

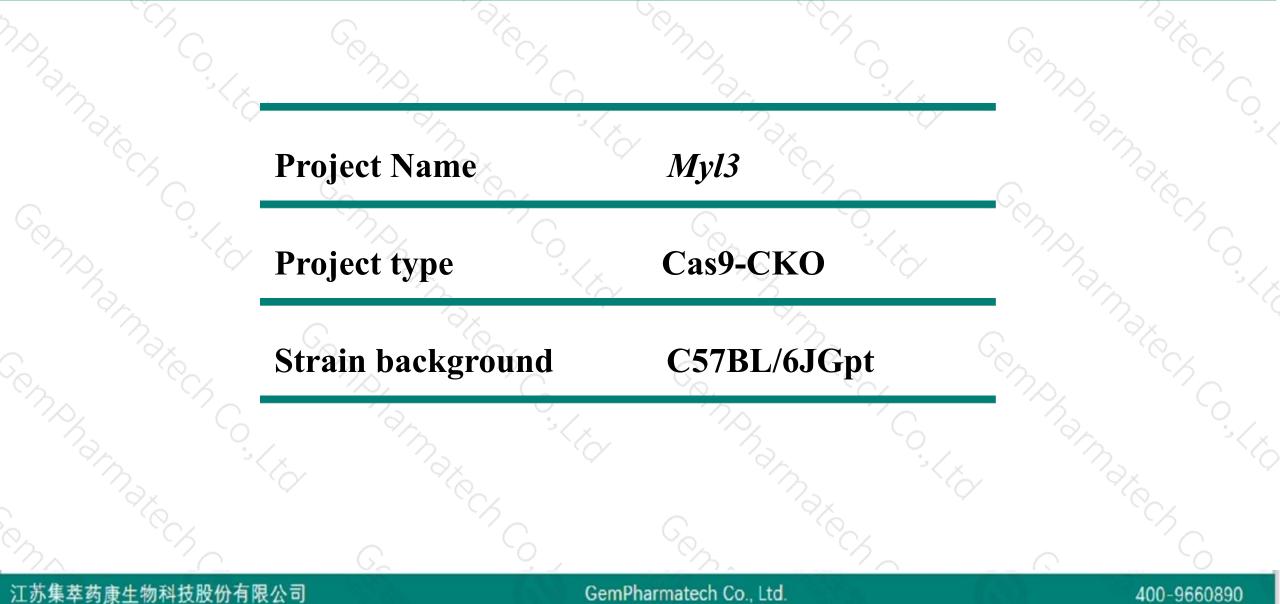
# Myl3 Cas9-CKO Strategy

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Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2020-1-19

# **Project Overview**

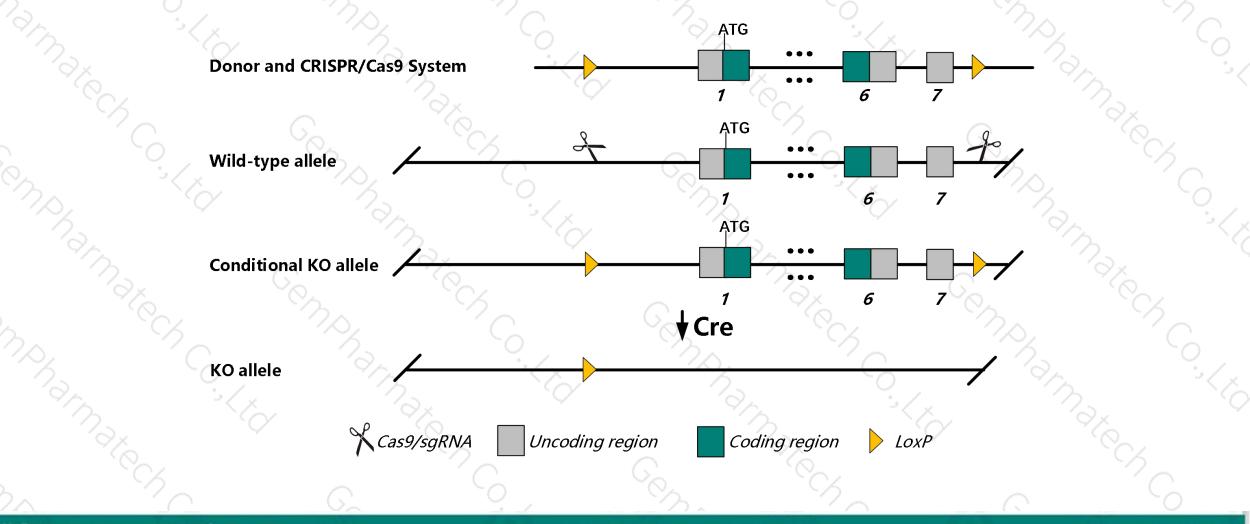




# **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Myl3* gene. The schematic diagram is as follows:



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The Myl3 gene has 5 transcripts. According to the structure of Myl3 gene, exon1-exon7 of Myl3-201 (ENSMUST00000079784.11) 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 *Myl3* 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.

### Notice



- The Myl3 gene is located on the Chr9. 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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| myosin, light                          | polypeptide 3 [   | Mus musculus (house mouse  | 2)]   |   |   |  |  |
|--|---|--|---|---|---|--|--|
| D: 17897, updated on                   | 1 24-Oct-2019   |  |   |   | ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~  |  |  |
| ummary                                 |   |  |   | * ?   |   |  |  |
| Official Symbol                        | MvI3 provided by MGI  |  |   |   |   |  |  |
| the second second second second second |   | de 3 provided by MGI   |   |   | 2.  |  |  |
|  |   | · · · · · · · · · · · · · · · · · · ·  |   |   |   |  |  |
| 0.5                                    |   | 0000059741   |   |   |   |  |  |
| Gene type                              | protein coding  |  |   |   | 5   |  |  |
| RefSeq status                          | VALIDATED   |  |   |   |   |  |  |
| Organism                               | Mus musculus  |  |   |   |   |  |  |
| Lineage                                | Eukaryota; Metazoa; C   | Chordata; Craniata; Vertebrata; Euteleostom  | ii; Mammal  | ia; Eutheria; Euarchontoglires; Glires; Rodentia;   |   |  |  |
|  | Myomorpha; Muroidea   | i; Muridae; Murinae; Mus; Mus  |   |   |   |  |  |
| Also known as                          | Mylc; VLC1; MLC1s; M  | /LC1v; MLC1SB  |   |   |   |  |  |
| Expression                             | Restricted expression toward heart adult (RPKM 3314.7) See more   |  |   |   |   |  |  |
| Orthologs                              | <u>human</u> all  |  |   |   |   |  |  |
| enomic context                         |   |  |   | * ?   |   |  |  |
| ation: 9 F2; 9 60.69                   | 9 cM  |  |   | See Myl3 in Genome Data Viewe   | er  |  |  |
| on count: 7                            |   |  |   |   | (   |  |  |
| notation release                       | Status  | Assembly   | Chr   | Location  | Sec.  |  |  |
| 8                                      | current   | GRCm38.p6 (GCF 000001635.26)   | 9   | NC 000075.6 (110763678110769802)  |   |  |  |
|  |   |  |   | 10_000013.0 (110103010110103002)  | 11/100  |  |  |
|  | D: 17897, updated on<br>ummary<br>Official Symbol<br>Official Full Name<br>Primary source<br>See related<br>Gene type<br>RefSeq status<br>Organism<br>Lineage<br>Also known as<br>Expression<br>Orthologs<br>enomic context<br>ation: 9 F2; 9 60.68<br>n count: 7 | D: 17897, updated on 24-Oct-2019<br>ummary<br>Official Symbol Myl3 provided by MGI<br>Official Full Name myosin, light polypepti<br>Primary source MGI:MGI:97268<br>See related Ensembl:ENSMUSGO<br>Gene type protein coding<br>RefSeq status VALIDATED<br>Organism Mus musculus<br>Lineage Eukaryota; Metazoa; O<br>Myomorpha; Muroidea<br>Also known as Mylc; VLC1; MLC1s; M<br>Expression Restricted expression<br>Orthologs human all<br>enomic context<br>ation: 9 F2; 9 60.69 cM<br>n count: 7 | D: 17897, updated on 24-Oct-2019<br>ummary<br>Official Symbol Myl3 provided by MGI<br>Official Full Name myosin, light polypeptide 3 provided by MGI<br>Primary source MGI:MGI:97268<br>See related Ensembl:ENSMUSG00000059741<br>Gene type protein coding<br>RefSeq status VALIDATED<br>Organism Mus musculus<br>Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostom<br>Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus<br>Also known as Mylc; VLC1; MLC1s; MLC1v; MLC1SB<br>Expression Restricted expression toward heart adult (RPKM 3314.7) See mor<br>Orthologs human all<br>enomic context<br>ation: 9 F2; 9 60.69 cM<br>n count: 7<br>notation release Status Assembly | Official Symbol Myl3 provided by MGI   Official Full Name myosin, light polypeptide 3 provided by MGI   Primary source MGI:MGI:97268   See related Ensembl:ENSMUSG00000059741   Gene type protein coding   RefSeq status VALIDATED   Organism Mus musculus   Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammal Myomorpha; Muroidea; Murinae; Mus; Mus   Also known as Mylc; VLC1; MLC1s; MLC1v; MLC1SB   Expression Restricted expression toward heart adult (RPKM 3314.7) See more Orthologs   human all enomic context   ation: 9 F2; 9 60.69 cM   n count: 7   notation release Status Assembly Chr | D: 17897, updated on 24-Oct-2019    ummary Image: Constraint of the symbol of the sym |  |  |

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# **Transcript information (Ensembl)**



The gene has 5 transcripts, all transcripts are shown below:

| i l' pre |                            |      | freeze       |                 |                    |                     |       | a de la compacta de l |             |
|----------|----------------------------|------|--------------|-----------------|--------------------|---------------------|-------|--|-------------|
| Name 🖕   | lame 🖕 🛛 Transcript ID 🖕 b |      | Protein 🖕    | Biotype 🍦       | CCDS               | UniProt 💧           | Flags |  |             |
| MyI3-201 | ENSMUST0000079784.11       | 937  | <u>204aa</u> | Protein coding  | <u>CCDS23571</u> & | <u>P09542</u> &     | TSL:1 | GENCODE basic  | APPRIS P2   |
| MyI3-205 | ENSMUST00000200011.4       | 737  | <u>184aa</u> | Protein coding  | -                  | <u>A0A0G2JDW2</u> & |       | CDS 3' incomplete  | TSL:5       |
| MyI3-203 | ENSMUST00000136695.2       | 614  | <u>127aa</u> | Protein coding  | ÷.                 | <u>A0A0G2JDM3</u> & | TSL:3 | GENCODE basic  | APPRIS ALT2 |
| MyI3-202 | ENSMUST00000124267.7       | 575  | <u>127aa</u> | Protein coding  | -                  | <u>A0A0G2JDM3</u> & | TSL:5 | GENCODE basic  | APPRIS ALT2 |
| MyI3-204 | ENSMUST00000153142.2       | 1125 | No protein   | Retained intron | 2                  | -                   |       | TSL:2  |             |
|          |                            | 1    |              |                 |                    | 1 <u>.</u>          |       |  |             |

The strategy is based on the design of Myl3-201 transcript, The transcription is shown below

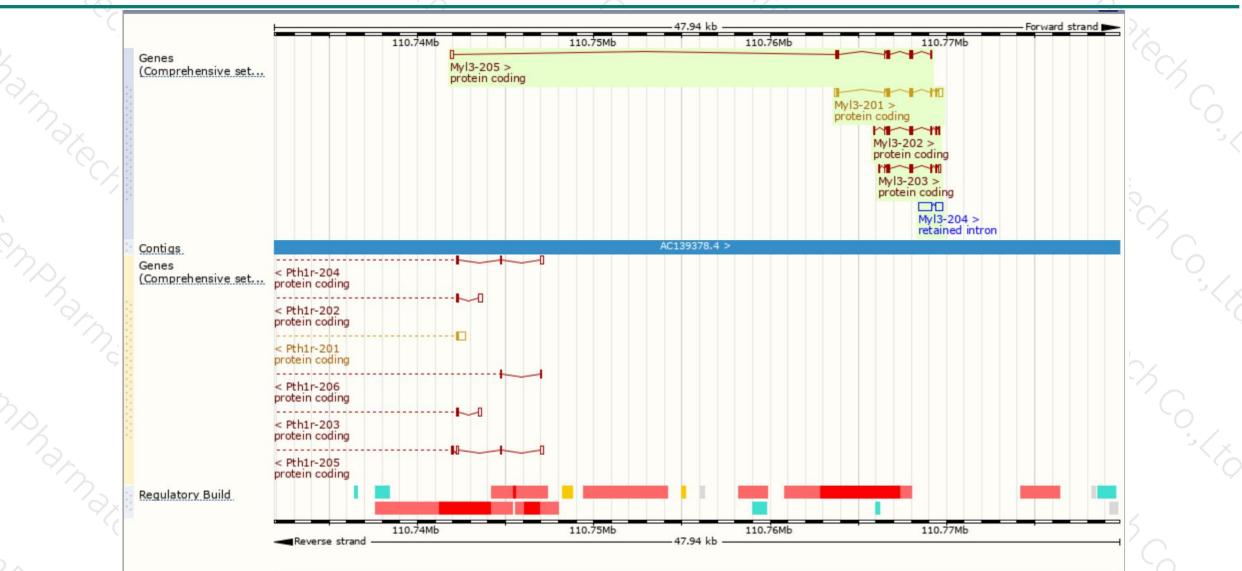
| Myl3-201 ><br>protein coding |   |     | 6.15 kb |      | Forward st | trand |
|------------------------------|---|-----|---------|------|------------|-------|
|                              | T provide the second | 1.3 | $\Box$  | N 20 | 1 J        |       |

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### **Genomic location distribution**





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### **Protein domain**



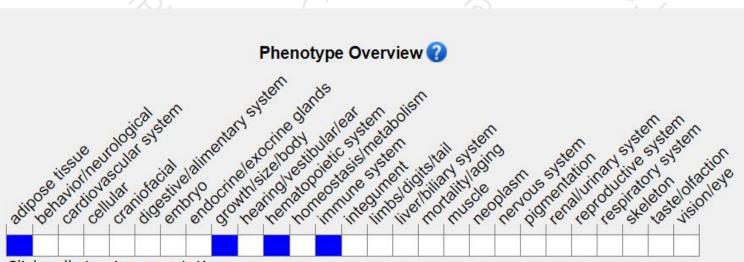
| nonarna.  |   | Gens,                                   | Steel Co                   |                |                 | S<br>S<br>S                            | Gensons                       |      |
|-----------|---|---|----------------------------|----------------|-----------------|--|-------------------------------|------|
|           | ENSMUSP00000078<br>MobiDB lite<br>Low complexity (Seg)<br>Superfamily<br>PROSITE profiles |   | EF-hand doma<br>EF-hand do |                |                 |  |                               | 2%   |
| Cenpha.   | Gene3D<br>CDD   | PTHR23048:SF2<br>PTHR23048              | 1.10.238.10                |                |                 | EF-hand domain                         |                               |      |
|           | All sequence SNPs/i<br>Variant Legend<br>Scale bar  | missense variant<br>synonymous variar   | NP and all other sources)  | 80             | splice region v |  | 160 180                       | 204  |
| Sempharme |   | Y X X X X X X X X X X X X X X X X X X X |                            | ,<br>,<br>,    | nsharns         | ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ | $\langle \rangle \rangle_{L}$ |      |
|           |   |   | S.C.                       | C <sub>O</sub> | ×<br>>.         | ×<br>Ch                                |                               | ~~~~ |

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# Mouse phenotype description(MGI)





Click cells to view annotations.

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



