

# ***Bcl9* Cas9-CKO Strategy**

**Designer: Lixin Lv**

**Reviewer: Shilei Zhu**

**Design Date: 2019-11-13**

# Project Overview

**Project Name**

*Bcl9*

**Project type**

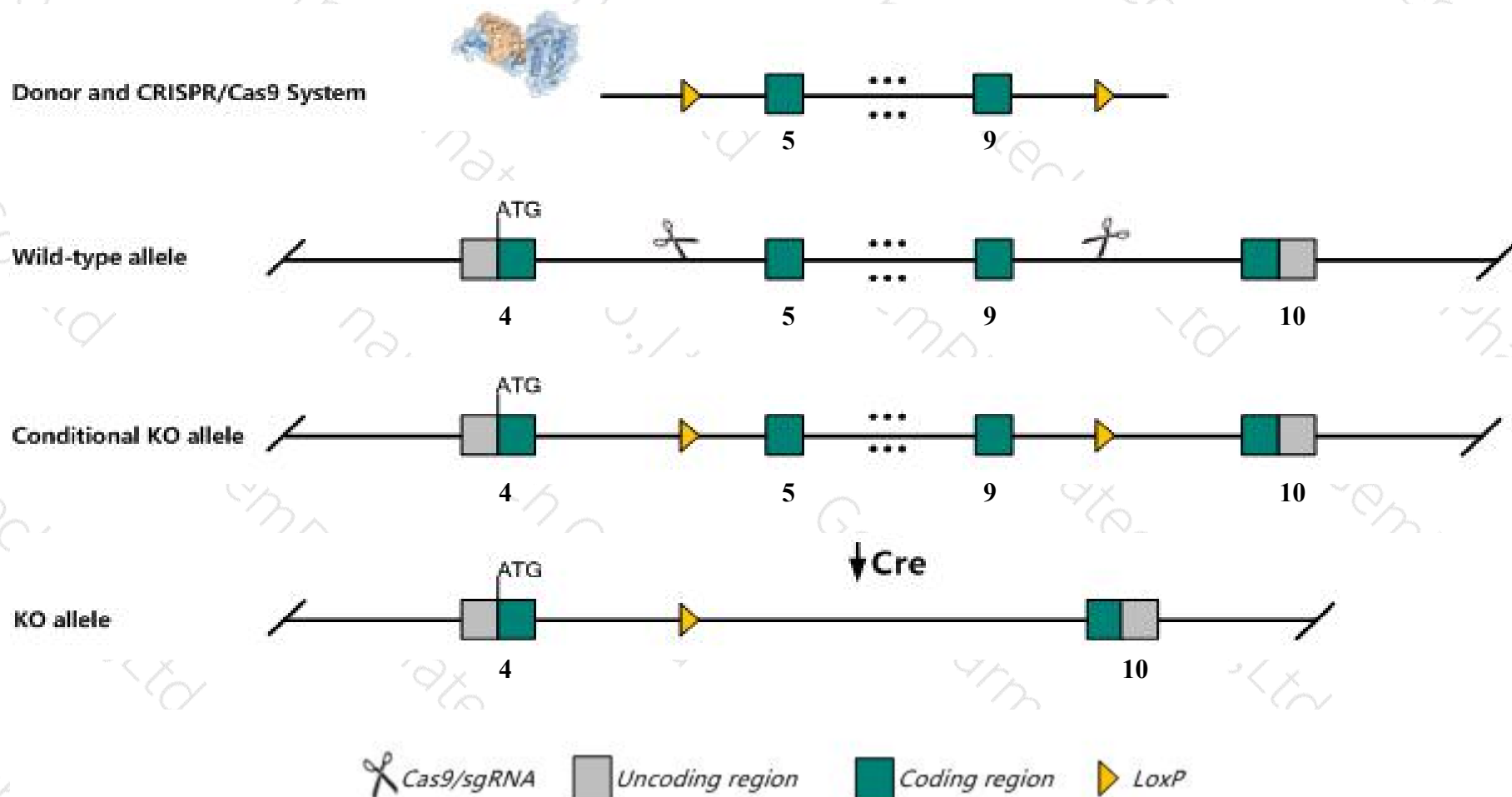
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Bcl9* gene. The schematic diagram is as follows:



# Technical routes

- The *Bcl9* gene has 8 transcripts. According to the structure of *Bcl9* gene, exon5-exon9 of *Bcl9*-201(ENSMUST00000046521.13) transcript is recommended as the knockout region. The region contains 3107bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Bcl9* 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 carrying homozygous floxed *Bcl9* and *Bcl9l* alleles, inactivated in muscle cells, exhibit impaired muscle regeneration due to increased apoptosis.
- The *Bcl9* gene is located on the Chr3. 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)

## Bcl9 B cell CLL/lymphoma 9 [Mus musculus (house mouse)]

Gene ID: 77578, updated on 31-Jan-2019

### Summary



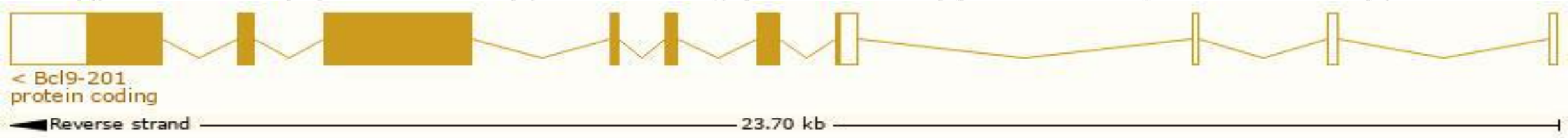
|                           |   |
|---------------------------|---|
| <b>Official Symbol</b>    | Bcl9 provided by <a href="#">MGI</a>  |
| <b>Official Full Name</b> | B cell CLL/lymphoma 9 provided by <a href="#">MGI</a>   |
| <b>Primary source</b>     | <a href="#">MGI:MGI:1924828</a>   |
| <b>See related</b>        | <a href="#">Ensembl:ENSMUSG00000038256</a>  |
| <b>Gene type</b>          | protein coding  |
| <b>RefSeq status</b>      | VALIDATED   |
| <b>Organism</b>           | <a href="#">Mus musculus</a>  |
| <b>Lineage</b>            | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| <b>Also known as</b>      | 2610202E01Rik, 8030475K17Rik, A330041G23Rik, Gm130  |
| <b>Expression</b>         | Ubiquitous expression in limb E14.5 (RPKM 13.7), CNS E11.5 (RPKM 12.3) and 28 other tissues <a href="#">See more</a>  |
| <b>Orthologs</b>          | <a href="#">human</a> <a href="#">all</a>   |

# Transcript information (Ensembl)

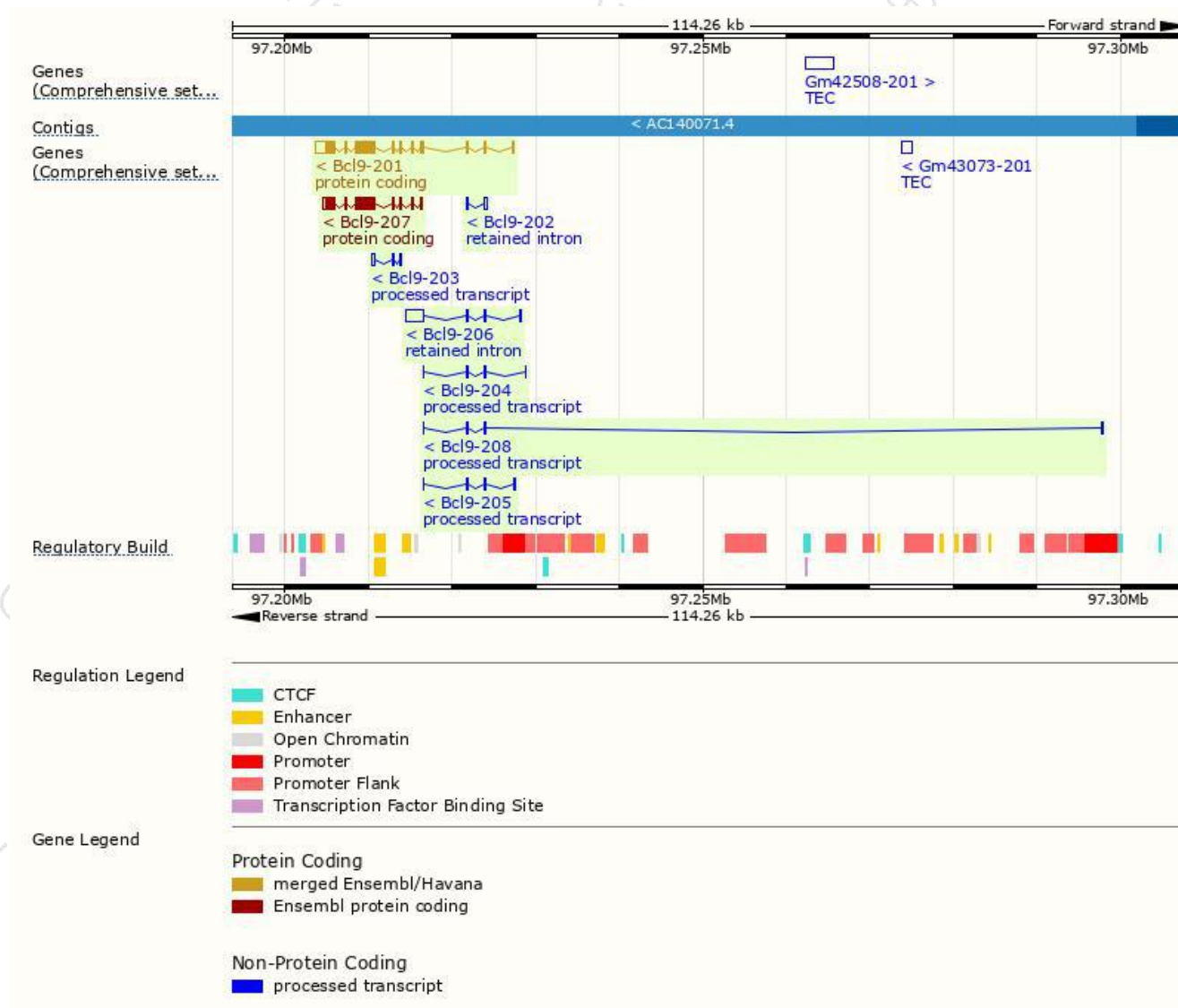
The gene has 8 transcripts,all transcripts are shown below:

| Name     | Transcript ID                         | bp   | Protein                | Biotype         | CCDS                      | UniProt                | Flags                         |
|----------|---------------------------------------|------|------------------------|-----------------|---------------------------|------------------------|-------------------------------|
| Bcl9-201 | <a href="#">ENSMUST00000046521.13</a> | 6102 | <a href="#">1425aa</a> | Protein coding  | <a href="#">CCDS17654</a> | <a href="#">Q9D219</a> | TSL:1 GENCODE basic APPRIS P1 |
| Bcl9-207 | <a href="#">ENSMUST00000166341.1</a>  | 4652 | <a href="#">1425aa</a> | Protein coding  | <a href="#">CCDS17654</a> | <a href="#">Q9D219</a> | TSL:1 GENCODE basic APPRIS P1 |
| Bcl9-206 | <a href="#">ENSMUST00000141861.7</a>  | 2500 | No protein             | Retained intron | -                         | -                      | TSL:1                         |
| Bcl9-202 | <a href="#">ENSMUST00000127319.1</a>  | 405  | No protein             | Retained intron | -                         | -                      | TSL:3                         |
| Bcl9-203 | <a href="#">ENSMUST00000132266.1</a>  | 679  | No protein             | lncRNA          | -                         | -                      | TSL:3                         |
| Bcl9-208 | <a href="#">ENSMUST00000196586.1</a>  | 453  | No protein             | lncRNA          | -                         | -                      | TSL:3                         |
| Bcl9-204 | <a href="#">ENSMUST00000134695.7</a>  | 421  | No protein             | lncRNA          | -                         | -                      | TSL:3                         |
| Bcl9-205 | <a href="#">ENSMUST00000135247.5</a>  | 353  | No protein             | lncRNA          | -                         | -                      | TSL:3                         |

The strategy is based on the design of *Bcl9-201* transcript,the transcription is shown below:

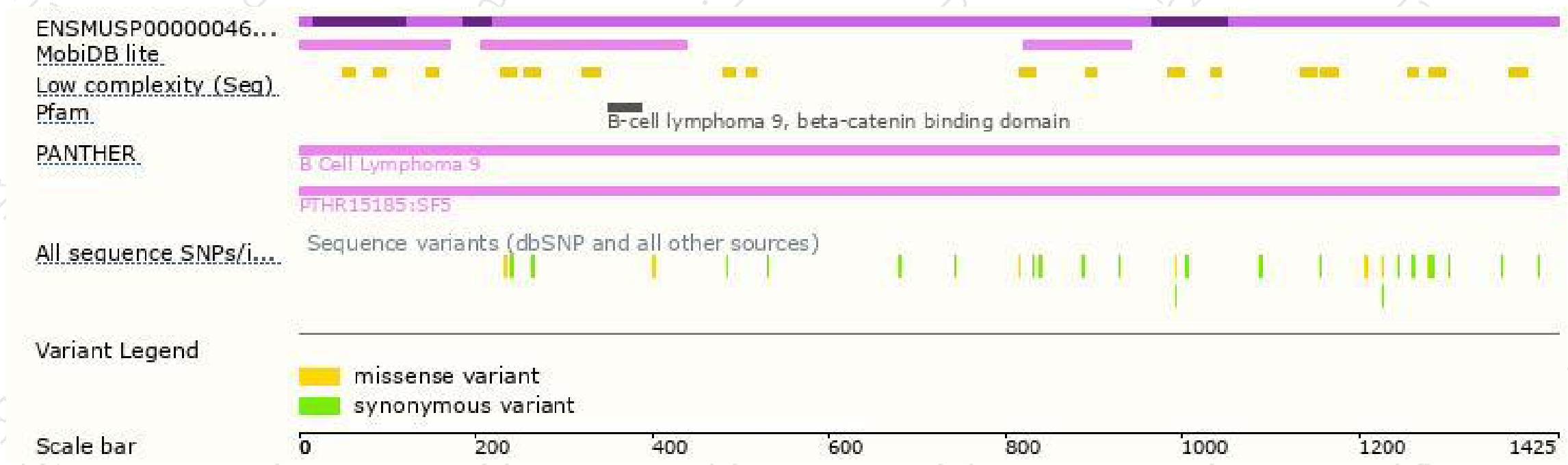


# Genomic location distribution





# Protein domain



If you have any questions, you are welcome to inquire.

Tel: 025-5864 1534

