

***Bag4* Cas9-KO Strategy**

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Reviewer:

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Project Overview

Project Name

Bag4

Project type

Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Bag4* gene. The schematic diagram is as follows:



- The *Bag4* gene has 3 transcripts. According to the structure of *Bag4* gene, exon2-exon5 of *Bag4-201* (ENSMUST00000038498.9) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Bag4* gene. The brief process is as follows: sgRNA was transcribed in vitro. Cas9 and sgRNA 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, Homozygous mutant animals may show enhanced cytokine response and increased IL-6 production following TNF challenge. Studies on two different alleles of this gene are not in agreement.
- The KO region overlaps with *Gm17484* gene and gene. Knockout the region may affect the function of *Gm17484* gene.
- The *Bag4* gene is located on the Chr8. 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.

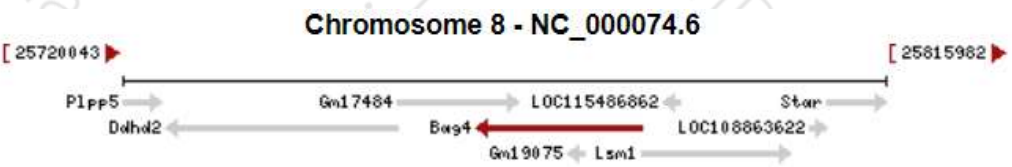
Gene information (NCBI)

Bag4 BCL2-associated athanogene 4 [*Mus musculus* (house mouse)]

Gene ID: 67384, updated on 12-Aug-2019

Summary

Official Symbol	Bag4 provided by MGI
Official Full Name	BCL2-associated athanogene 4 provided by MGI
Primary source	MGI:MGI:1914634
See related	Ensembl:ENSMUSG00000037316
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<i>Mus musculus</i>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	SODD; 2410112I15Rik
Expression	Ubiquitous expression in adrenal adult (RPKM 6.8), mammary gland adult (RPKM 6.2) and 28 other tissues See more
Orthologs	human all

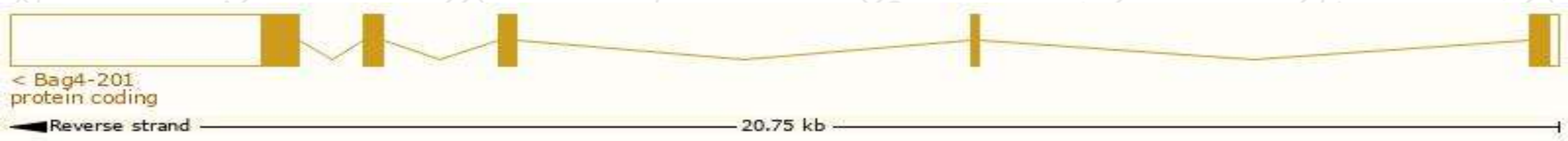


Transcript information (Ensembl)

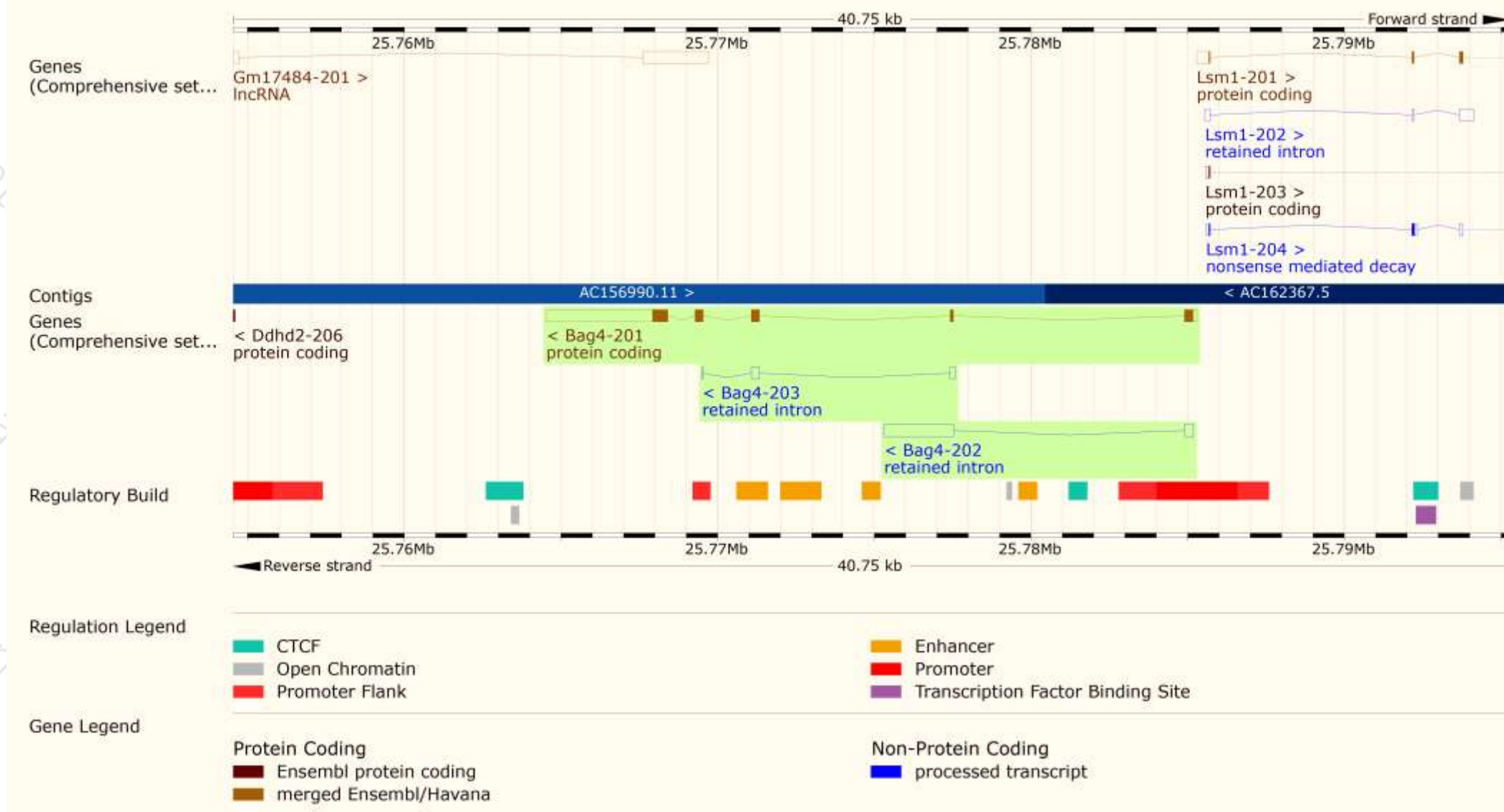
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Bag4-201	ENSMUST00000038498.9	4876	457aa	ENSMUSP00000044725.8	Protein coding	CCDS22201	A6H6S8 Q8CI61	TSL:1 Gencode basic APPRIS P1
Bag4-202	ENSMUST00000209948.1	2506	No protein	-	Retained intron	-	-	TSL:2
Bag4-203	ENSMUST00000210103.1	441	No protein	-	Retained intron	-	-	TSL:2

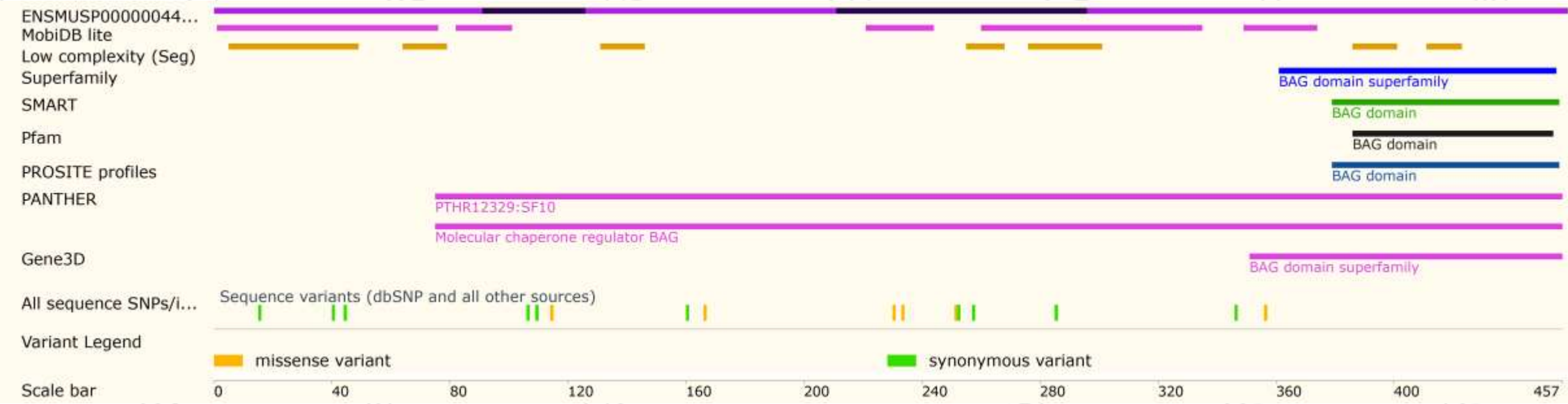
The strategy is based on the design of *Bag4-201* transcript,The transcription is shown below



Genomic location distribution

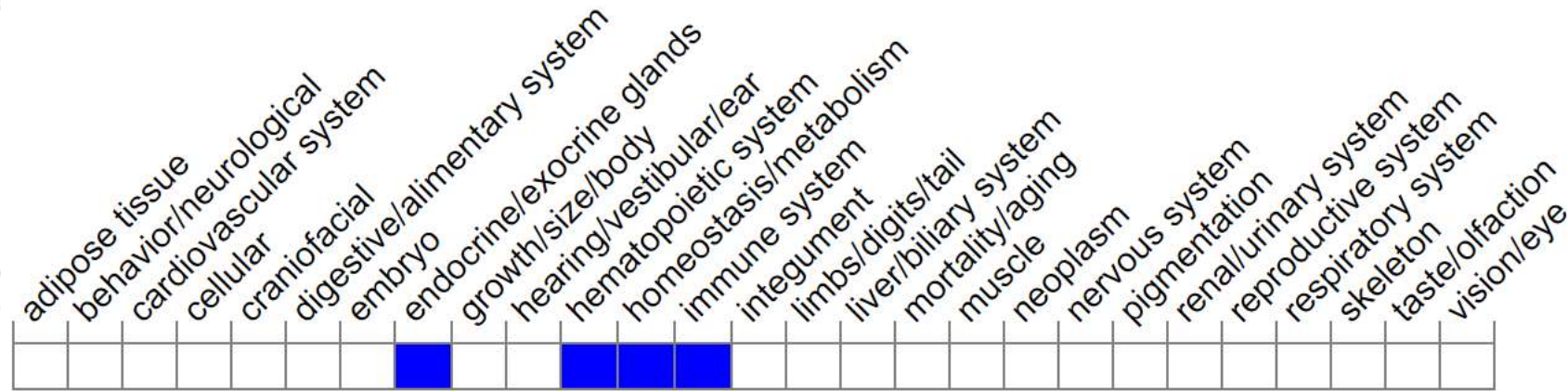


Protein domain



Mouse phenotype description(MGI)

Phenotype Overview ?



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, Homozygous mutant animals may show enhanced cytokine response and increased IL-6 production following TNF challenge. Studies on two different alleles of this gene are not in agreement.

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

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