

# ***Ngef Cas9-CKO Strategy***

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# Project Overview

**Project Name**

*Ngef*

**Project type**

**Cas9-CKO**

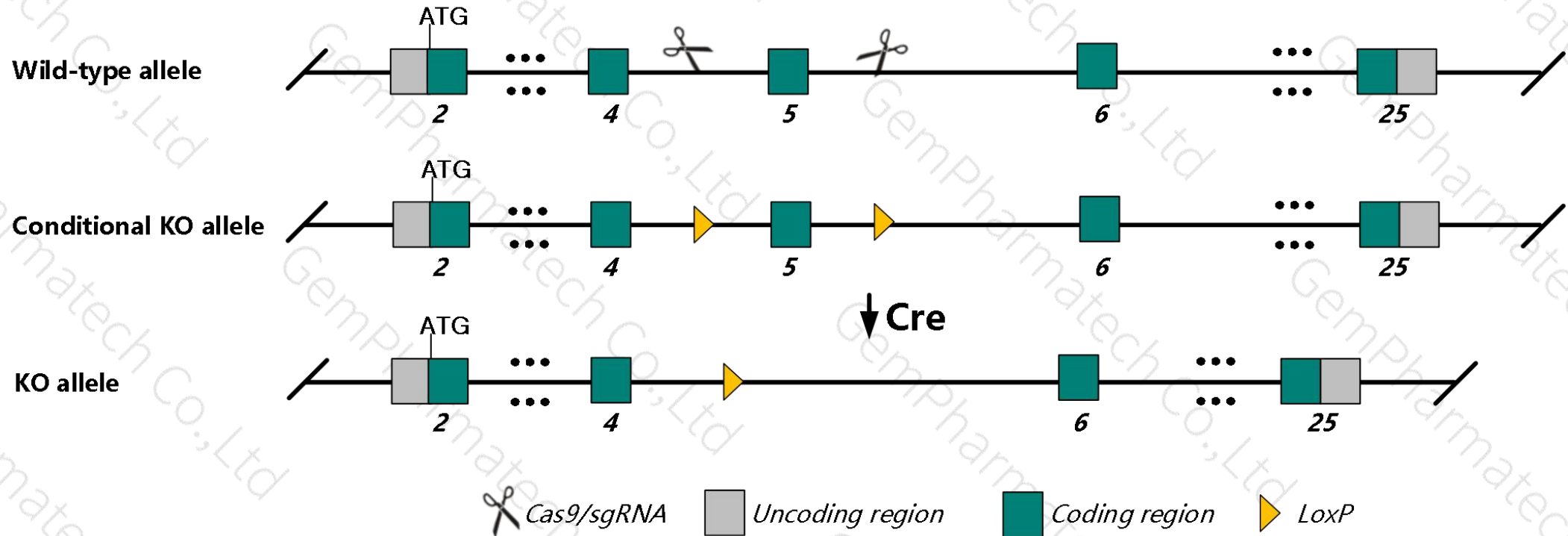
**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

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

Donor and CRISPR/Cas9 System



# Technical routes

- The *Ngef* gene has 4 transcripts. According to the structure of *Ngef* gene, exon5 of *Ngef*-202 (ENSMUST00000068681.11) transcript is recommended as the knockout region. The region contains 308bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ngef* 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.

- According to the MGI date, mice homozygous for a knock-out allele are viable and fertile and show no overt axonal phenotype; however, cultured retinal ganglion cells display defects in axonal outgrowth and ephrin-induced growth cone collapse.
- The transcript *Ngef-204* is incomplete, so the effect on it is unknown.
- The *Ngef* 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)

## Ngef neuronal guanine nucleotide exchange factor [ *Mus musculus* (house mouse) ]

Gene ID: 53972, updated on 13-Mar-2020

### Summary

- Official Symbol** Ngef provided by [MGI](#)
- Official Full Name** neuronal guanine nucleotide exchange factor provided by [MGI](#)
- Primary source** [MGI:MGI:1858414](#)
- See related** [Ensembl:ENSMUSG00000026259](#)
- 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** Tims2; ephexin
- Expression** Biased expression in cortex adult (RPKM 59.5), frontal lobe adult (RPKM 38.3) and 10 other tissues [See more](#)
- Orthologs** [human](#) [all](#)

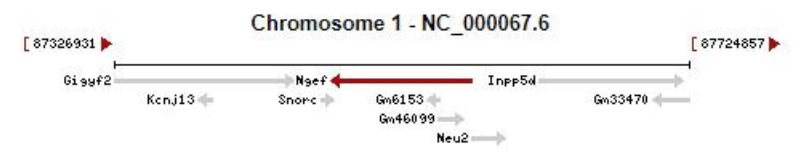
### Genomic context

Location: 1 D; 1 44.42 cM

See Ngef in [Genome Data Viewer](#)

Exon count: 16

Annotation release	Status	Assembly	Chr	Location
<a href="#">108</a>	current	GRCm38.p6 ( <a href="#">GCF_000001635.26</a> )	1	NC_000067.6 (87476829..87573870, complement)
Build 37.2	previous assembly	MGSCv37 ( <a href="#">GCF_000001635.18</a> )	1	NC_000067.5 (89373404..89470445, complement)

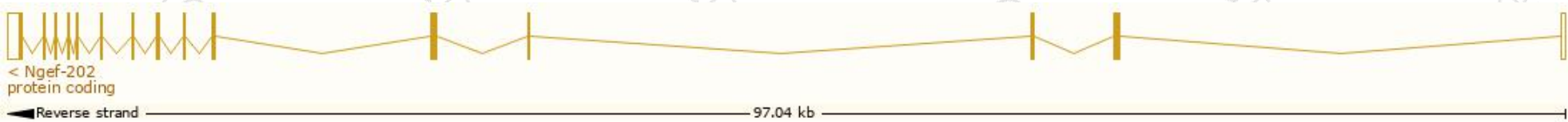


# Transcript information (Ensembl)

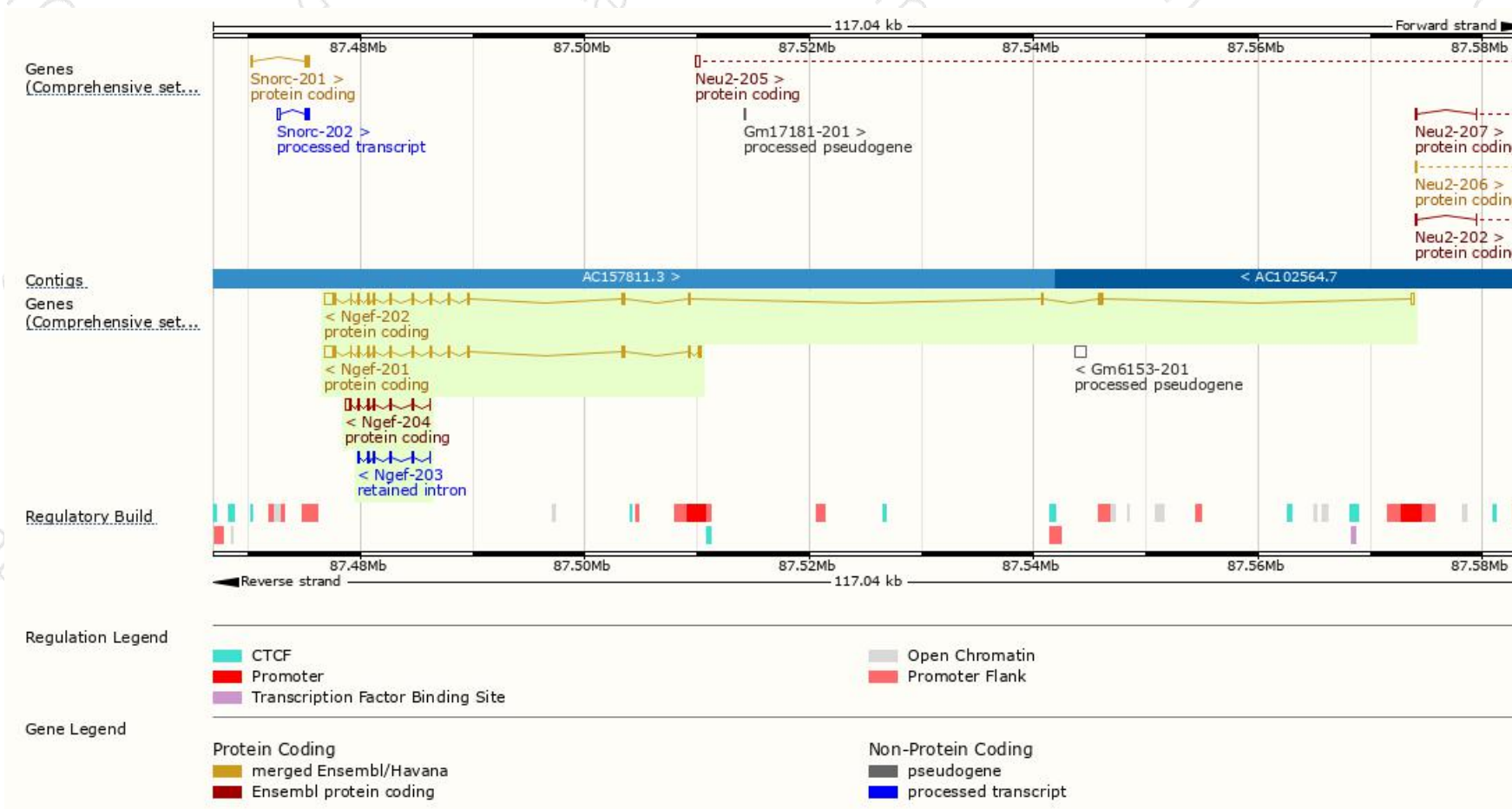
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ngef-202	<a href="#">ENSMUST00000068681.11</a>	3109	<a href="#">710aa</a>	Protein coding	<a href="#">CCDS48307</a>	<a href="#">E9QK62</a>	TSL:1 GENCODE basic APPRIS P4
Ngef-201	<a href="#">ENSMUST00000027477.14</a>	2746	<a href="#">620aa</a>	Protein coding	<a href="#">CCDS48306</a>	<a href="#">Q8CHT1</a>	TSL:1 GENCODE basic APPRIS ALT2
Ngef-204	<a href="#">ENSMUST00000168235.1</a>	1110	<a href="#">245aa</a>	Protein coding	-	<a href="#">F6SWQ9</a>	CDS 5' incomplete TSL:3
Ngef-203	<a href="#">ENSMUST00000166463.1</a>	735	No protein	Retained intron	-	-	TSL:2

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



# Genomic location distribution

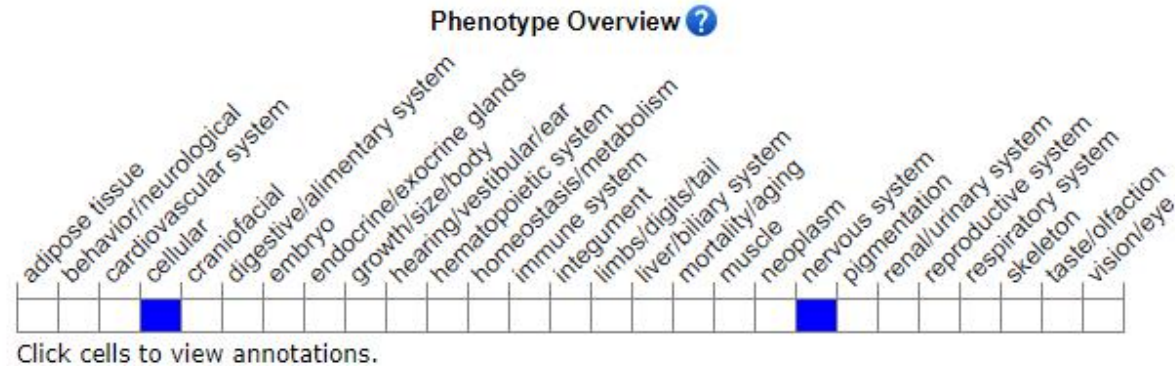




# Protein domain



# Mouse phenotype description(MGI)



*Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).*

According to the existing MGI data, mice homozygous for a knock-out allele are viable and fertile and show no overt axonal phenotype; however, cultured retinal ganglion cells display defects in axonal outgrowth and ephrin-induced growth cone collapse.

If you have any questions, you are welcome to inquire.

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