

Itgam Cas9-CKO Strategy

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Reviewer :

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Project Overview

Project Name

Itgam

Project type

Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

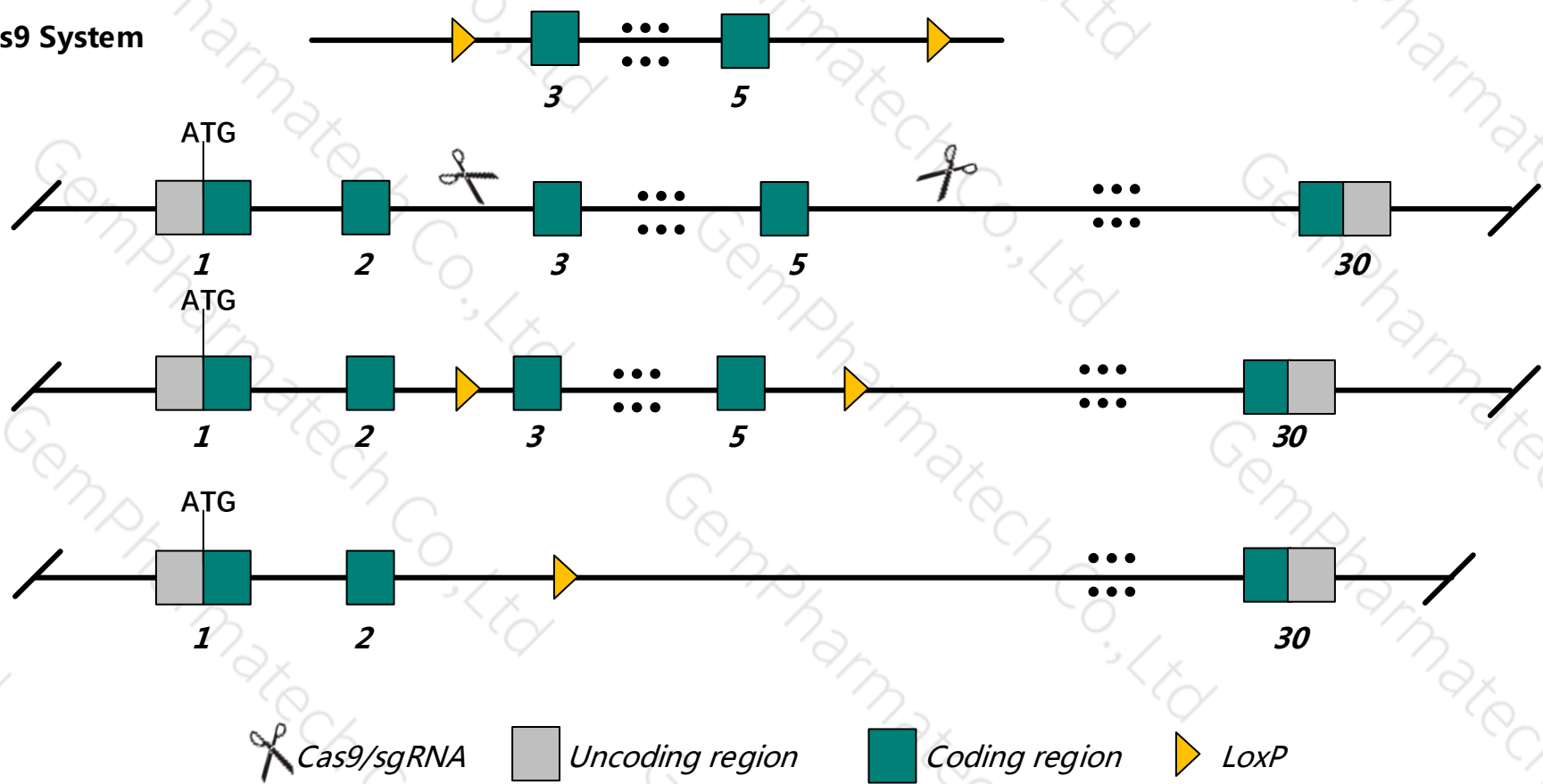
This model will use CRISPR/Cas9 technology to edit the *Itgam* gene. The schematic diagram is as follows:

Donor and CRISPR/Cas9 System

Wild-type allele

Conditional KO allele

KO allele



- The *Itgam* gene has 6 transcripts. According to the structure of *Itgam* gene, exon3-exon5 of *Itgam*-201 (ENSMUST00000064821.13) transcript is recommended as the knockout region. The region contains 293bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Itgam* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

- According to the existing MGI data , Homozygous null mice exhibit reduced staphylococcal enterotoxin- induced T cell proliferation, reduced neutrophil adhesion to fibrinogen, and defective homotypic aggregation and reduced degranulation of neutrophils.
- The KO region contains functional region of the *Gm49368* gene. Knockout the region will affect the function of *Gm49368* gene.
- The *Itgam* gene is located on the Chr7. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Itgam integrin alpha M [*Mus musculus* (house mouse)]

Gene ID: 16409, updated on 28-Feb-2020

Summary

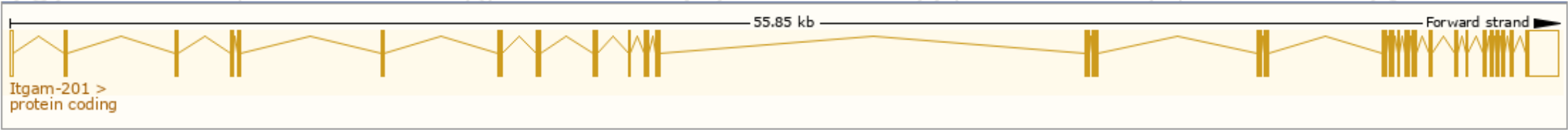
Official Symbol	Itgam provided by MGI
Official Full Name	integrin alpha M provided by MGI
Primary source	MGI:MGI:96607
See related	Ensembl:ENSMUSG00000030786
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	CR3; CR3A; MAC1; Cd11b; Ly-40; Mac-1; Mac-1a; CD11b/CD18; F730045J24Rik
Expression	Broad expression in liver E18 (RPKM 8.3), mammary gland adult (RPKM 7.7) and 23 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

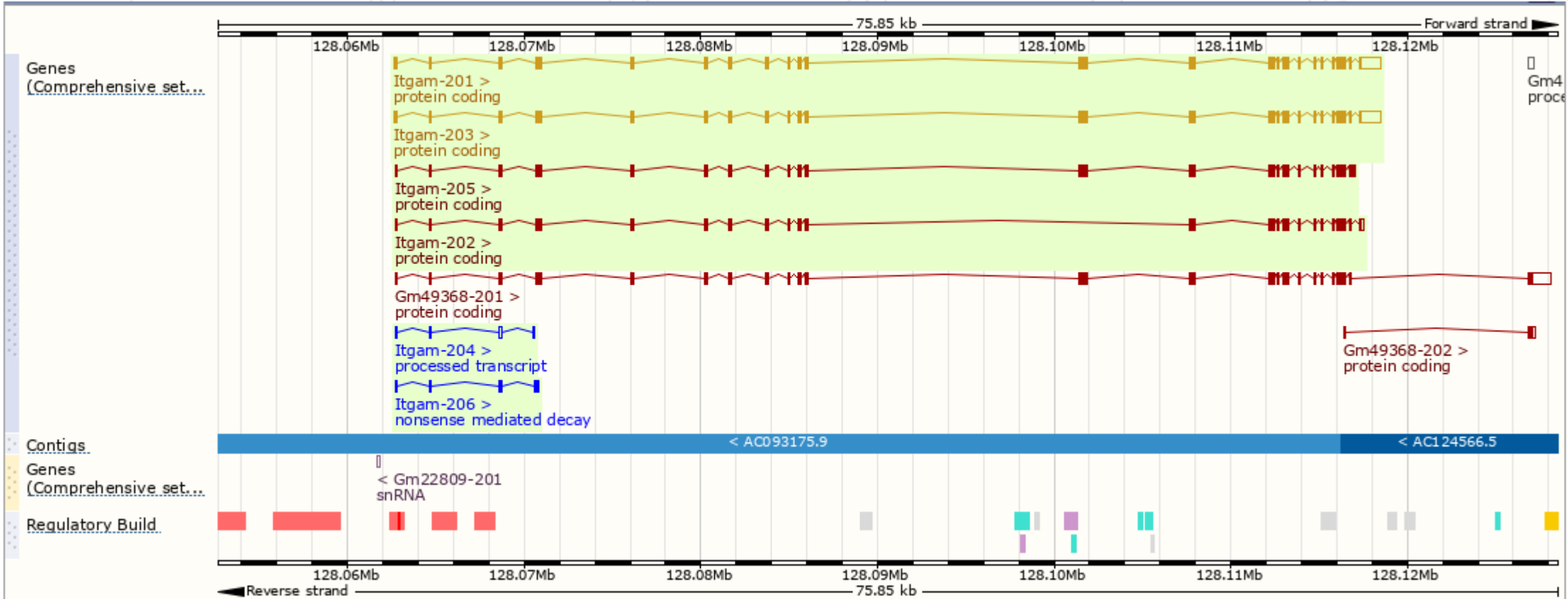
The gene has 6 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Itgam-201	ENSMUST00000064821.13	4682	1154aa	Protein coding	CCDS40149	G5E8F1	TSL:5 GENCODE basic APPRIS ALT2
Itgam-203	ENSMUST00000106242.9	4649	1153aa	Protein coding	CCDS21889	E9Q604	TSL:1 GENCODE basic APPRIS P3
Itgam-205	ENSMUST00000120355.7	3701	1167aa	Protein coding	-	E9Q5K8	TSL:5 GENCODE basic APPRIS ALT2
Itgam-202	ENSMUST00000106240.8	3325	1036aa	Protein coding	-	A0A0R4J1B4	TSL:1 GENCODE basic
Itgam-206	ENSMUST00000156593.2	349	94aa	Nonsense mediated decay	-	D6RJ73	TSL:3
Itgam-204	ENSMUST00000119696.7	338	No protein	Processed transcript	-	-	TSL:3

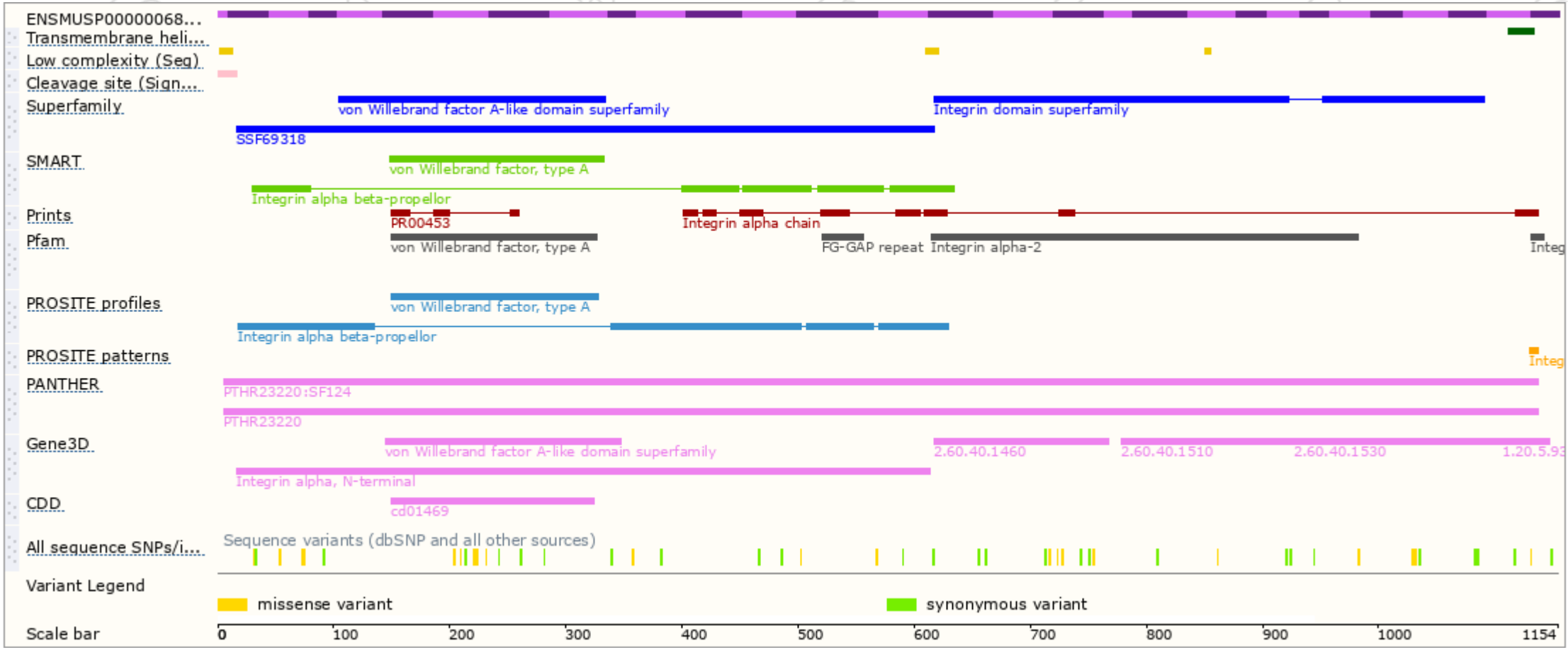
The strategy is based on the design of *Itgam-201* transcript, The transcription is shown below



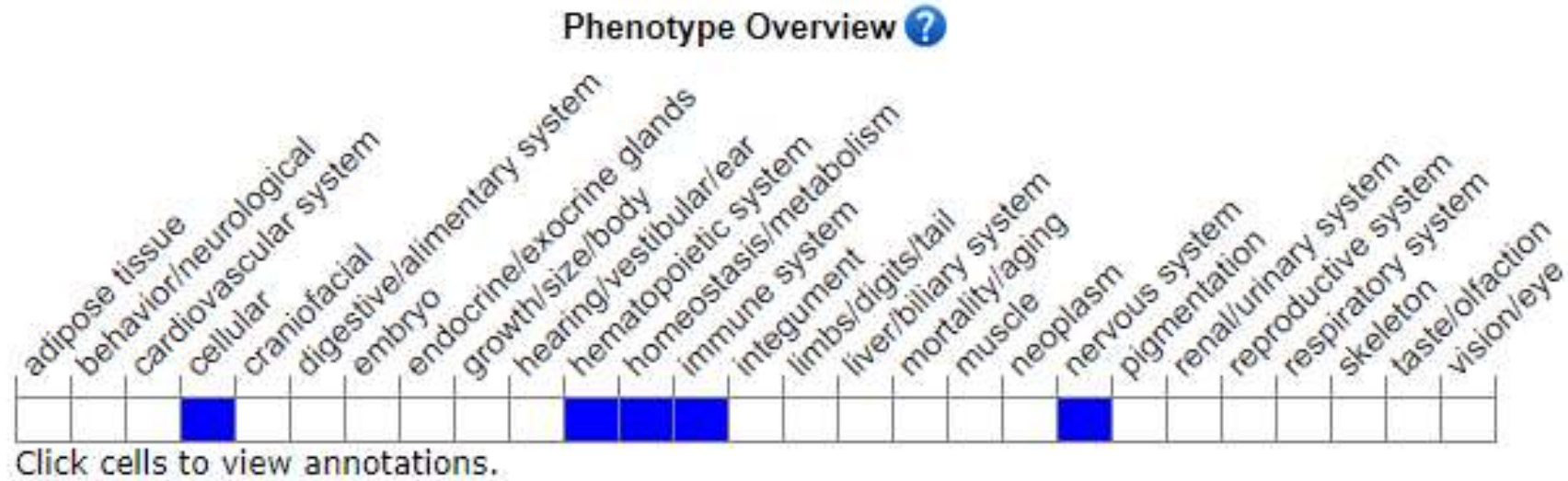
Genomic location distribution



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, Homozygous null mice exhibit reduced staphylococcal enterotoxin- induced T cell proliferation, reduced neutrophil adhesion to fibrinogen, and defective homotypic aggregation and reduced degranulation of neutrophils.

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