

Pclo Cas9-CKO Strategy

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Project Overview

Project Name

Pclo

Project type

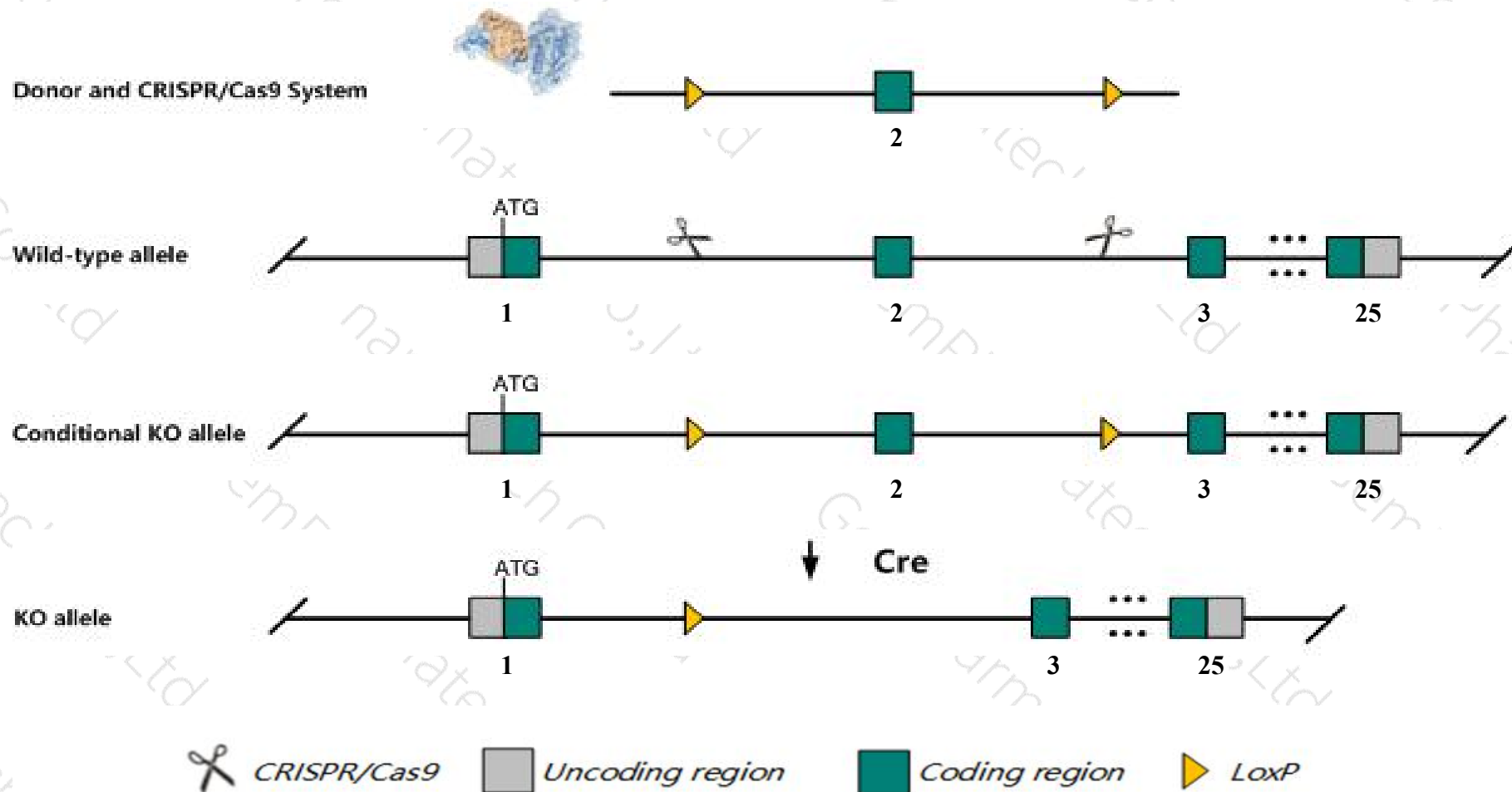
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



Technical routes

- The *Pclo* gene has 4 transcripts. According to the structure of *Pclo* gene, exon2 of *Pclo-201*(ENSMUST00000030691.16) transcript is recommended as the knockout region. The region contains 1483bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Pclo* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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.

- According to the existing MGI data, mice homozygous for one deletion of *Pclo* are viable and fertile, and display no overt abnormal phenotype. Mice homozygous for another knock-out allele exhibit some premature lethality, decreased body size, and abnormal synaptic vesicle number.
- Transcript *Pclo*-204 may not be affected.
- The KO region contains functional region of the *Gm26918* gene. Knockout the region may affect the function of *Gm26918* gene.
- The *Pclo* gene is located on the Chr5. 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)

Pclo piccolo (presynaptic cytomatrix protein) [*Mus musculus* (house mouse)]

Gene ID: 26875, updated on 26-Jun-2020

Summary



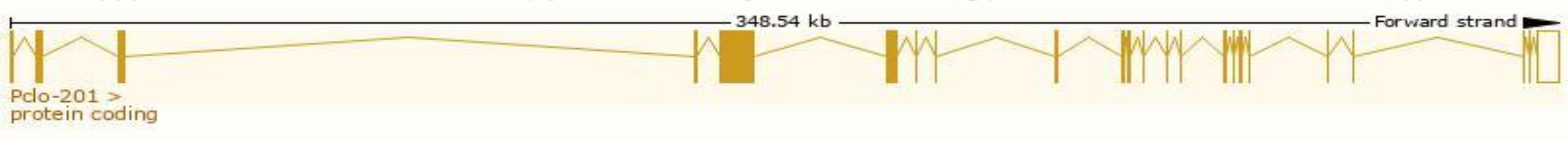
| | |
|--------------------|---|
| Official Symbol | Pclo provided by MGI |
| Official Full Name | piccolo (presynaptic cytomatrix protein) provided by MGI |
| Primary source | MGI:MGI:1349390 |
| See related | Ensembl:ENSMUSG000000061601 |
| Gene type | protein coding |
| RefSeq status | VALIDATED |
| Organism | Mus musculus |
| Lineage | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| Also known as | Acz; Pico |
| Expression | Biased expression in cerebellum adult (RPKM 10.3), cortex adult (RPKM 8.0) and 5 other tissues See more |
| Orthologs | human all |

Transcript information (Ensembl)

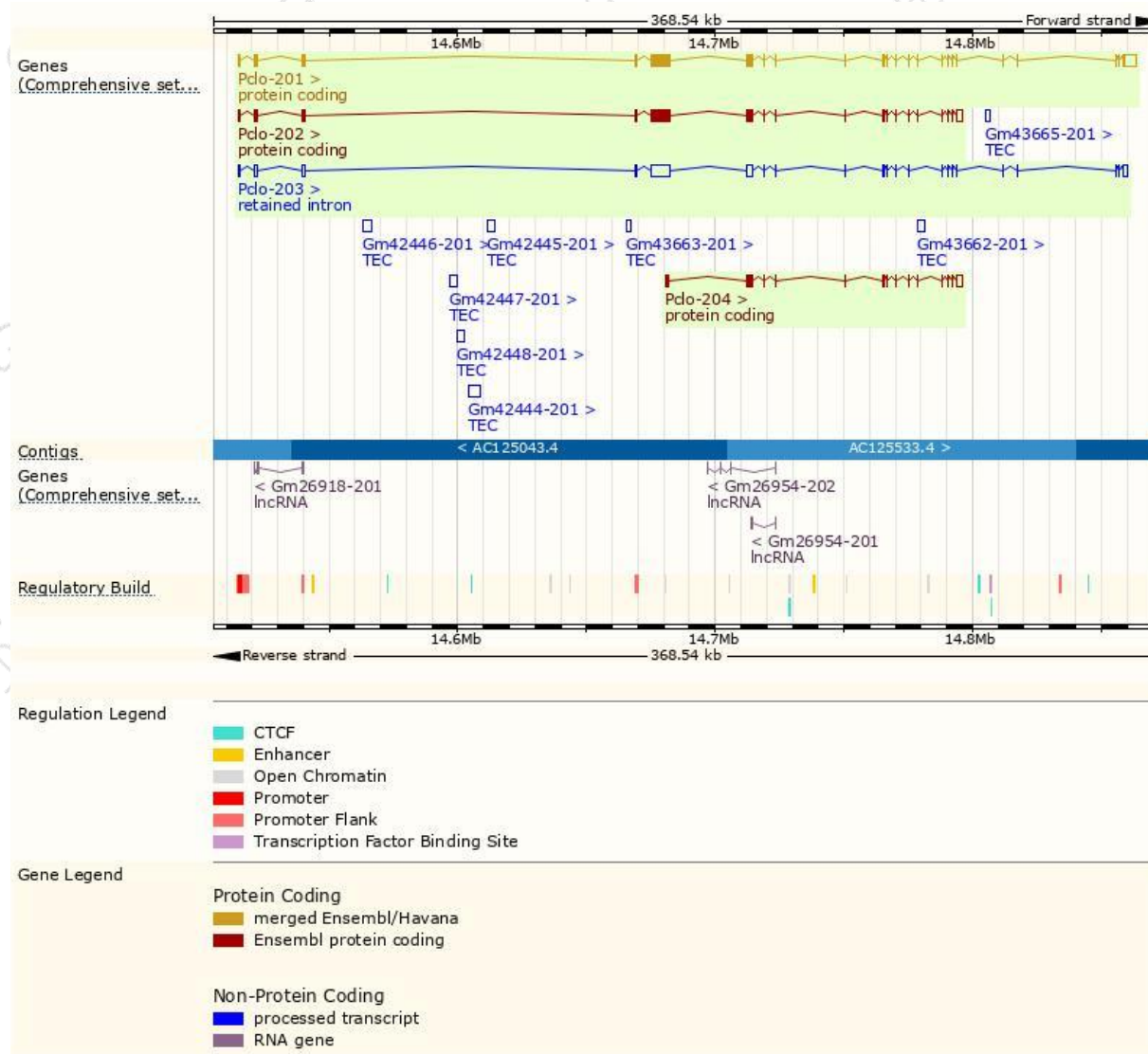
The gene has 4 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|----------|---------------------------------------|-------|------------------------|-----------------|---------------------------|------------------------|-------------------------------|
| Pclo-201 | ENSMUST00000030691.16 | 20084 | 5068aa | Protein coding | CCDS51415 | Q9QYX7 | TSL:5 GENCODE basic APPRIS P1 |
| Pclo-202 | ENSMUST00000182407.7 | 16846 | 4863aa | Protein coding | CCDS59667 | Q9QYX7 | TSL:5 GENCODE basic |
| Pclo-204 | ENSMUST00000182915.1 | 6738 | 1592aa | Protein coding | - | S4R2E0 | CDS 5' incomplete TSL:1 |
| Pclo-203 | ENSMUST00000182426.2 | 17063 | No protein | Retained intron | - | - | TSL:2 |

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



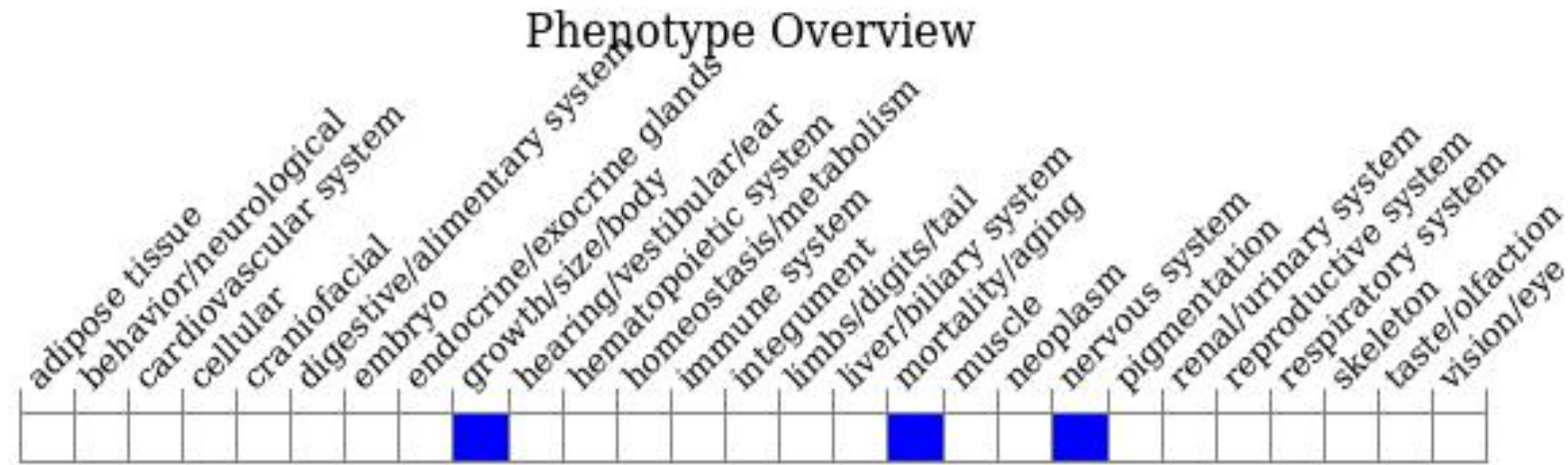
Genomic location distribution



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, mice homozygous for one deletion of *Pc10* are viable and fertile, and display no overt abnormal phenotype. Mice homozygous for another knock-out allele exhibit some premature lethality, decreased body size, and abnormal synaptic vesicle number.

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

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