

***Lag3-p.I447T* Mouse Model Strategy**

-CRISPR/Cas9 technology

Designer: Xiaojing Li

Reviewer: Yanhua Shen

Design Date: 2021-1-7

Project Overview

Project Name

Lag3-p.I447T

Project type

Cas9-ki(PM)

Strain background

C57BL/6JGpt

Technical Description

- The mouse *Lag3* gene has 2 transcripts.
- According to the structure of *Lag3* gene and requirements of customer, This project produced *Lag3*-p.I447T point mutation on exon7 of the transcript of *Lag3*-201(ENSMUST00000032217.1),The 447th amino acid will be mutated from I to T.
- The mouse *Lag3*-201 transcript contains 8 exons. The translation initiation site ATG is located at exon1, and the translation termination site TGA is located at exon8, encoding 521aa.
- In this project, *Lag3* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: In vitro, sgRNA and donor vectors were constructed. Cas9, sgRNA and donor were injected into the fertilized eggs of C57BL/6JGpt mice for homologous recombination, and obtained positive F0 mice identified by PCR and sequencing analysis. The stable inheritable positive F1 mice model was obtained by mating F0 mice with C57BL/6JGpt mice.

Mutation Site

Before mutation

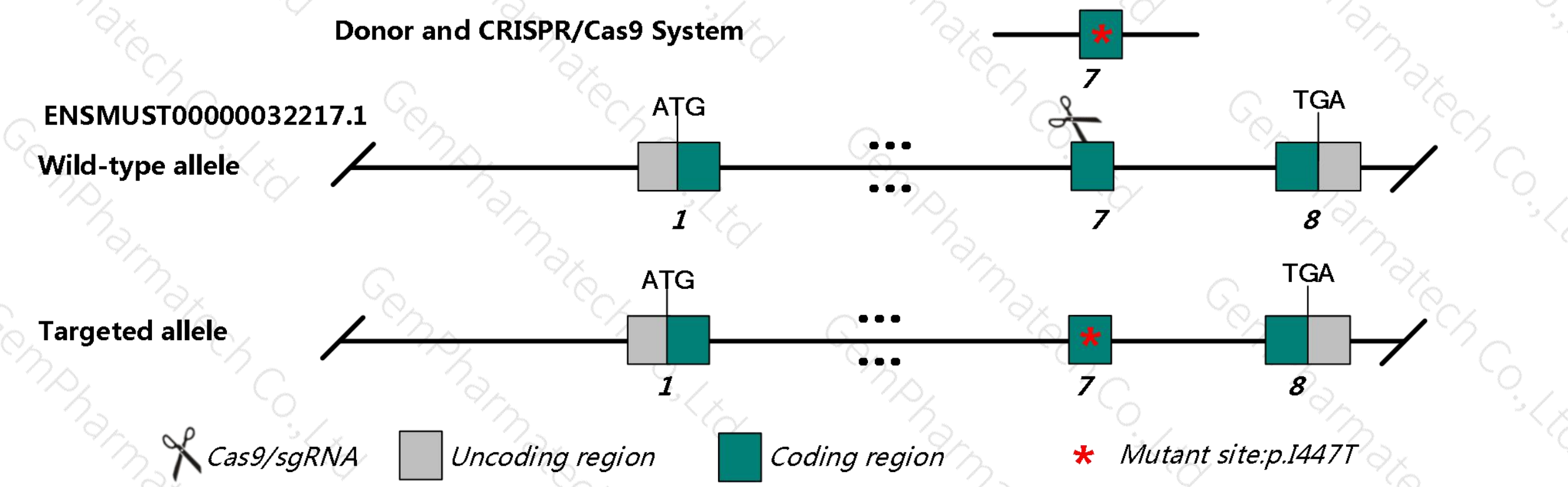
	+1	A H S A R R I S G D L K G G H L V L V L I ?																	
8701	CTCCAACCCC	GGATCTAACC	CAGTGCTTTC	TTCACAGGCG	CCCACAGTGC	TAGGAGAATC	TCAGGTGACC	TTAAAGGAGG	CCATCTCGTT	CTCGTTCTCA									
	GAGGTTGGGG	CCTAGATTGG	GTCACGAAAG	AAGTGTCGCG	GGGTGTCACG	ATCCTCTTAG	AGTCCACTGG	AATTTCCTCC	GGTAGAGCAA	GAGCAAGAGT									
	+1	? I L G A L S L F L L V A G A F G F H W W R K Q																	
8801	TCCTTGGTGC	CCTCTCCCTG	TTCCTTTTGG	TGGCCGGGGC	CTTTGGCTTT	CACTGGTGGA	GAAAACAGGT	GAGACAGACC	TCAGAGTGCT	GTGTGGGACC									
	AGGAACCACG	GGAGAGGGAC	AAGGAAAACC	ACCGGCCCCG	GAAACCGAAA	GTGACCACCT	CTTTTGTCCT	CTCTGTCTGG	AGTCTCACGA	CACACCCTGG									

After mutation

	+1	A H S A R R I S G D L K G G H L V L V L T ?																	
8701	CTCCAACCCC	GGATCTAACC	CAGTGCTTTC	TTCACAGGCG	CCCACAGTGC	TAGGAGAATC	TCAGGTGACC	TTAAAGGAGG	CCATCTCGTT	CTCGTTCTCA									
	GAGGTTGGGG	CCTAGATTGG	GTCACGAAAG	AAGTGTCGCG	GGGTGTCACG	ATCCTCTTAG	AGTCCACTGG	AATTTCCTCC	GGTAGAGCAA	GAGCAAGAGT									
	+1	? T L G A L S L F L L V A G A F G F H W W R K Q																	
8801	CCCTTGGTGC	CCTCTCCCTG	TTCCTTTTGG	TGGCCGGGGC	CTTTGGCTTT	CACTGGTGGA	GAAAACAGGT	GAGACAGACC	TCAGAGTGCT	GTGTGGGACC									
	GGGAACCACG	GGAGAGGGAC	AAGGAAAACC	ACCGGCCCCG	GAAACCGAAA	GTGACCACCT	CTTTTGTCCT	CTCTGTCTGG	AGTCTCACGA	CACACCCTGG									

The green region is exon7 of *Lag3-201*, the red region represents the mutation site (ATC>ACC) .

This model uses CRISPR/Cas9 technology to edit the *Lag3* gene and the schematic diagram is as follow:



- According to the data of MGI , Mice homozygous for disruptions in this gene have a generally normal phenotype but do display reduced natural killer cell activity and increased T cell response to infection.
- One or Two synonymous mutations of amino acids will be introduced on exon7 of *Lag3*.
- Mouse *Lag3* gene is located on Chr6. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr6, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

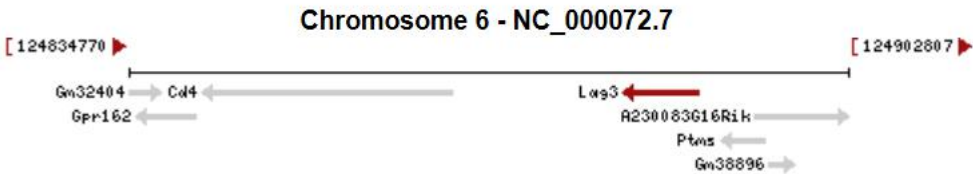
Gene name and location (NCBI)

Lag3 lymphocyte-activation gene 3 [*Mus musculus* (house mouse)]

Gene ID: 16768, updated on 25-Sep-2020

Summary

Official Symbol	Lag3 provided by MGI
Official Full Name	lymphocyte-activation gene 3 provided by MGI
Primary source	MGI:MGI:106588
See related	Ensembl:ENSMUSG00000030124
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	LAG; Ly6; Ly66; CD223; LAG-3
Expression	Broad expression in thymus adult (RPKM 7.2), spleen adult (RPKM 4.9) and 19 other tissues See more
Orthologs	human all

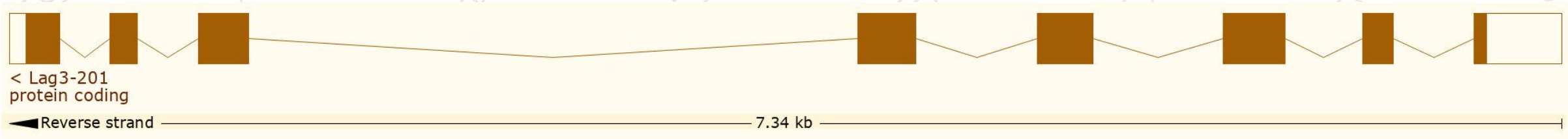


Transcript information (Ensembl)

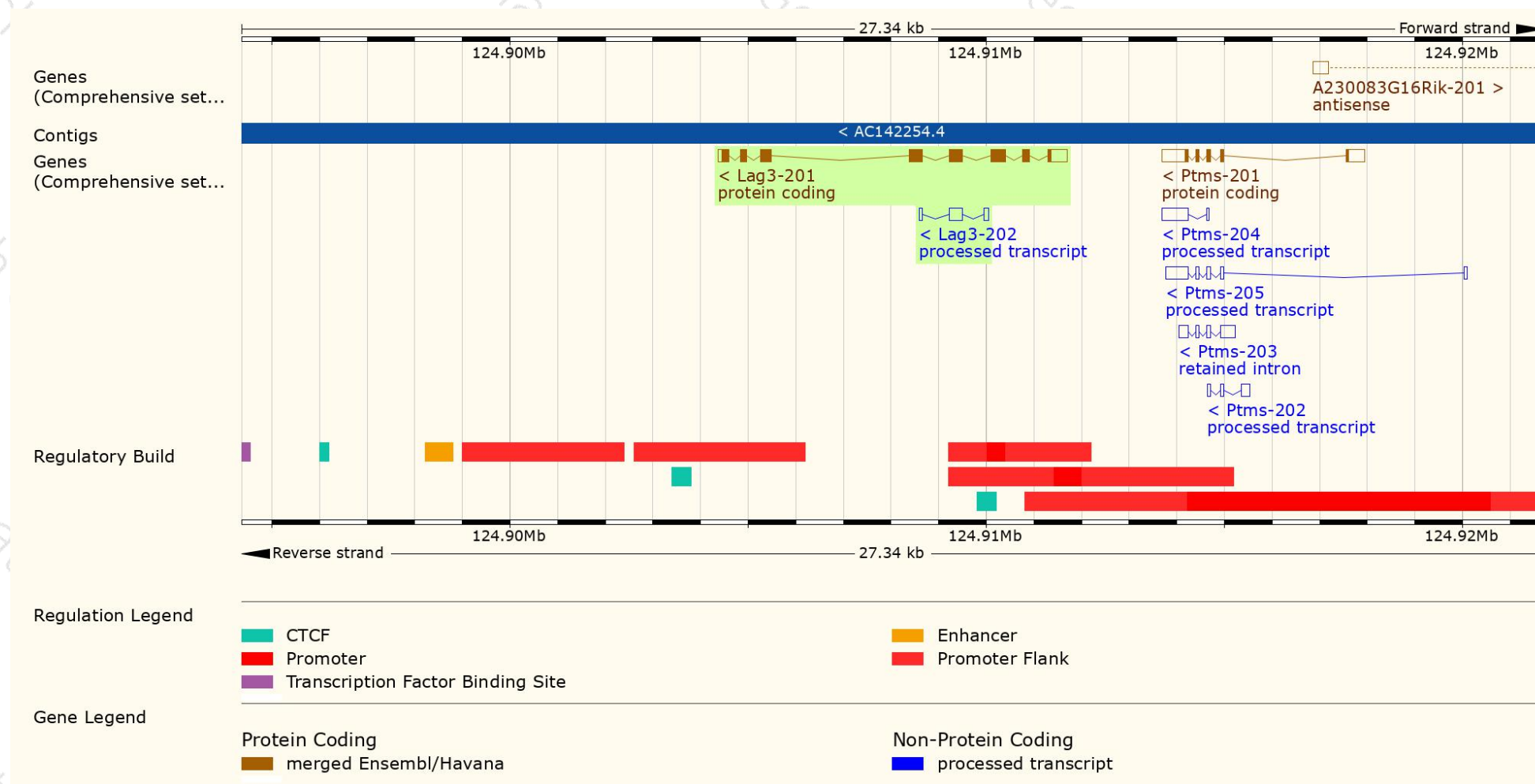
The gene has 2 transcripts, and all transcripts are shown below:

Name ▲	Transcript ID ▲	bp ▲	Protein ▲	Biotype ▲	CCDS ▲	UniProt Match ▲	Flags ▲
Lag3-201	ENSMUST00000032217.1	2001	521aa	Protein coding	CCDS20536	Q61790	TSL:1 GENCODE basic APPRIS P1
Lag3-202	ENSMUST00000139571.1	426	No protein	Processed transcript	-	-	TSL:3

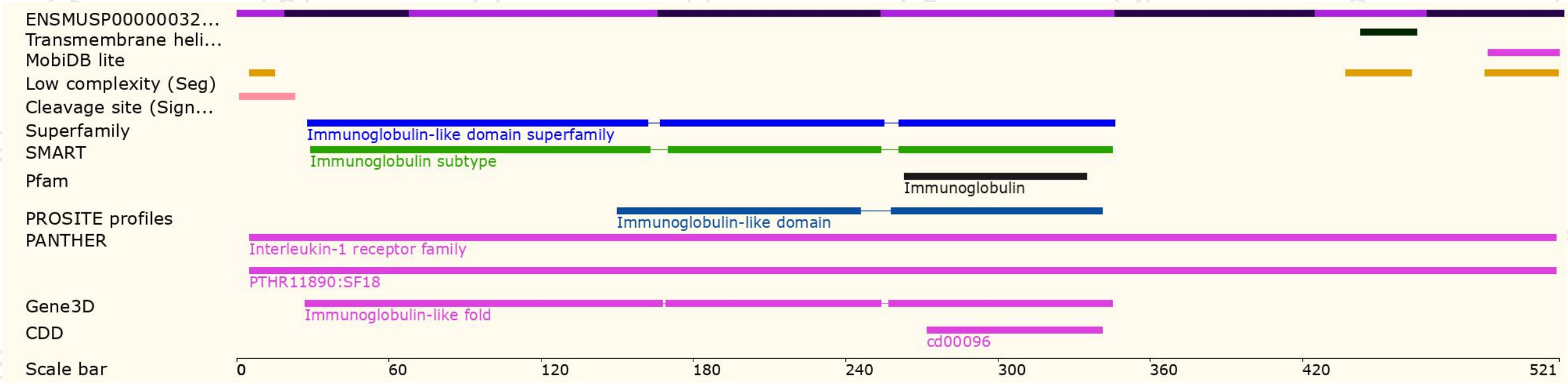
The strategy is based on the design of *Lag3*-201 transcript, the transcription is shown below:



Genomic location distribution



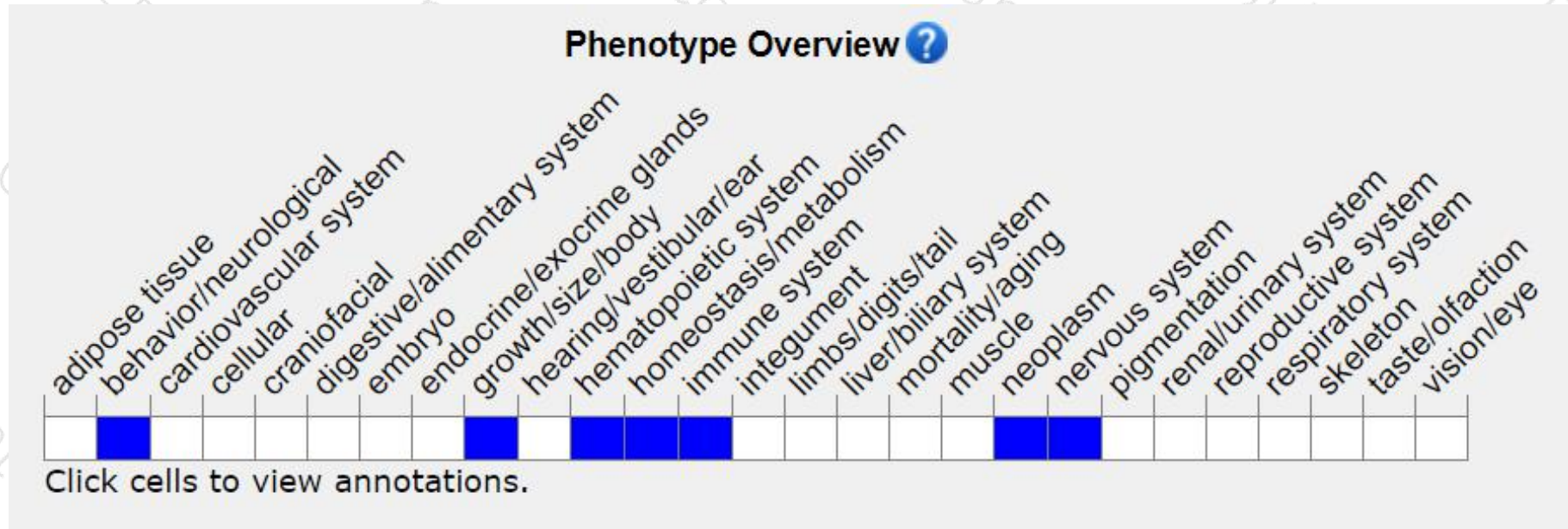
Protein domain



Mouse phenotype description(MGI)

URL link is as follows:

<http://www.informatics.jax.org/marker/MGI:106588>



Mice homozygous for disruptions in this gene have a generally normal phenotype but do display reduced natural killer cell activity and increased T cell response to infection.

If you have any questions, please feel free to contact us.

Tel: 025-5864 1534



集萃药康生物科技
GemPharmatech Co.,Ltd

