

Rag1&Il2rg Cas9-KO Strategy

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Reviewer: Yanhua Shen

Design Date: 2021-08-25

Project Overview

Project Name

Rag1&Il2rg

Project type

Cas9-KO

Strain background

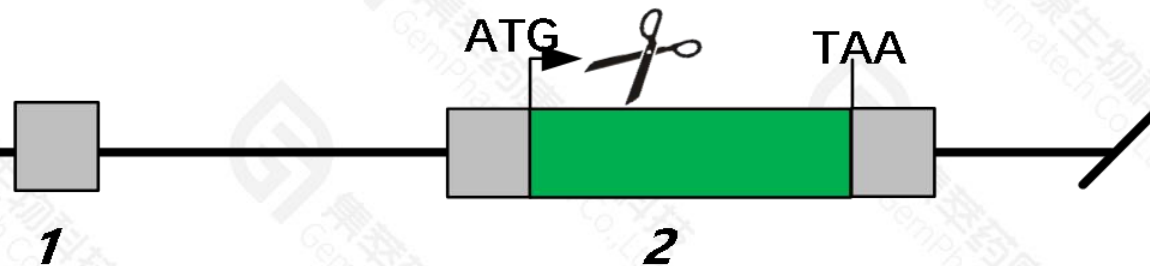
NOD/ShiLtJGpt

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rag1* and *Il2rg* gene. The schematic diagram is as follows:

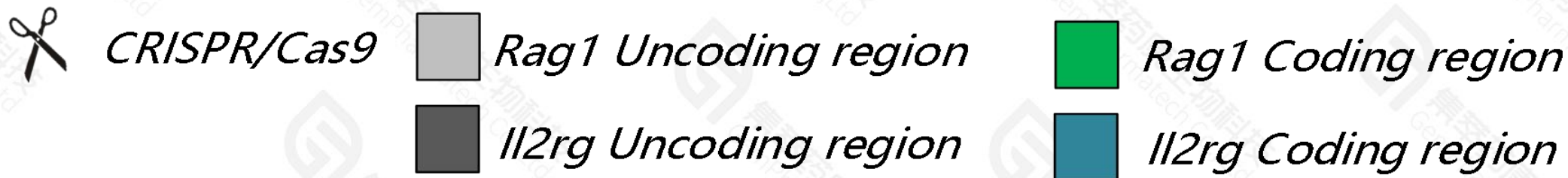
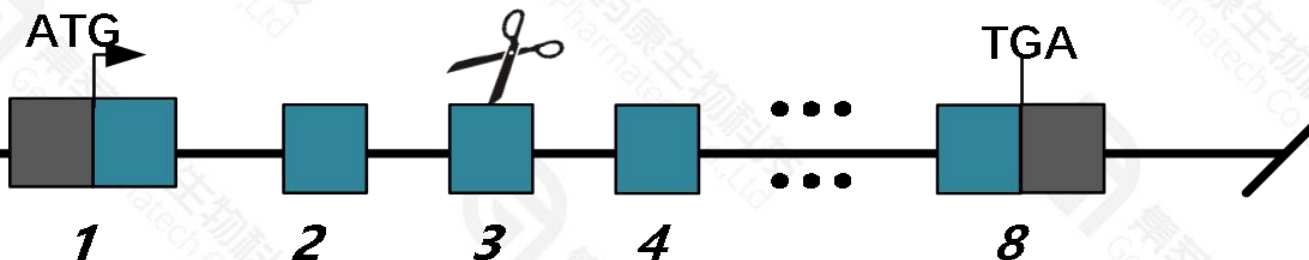
MGP_NODShiLtJ_T0056045.1

Wild-type allele



MGP_NODShiLtJ_T0096480.1

Wild-type allele



- The *Rag1* gene has 1 transcript. According to the structure of *Rag1* gene, partial coding sequence of exon1 of MGP_NODShiLtJ_T0056045.1 transcript is recommended as the knockout region. Knock out the region will result in disruption of protein function.
- The *Il2rg* gene has 4 transcripts. According to the structure of *Il2rg* gene, partial coding sequence of exon3 of MGP_NODShiLtJ_T0096480.1 transcript is recommended as the knockout region. The region contains 185bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Rag1* and *Il2rg* gene. The brief process is as follows:
CRISPR/Cas9 system 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with NOD/ShiLtJGpt mice.

- According to the existing MGI data, 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.
- Because *B230118H07Rik* gene overlap with *Rag1* gene, the deletion of *Rag1* gene may also disrupt *B230118H07Rik* gene.
- The *Rag1* 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.

- According to the existing MGI data, males hemizygous for targeted null mutations exhibit markedly reduced numbers of T and B cells, lack NK cells and Peyer's patches, and have severely reduced numbers of gut-associated intraepithelial lymphocytes.
- The deleted region of *Il2rg* gene is near to the 5'UTR of *Med12* gene, and the deletion of *Il2rg* gene may influence the normal expression of the *Med12* gene.
- Because *Gm20489* gene overlap with *Il2rg* gene, the deletion of *Il2rg* gene can also disrupt *Gm20489* gene.
- The *Il2rg* gene is located on the ChrX. 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)-Rag1

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

Gene ID: 19373, updated on 13-Mar-2020

Summary



Official Symbol Rag1 provided by [MGI](#)

Official Full Name recombination activating 1 provided by [MGI](#)

Primary source [MGI:MGI:97848](#)

See related [Ensembl:ENSMUSG00000061311](#)

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](#)

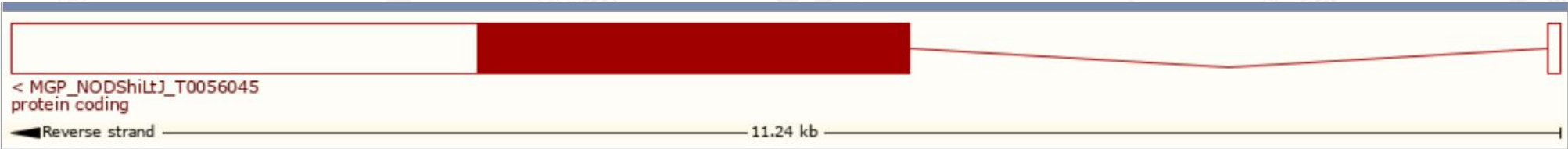
Orthologs [human](#) [all](#)

Transcript information(Ensembl)-Rag1

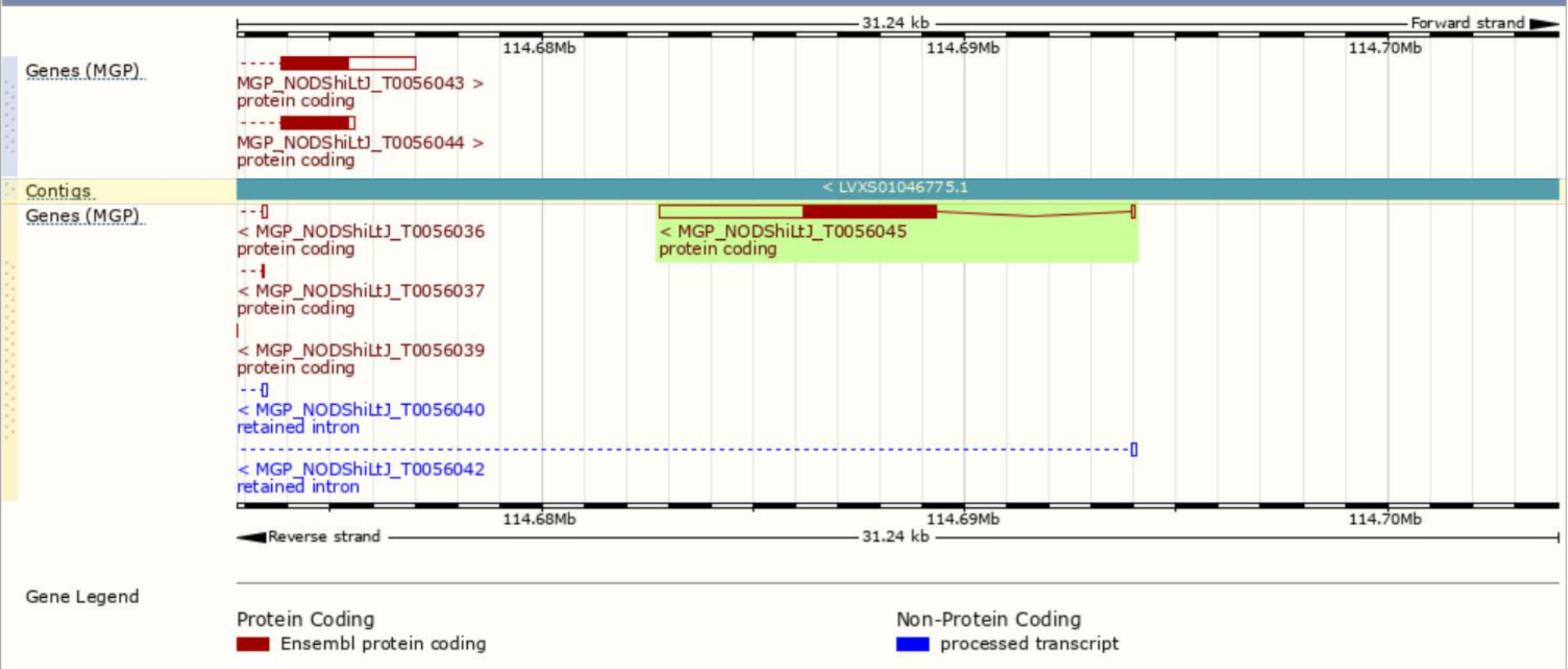
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
-	MGP_NODShiLtJ_T0056045.1	6608	1040aa	Protein coding	CCDS16463	P15919	-

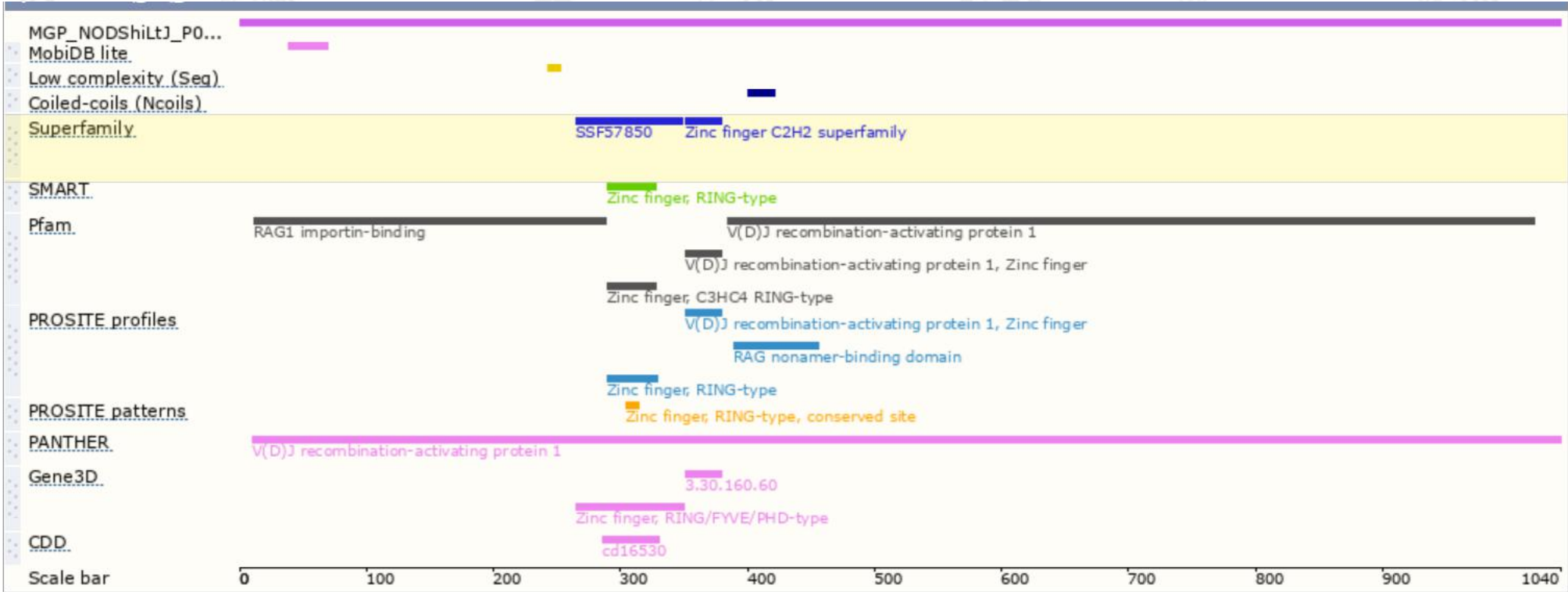
The strategy is based on the design of *MGP_NODShiLtJ_T0056045.1* transcript,the transcription is shown below:



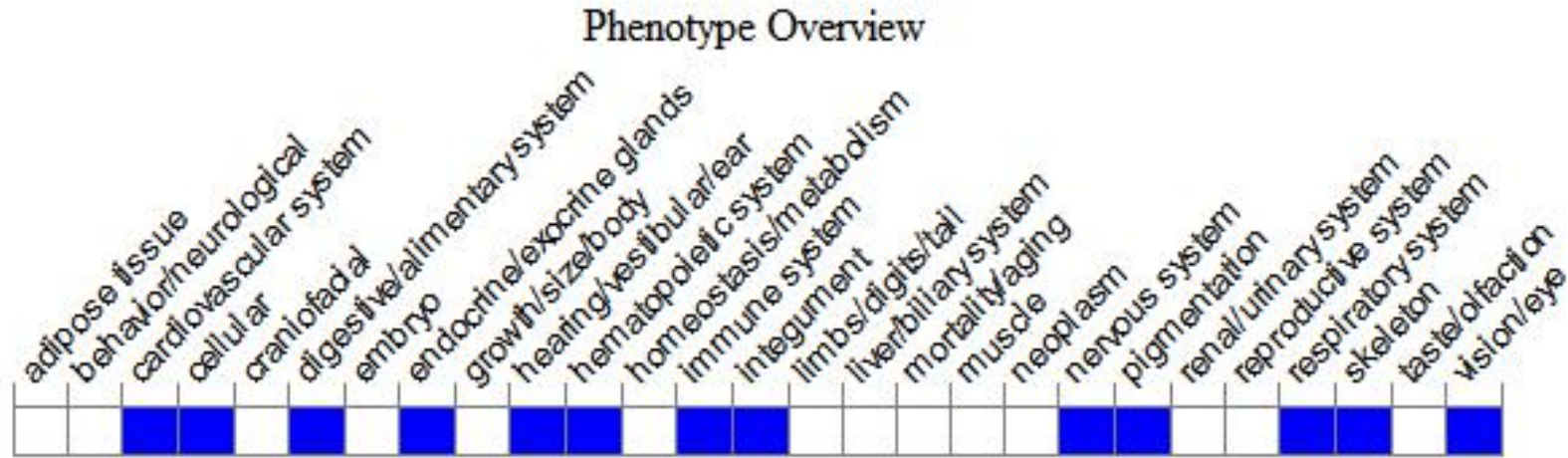
Genomic location distribution-Rag1



Protein domain-Rag1



Mouse phenotype description(MGI)-Rag1



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, 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.

Gene information (NCBI)-Il2rg

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

Gene ID: 19373, updated on 13-Mar-2020

Summary



Official Symbol Rag1 provided by [MGI](#)

Official Full Name recombination activating 1 provided by [MGI](#)

Primary source [MGI:MGI:97848](#)

See related [Ensembl:ENSMUSG00000061311](#)

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](#)

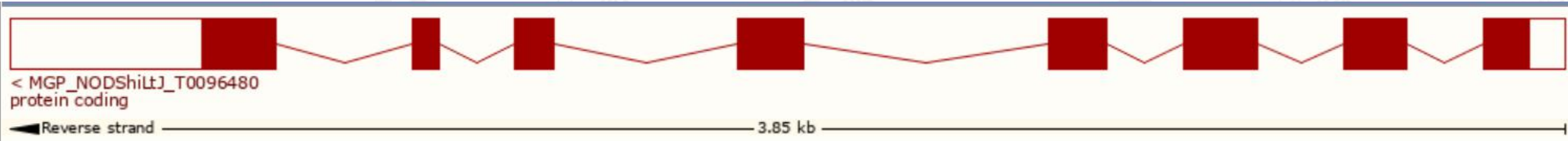
Orthologs [human](#) [all](#)

Transcript information(Ensembl)-Il2rg

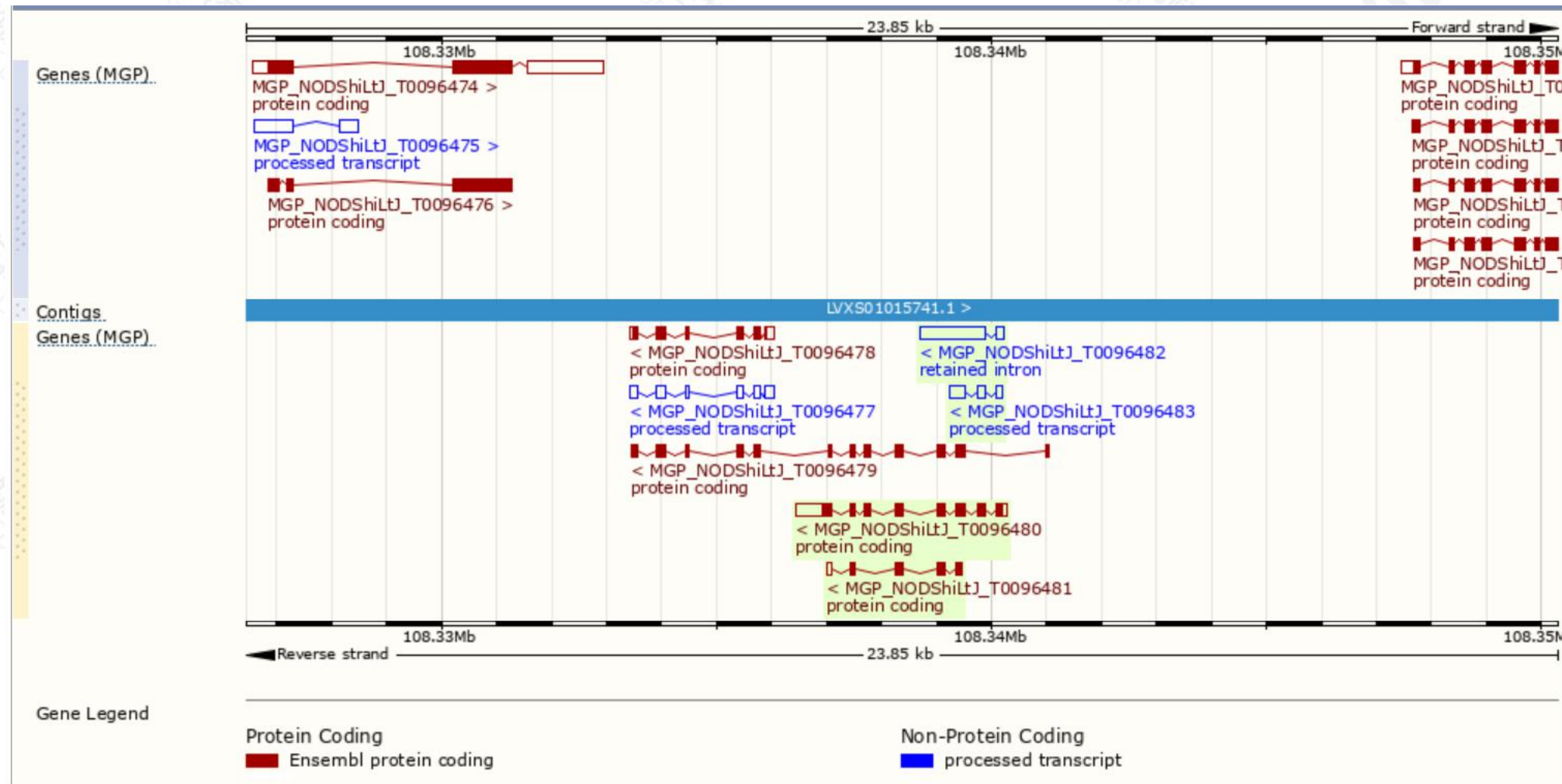
The gene has 4 transcripts,and all the transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
-	MGP_NODShiLtJ_T0096480.1	1670	369aa	Protein coding	CCDS30312	B0QZX1 P34902 Q3UPA9	-
-	MGP_NODShiLtJ_T0096481.1	597	153aa	Protein coding	-	-	-
-	MGP_NODShiLtJ_T0096483.1	558	No protein	Processed transcript	-	-	-
-	MGP_NODShiLtJ_T0096482.1	1335	No protein	Retained intron	-	-	-

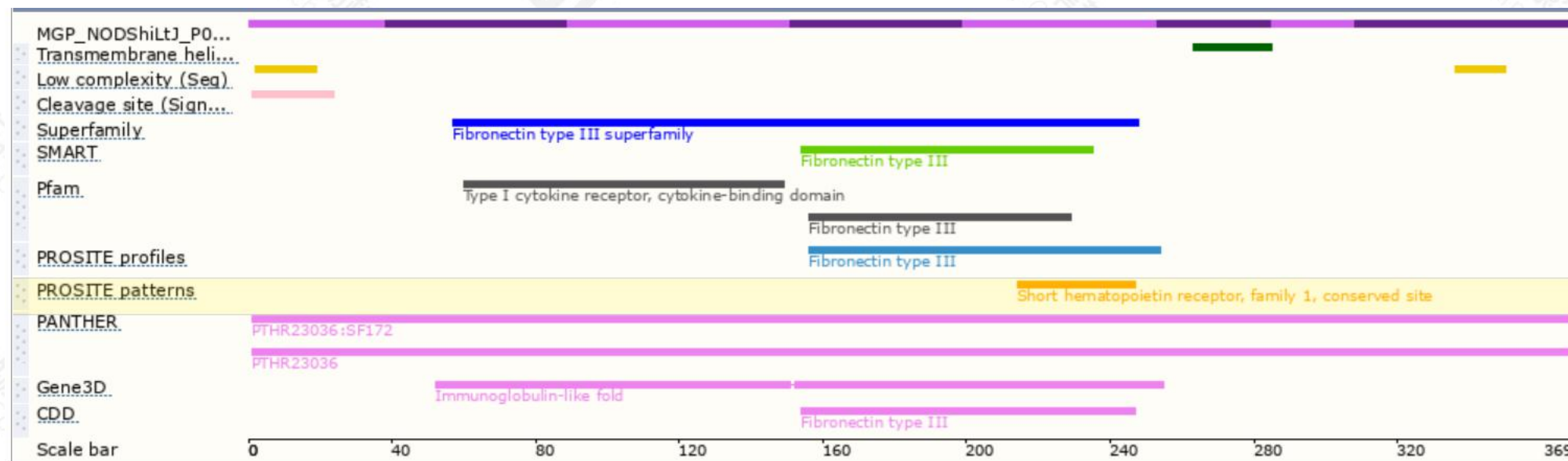
The strategy is based on the design of *MGP_NODShiLtJ_T0096480.1* transcript,the transcription is shown below:



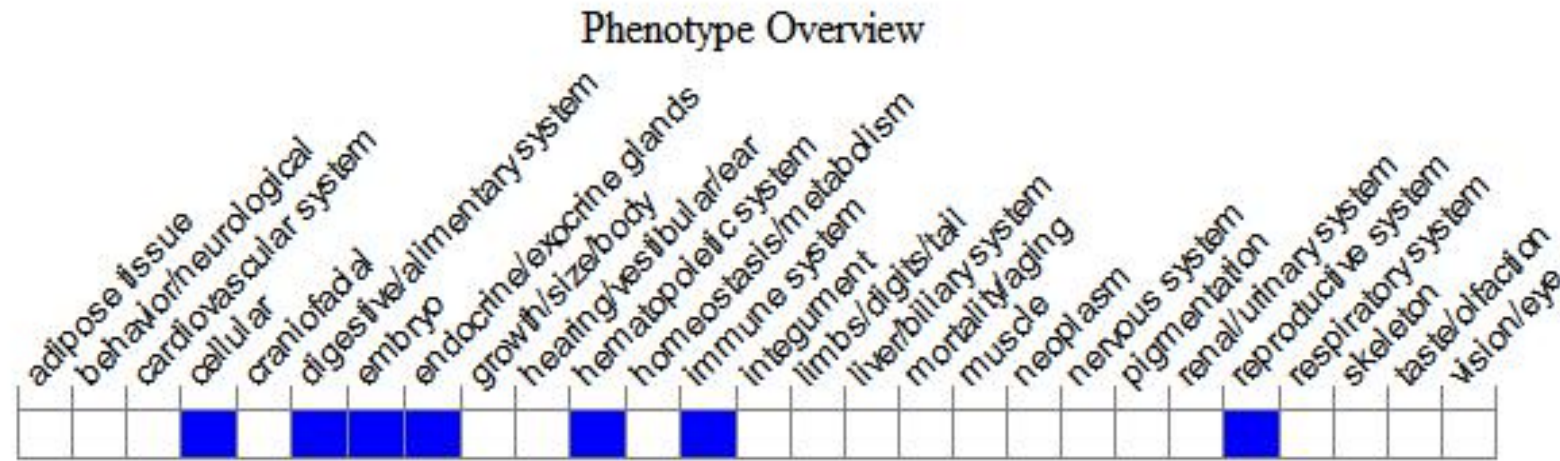
Genomic location distribution-Il2rg



Protein domain-II2rg



Mouse phenotype description(MGI)-Il2rg



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, males hemizygous for targeted null mutations exhibit markedly reduced numbers of T and B cells, lack NK cells and Peyer's patches, and have severely reduced numbers of gut-associated intraepithelial lymphocytes.

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