GemPharmatech™

# Rag1 Cas9-KO Strategy

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# Overview

## Target Gene Name

- Rag1

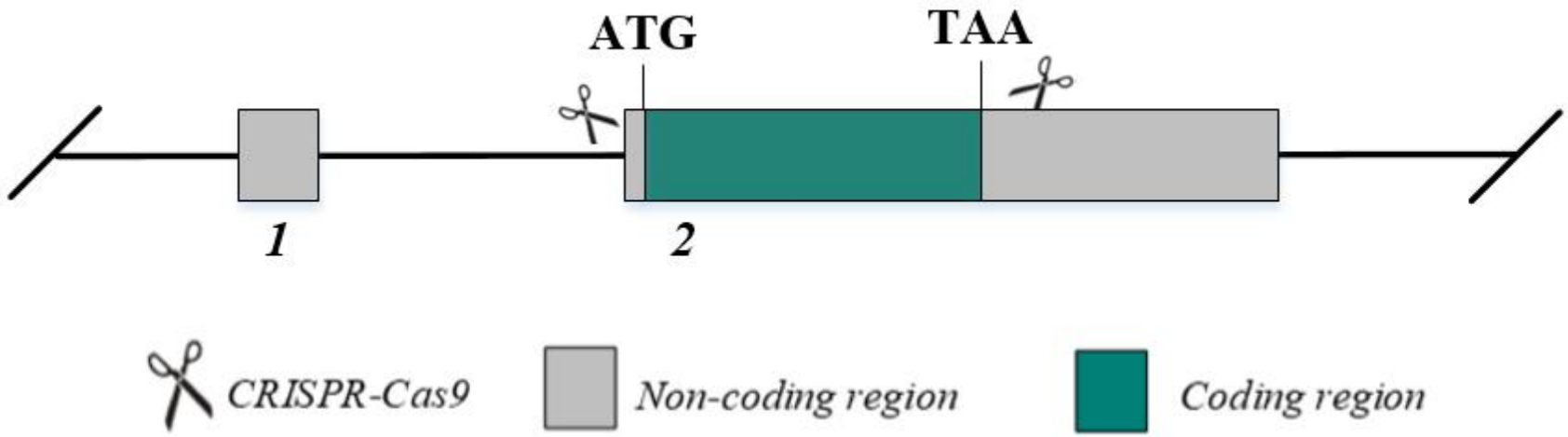
## Project Type

- Cas9-KO

## Genetic Background

- NOD/ShiLtJGpt

# Strain Strategy




Schematic representation of CRISPR-Cas9 engineering used to edit the *Rag1* gene.

# Technical Information

- The *Rag1* gene has 1 transcript. According to the structure of *Rag1* gene, exon2 of *MGP_NODShiLtJ_T0056045.1* transcript is recommended as the knockout region. The region contains all of the coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Rag1* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs were microinjected into the fertilized eggs of NOD/ShiLtJGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with NOD/ShiLtJGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

# Gene Information

**Rag1 recombination activating gene 1 [ *Mus musculus* (house mouse) ]**

Gene ID: 19373, updated on 11-Apr-2018

**Summary**

| | |
|---|---|
| **Official Symbol** | Rag1 provided by MGI |
| **Official Full Name** | recombination activating gene 1 provided by MGI |
| **Primary source** | MGI:MGI:97848 |
| **See related** | Ensembl:ENSMUSG00000061311 Vega:OTTMUSG00000014584 |
| **Gene type** | protein coding |
| **RefSeq status** | VALIDATED |
| **Organism** | Mus musculus |
| **Lineage** | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| **Also known as** | Rag-1 |
| **Expression** | Restricted expression toward thymus adult (RPKM 117.3) See more |
| **Orthologs** | human all |

**Genomic context**

**Location:** 2 E2; 2 53.88 cM

See Rag1 in Genome Data Viewer Map Viewer

**Exon count:** 3

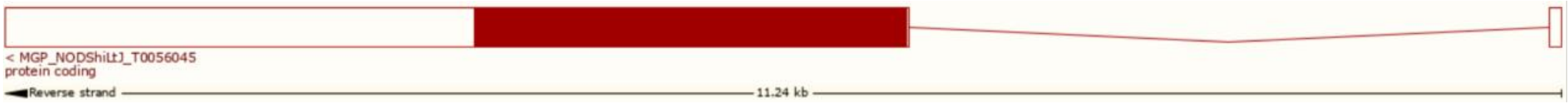
Source: https://www.ncbi.nlm.nih.gov/

# Transcript Information

The gene has 1 transcript,and the transcript is shown below:

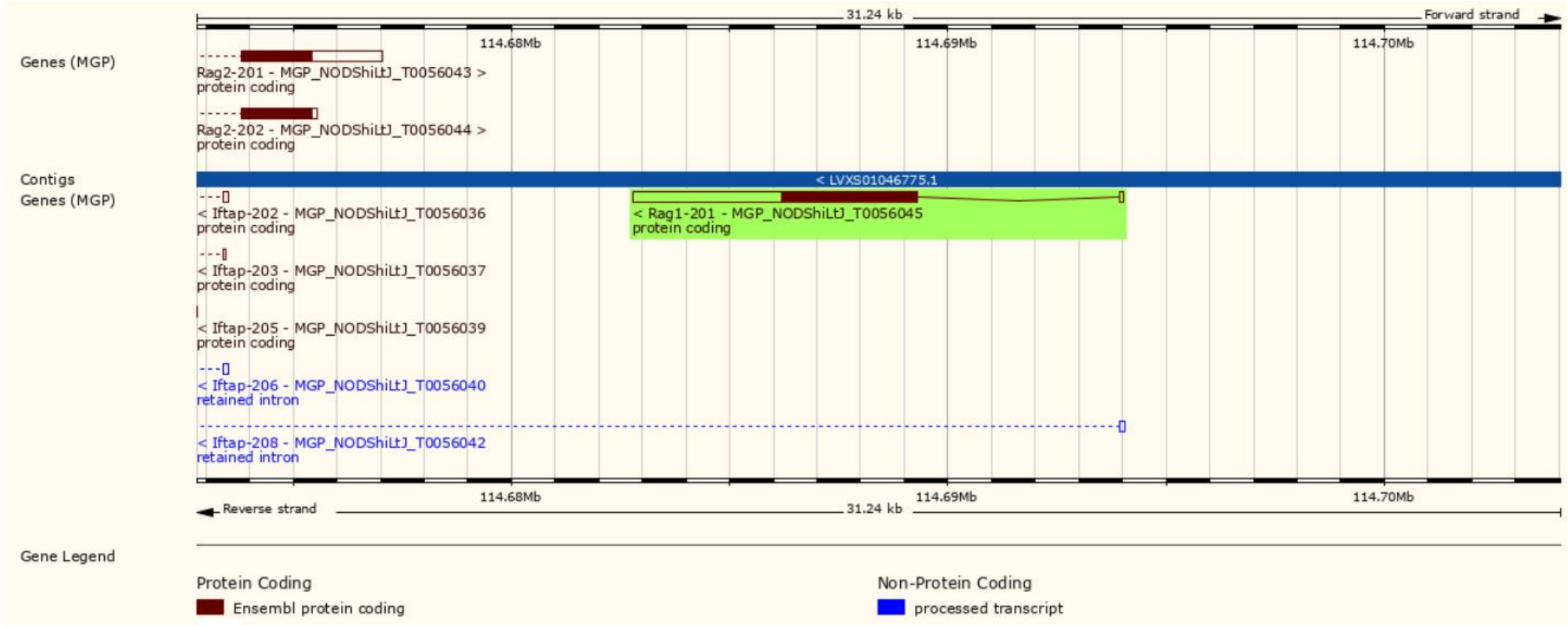
| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | RefSeq | Flags |
|---|---|---|---|---|---|---|---|---|
| - | MGP_NODShiLtJ_T0056045.1 | 6608 | 1040aa | Protein coding | CCDS16463 | P15919 | NM_009019<br>NP_033045 | |

The strategy is based on the design of *MGP_NODShiLtJ_T0056045.1* transcript, the transcription is shown below:




Source: https://www.ensembl.org

# Genomic Information



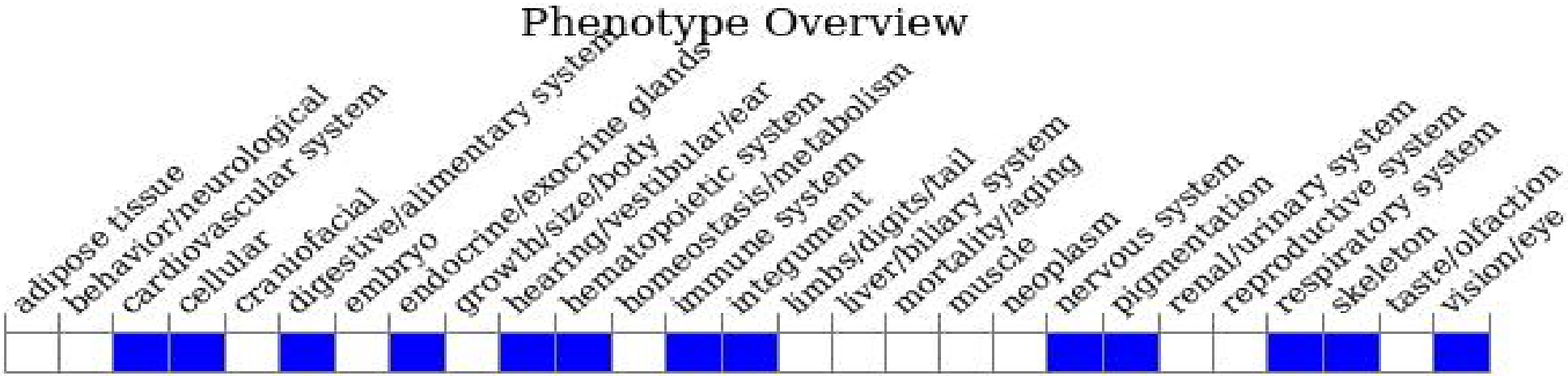

Source: : https://www.ensembl.org

# Protein Information




Source: : https://www.ensembl.org

# Mouse Phenotype Information (MGI)




- Homozygotes for targeted null mutations exhibit arrested development of T and B cell maturation at the CD4-8- thymocyte or B220+/CD43+pro-B cell stage due to inability to undergo V(D)J recombination.



Source: https://www.informatics.jax.org

# Important Information

- *Rag1* is located on Chr2. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risks of the mutation on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.