Ntn1 Cas9-CKO Strategy

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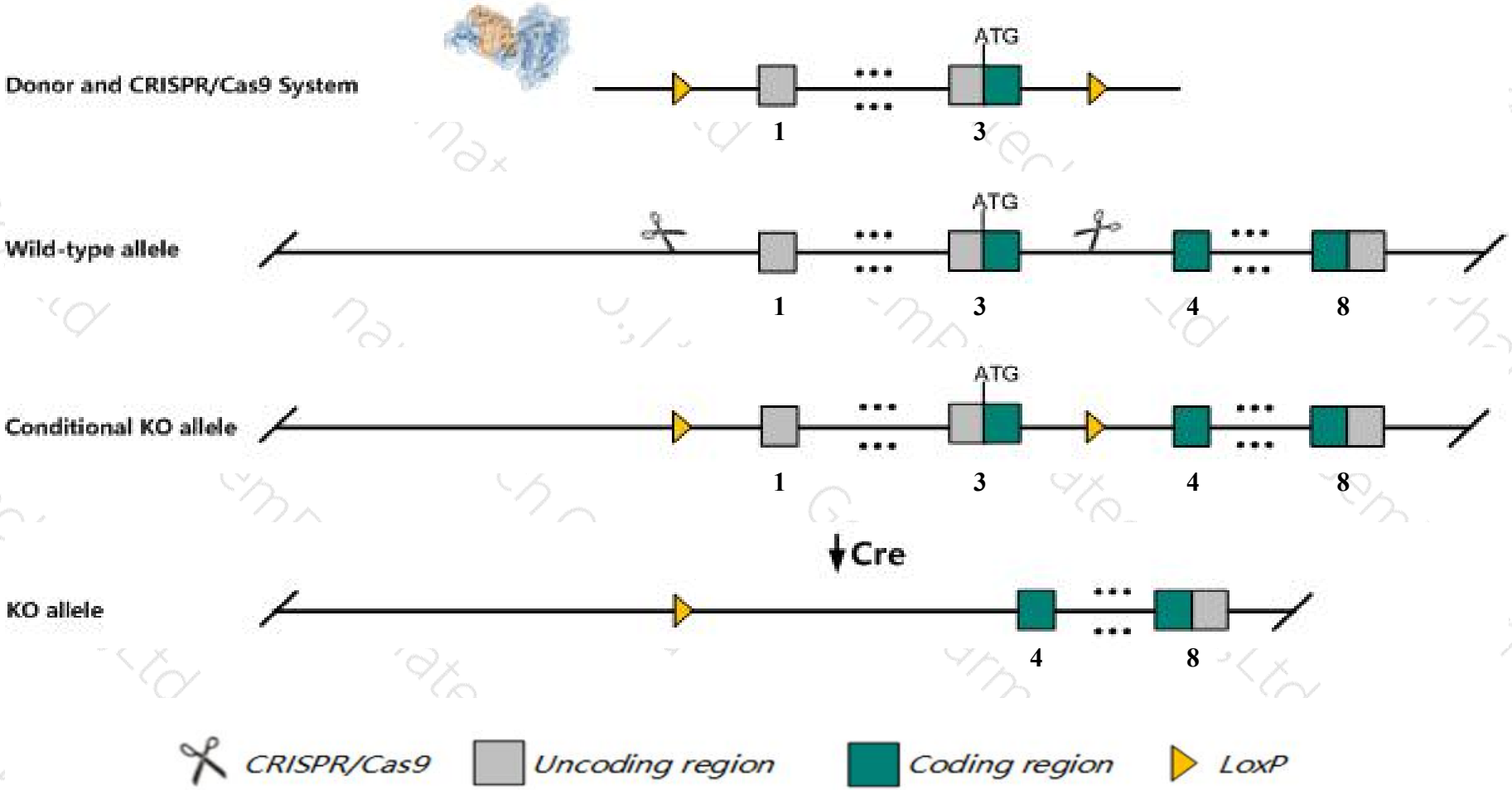
Project Overview

Project Name	***Ntn1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ntn1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ntn1* gene has 3 transcripts. According to the structure of *Ntn1* gene, exon1-exon3 of *Ntn1-202*(ENSMUST00000108674.8) transcript is recommended as the knockout region.The region contains start codon ATG. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ntn1* 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,homozygotes for targeted mutations exhibit impaired axonal migration, abnormal semicircular canals, lack of corpus callosum, aberrant commissures, hypoplasia of the optic nerve, motor and balance defects, failure to suckle, and neonatal death.

➢ The *Ntn1* gene is located on the Chr11. 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）

集萃药康 GemPharmatech

Ntn1 netrin 1 [Mus musculus (house mouse)]

Gene ID: 18208, updated on 13-Mar-2020

Summary

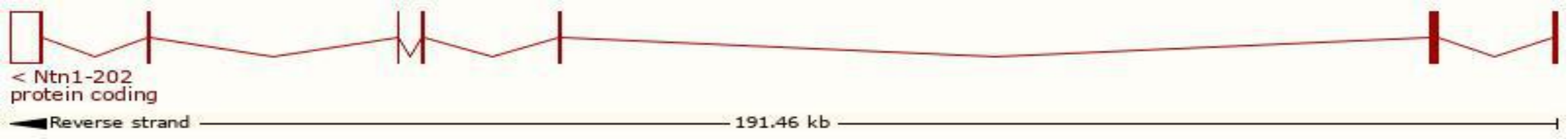
Official Symbol	Ntn1 provided by MGI
Official Full Name	netrin 1 provided by MGI
Primary source	MGI:MGI:105088
See related	Ensembl:ENSMUSG00000020902
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI561871, BC019633, Netrin-1
Expression	Ubiquitous expression in lung adult (RPKM 25.3), colon adult (RPKM 12.9) and 23 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

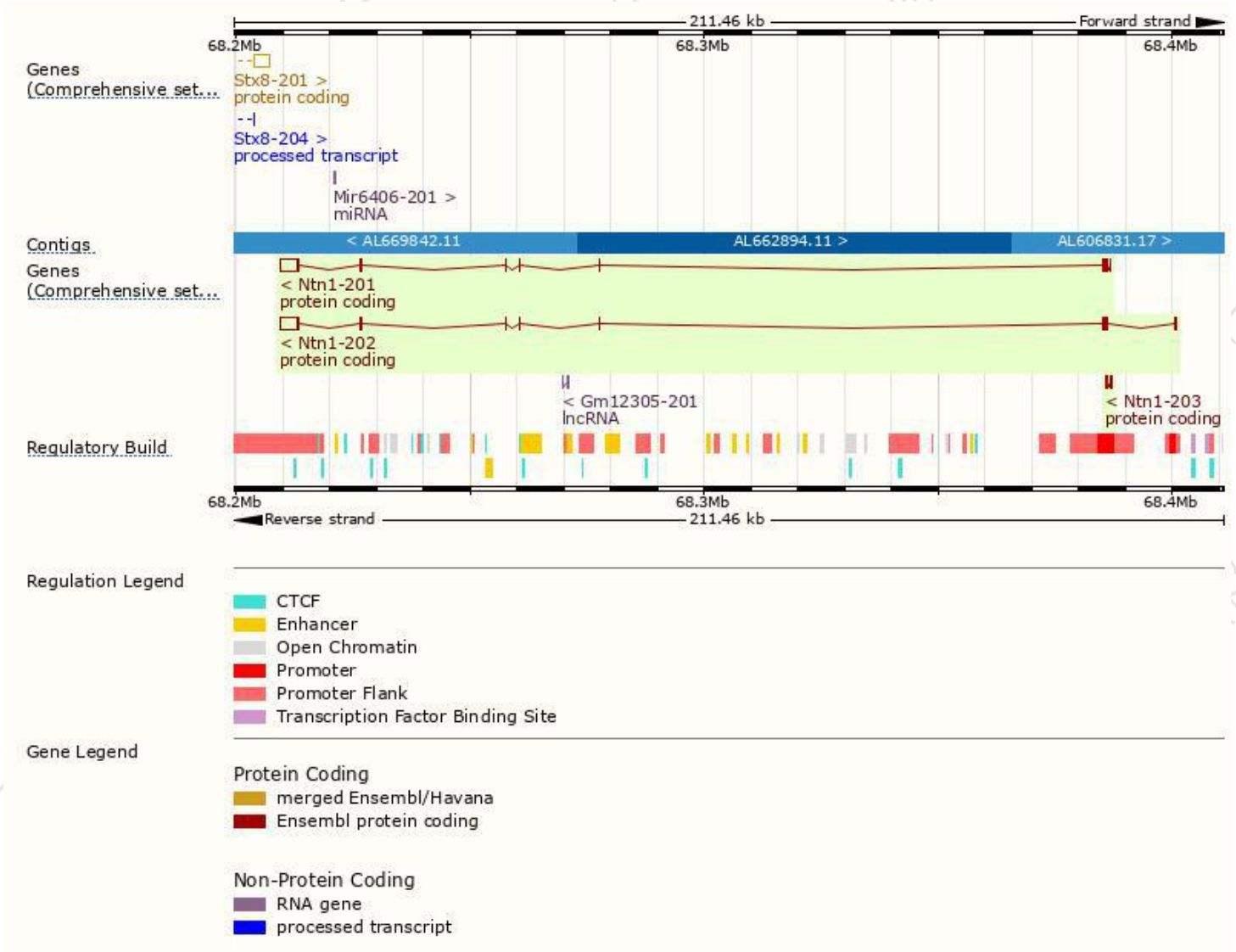
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ntn1-202	ENSMUST00000108674.8	5874	604aa	Protein coding	CCDS24864	O09118	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Ntn1-201	ENSMUST00000021284.3	5738	604aa	Protein coding	CCDS24864	O09118	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Ntn1-203	ENSMUST00000135141.1	944	159aa	Protein coding	-	B0QZL0	CDS 3' incomplete TSL:2

The strategy is based on the design of *Ntn1-202* transcript,the transcription is shown below:

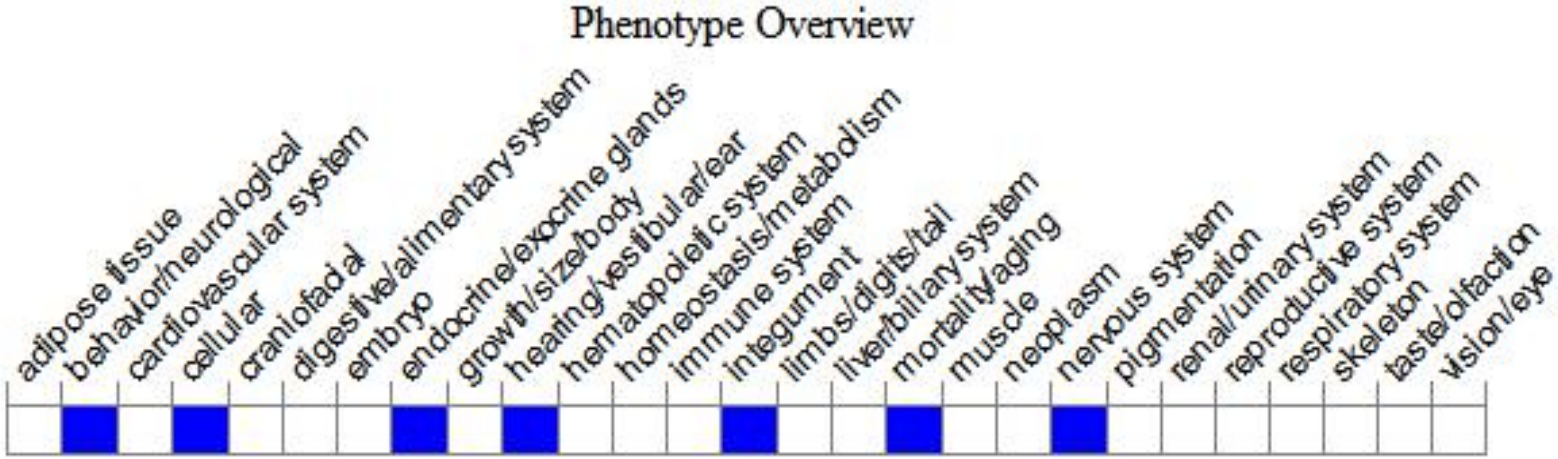


Genomic location distribution



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

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