Ntrk3 Cas9-CKO Strategy

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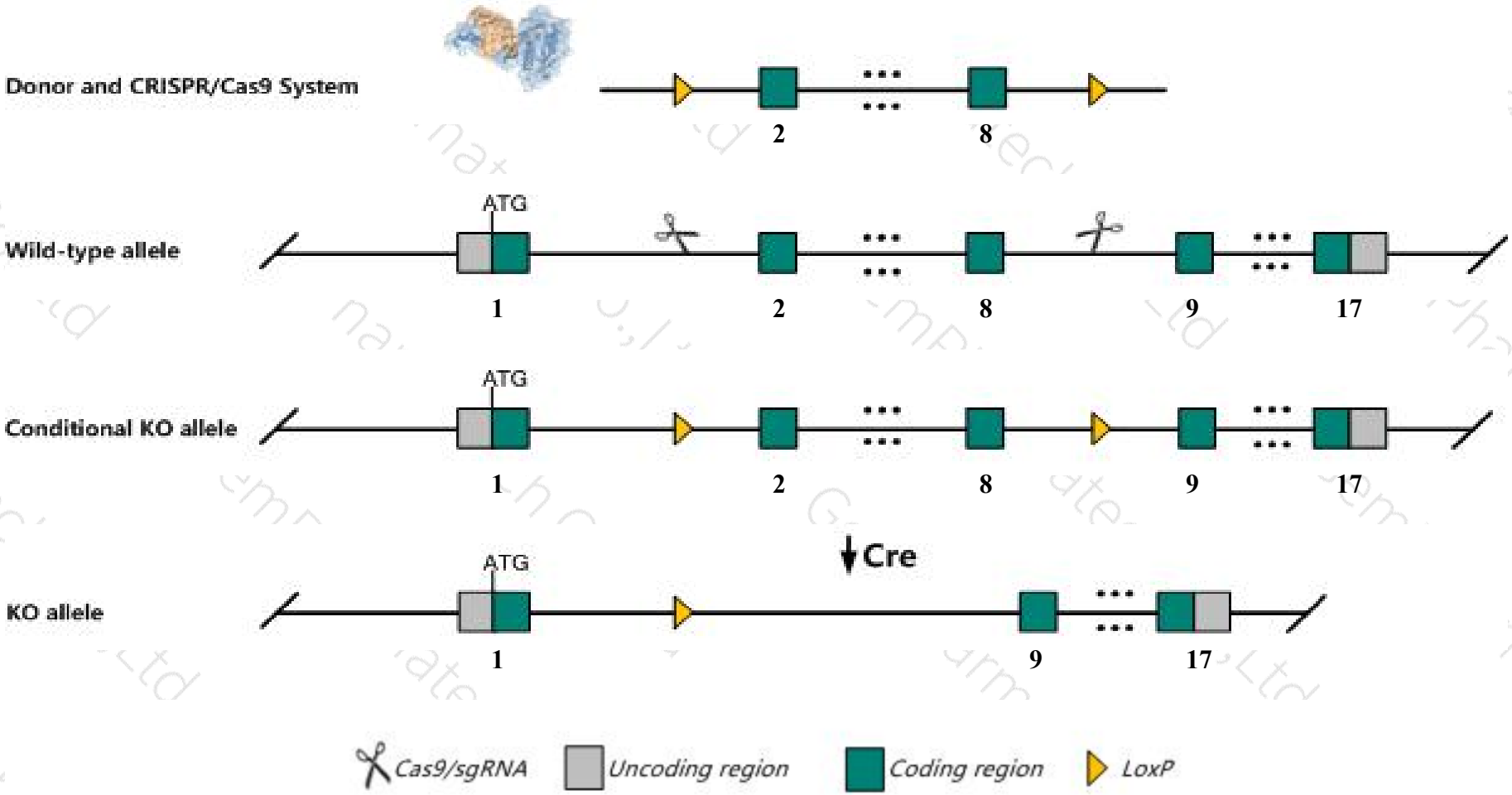
Project Overview

Project Name	***Ntrk3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ntrk3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ntrk3* gene has 20 transcripts. According to the structure of *Ntrk3* gene, exon2-exon8 of *Ntrk3-201*(ENSMUST00000039431.13) transcript is recommended as the knockout region.The region contains 956bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ntrk3* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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 show a range of phenotypes including postnatal death at 2-21 days, cardiac defects, reduced numbers of dorsal root ganglia neurons and germ cells, abnormal motor coordination and posture and abnormal sensory innervation.

➢ Transcript *Ntrk3-212* may not be affected.

➢ The *Ntrk3* gene is located on the Chr7. 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）

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Ntrk3 neurotrophic tyrosine kinase, receptor, type 3 [Mus musculus (house mouse)]

Gene ID: 18213, updated on 13-Mar-2020

Summary

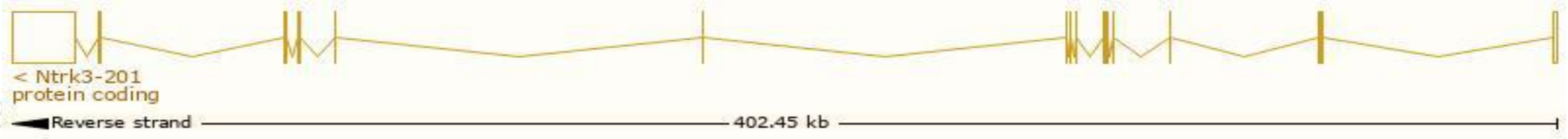
Official Symbol	Ntrk3 provided by MGI
Official Full Name	neurotrophic tyrosine kinase, receptor, type 3 provided byMGI
Primary source	MGI:MGI:97385
See related	Ensembl:ENSMUSG00000059146
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW125844, Ntrk3_tv3, Trk, TrkC
Expression	Broad expression in cerebellum adult (RPKM 7.6), CNS E18 (RPKM 6.3) and 16 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

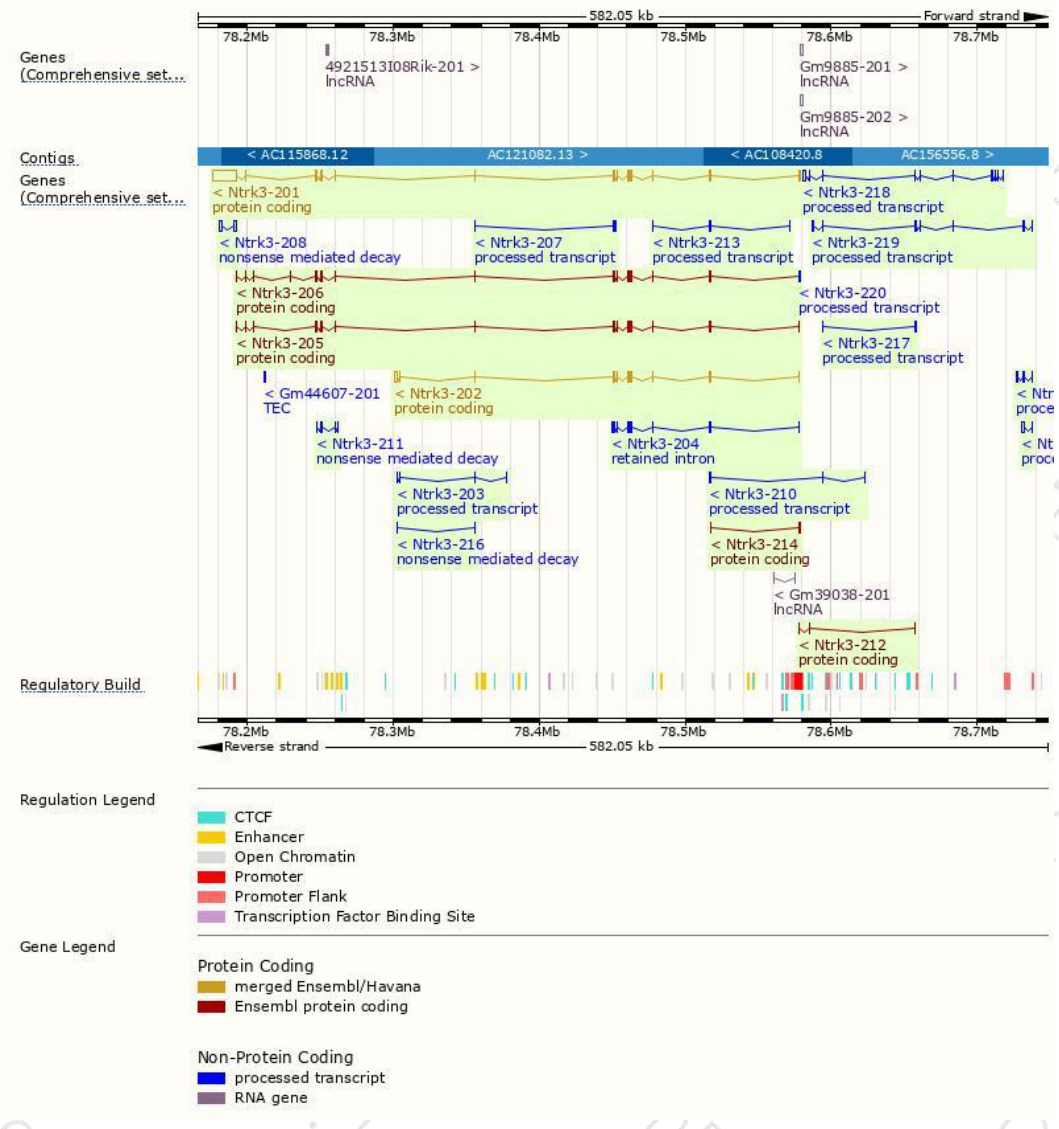
The gene has 20 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ntrk3-201	ENSMUST00000039431.13	19703	825aa	Protein coding	CCDS21371	Q6VNS1	TSL:1 GENCODE basic APPRIS P2
Ntrk3-202	ENSMUST00000039438.8	3977	612aa	Protein coding	CCDS21372	Q6VNS1	TSL:1 GENCODE basic
Ntrk3-206	ENSMUST00000195262.5	2774	864aa	Protein coding	-	A0A0A6YWL7	TSL:5 GENCODE basic APPRIS ALT2
Ntrk3-205	ENSMUST00000193002.5	2678	839aa	Protein coding	-	A0A0A6YWF9	TSL:5 GENCODE basic APPRIS ALT2
Ntrk3-214	ENSMUST00000206268.1	426	86aa	Protein coding	-	A0A0U1RPZ9	CDS 3' incomplete TSL:5
Ntrk3-212	ENSMUST00000206091.1	423	82aa	Protein coding	-	A0A0U1RPF1	CDS 3' incomplete TSL:3
Ntrk3-208	ENSMUST00000205354.1	4386	61aa	Nonsense mediated decay	-	A0A0U1RNS2	CDS 5' incomplete TSL:1
Ntrk3-211	ENSMUST00000205868.1	395	38aa	Nonsense mediated decay	-	A0A0U1RNS9	CDS 5' incomplete TSL:5
Ntrk3-216	ENSMUST00000206599.1	224	12aa	Nonsense mediated decay	-	A0A0U1RP04	CDS 5' incomplete TSL:5
Ntrk3-218	ENSMUST00000206849.1	3091	No protein	Processed transcript	-	-	TSL:1
Ntrk3-209	ENSMUST00000205411.1	1549	No protein	Processed transcript	-	-	TSL:1
Ntrk3-219	ENSMUST00000206877.1	1435	No protein	Processed transcript	-	-	TSL:1
Ntrk3-215	ENSMUST00000206500.1	1115	No protein	Processed transcript	-	-	TSL:1
Ntrk3-203	ENSMUST00000151885.2	866	No protein	Processed transcript	-	-	TSL:1
Ntrk3-217	ENSMUST00000206752.1	511	No protein	Processed transcript	-	-	TSL:3
Ntrk3-220	ENSMUST00000206949.1	435	No protein	Processed transcript	-	-	TSL:2
Ntrk3-210	ENSMUST00000205700.1	431	No protein	Processed transcript	-	-	TSL:5
Ntrk3-213	ENSMUST00000206167.1	390	No protein	Processed transcript	-	-	TSL:5
Ntrk3-207	ENSMUST00000205312.1	364	No protein	Processed transcript	-	-	TSL:3
Ntrk3-204	ENSMUST00000155795.2	2850	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Ntrk3-201* transcript,the transcription is shown below:

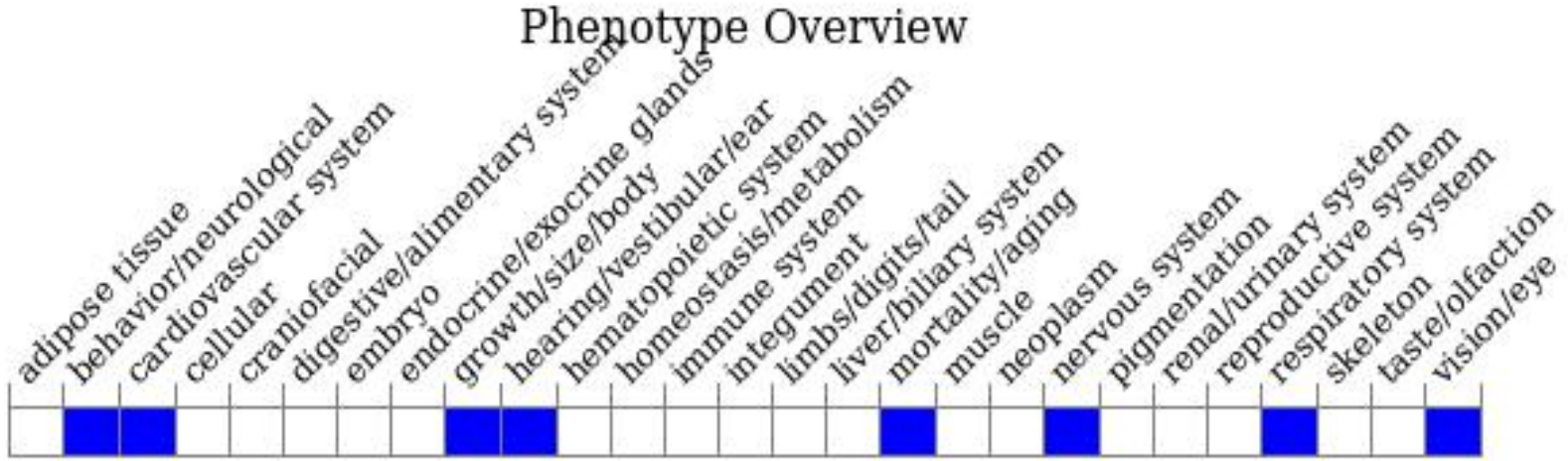


Genomic location distribution



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,homozygotes for targeted mutations show a range of phenotypes including postnatal death at 2-21 days, cardiac defects, reduced numbers of dorsal root ganglia neurons and germ cells, abnormal motor coordination and posture and abnormal sensory innervation.

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

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