

Gpr33 Cas9-CKO Strategy

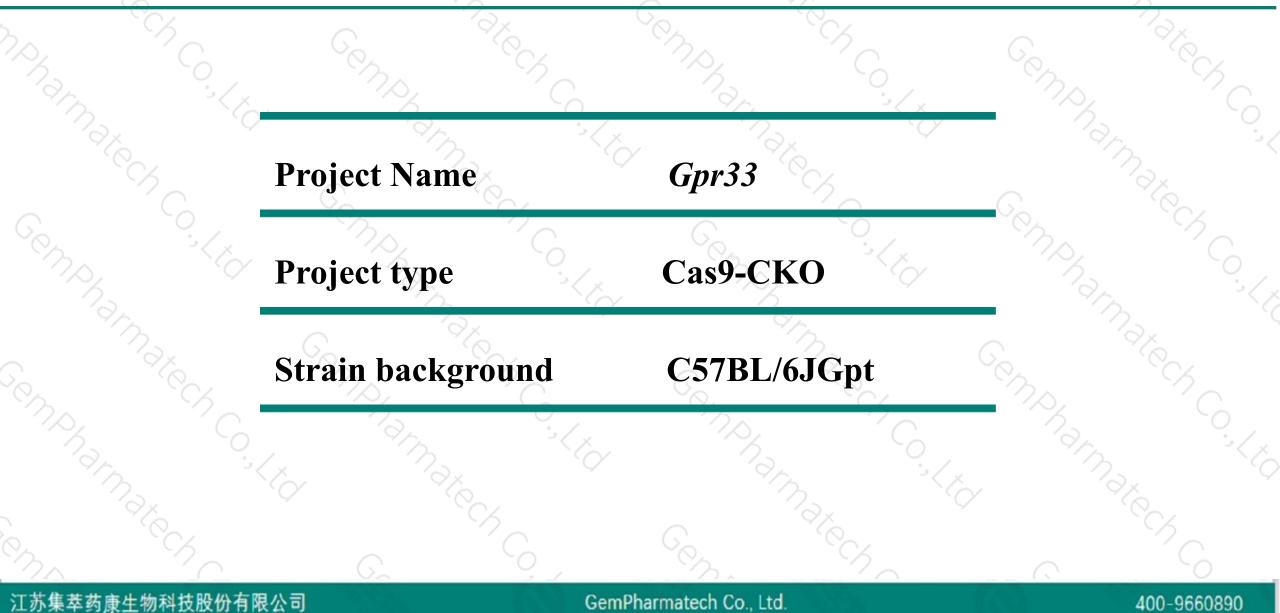
Designer: Reviewer:

Design Date:

Lingyan Wu Jiayuan Yao 2019-7-22

Project Overview





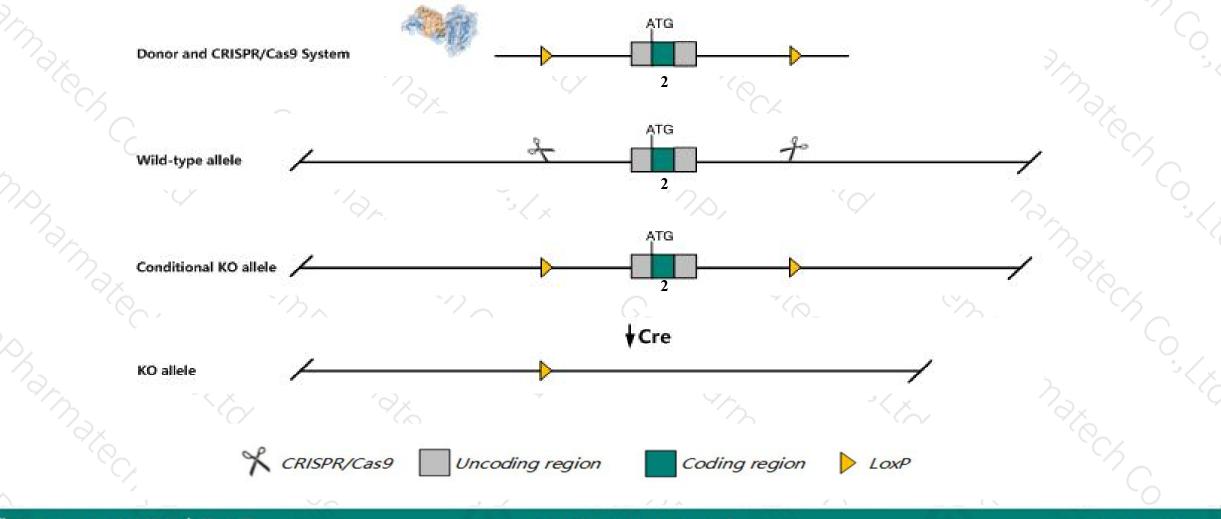
Conditional Knockout strategy

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400-9660890

This model will use CRISPR/Cas9 technology to edit the *Gpr33* gene. The schematic diagram is as follows:



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The Gpr33 gene has 1 transcript. According to the structure of Gpr33 gene, exon2 of Gpr33-201 (ENSMUST00000040161.4) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Gpr33* 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.



- The Gpr33 gene is located on the Chr12. 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)



☆ ?

Gpr33 G protein-coupled receptor 33 [Mus musculus (house mouse)]

Gene ID: 14762, updated on 31-Jan-2019

Summary

| Official Symbol | Gpr33 provided by MGI |
|--------------------|--|
| Official Full Name | G protein-coupled receptor 33 provided by MGI |
| Primary source | MGI:MGI:1277106 |
| See related | Ensembl:ENSMUSG00000035148 |
| 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 |
| Expression | Low expression observed in reference datasetSee more |
| Orthologs | human all |

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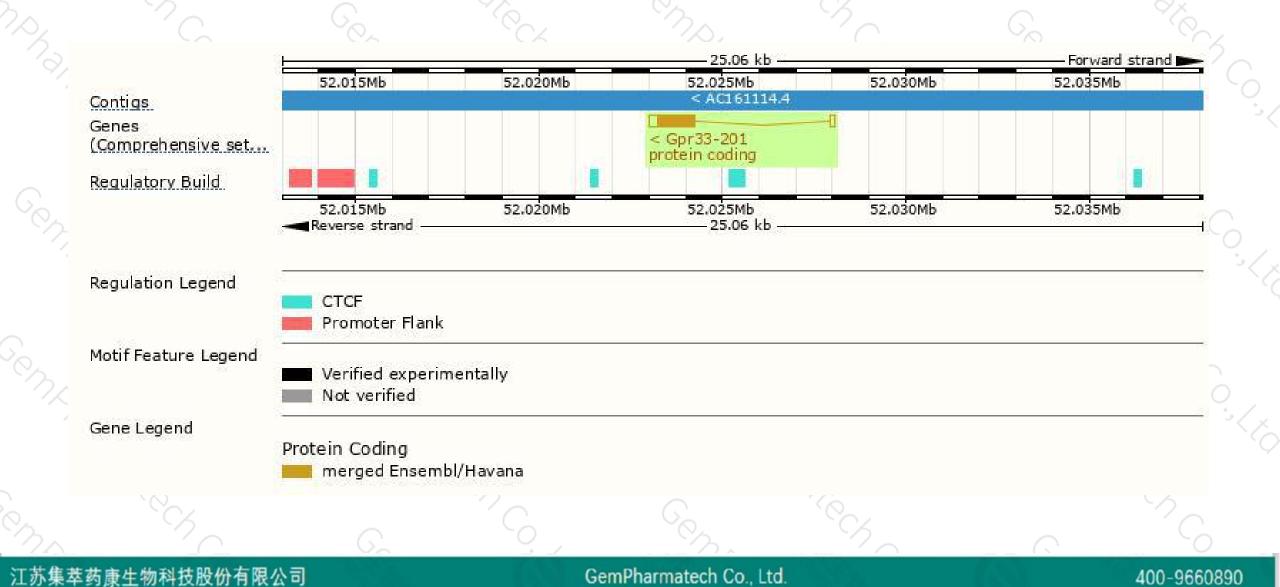


The gene has 1 transcript, and the transcript is shown below:

| 1 Mar | 7667 | C 8 A | | | | 6 / | I has I | 1 |
|--|---------------|---------------|------------------|----------------|------------------|----------------|------------------------------|-----------|
| Name Transcript ID Gpr33-201 ENSMUST0000040161.4 | | bp | Protein | tein Biotype | CCDS | UniProt | Flags | |
| | | 1388 | <u>339aa</u> | Protein coding | CCDS25904 | D8VER2 088416 | TSL:1 GENCODE basic APPRIS | APPRIS P1 |
| Cenphan Seno | A Contraction | | | | Ceno, | Ch Co. (r.) | Cemphanatech Cemphanatech | |
| Copr33-201 protein coding Reverse st | 9 | of <i>Gpi</i> | <i>~33-201</i> t | ranscript,The | | is shown below | | |
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Genomic location distribution





Protein domain



|), | | | °Co. | | | | | | | |
|-----------------------------|--|----------------------|-----------------------|------------------|------|-----|-----|-------|----------------|---|
| 22/ | ENSMUSP00000048 Transmembrane heli Low complexity (Seg) Conserved Domains | | | | | | | | ³ Q |) |
| | hmmpanther | Formyl peptide recei | ptor-related | | | | | | | |
| | | PTHR:24225:SF5 | | | | | | | | |
| | Superfamily domains | SSF81321 | | | | | | | | |
| | Prints domain | | PR00526 | | | | | | S | |
| | 5745) (X) (X) | G protei | n-coupled receptor, r | hod opsin-like | | | | • • • | | |
| | Pfam domain | G | protein-coupled rece | ptor, rhodopsin- | like | | | | | |
| | PROSITE profiles | GP | CR, rhodopsin-like, | 7TM | | | | | | |
| | Gene3D | 1.20,1070,10 | | | | | | | | |
| $\mathcal{F}_{\mathcal{F}}$ | All sequence SNPs/i | Sequence variant | s (dbSNP and all ot | ther sources) | | 10 | 4 | | <u> 111</u> | |
| | Variant Legend | missense val | | | | | | | -< | |
| | Scale bar | o 40 | 80 | 120 | 160 | 200 | 240 | 280 | 339 | |
| 2 | ° CA | | 6 | | | | | | 6 | |

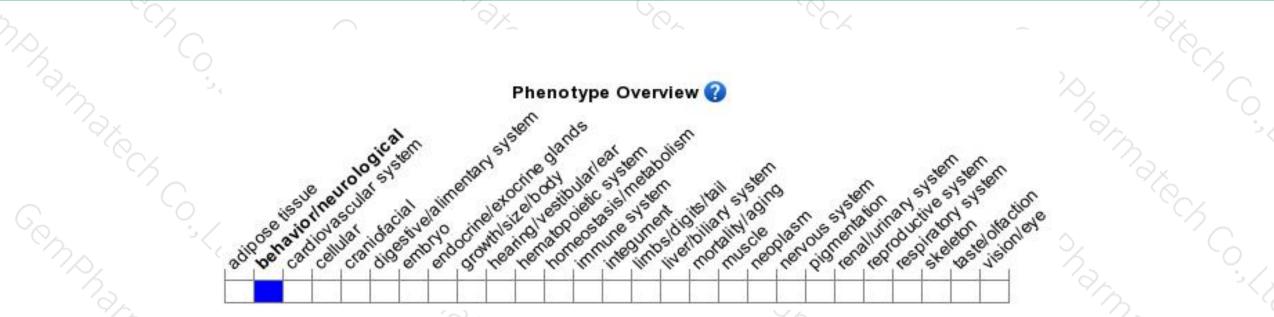
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Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).



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



