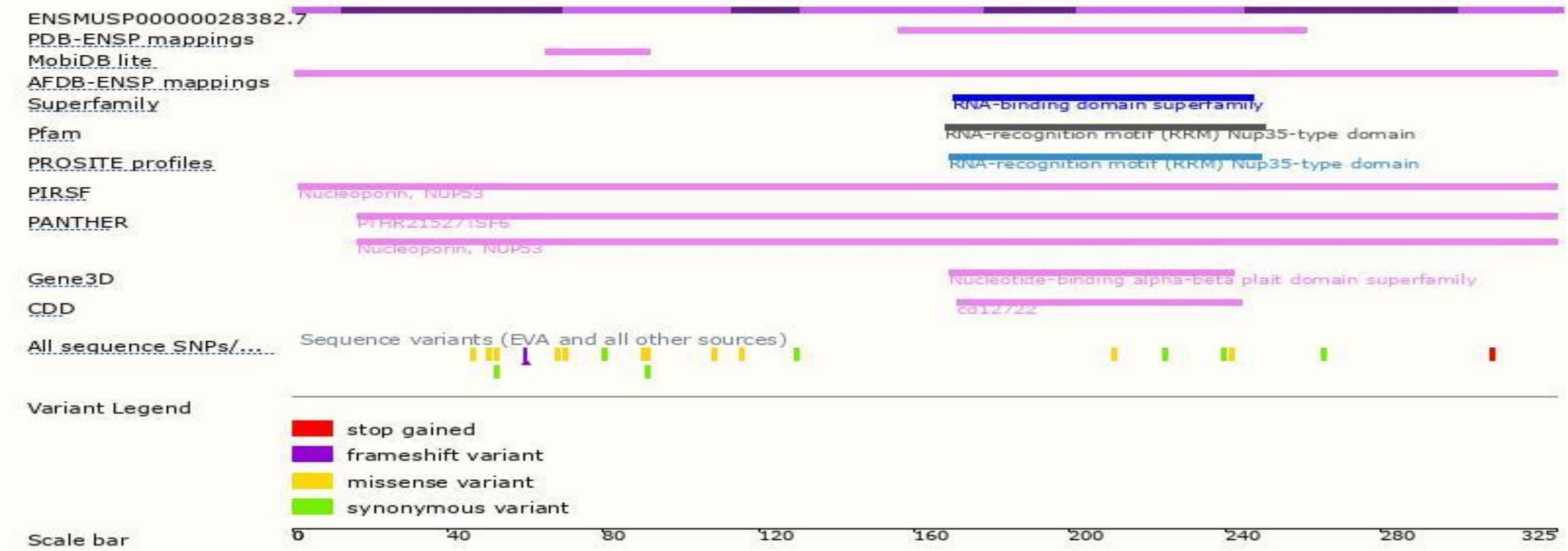
Source: https://www.ensembl.org

Genomic Information



Source: : https://www.ensembl.org

Protein Information



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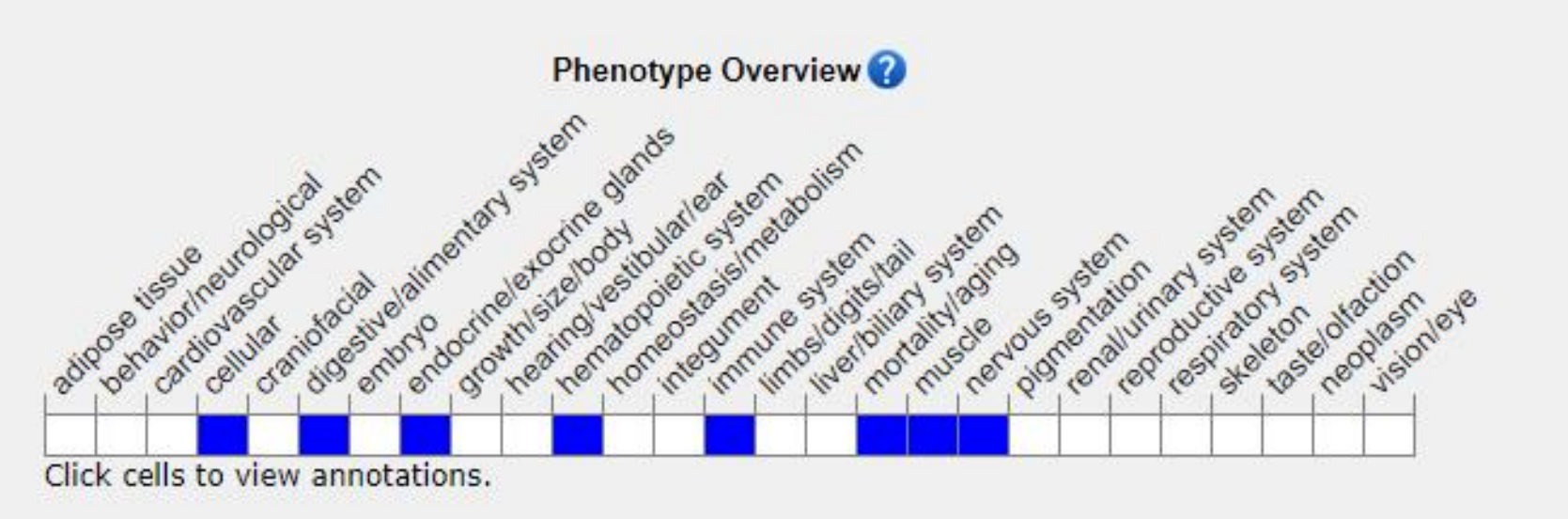
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Homozygous knockout through a point mutation in a critical functional domain leads to early death as a result of megacolon caused by colon myopathy.

Source: https://www.informatics.jax.org

Important Information

- *Nup35* is located on Chr2. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

References

Targeted (Conditional ready, Null/knockout, Reporter)

Insertion *Vector:* L1L2_Bact_P

Mutation details: The L1L2_Bact_P cassette was inserted at position 80473824 of Chromosome 2 upstream of the critical exon(s) (Build GRCm39). The cassette is composed of an FRT site followed by lacZ sequence and a loxP site. This first loxP site is followed by a neomycin resistance gene under the control of the human beta-actin promoter, SV40 polyA, a second FRT site and a second loxP site. A third loxP site is inserted downstream of the targeted exon(s) at position 80474761. The critical exon(s) is/are thus flanked by loxP sites. A "conditional ready" (floxed) allele can be created by flp recombinase expression in mice carrying this allele. Subsequent cre expression results in a knockout mouse. If cre expression occurs without flp expression, a reporter knockout mouse will be created. Further information on targeting strategies used for this and other IKMC alleles can be found at http://www.informatics.jax.org/mgihome/nomen/IKMC_schematics.shtml (*J:155845, J:173534*)

https://www.informatics.jax.org/allele/MGI:5008019