

# Arid5b Cas9-CKO Strategy

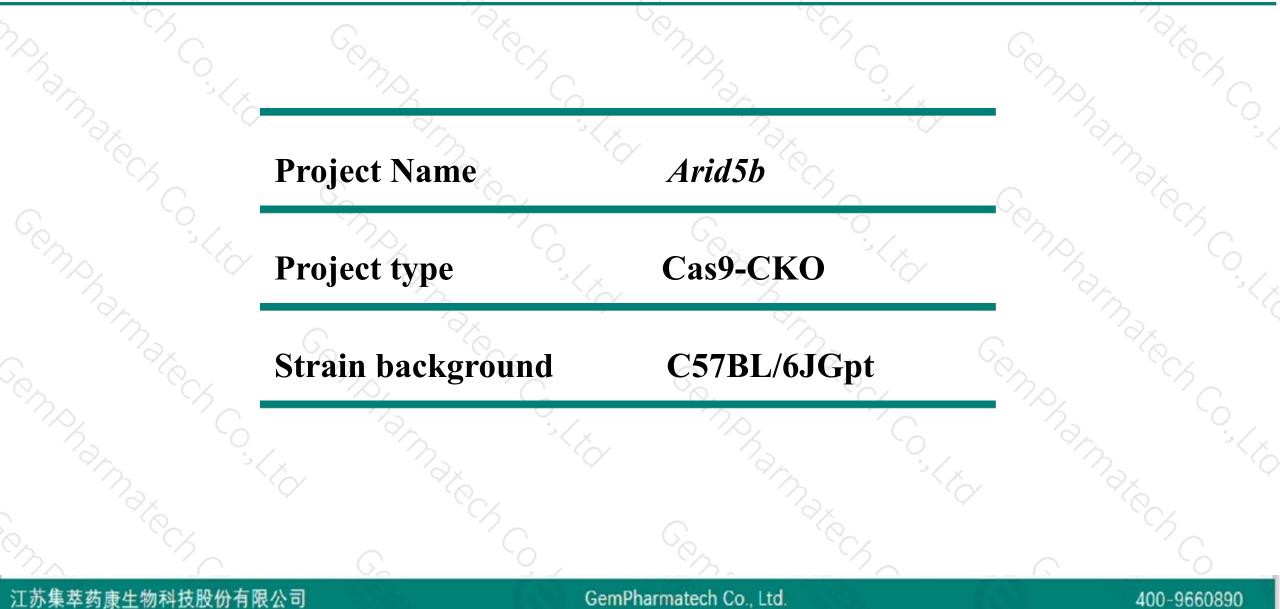
Designer: Reviewer:

Design Date:

Huan Fan Huan Wang 2019-12-11

# **Project Overview**



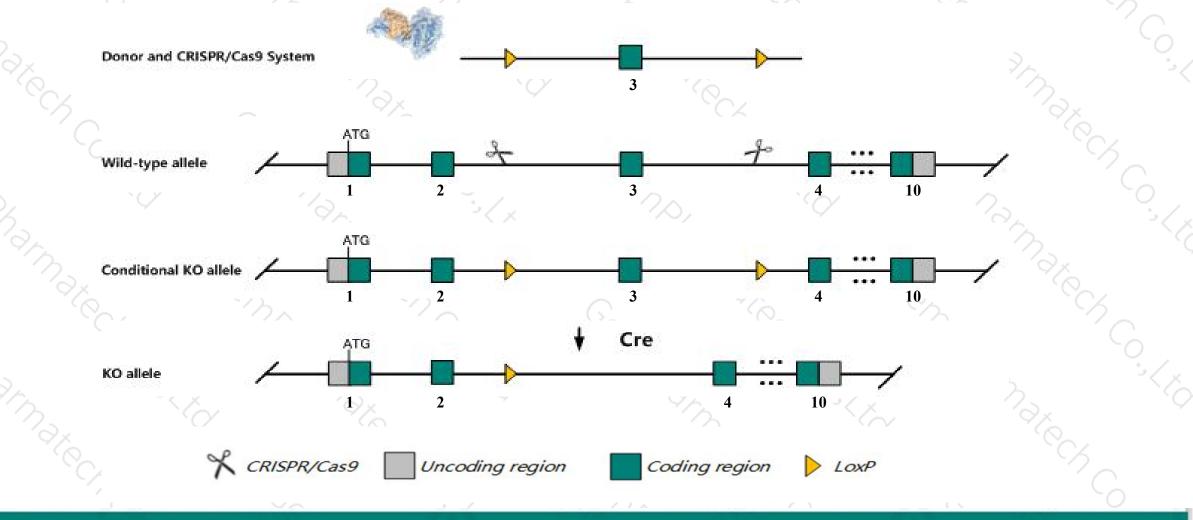


## **Conditional Knockout strategy**



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Arid5b* gene. The schematic diagram is as follows:



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The Arid5b gene has 4 transcripts. According to the structure of Arid5b gene, exon3 of Arid5b-204 (ENSMUST00000219238.1) transcript is recommended as the knockout region. The region contains 226bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Arid5b* 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 disruptions in this gene experience a high level of mortality perinatally or earlier. Growth rates are low and mice remain small throughout live. There are abnormalities in various organ systems as well as the reproductive system. Fertility is reduced.
- ≻Transcript *Arid5b-203* may not be affected.
- The Arid5b gene is located on the Chr10. 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)**



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Arid5b AT rich interactive domain 5B (MRF1-like) [Mus musculus (house mouse)]

Gene ID: 71371, updated on 31-Jan-2019

#### Summary

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# **Transcript information (Ensembl)**



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

| Name       | Transcript ID        | bp   | Protein       | Biotype                 | CCDS      | UniProt    | Flags                           |
|------------|----------------------|------|---------------|-------------------------|-----------|------------|---------------------------------|
| Arid5b-204 | ENSMUST00000219238.1 | 7581 | <u>1188aa</u> | Protein coding          | CCDS35929 | Q8BM75     | TSL:1 GENCODE basic APPRIS P2   |
| Arid5b-203 | ENSMUST00000218532.1 | 3025 | <u>945aa</u>  | Protein coding          | -8        | Q8BM75     | TSL:1 GENCODE basic APPRIS ALT2 |
| Arid5b-201 | ENSMUST00000020106.8 | 4202 | <u>254aa</u>  | Nonsense mediated decay | -         | A0A1X7SB62 | TSL:1                           |
| Arid5b-202 | ENSMUST00000218425.1 | 2946 | No protein    | Retained intron         | 2         | 82         | TSL:1                           |

The strategy is based on the design of Arid5b-204 transcript, The transcription is shown below

#### < Arid5b-204 protein coding

Reverse strand ·

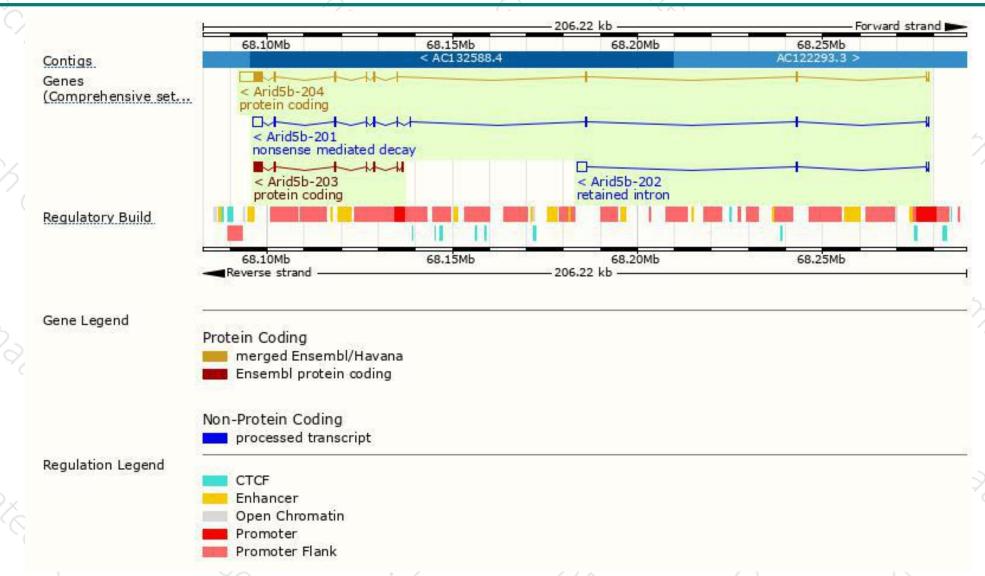
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186.22 kb

### **Genomic location distribution**





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# **Protein domain**

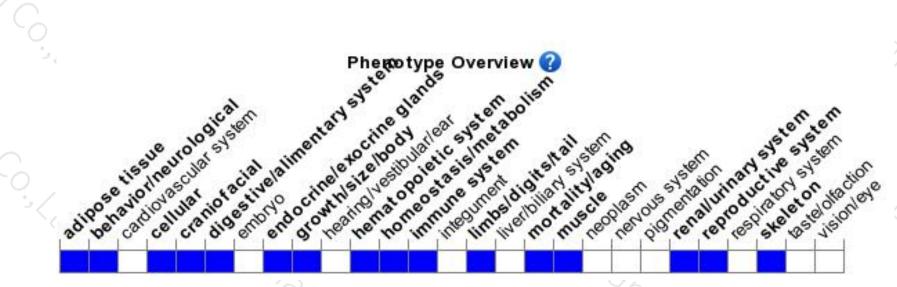
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|    | Variant Legend                                     | <u> </u>                 |              |                           |             | 00 (0     | 618 |  | 1.0.1 |  | h. 1911. | 0./. |
|----|--|--------------------------|--------------|---------------------------|-------------|-----------|-----|--|-------|--|----------|------|
|    | All sequence SNPs/i                                | Sequence variant         | s (dbSNP and |                           |             |           |     |  |       |  | 5 MG     |      |
|    | Gene3D   | PTHR13964<br>2.30.30.490 |              | ARID DNA-bindi            |             |           |     |  |       |  |          | - 34 |
|    | PROSITE profiles<br>PANTHER                        |                          |              |                           |             |           |     |  |       |  | _        |      |
|    |  |                          |              | ARID DNA-bind             |             |           |     |  |       |  |          |      |
|    |  |                          |              | SM01014<br>ARID DNA-bindi | ng domain   |           |     |  |       |  |          |      |
|    | Superfamily<br>SMART                               |                          |              | RID DNA-binding           | g domain su | perfamily |     |  |       |  |          | 0    |
| 2. | MobiDB lite<br>Low complexity (Seg)<br>Superfamily |                          |              |                           |             |           |     |  |       |  |          |      |

### 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 disruptions in this gene experience a high level of mortality perinatally or earlier. Growth rates are low and mice remain small throughout live. There are abnormalities in various organ systems as well as the reproductive system. Fertility is reduced.

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



