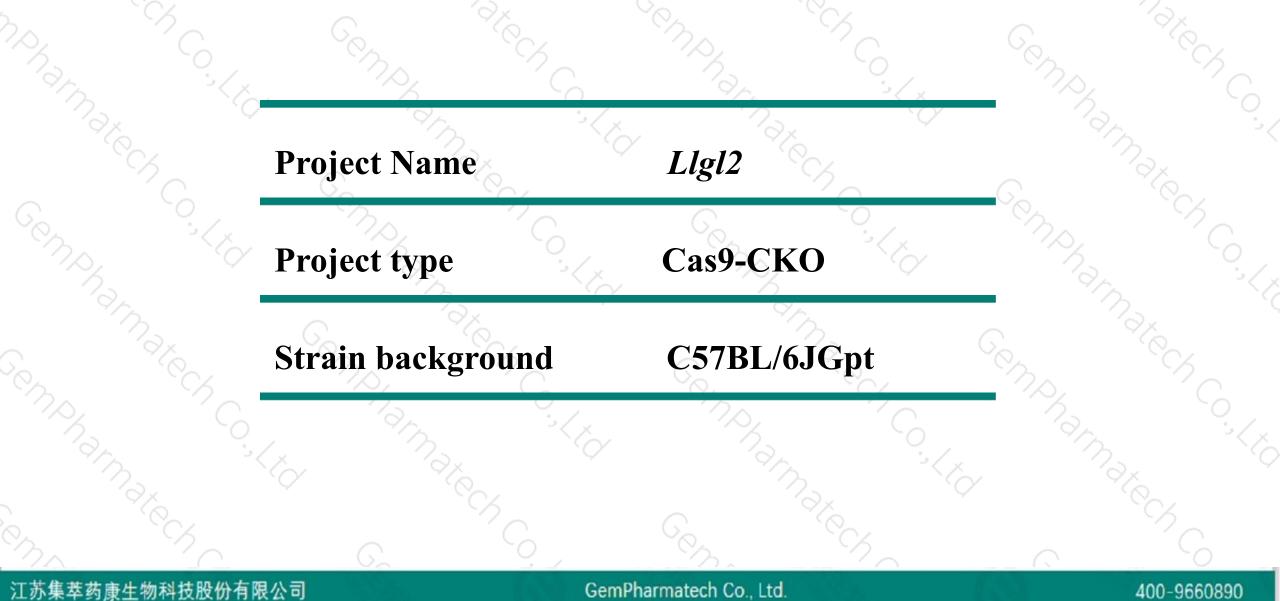


# Llgl2 Cas9-CKO Strategy

Designer: Xiaojing Li Design Date: 2019-9-11 Reviewer: JiaYu

# **Project Overview**

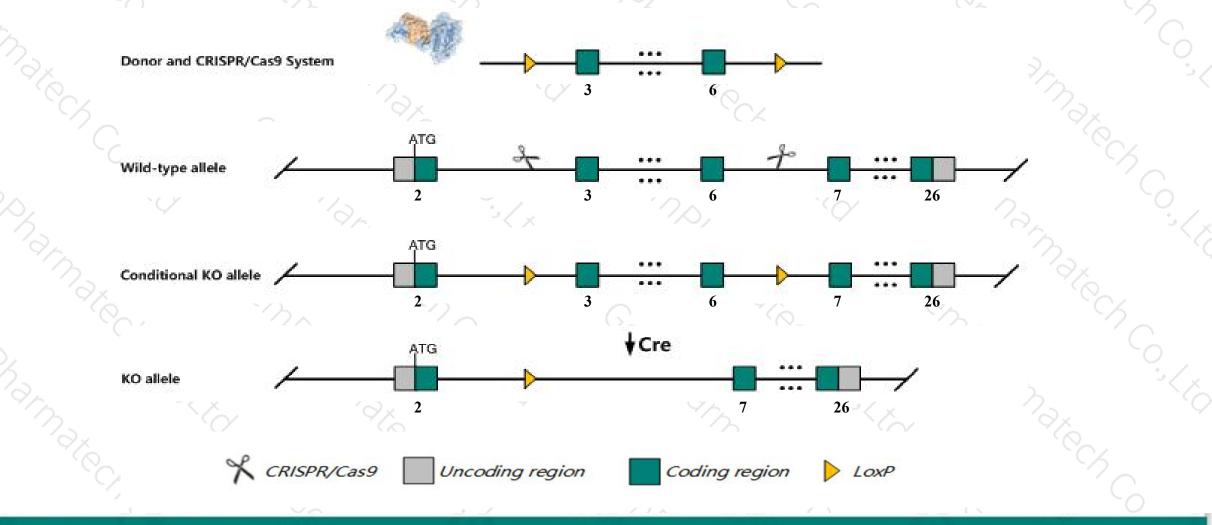




## **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Llgl2* gene. The schematic diagram is as follows:



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The Llgl2 gene has 12 transcripts. According to the structure of Llgl2 gene, exon3-exon6 of Llgl2-212 (ENSMUST00000177736.7) transcript is recommended as the knockout region. The region contains 455bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Llgl2* 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.



- According to the existing MGI data, Mice homozygous for a gene-trapped allele exhibit abnormal branching morphogenesis of the placental labyrinth layer and are born as runts but catch up in size by adulthood.
- The *Llgl2* gene is located on the Chr11. 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)**



\$ ?

#### LIgI2 LLGL2 scribble cell polarity complex component [Mus musculus (house mouse)]

Gene ID: 217325, updated on 31-Jan-2019

#### Summary

| Official Symbol       | LIgI2 provided by MGI  |
|-----------------------|--|
| Official Full Name    | LLGL2 scribble cell polarity complex component provided by MGI   |
| <b>Primary source</b> | MGI:MGI:1918843  |
| See related           | Ensembl:ENSMUSG0000020782  |
| Gene type             | protein coding   |
| <b>RefSeq status</b>  | 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         | 9130006H11Rik, Llglh2  |
| Expression            | Broad expression in colon adult (RPKM 75.1), duodenum adult (RPKM 44.0) and 16 other tissues See more                                |
| Orthologs             | human all  |

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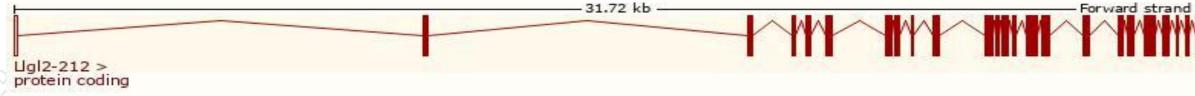
# **Transcript information (Ensembl)**



#### The gene has 12 transcripts, all transcripts are shown below:

| Name      | Transcript ID         | bp   | Protein       | Biotype         | CCDS      | UniProt       | Flags                           |
|-----------|-----------------------|------|---------------|-----------------|-----------|---------------|---------------------------------|
| Llgl2-212 | ENSMUST00000177736.7  | 3612 | <u>1051aa</u> | Protein coding  | CCDS56820 | <u>J3QJU5</u> | TSL:2 GENCODE basic APPRIS ALT2 |
| Llgl2-201 | ENSMUST00000103032.10 | 3551 | <u>1027aa</u> | Protein coding  | CCDS25649 | <u>Q3TJ91</u> | TSL:1 GENCODE basic APPRIS P3   |
| Llg12-205 | ENSMUST00000133250.7  | 847  | <u>146aa</u>  | Protein coding  | 43        | B1ATA8        | CDS 3' incomplete TSL:5         |
| LIgI2-202 | ENSMUST00000128826.1  | 791  | <u>161aa</u>  | Protein coding  | 20<br>20  | <u>F6X6X1</u> | CDS 5' incomplete TSL:3         |
| Llgl2-211 | ENSMUST00000173289.7  | 643  | <u>148aa</u>  | Protein coding  |           | G3UXR0        | CDS 3' incomplete TSL:5         |
| Llgl2-210 | ENSMUST00000172552.7  | 550  | <u>101aa</u>  | Protein coding  |           | G3UXS2        | CDS 3' incomplete TSL:3         |
| LIgI2-206 | ENSMUST00000137900.1  | 548  | <u>158aa</u>  | Protein coding  | 20        | B1ATA9        | CDS 3' incomplete TSL:2         |
| LIgI2-209 | ENSMUST00000155878.7  | 545  | <u>128aa</u>  | Protein coding  | 10<br>20  | B1ATA7        | CDS 3' incomplete TSL:3         |
| LIgI2-208 | ENSMUST00000147878.7  | 863  | No protein    | Retained intron |           |               | TSL:3                           |
| LIgI2-207 | ENSMUST00000137951.1  | 576  | No protein    | Retained intron | -         | -             | TSL:2                           |
| LIgI2-204 | ENSMUST00000132999.1  | 451  | No protein    | Retained intron | 20        | -             | TSL:2                           |
| Llgl2-203 | ENSMUST00000130518.1  | 566  | No protein    | IncRNA          |           | -             | TSL:2                           |
|           |                       |      |               |                 |           |               |                                 |

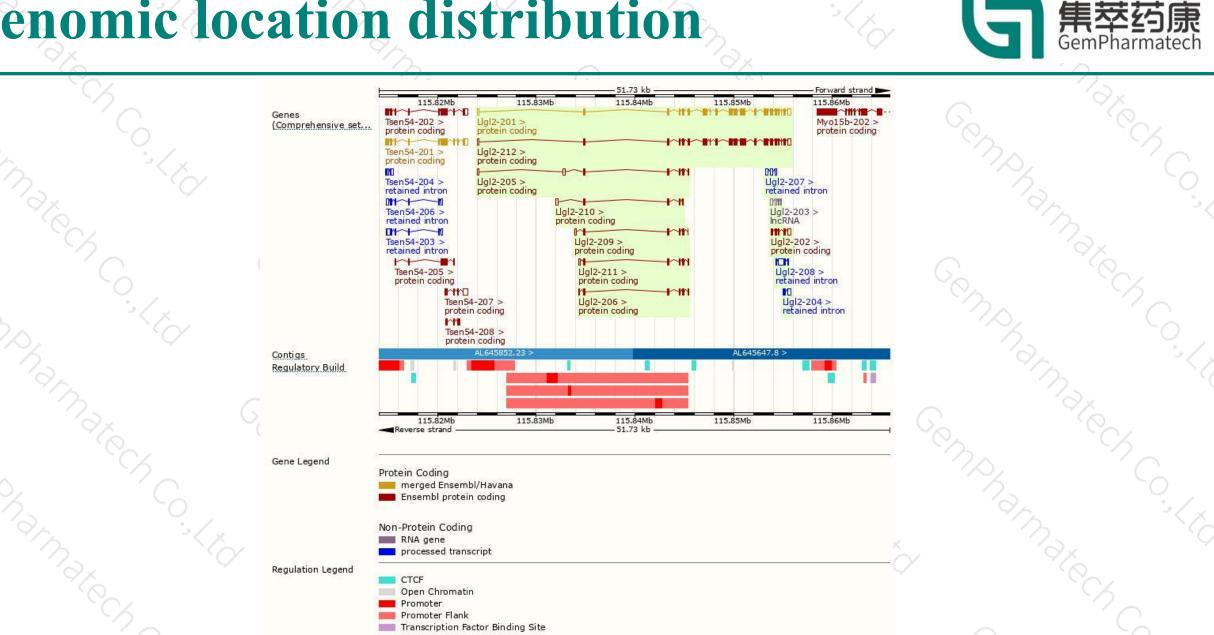
The strategy is based on the design of *Llgl2-212* transcript, The transcription is shown below



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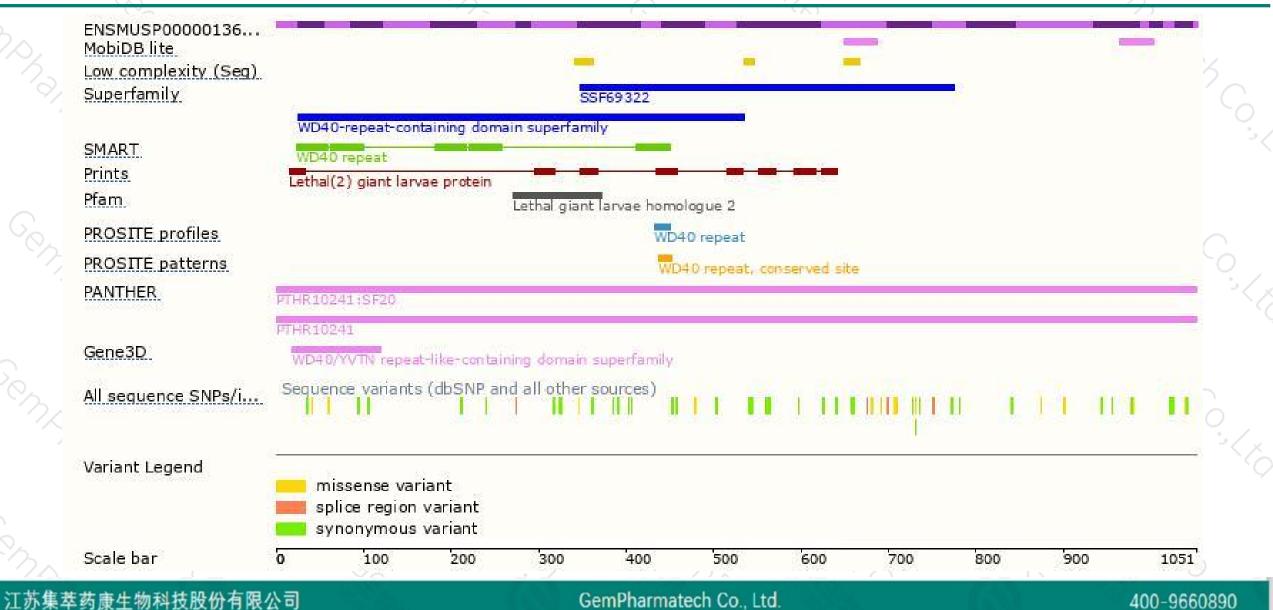
### **Genomic location distribution**



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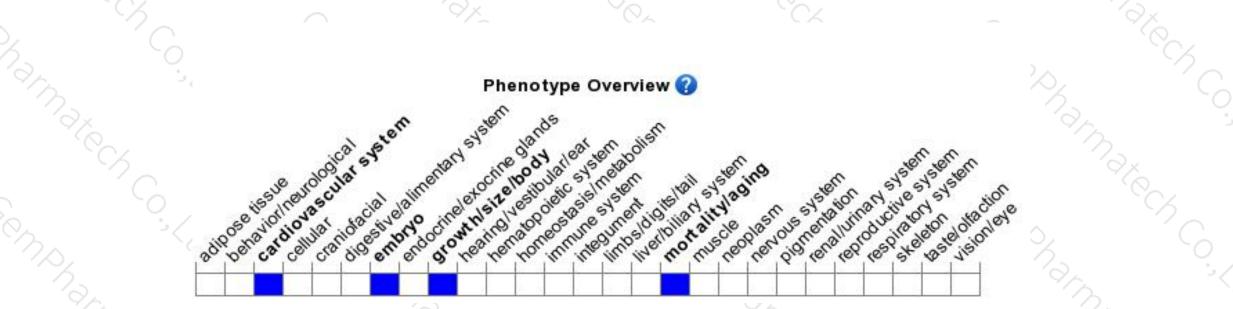
### **Protein domain**





## 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 gene-trapped allele exhibit abnormal branching morphogenesis of the placental labyrinth layer and are born as runts but catch up in size by adulthood.



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



