

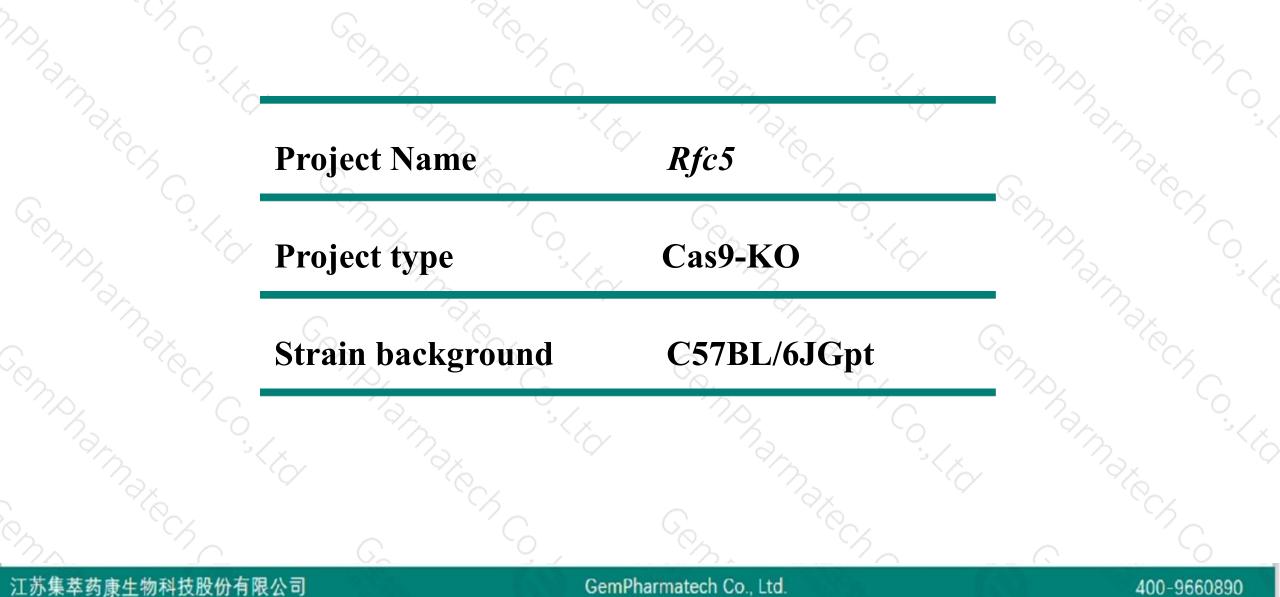
Rfc5 Cas9-KO Strategy

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Project Overview

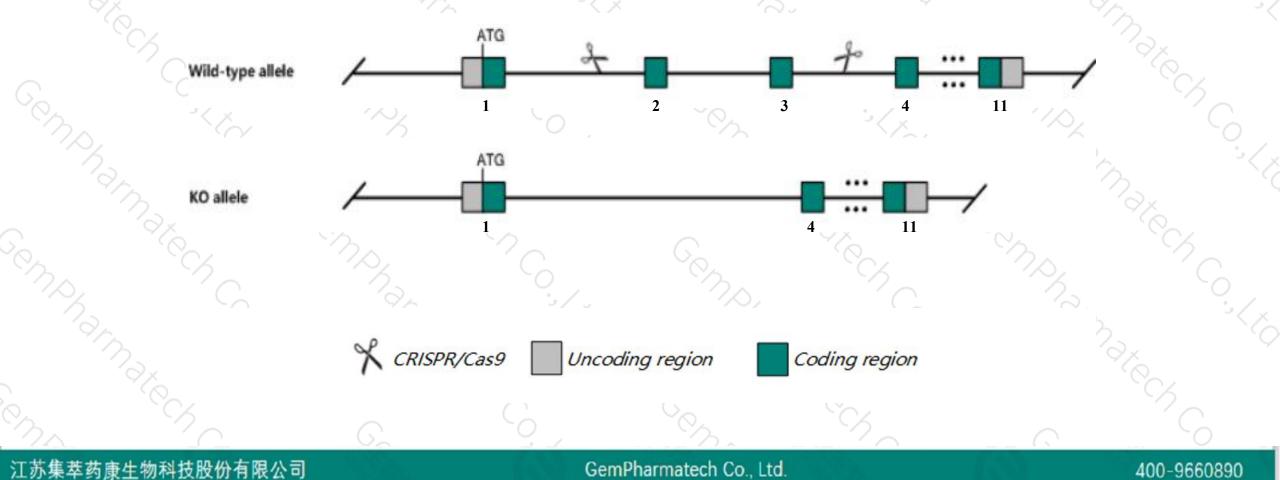




Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Rfc5* gene. The schematic diagram is as follows:





- The Rfc5 gene has 6 transcripts. According to the structure of Rfc5 gene, exon2-exon3 of Rfc5-201 (ENSMUST0000086461.12) transcript is recommended as the knockout region. The region contains 202bp coding sequence. Knock out the region will result in disruption of protein function.
- > In this project we use CRISPR/Cas9 technology to modify Rfc5 gene. The brief process is as follows: CRISPR/Cas9 system v



- ➤ Transcript *Rfc5*-203&205 may not be affected.
- The knockout region is near to the N-terminal of *Gm15728* gene, this strategy may influence the regulatory function of the N-terminal of *Gm15728* gene.
- The *Rfc5* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

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Gene information (NCBI)

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Transcript information (Ensembl)



The gene has 6 transcripts, and al the transcripts are shown below:

| Name | Transcript ID | bp 🔺 | Protein 💧 | Biotype 💧 | CCDS | UniProt 🔺 | Flags |
|----------|----------------------|--------------------|--------------|-----------------|--------------------|---------------------------------|-------------------------------|
| Rfc5-201 | ENSMUST0000086461.12 | 1000 000 000 000 0 | <u>339aa</u> | Protein coding | <u>CCDS39235</u> & | <u>Q5HZI8</u> & <u>Q9D0F6</u> & | TSL:1 GENCODE basic APPRIS P1 |
| Rfc5-202 | ENSMUST00000111953.1 | 772 | <u>112aa</u> | Protein coding | - | <u>D3Z1Y6</u> & | TSL:1 GENCODE basic |
| Rfc5-206 | ENSMUST00000150962.7 | 3751 | No protein | Retained intron | - | - | TSL:2 |
| Rfc5-204 | ENSMUST00000126262.1 | 1256 | No protein | Retained intron | - | - | TSL:2 |
| Rfc5-205 | ENSMUST00000129369.1 | 375 | No protein | Retained intron | - | | TSL:2 |
| Rfc5-203 | ENSMUST00000123392.1 | 264 | No protein | Retained intron | - | - | TSL:5 |

The strategy is based on the design of *Rfc5-201* transcript, The transcription is shown below

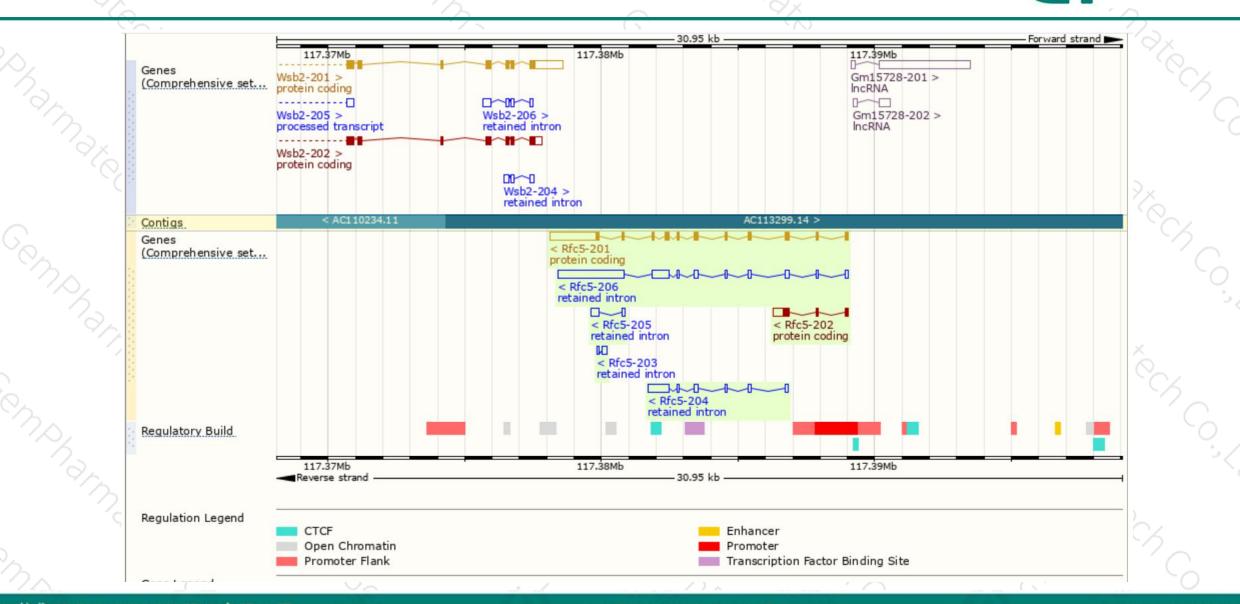
| < Rfc5-201 protein coding | | | | | |
|------------------------------|--------|--------------|------|---|-----|
| Reverse strand - | | — 10.95 kb — | | | -12 |
| 0. | B. | | - 25 | 0 | |

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Genomic location distribution



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Protein domain



| $\gamma_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}}}}$ | ъ С | G | A P | | 00ms | | °° | | G | | Q |
|--|--|----------------------------|---|-------------|------|----------------------|-----------------|--|----------------------|--------------|-----|
| "Charmar | ENSMUSP0000083 Low complexity (Seg) Coiled-coils (Ncoils) Superfamily | P-loop containing nu | cleoside triphosphate hyd | drolase | | | | DNA polymerase I | II, clamp loader cor | nplex, gamma | |
| 2 | SMART Pfam | 13.05 | A+ ATPase domain TPase, AAA-type, core | | | - | | Replication factor (| C, C-terminal | _ | X |
| Comphan | PANTHER. | PTHR11669:SF9 PTHR11669 | | | | | | | | | |
| | Gene3D CDD | 3.40.50.300 cd00009 | | | | 1.10.8.60 cd18140 | | 1.20.272.10 | | | × |
| George (| All sequence SNPs/i Variant Legend | Sequence variants (dbSNF | and all other sources | 1 | 1.11 | synonymo | lus variant | | 1 | 1 | Ċ, |
| $\gamma_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}_{\mathcal{O}}}}}$ | Scale bar | 0 40 | 80 | 120 | 160 | 20 | | 240 | 280 | 339 | |
| Sensharn. | | | | <u>`</u> {} | | 7317 | 5. | ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ | 1 | 339 | |
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If you have any questions, you are welcome to inquire. Tel: 400-9660890



