Plxna4 Cas9-CKO Strategy

Designer: Yang Zeng
Reviewer: Jia Yu
Design Date: 2020-2-10

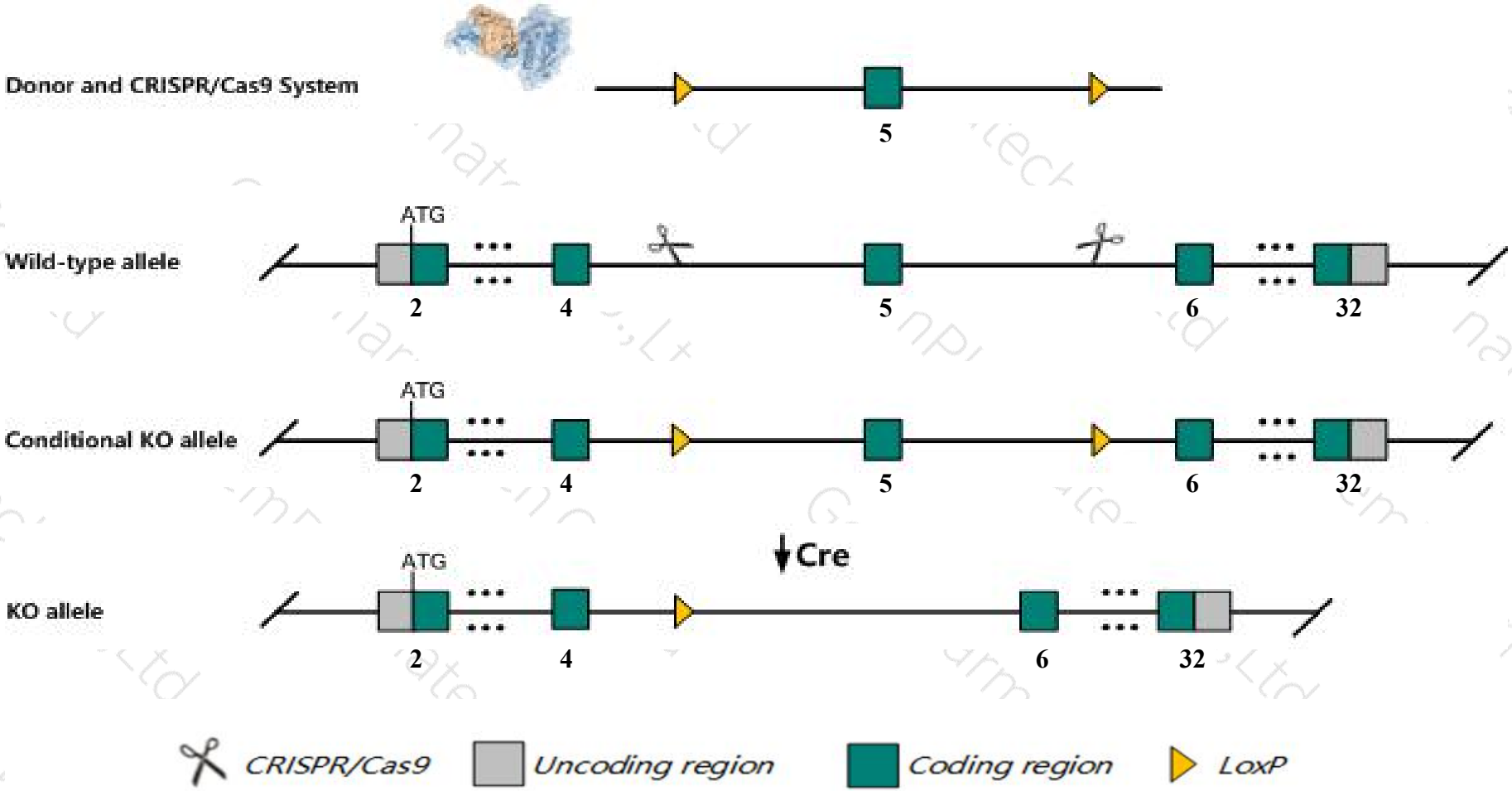
集萃药康 GemPharmatech
GemPharmatech Co.,Ltd

Project Overview

Project Name	***Plxna4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Plxna4* gene. The schematic diagram is as follows:



Technical routes

- The *Plxna4* gene has 2 transcripts. According to the structure of *Plxna4* gene, exon5 of *Plxna4-201* (ENSMUST00000115096.3) transcript is recommended as the knockout region.The region contains 101bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Plxna4* 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,Homozygous null mice exhibit defective trajecotory and projection of peripheral sensory axons and sympathetic ganglion axons and the formation of the anterior commissure and the barrels.

➢ Some amino acids will remain at the N-terminus and some functions may be retained.

➢ The *Plxna4* 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）

集萃药康 GemPharmatech

Plxna4 plexin A4 [*Mus musculus* (house mouse)]

Gene ID: 243743, updated on 17-Sep-2019

Summary

Official Symbol	Plxna4 provided by MGI
Official Full Name	plexin A4 provided by MGI
Primary source	MGI:MGI:2179061
See related	Ensembl:ENSMUSG00000029765
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	Plxa4; mKIAA1550; 9330117B14
Expression	Broad expression in CNS E18 (RPKM 7.4), CNS E14 (RPKM 6.4) and 16 other tissues See more
Orthologs	human all

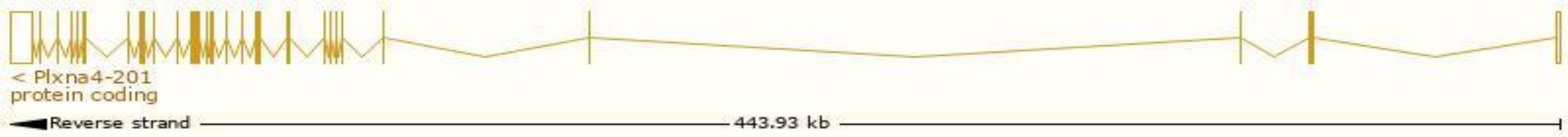
Chromosome 6 - NC_000072.6

Transcript information（Ensembl）

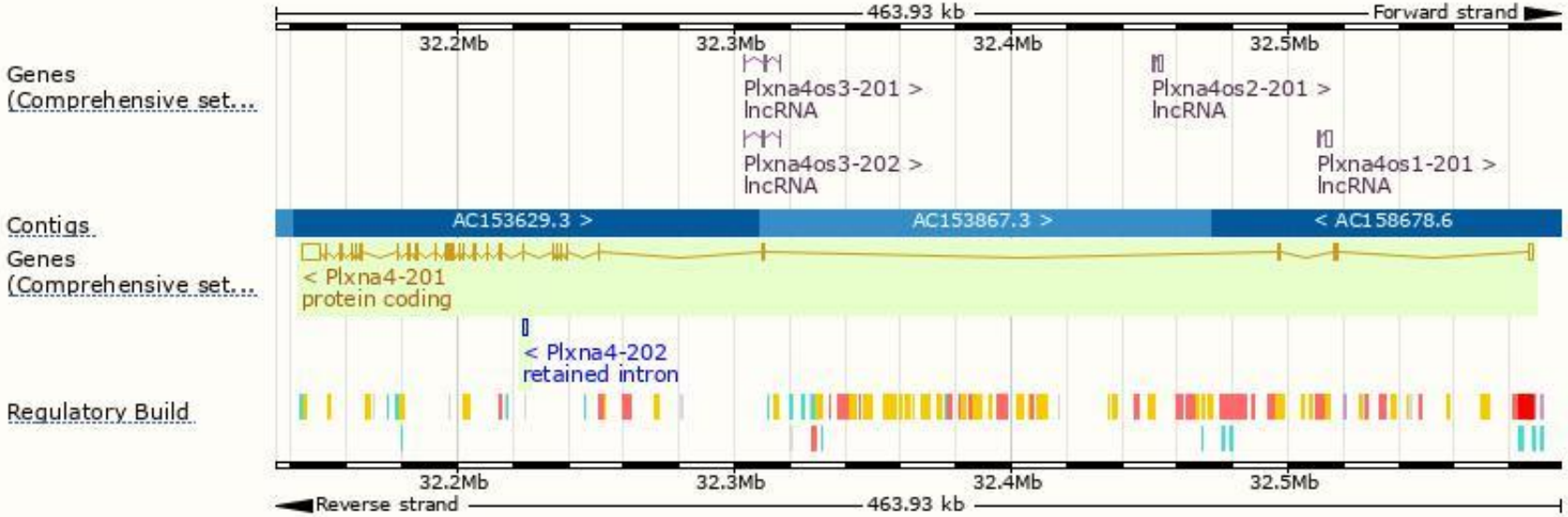
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Plxna4-201	ENSMUST00000115096.3	12877	1893aa	ENSMUSP00000110748.2	Protein coding	CCDS19985	Q80UG2	TSL:1 GENCODE basic APPRIS P1
Plxna4-202	ENSMUST00000200732.1	1427	No protein	-	Retained intron	-	-	TSL:NA

The strategy is based on the design of *Plxna4-201* transcript,The transcription is shown below



Genomic location distribution



Gene Legend

Protein Coding
- merged Ensembl/Havana

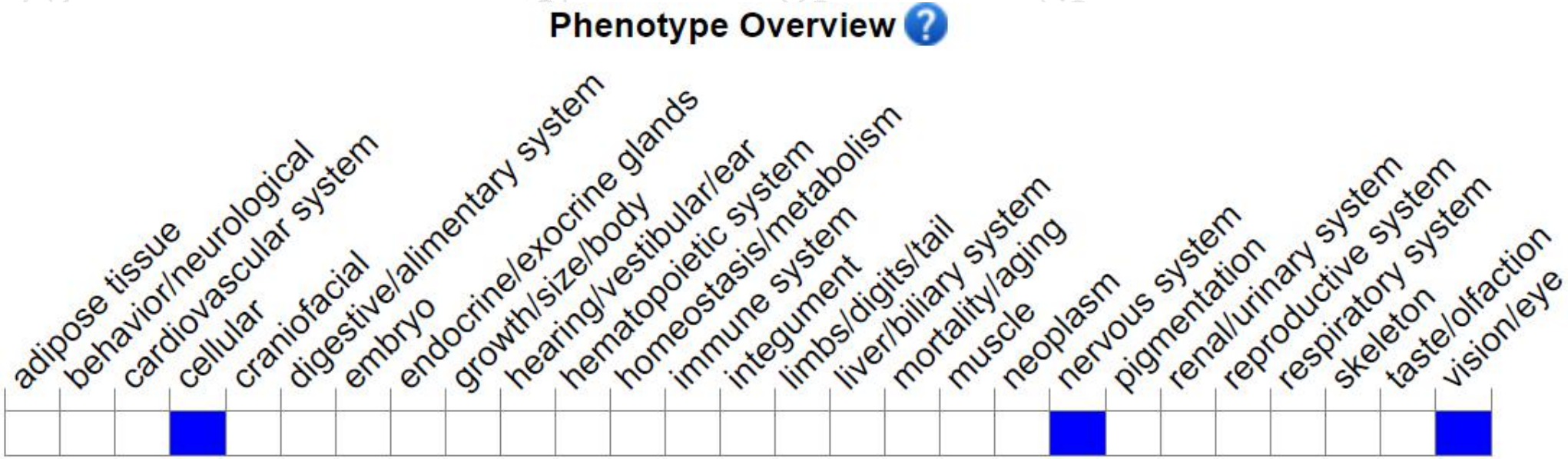
Non-Protein Coding
- RNA gene
- processed transcript

Regulation Legend

- CTCF
- Enhancer
- Open Chromatin
- Promoter
- Promoter Flank
- Transcription Factor Binding Site

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,Homozygous null mice exhibit defective trajecotory and projection of peripheral sensory axons and sympathetic ganglion axons and the formation of the anterior commissure and the barrels.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd

GemPharmatech Co.,Ltd