Ntf3 Cas9-KO Strategy

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Reviewer: Shuang Zhang

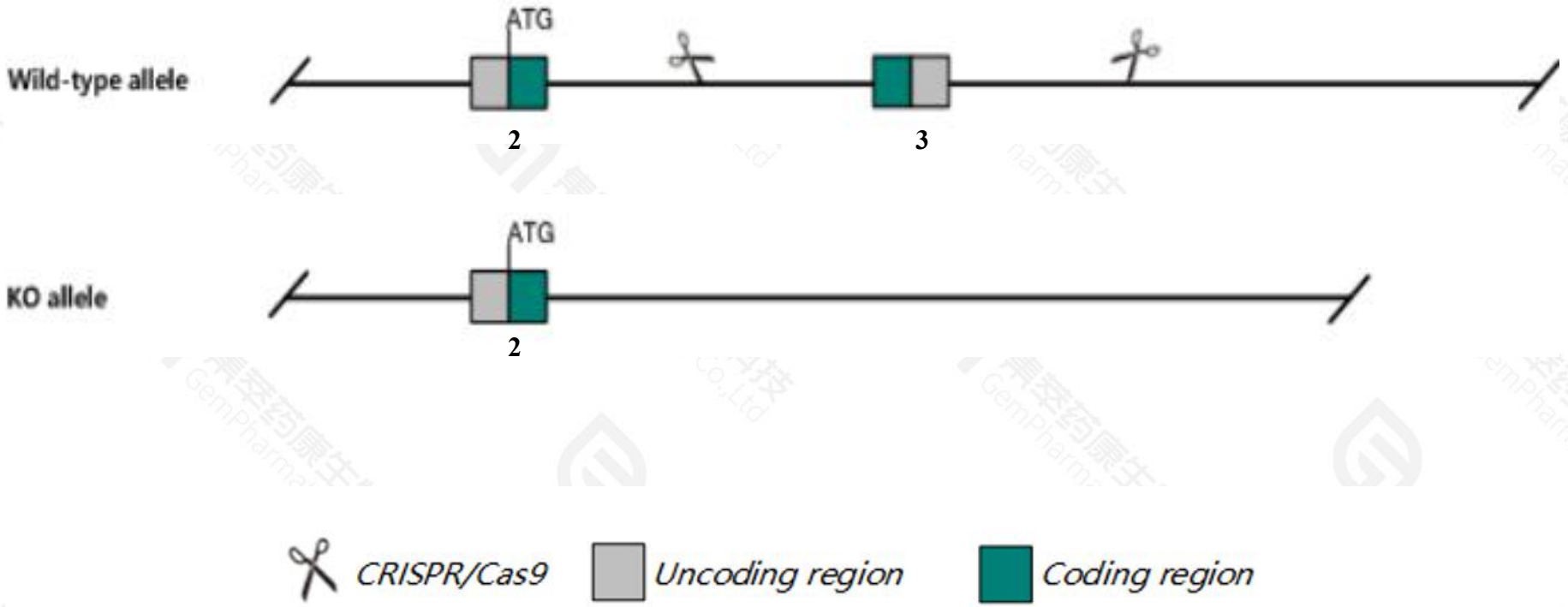
Design Date: 2021-5-19

Project Overview

Project Name	***Ntf3***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ntf3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ntf3* gene has 3 transcripts. According to the structure of *Ntf3* gene, exon3 of *Ntf3-202*(ENSMUST00000112244.8) transcript is recommended as the knockout region.The region contains most 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 *Ntf3* 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.

Notice

➢ According to the existing MGI data,homozygotes for targeted null mutations exhibit loss of peripheral sensory and sympathetic neurons, lack of spinal propioceptive afferents and their sense organs, impaired suckling and movement, and postnatal lethality. Heterozygotes show mild defects.

➢ The *Ntf3* gene is located on the Chr6. 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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Ntf3 neurotrophin 3 [Mus musculus (house mouse)]

Gene ID: 18205, updated on 17-Dec-2020

Summary

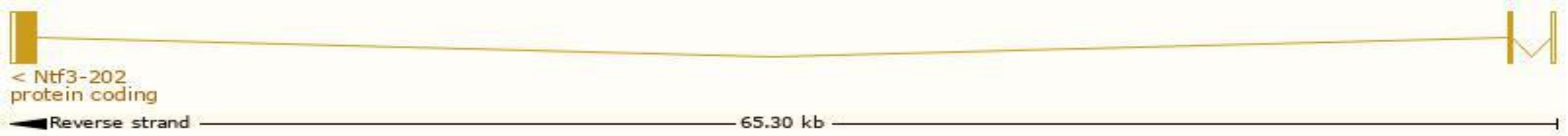
Official Symbol	Ntf3 provided by MGI
Official Full Name	neurotrophin 3 provided by MGI
Primary source	MGI:MGI:97380
See related	Ensembl:ENSMUSG00000049107
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI316846, AI835689, HDNF, NGF-2, NT, NT-, Nt3, Ntf-, Ntf-3
Summary	This gene encodes a member of the neurotrophins that have a wide variety of functions in both neural and non-neural tissues. The encoded preproprotein undergoes proteolytic processing to generate a noncovalently linked homodimeric mature protein that can bind to the transmembrane receptor tyrosine kinases to initiate a series of signaling events. Mice lacking the encoded protein exhibit severe defects in the peripheral nervous system including a complete lack of spinal proprioceptive afferents and their peripheral sense organs. [provided by RefSeq, Sep 2016]
Expression	Broad expression in bladder adult (RPKM 4.6), limb E14.5 (RPKM 4.0) and 21 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

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

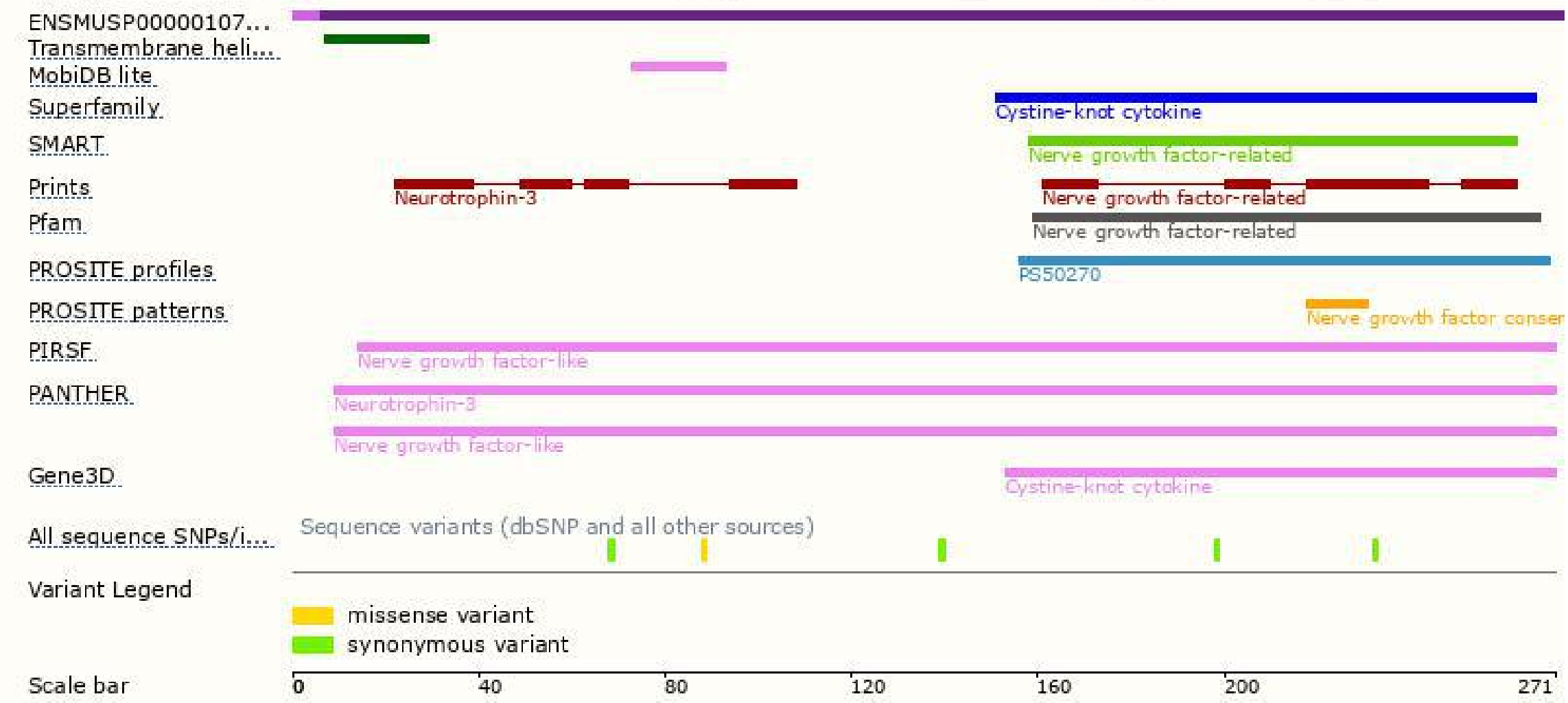
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ntf3-201	ENSMUST00000050484.9	1483	258aa	Protein coding	CCDS20553		TSL:2 , GENCODE basic , APPRIS P3 ,
Ntf3-202	ENSMUST00000112244.8	1399	271aa	Protein coding	CCDS51914		TSL:3 , GENCODE basic , APPRIS ALT1 ,
Ntf3-203	ENSMUST00000204542.3	1343	271aa	Protein coding	CCDS51914		TSL:1 , GENCODE basic , APPRIS ALT1 ,

The strategy is based on the design of *Ntf3-202* transcript,the transcription is shown below:

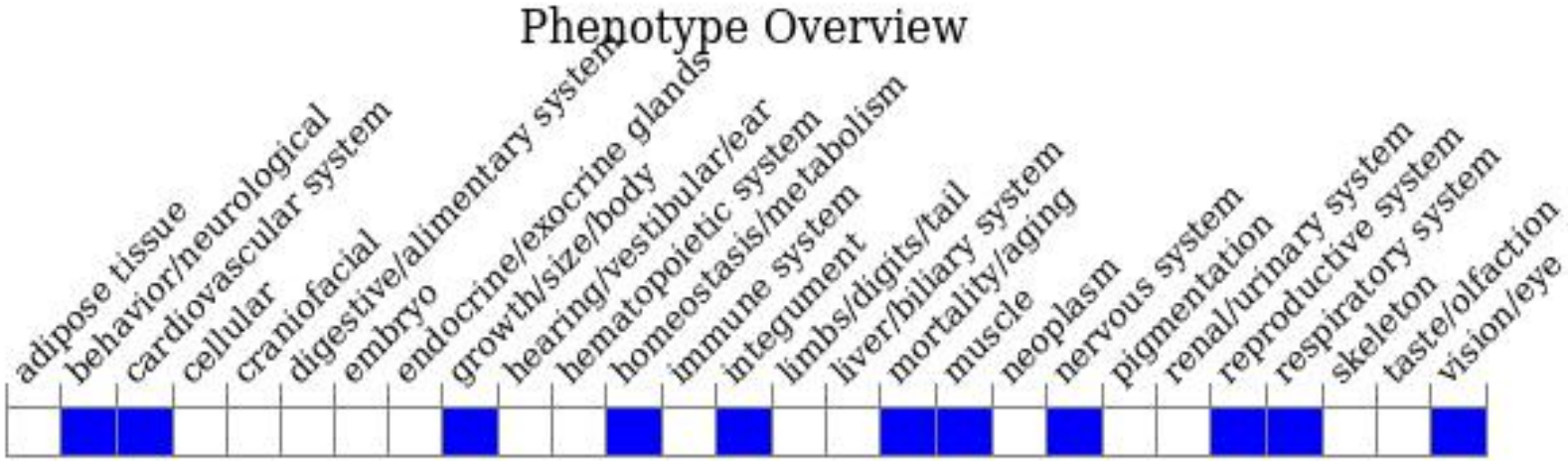


Genomic location distribution

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/).

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