

# Card9 Cas9-CKO Strategy

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# **Project Overview**



**Project Name** 

Card9

**Project type** 

Cas9-CKO

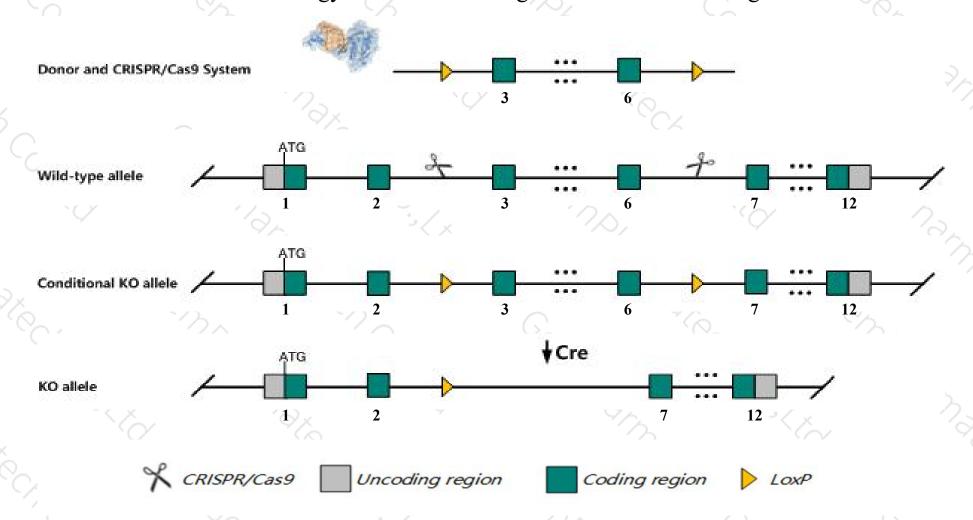
Strain background

C57BL/6JGpt

# Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Card9 gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Card9* gene has 3 transcripts. According to the structure of *Card9* gene, exon3-exon6 of *Card9-202* (ENSMUST00000100303.9) transcript is recommended as the knockout region. The region contains 755bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Card9* 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**



- > According to the existing MGI data, Mice homozygous for one allele of this gene display impaired immune responses to fungal infection but normal rates of bacterial clearance. However, homozygotes for a second allele display impaired bacterial clearance and impaired early innate immune responses.
- $\triangleright$  The partial intron of Gm13562 gene will be deleted together in this strategy.
- The floxed region is near to the N-terminal of *Dnlz* gene, this strategy may influence the regulatory function of the N-terminal of *Dnlz* gene.
- The *Card9* gene is located on the Chr2. 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)



#### Card9 caspase recruitment domain family, member 9 [ Mus musculus (house mouse) ]

Gene ID: 332579, updated on 8-Oct-2019

#### Summary

△ ?

Official Symbol Card9 provided by MGI

Official Full Name caspase recruitment domain family, member 9 provided by MGI

Primary source MGI:MGI:2685628

See related Ensembl: ENSMUSG00000026928

Gene type protein coding
RefSeq status VALIDATED
Organism <u>Mus musculus</u>

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;

Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

Also known as Gm782

Expression Broad expression in large intestine adult (RPKM 6.7), lung adult (RPKM 4.8) and 27 other tissues See more

Orthologs human all

#### Genomic context

☆ ?

Location: 2; 2 A3

See Card9 in Genome Data Viewer

Exon count: 13

| Annotation release | Status            | Assembly                     | Chr | Location                                   |  |
|--------------------|-------------------|------------------------------|-----|--|--|
| 108                | current           | GRCm38.p6 (GCF_000001635.26) | 2   | NC_000068.7 (2635217126360945, complement) |  |
| Build 37.2         | previous assembly | MGSCv37 (GCF_000001635.18)   | 2   | NC_000068.6 (2620783226215067, complement) |  |

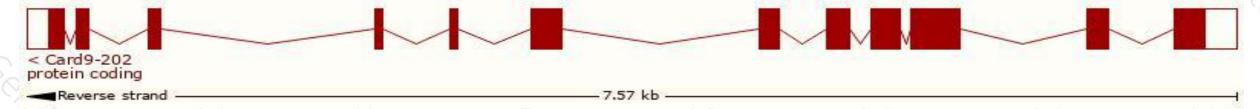
# Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

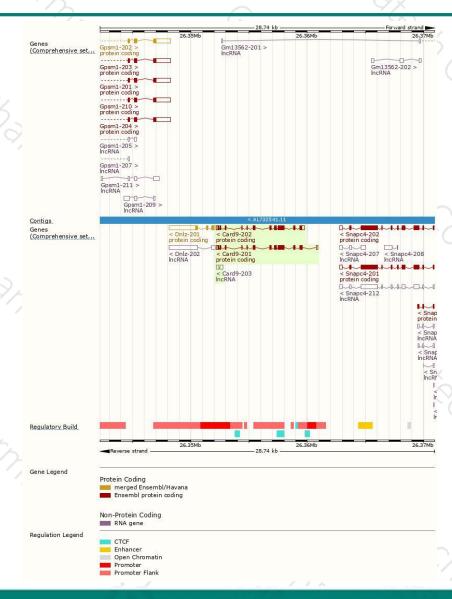
| Name      | Transcript ID        | bp   | Protein      | Biotype        | ccds      | UniProt | Flags                         |
|-----------|----------------------|------|--------------|----------------|-----------|---------|-------------------------------|
| Card9-202 | ENSMUST00000100303.9 | 1947 | <u>536aa</u> | Protein coding | CCDS38084 | A2AIV8  | TSL:1 GENCODE basic APPRIS P1 |
| Card9-201 | ENSMUST00000028294.6 | 1880 | <u>536aa</u> | Protein coding | CCDS38084 | A2AIV8  | TSL:5 GENCODE basic APPRIS P1 |
| Card9-203 | ENSMUST00000153370.1 | 516  | No protein   | IncRNA         | ų.        | 120     | TSL:1                         |

The strategy is based on the design of Card9-202 transcript, The transcription is shown below



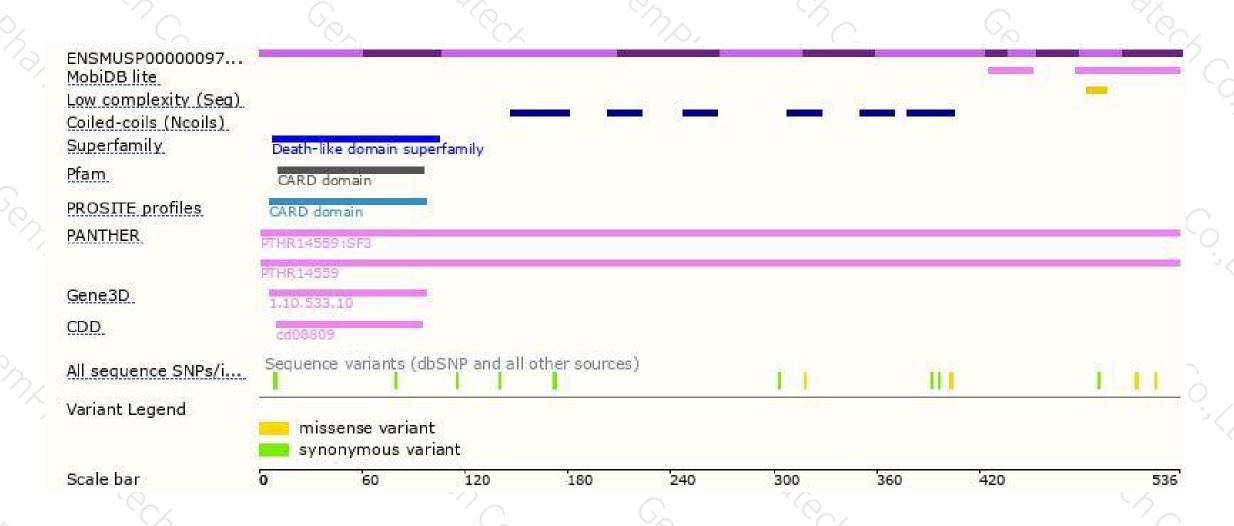
## Genomic location distribution





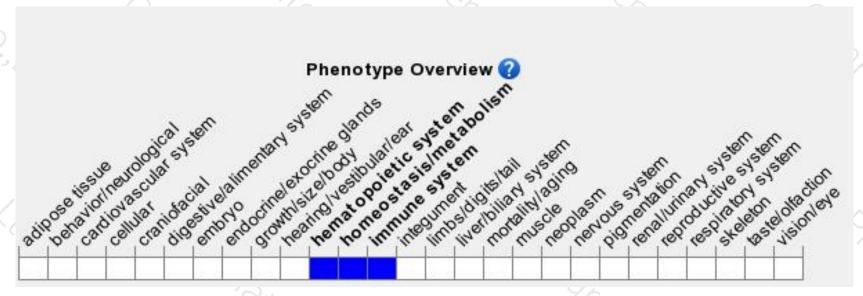
### Protein domain





# 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 one allele of this gene display impaired immune responses to fungal infection but normal rates of bacterial clearance. However, homozygotes for a second allele display impaired bacterial clearance and impaired early innate immune responses.



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





