Plxna2 Cas9-KO Strategy make ch Co-Lity Conphanatech Co. 14

Designer: Enphamaten C. It

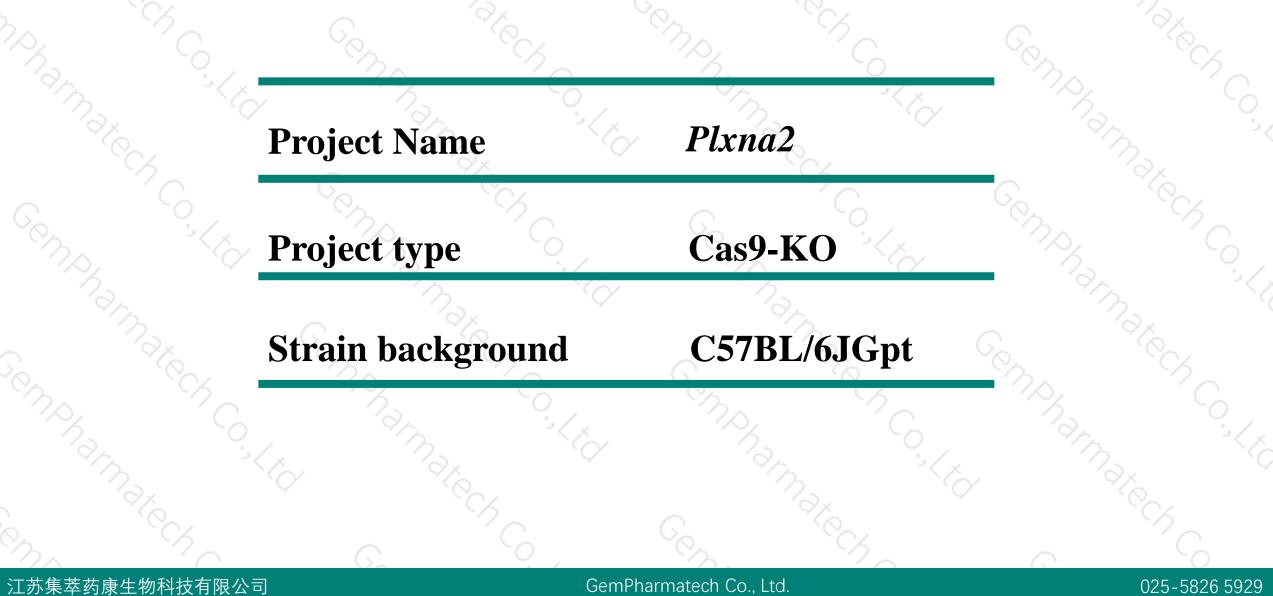
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Project Overview





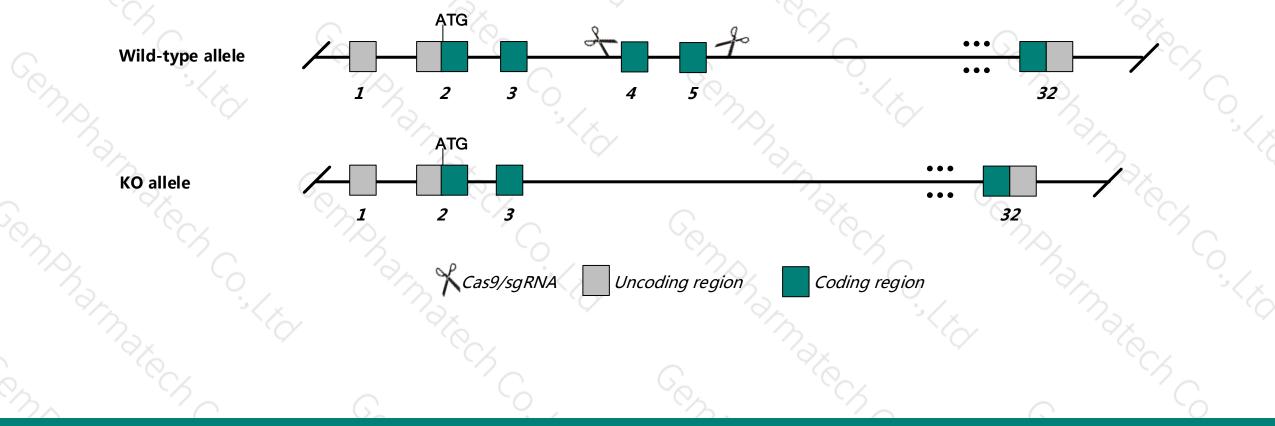
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Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Plxna2* gene. The schematic diagram is as follows:



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Technical routes



- The *Plxna2* gene has 5 transcripts. According to the structure of *Plxna2* gene, exon4-exon5 of *Plxna2*-201 (
 ENSMUST00000027952.11) transcript is recommended as the knockout region. The region contains 236bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Plxna2* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice



- According to the existing MGI data, Mice homozygous for a knock-out allele show abnormal granule cell migration in the adult cerebellum and aberrant projection of mossy fibers in hippocampal slices. Mice homozygous for an ENU-induced allele are smaller and show granule cell migration defects and mild ataxia with incomplete penetrance.
- The KO region contains functional region of the 2900035J10Rik gene.Knockout the region may affect the function of 2900035J10Rik gene.
- The *Plxna2* 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, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



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PIxna2 plexin A2 [Mus musculus (house mouse)]

Gene ID: 18845, updated on 9-Jun-2019

Summary

Official SymbolPlxna2 provided by MGIOfficial Full Nameplexin A2 provided by MGIOfficial Full NameMGI:MGI:107684Primary sourceMGI:MGI:107684See relatedEnsembl:ENSMUSG0000026640Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
Muroidea; Murinae; Mus; MusAlso known asOCT; Plxn2; PlexA2; AA589422; AW457381; mKIAA0463; 2810428A13RikExpressionBroad expression in CNS E14 (RPKM 20.1), whole brain E14.5 (RPKM 19.4) and 25 other tissues See more
human all

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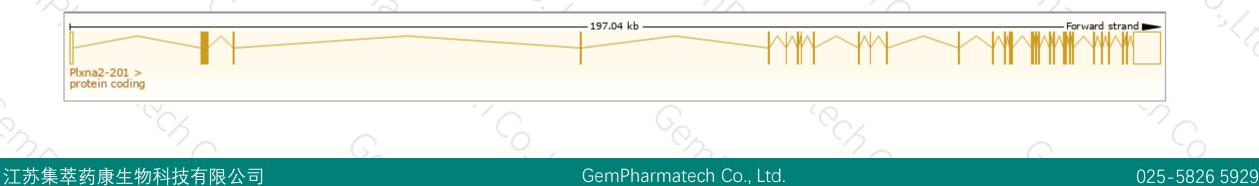
Transcript information (Ensembl)



The gene has 5 transcripts, and all transcripts are shown below:

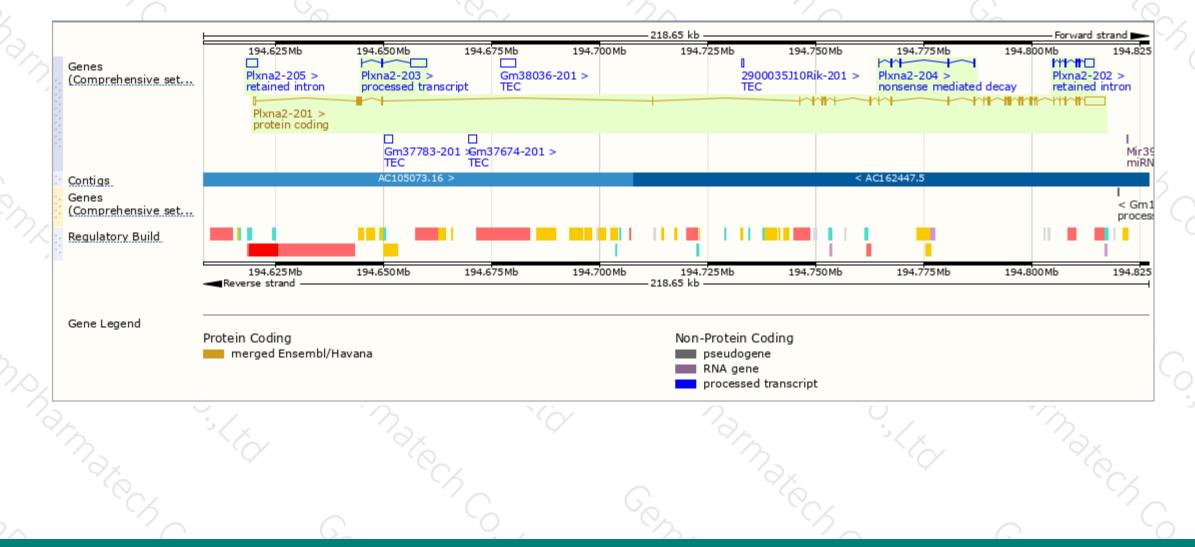
| Show/hide | columns (1 hidden) | Filter | | | | | | |
|------------|----------------------|--------|---------------|-------------------------|--------------------|-----------------|-------------------------------|--|
| Name 🍦 | Transcript ID 🛛 🍦 | bp 🌲 | Protein 🖕 | Biotype | CCDS 🍦 | UniProt 🖕 | Flags 🍦 | |
| Plxna2-201 | ENSMUST0000027952.11 | 11040 | <u>1894aa</u> | Protein coding | <u>CCDS35827</u> & | <u>P70207</u> & | TSL:1 GENCODE basic APPRIS P1 | |
| Plxna2-204 | ENSMUST00000135664.1 | 605 | <u>83aa</u> | Nonsense mediated decay | - | <u>F6VSI0</u> & | CDS 5' incomplete TSL:5 | |
| Plxna2-203 | ENSMUST00000125381.1 | 3951 | No protein | Processed transcript | - | - | TSL:2 | |
| Plxna2-202 | ENSMUST00000124785.1 | 3180 | No protein | Retained intron | - | - | TSL:1 | |
| Plxna2-205 | ENSMUST00000194398.1 | 2650 | No protein | Retained intron | - | - | TSL:NA | |

The strategy is based on the design of *Plxna2*-201 transcript, The transcription is shown below



Genomic location distribution





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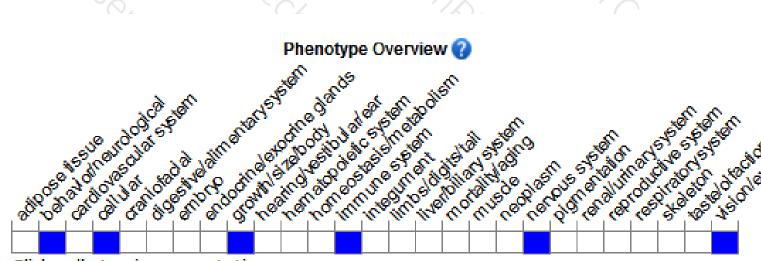
Protein domain



| | 5 | C | | \sim | `С | | \bigcirc | (QX | |
|------------|---|--|---|--------------------|-------------|-----------------------------------|--------------|---------|-------------------------|
| 2 | ENSMUSP00000027 Transmembrane heli Low complexity (Seg) Conserved Domains Coiled-coils (Ncoils) hmmpanther | | • | | | _ | - | | r S C |
| | nmmpantner | Plexin family PTHR22625:SF37 | | | | | | | |
| | Superfamily domains | Sema domain superfamily | SSF103575 | Immunoglot | oulin E-set | Rho GTPase activa | tion protein | | |
| | SMART domains | Sema domain | PSI domain | IPT domain | | | | , | |
| $\langle $ | Pfam domain Sema domain | | Plexin repeat Plexin, TIG domain 2 | | | Plexin, cytoplasmic RasGAP domain | | | |
| | PROSITE profiles | Sema domain | Plexin, TIG do | omain 1 IPT domain | | | | | - () , / |
| | Gene3D | WD40/YVTN repeat-like-containi | ing domain superfamily Immunoglobulin-li | | | | | | |
| | All sequence SNPs/i | Sequence variants (dbSNP and | | | | 1.1 | 1 11 | 1.1.1 | |
| | Variant Legend | missense variant synonymous variant | | - | | | | 6 | |
| 2 | Scale bar | 0 200 | 400 600 | 800 | 1000 1200 | 1400 | 1600 | 1894 | - 34 |
| | Mar Contraction | | ALC A | - | armax | · · / × / | | nate Ct | |
| | C. C. | G | í Co | C. | °°, | 5 | C | \sim | $\overline{\mathbf{O}}$ |

Mouse phenotype description(MGI)





Click cells to view annotations.

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 show abnormal granule cell migration in the adult cerebellum and aberrant projection of mossy fibers in hippocampal slices. Mice homozygous for an ENU-induced allele are smaller and show granule cell migration defects and mild ataxia with incomplete penetrance.

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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



