

# Zswim6 Cas9-CKO Strategy

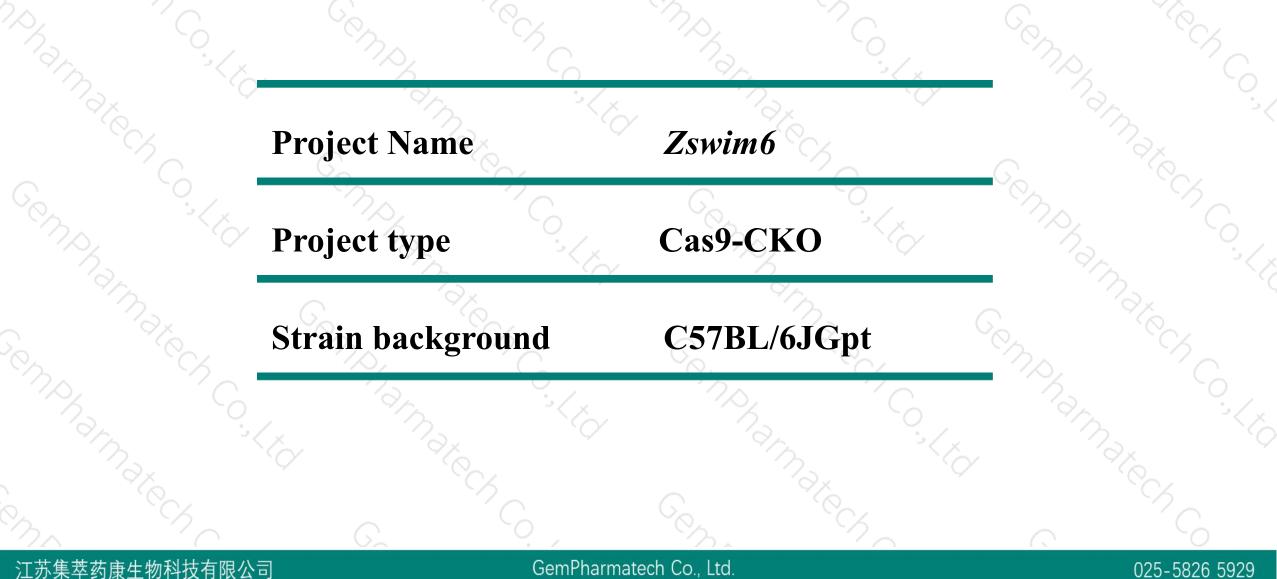
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**Reviewer: JiaYu** 

**Design Date: 2020-7-29** 

# **Project Overview**



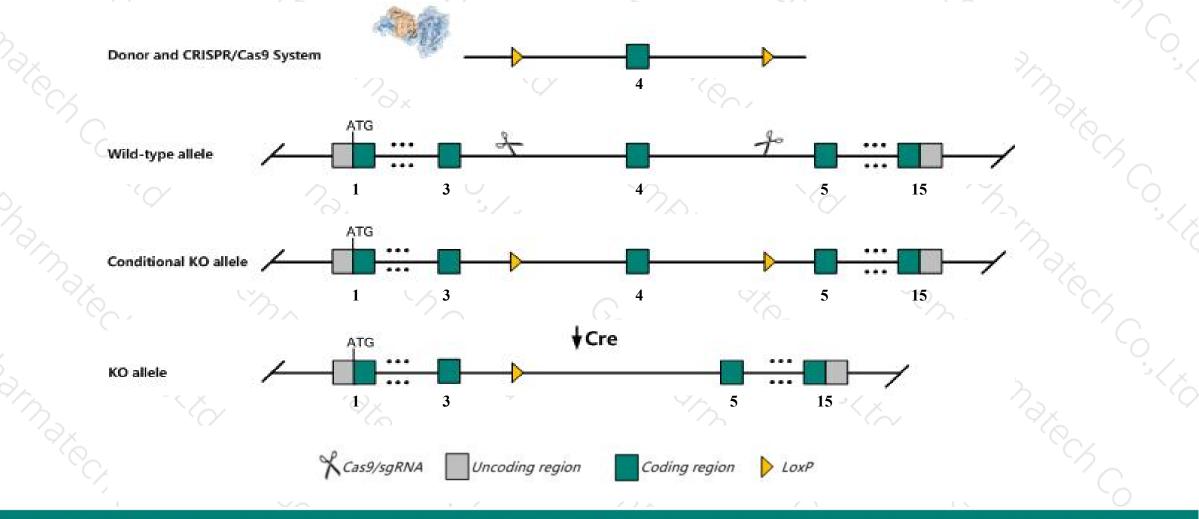


## **Conditional Knockout strategy**



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This model will use CRISPR/Cas9 technology to edit the Zswim6 gene. The schematic diagram is as follows:



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The Zswim6 gene has 4 transcripts. According to the structure of Zswim6 gene, exon4 of Zswim6-201(ENSMUST00000105097.2) transcript is recommended as the knockout region. The region contains 149bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Zswim6* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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 knock-out allele exhibit partial postnatal lethality, decreased striatal volume, abnormal medium spiny neuron morphology, and altered motor control including hyperactivity, impaired rotarod performance, repetitive movements, and behavioral hyperresponsiveness to amphetamine.
The Zswim6 gene is located on the Chr13. 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)**



☆ ?

### Zswim6 zinc finger SWIM-type containing 6 [Mus musculus (house mouse)]

Gene ID: 67263, updated on 13-Mar-2020

#### Summary

| Official Symbol           | Zswim6 provided by MGI   |  |  |  |  |  |
|---------------------------|--|--|--|--|--|--|
| <b>Official Full Name</b> | zinc finger SWIM-type containing 6 provided by <u>MGI</u>  |  |  |  |  |  |
| <b>Primary source</b>     | MGI:MGI:1914513  |  |  |  |  |  |
| See related               | Ensembl:ENSMUSG0000032846  |  |  |  |  |  |
| Gene type                 | protein coding   |  |  |  |  |  |
| RefSeq status             | VALIDATED  |  |  |  |  |  |
| Organism                  | Mus musculus   |  |  |  |  |  |
| Lineage                   | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;<br>Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |  |  |  |  |  |
| Also known as             | 2900036G02Rik, mKIAA1577   |  |  |  |  |  |
| Expression                | Ubiquitous expression in whole brain E14.5 (RPKM 11.2), CNS E14 (RPKM 10.4) and 28 other tissuesSee more   |  |  |  |  |  |
| Orthologs                 | human all  |  |  |  |  |  |

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# **Transcript information (Ensembl)**

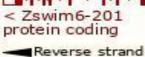


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The gene has 4 transcripts, all transcripts are shown below:

| Name       | Transcript ID        | bp   | Protein       | Biotype              | CCDS | UniProt       | Flags                         |
|------------|----------------------|------|---------------|----------------------|------|---------------|-------------------------------|
| Zswim6-201 | ENSMUST00000105097.2 | 5456 | <u>1207aa</u> | Protein coding       | (50  | <u>Q80TB7</u> | TSL:5 GENCODE basic APPRIS P1 |
| Zswim6-204 | ENSMUST00000225822.1 | 5311 | No protein    | Processed transcript |      | -             |                               |
| Zswim6-203 | ENSMUST00000225197.1 | 3873 | No protein    | Processed transcript | 1251 | -             |                               |
| Zswim6-202 | ENSMUST00000224719.1 | 2126 | No protein    | Retained intron      |      |               |                               |

The strategy is based on the design of Zswim6-201 transcript, the transcription is shown below:



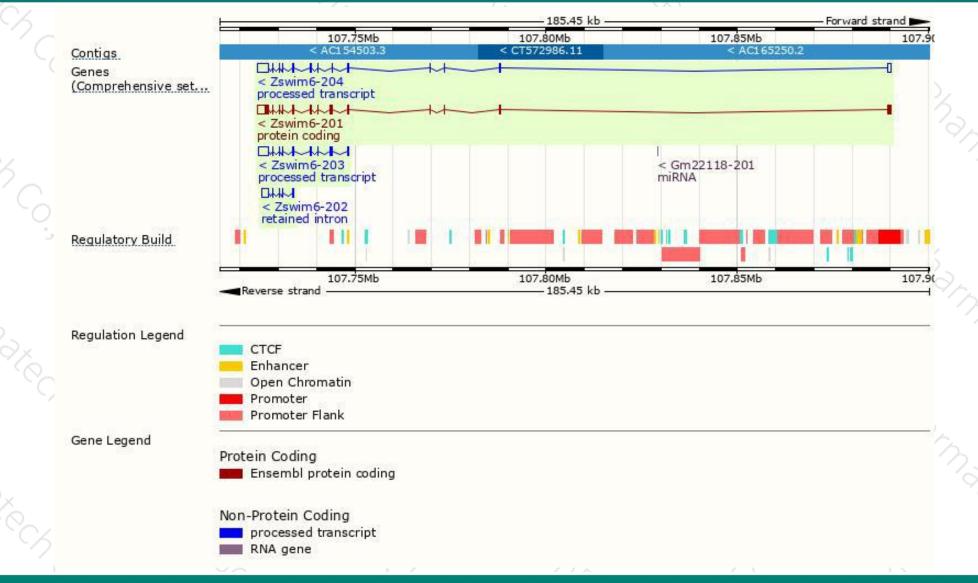
- 165.45 kb -

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### **Genomic location distribution**



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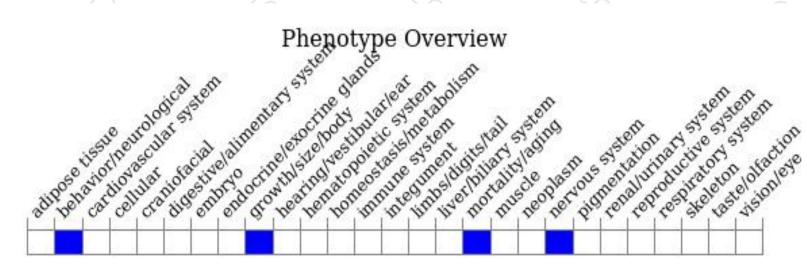
## **Protein domain**



|                        | 6   |                         |                 |                   | 7.S.                       |      |              | °°S -         |
|------------------------|---|-------------------------|-----------------|-------------------|----------------------------|------|--------------|---------------|
| ×1.                    | ENSMUSP00000158<br>MobiDB lite<br>Low complexity (Seg)<br>Coiled-coils (Ncoils) |                         |                 |                   |                            | -    |              |               |
|                        | PROSITE profiles  |                         | Zinc finger     | ; SWIM-type       |                            |      |              |               |
| 6                      | PANTHER   |                         | PTHR22619 ISF   | 3                 |                            |      |              |               |
| ~^ <u>&gt;</u>         |   |                         | PTHR22619       |                   |                            |      |              | G             |
|                        | All sequence SNPs/i   | Seguence varian         | ts (dbSNP and a | Il other sources) | 5 100                      | i II | 0            | <u>n</u> 34   |
|                        | Variant Legend  |                         |                 |                   |                            |      |              |               |
|                        |   | splice accep            |                 |                   |                            |      |              |               |
|                        |   | frameshift v            | - X.C.          |                   |                            |      |              | <u></u>       |
| N                      |   | missense va             | 2.45 2.55       |                   |                            |      |              |               |
|                        |   | splice region synonymou |                 |                   |                            |      |              |               |
|                        | Scale bar   | 6                       | 200             | 400               | 600                        | 800  | 1000         | 1207          |
|                        | 97  |                         |                 |                   | $\langle \diamond \rangle$ | ×    | $\checkmark$ | Sec.          |
|                        |   |                         |                 |                   |                            |      |              | ~~~~          |
| $\mathcal{D}_{\Delta}$ |   |                         | $\sim 0$        |                   | Sh.                        | 20   |              |               |
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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/).

According to the existing MGI data,mice homozygous for a knock-out allele exhibit partial postnatal lethality, decreased striatal volume, abnormal medium spiny neuron morphology, and altered motor control including hyperactivity, impaired rotarod performance, repetitive movements, and behavioral hyperresponsiveness to amphetamine.

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



