

Wnt5b Cas9-CKO Strategy

Designer:

Reviewer:

Design Date:

Yang Zeng

Xueting Zhang

2019-11-29

Project Overview



Project Name

Wnt5b

Project type

Cas9-CKO

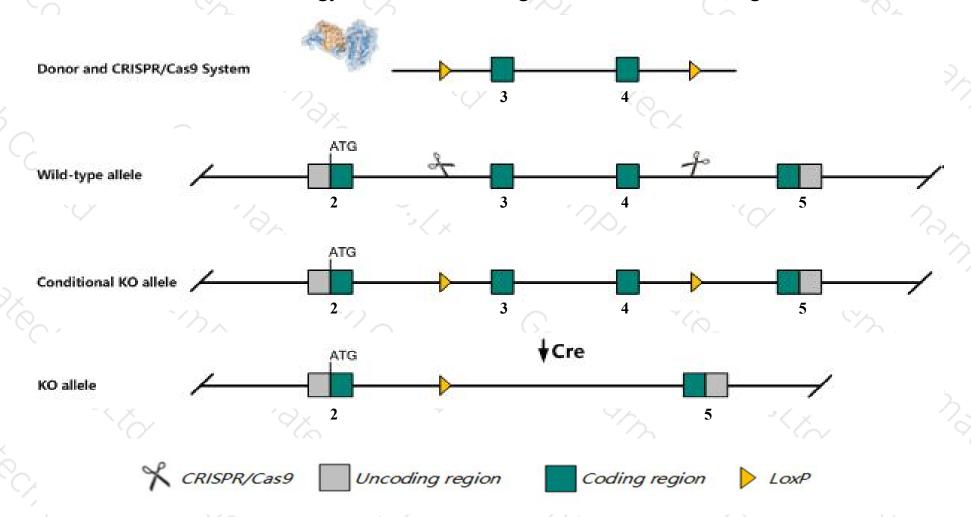
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Wnt5b* gene. The schematic diagram is as follows:



Technical routes



- The *Wnt5b* gene has 4 transcripts. According to the structure of *Wnt5b* gene, exon3-exon4 of *Wnt5b-204* (ENSMUST00000178696.7) transcript is recommended as the knockout region. The region contains 541bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Wnt5b* 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, Mice homozygous for a knock-out allele exhibit normal numbers of thoracic motor neurons and proportions of motor columnar subtypes.
- The *Wnt5b* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)



Wnt5b wingless-type MMTV integration site family, member 5B [Mus musculus (house mouse)]

Gene ID: 22419, updated on 12-Aug-2019

Summary

Official Symbol Wnt5b provided by MGI

Official Full Name wingless-type MMTV integration site family, member 5B provided by MGI

Primary source MGI:MGI:98959

See related Ensembl:ENSMUSG00000030170

Gene type protein coding
RefSeq status VALIDATED

Organism Mus musculus

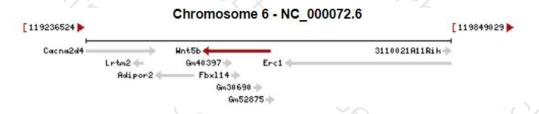
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Muroidea; Muridae; Murinae; Mus; Mus

Also known as Wnt-5b; AW545702

Expression Ubiquitous expression in ovary adult (RPKM 4.7), limb E14.5 (RPKM 3.9) and 25 other tissues <u>See more</u>

Orthologs human all



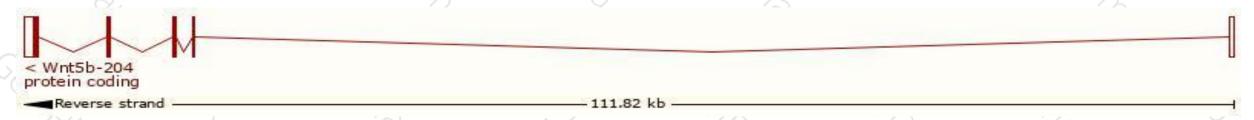
Transcript information (Ensembl)



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

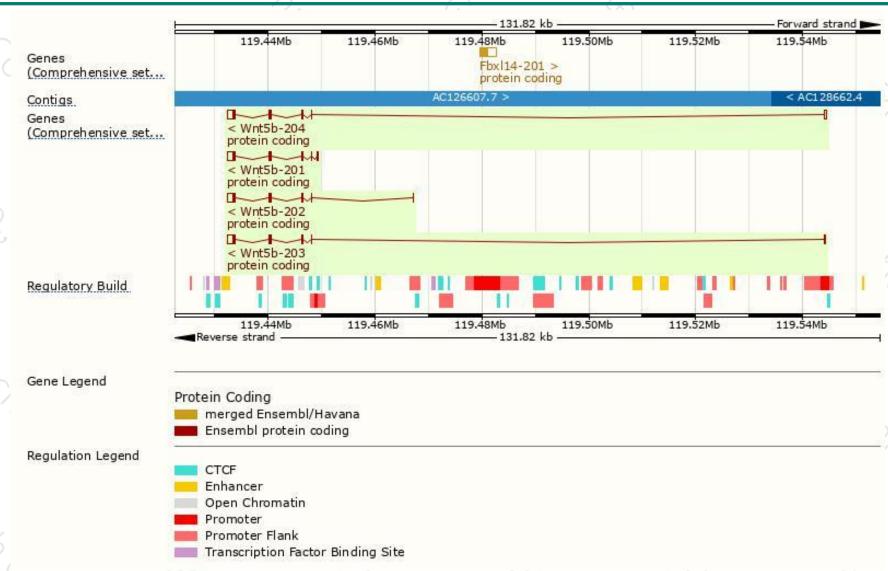
Name 🍦	Transcript ID 👙	bp 🌲	Protein 🍦	Translation ID 🗼	Biotype 🍦	CCDS 🍦	UniProt 🍦	Flags
Wnt5b-204	ENSMUST00000178696.7	2371	<u>359aa</u>	ENSMUSP00000137065.1	Protein coding	CCDS20474₽	P22726译	TSL:1 GENCODE basic APPRIS P2
Wnt5b-201	ENSMUST00000117171.7	2174	359aa	ENSMUSP00000113188.1	Protein coding	CCDS20474₽	P22726₽	TSL:1 GENCODE basic APPRIS P2
Wnt5b-203	ENSMUST00000119369.1	2339	372aa	ENSMUSP00000112448.1	Protein coding	-	A0A0R4J1M1®	TSL:1 GENCODE basic APPRIS ALT2
Wnt5b-202	ENSMUST00000118120.7	2115	<u>321aa</u>	ENSMUSP00000112819.1	Protein coding	-	D3YTW9₽	TSL:1 GENCODE basic APPRIS ALT2

The strategy is based on the design of Wnt5b-204 transcript, The transcription is shown below



Genomic location distribution





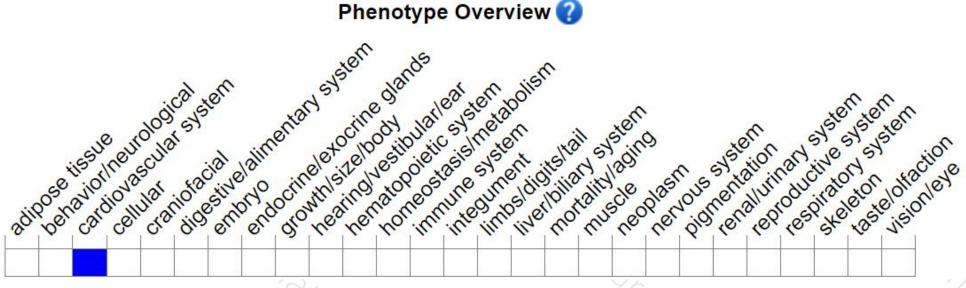
Protein domain





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 exhibit normal numbers of thoracic motor neurons and proportions of motor columnar subtypes.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





