

# *Card9* Cas9-KO Strategy

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# Project Overview

**Project Name**

*Card9*

**Project type**

**Cas9-KO**

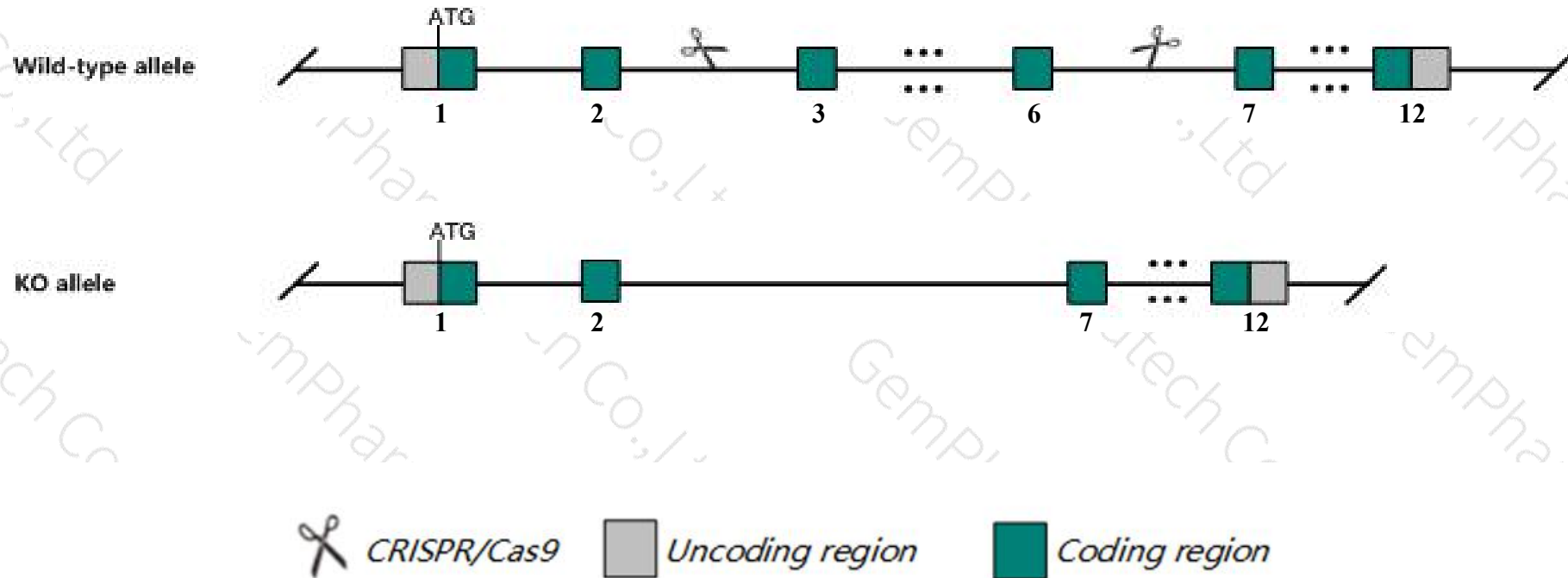
**Strain background**

**C57BL/6JGpt**



# Knockout strategy

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





- 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: gRNA was transcribed in vitro. Cas9 and gRNA 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.



- 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.
- The partial intron of *Gm13562* gene will be deleted together in this strategy.
- The knockout 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology 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 <a href="#">MGI</a>
Official Full Name	caspase recruitment domain family, member 9 provided by <a href="#">MGI</a>
Primary source	<a href="#">MGI:MGI:2685628</a>
See related	<a href="#">Ensembl:ENSMUSG00000026928</a>
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<a href="#">Mus musculus</a>
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 <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

### Genomic context

Location: 2; 2 A3 [See Card9 in Genome Data Viewer](#)

Exon count: 13

Annotation release	Status	Assembly	Chr	Location
<a href="#">108</a>	current	GRCm38.p6 ( <a href="#">GCF_000001635.26</a> )	2	NC_000068.7 (26352171..26360945, complement)
Build 37.2	previous assembly	MGSCv37 ( <a href="#">GCF_000001635.18</a> )	2	NC_000068.6 (26207832..26215067, complement)

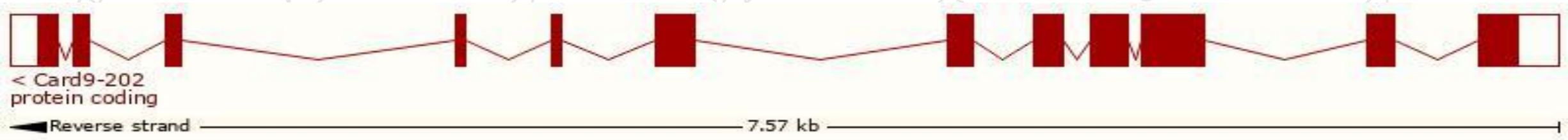


# Transcript information (Ensembl)

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

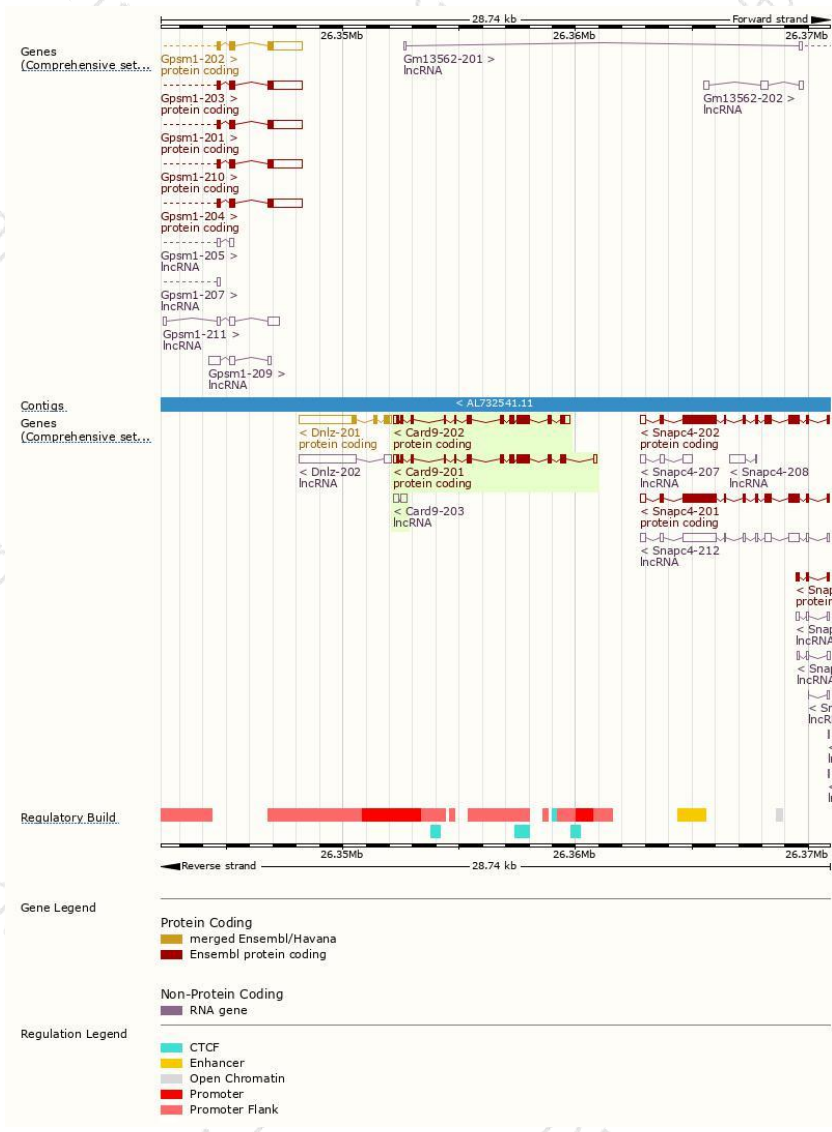
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Card9-202	<a href="#">ENSMUST00000100303.9</a>	1947	<a href="#">536aa</a>	Protein coding	<a href="#">CCDS38084</a>	<a href="#">A2AIV8</a>	TSL:1 GENCODE basic APPRIS P1
Card9-201	<a href="#">ENSMUST00000028294.6</a>	1880	<a href="#">536aa</a>	Protein coding	<a href="#">CCDS38084</a>	<a href="#">A2AIV8</a>	TSL:5 GENCODE basic APPRIS P1
Card9-203	<a href="#">ENSMUST00000153370.1</a>	516	No protein	lncRNA	-	-	TSL:1

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



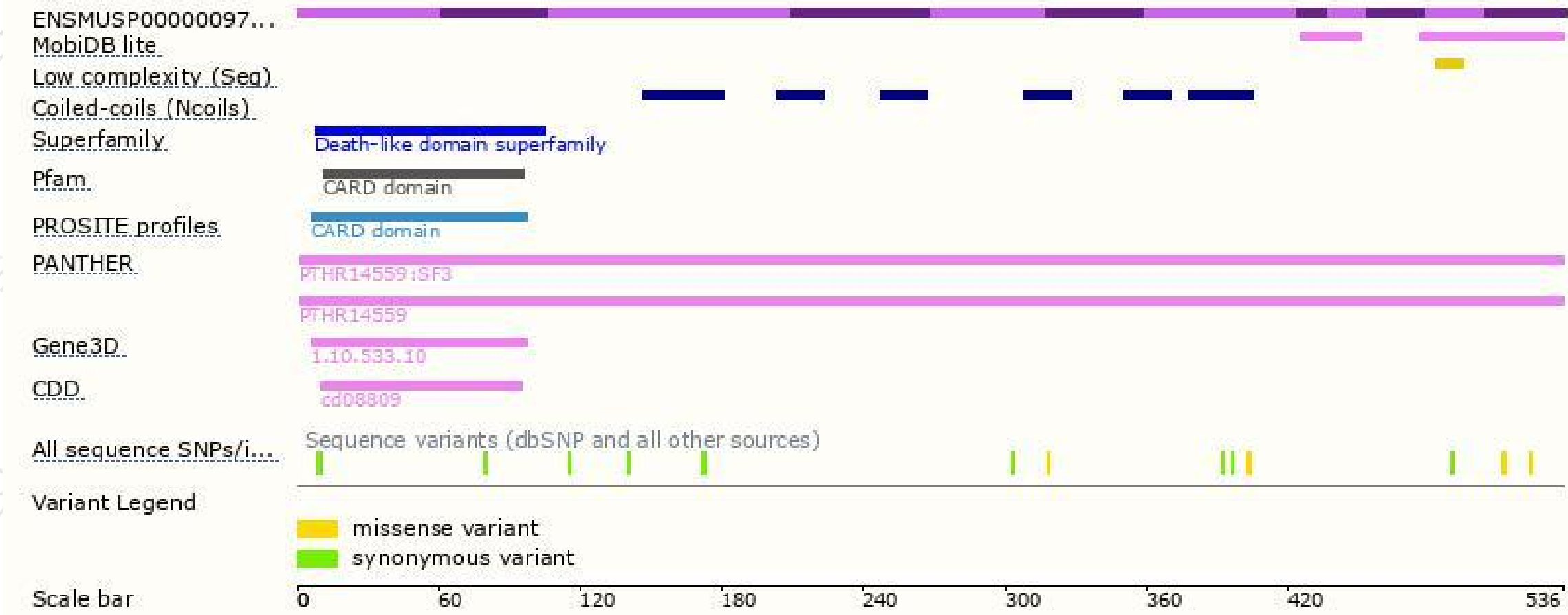


# Genomic location distribution



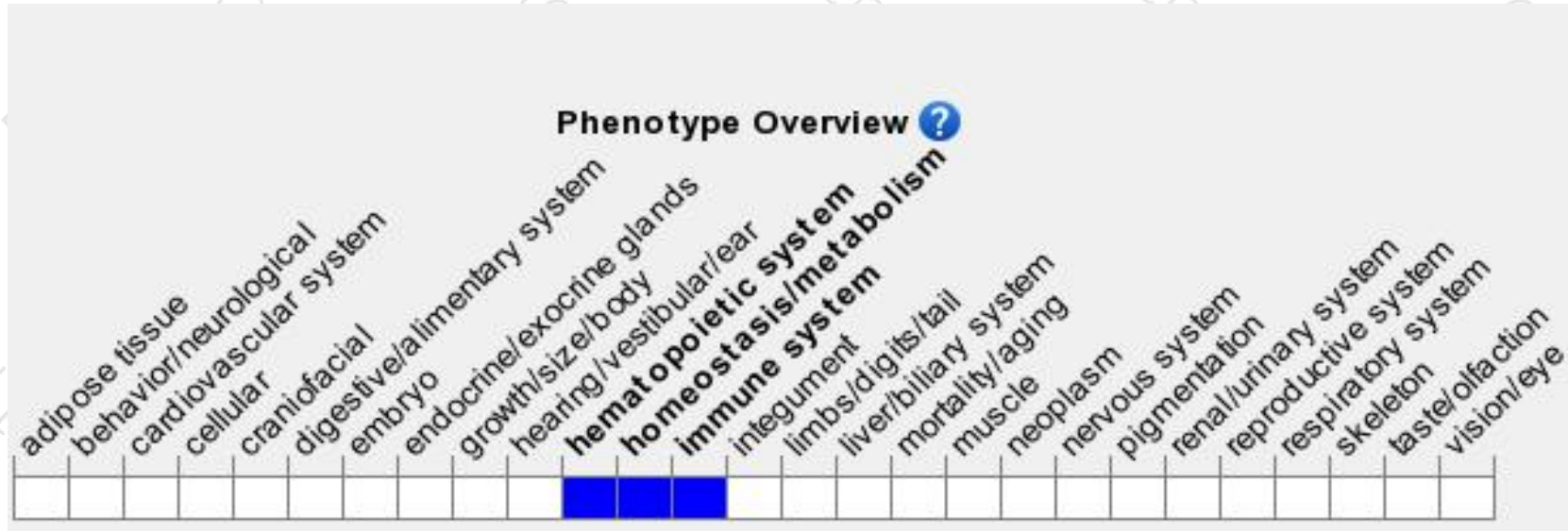


# Protein domain





# 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.

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