

Lag3 Cas9-CKO Strategy

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Design Date: 2019-7-29

Project Overview



Project Name

Lag3

Project type

Cas9-CKO

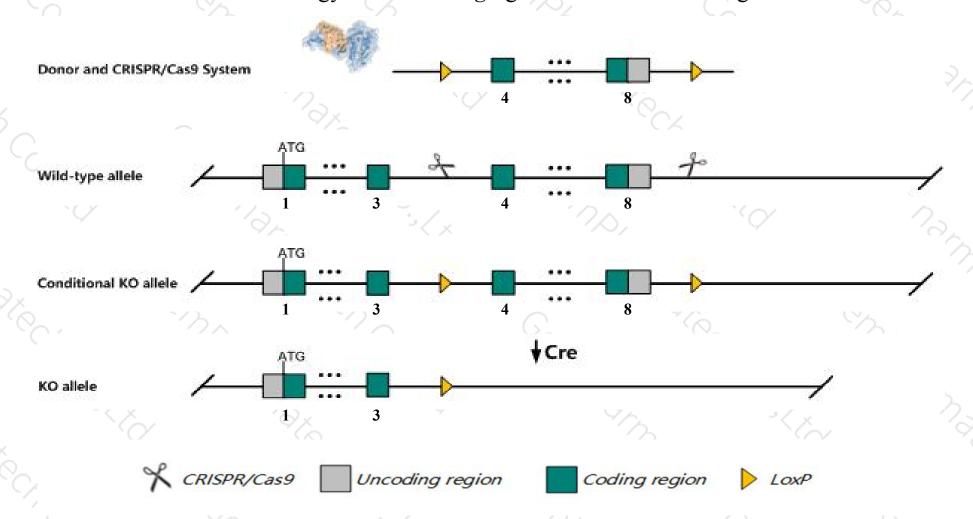
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Lag3* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Lag3* gene has 2 transcripts. According to the structure of *Lag3* gene, exon4-exon8 of *Lag3-201*(ENSMUST00000032217.1) transcript is recommended as the knockout region. The region contains 1067bp coding sequence.

 Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Lag3* 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 disruptions in this gene have a generally normal phenotype but do display reduced natural killer cell activity and increased T cell response to infection.
- The *Lag3* gene is located on the Chr6. 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)



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

Gene ID: 16768, updated on 12-Mar-2019

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 CD223, LAG-3, Ly66

Expression Broad expression in thymus adult (RPKM 7.2), spleen adult (RPKM 4.9) and 19 other tissuesSee more

Orthologs <u>human all</u>

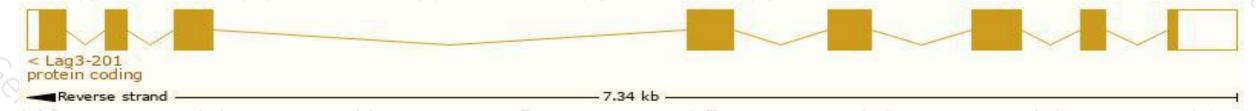
Transcript information (Ensembl)



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

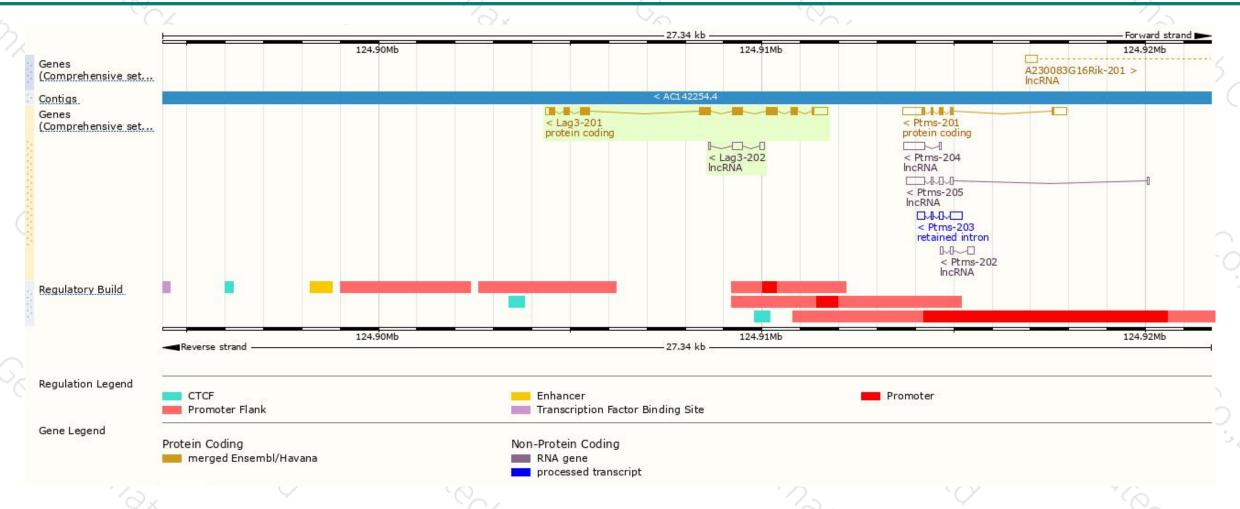
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lag3-201	ENSMUST00000032217.1	2001	521aa	Protein coding	CCDS20536	Q61790	TSL:1 GENCODE basic APPRIS P1
Lag3-202	ENSMUST00000139571.1	426	No protein	Processed transcript	6-8		TSL:3

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



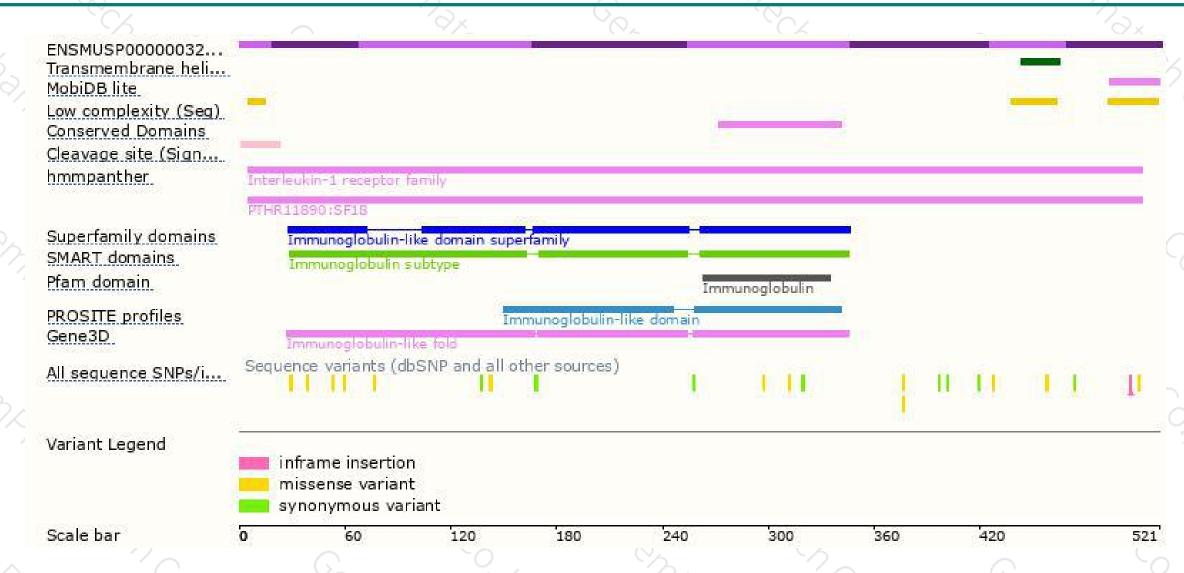
Genomic location distribution





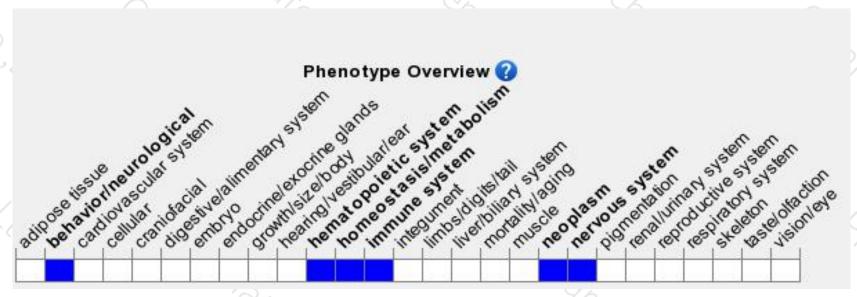
Protein domain





Mouse phenotype description(MGI)





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 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, you are welcome to inquire. Tel: 400-9660890





