Gzmb Cas9-CKO Strategy

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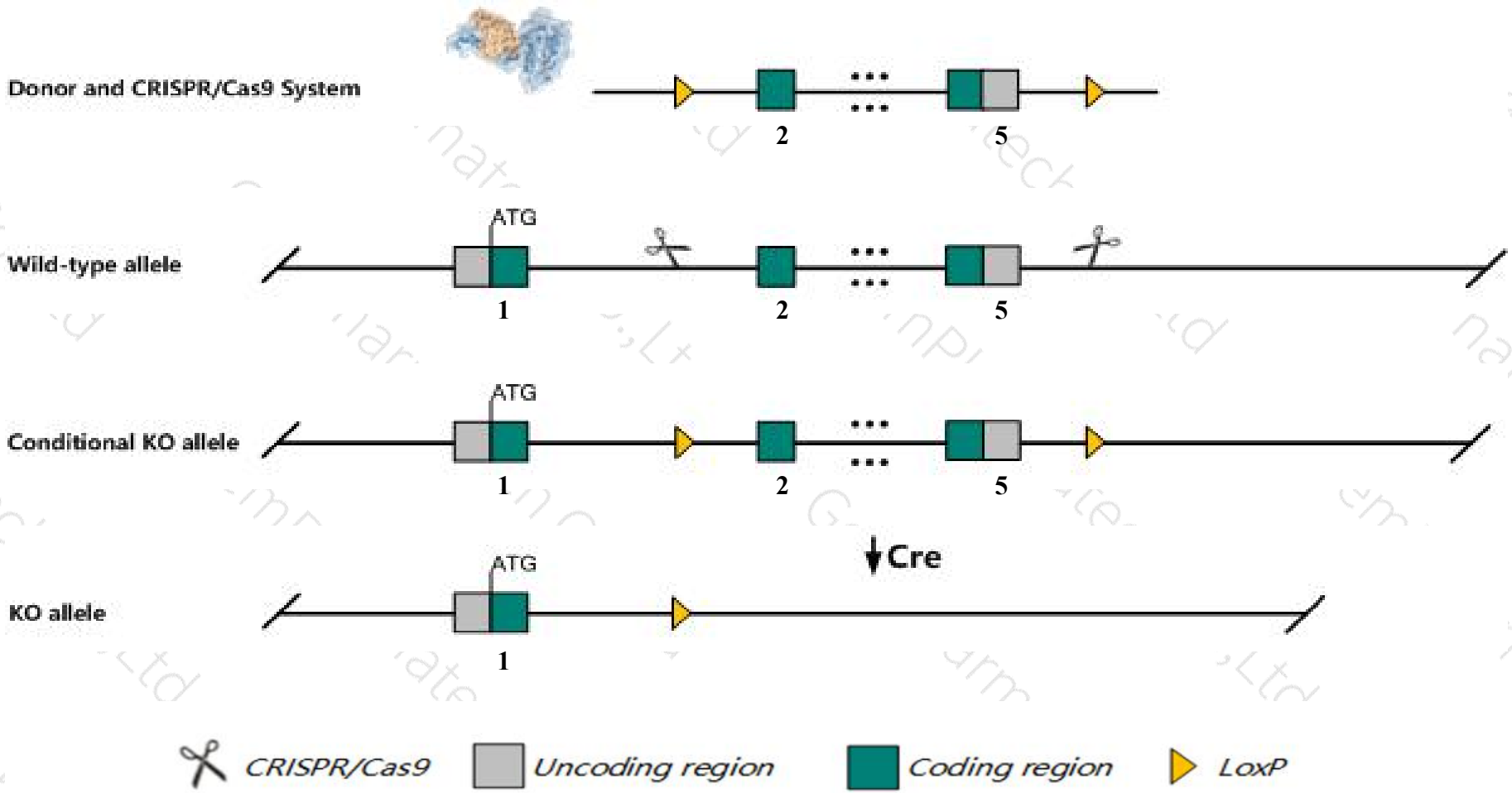
Project Overview

Project Name	***Gzmb***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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



Technical routes

- The *Gzmb* gene has 1 transcript. According to the structure of *Gzmb* gene, exon2-exon5 of *Gzmb-201* (ENSMUST00000015581.5) transcript is recommended as the knockout region.The region contains 689bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Gzmb* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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,Homozygotes for a null allele show impaired CTL and NK cell cytolysis, and enhanced clearance of allogeneic and syngeneic tumor cells. Homozygotes for another null allele have defective CTL cytolysis and show impaired clearance of allogeneic tumor cells only if the selection cassette is retained.

- The *Gzmb* gene is located on the Chr14. 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）

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Gzmb granzyme B [Mus musculus (house mouse)]

Gene ID: 14939, updated on 19-Mar-2019

Summary

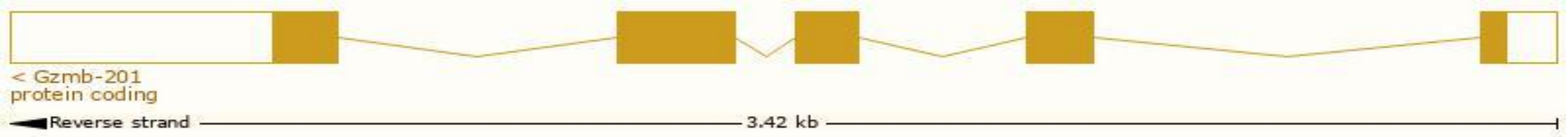
Official Symbol	Gzmb provided by MGI
Official Full Name	granzyme B provided by MGI
Primary source	MGI:MGI:109267
See related	Ensembl:ENSMUSG00000015437
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI553453, CCP-1/C11, CCP1, Ctla-1, Ctla1, GZB
Summary	This gene encodes a member of the granzyme subfamily of proteins, part of the peptidase S1 family of serine proteases. The encoded preproprotein is secreted by natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) and proteolytically processed to generate the active protease, which induces target cell apoptosis. This protein also processes cytokines and degrades extracellular matrix proteins, and these roles are implicated in chronic inflammation and wound healing. Mice lacking a functional copy of this gene exhibit impaired immune cell-mediated cytolysis. [provided by RefSeq, Sep 2016]
Expression	Biased expression in large intestine adult (RPKM 11.7), small intestine adult (RPKM 4.9) and 8 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

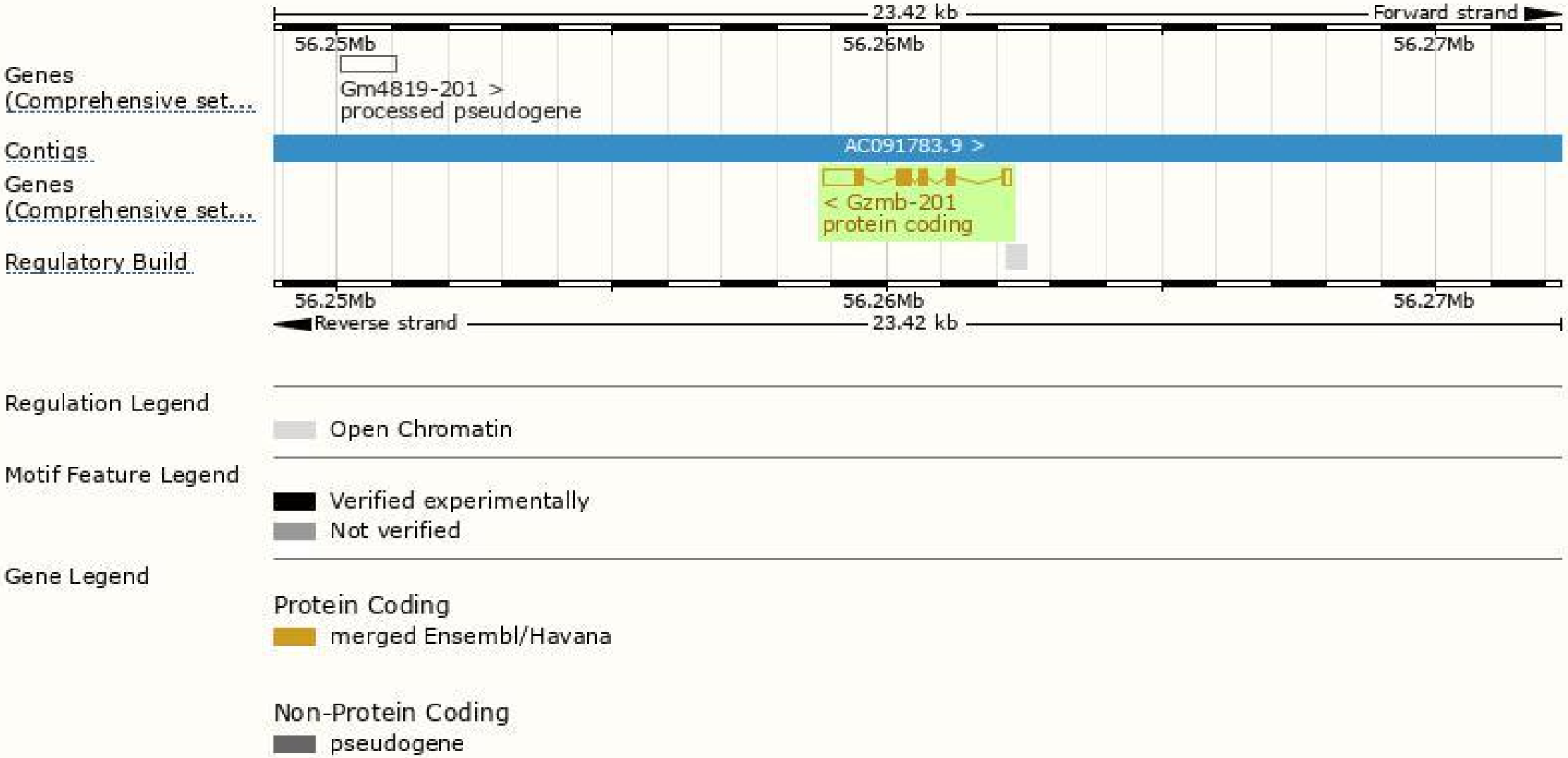
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gzmb-201	ENSMUST00000015581.5	1436	247aa	Protein coding	CCDS27147	P04187 Q3TZH4	TSL:1 GENCODE basic APPRIS P1

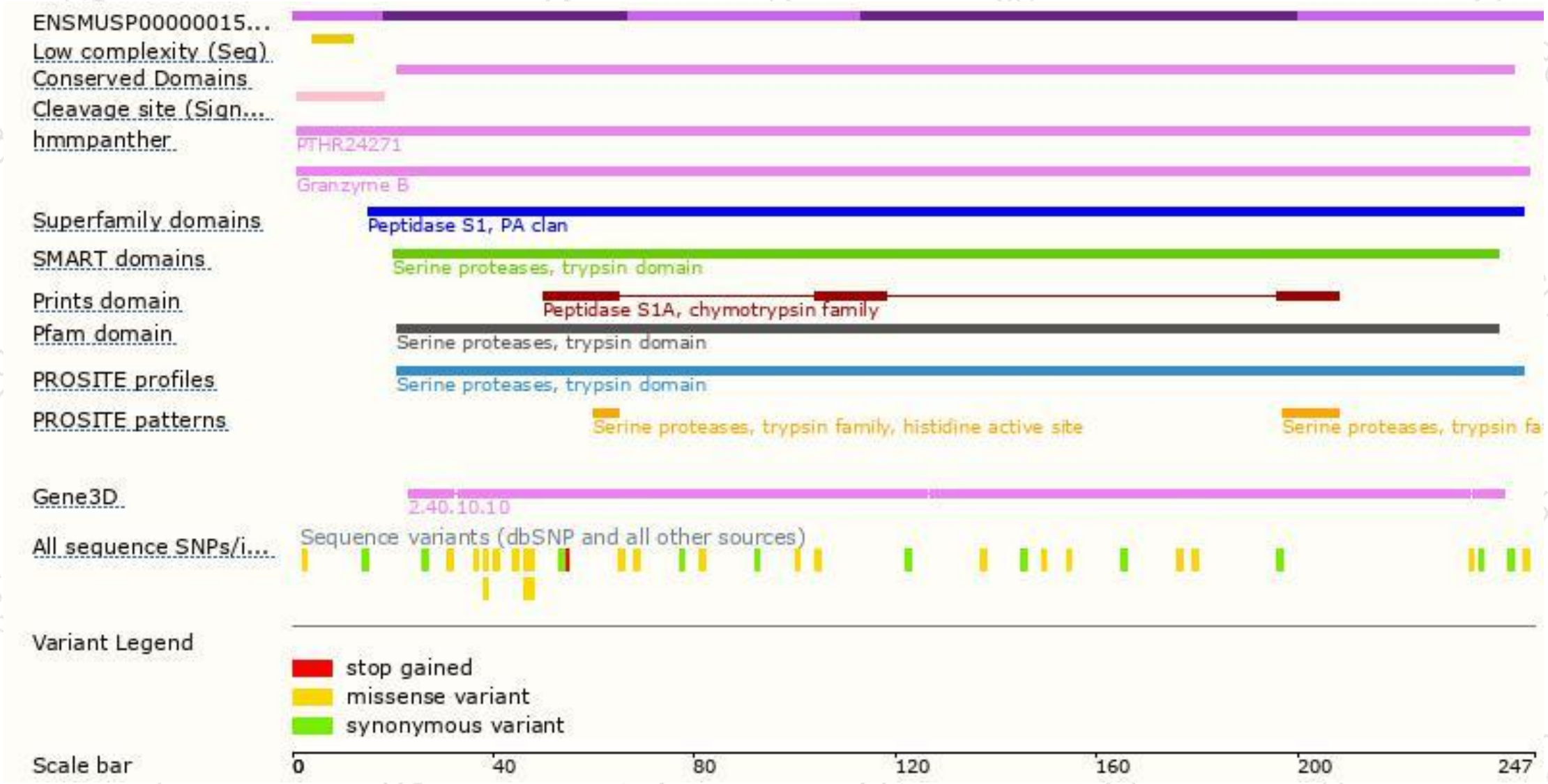
The strategy is based on the design of *Gzmb-201* transcript,The transcription is shown below



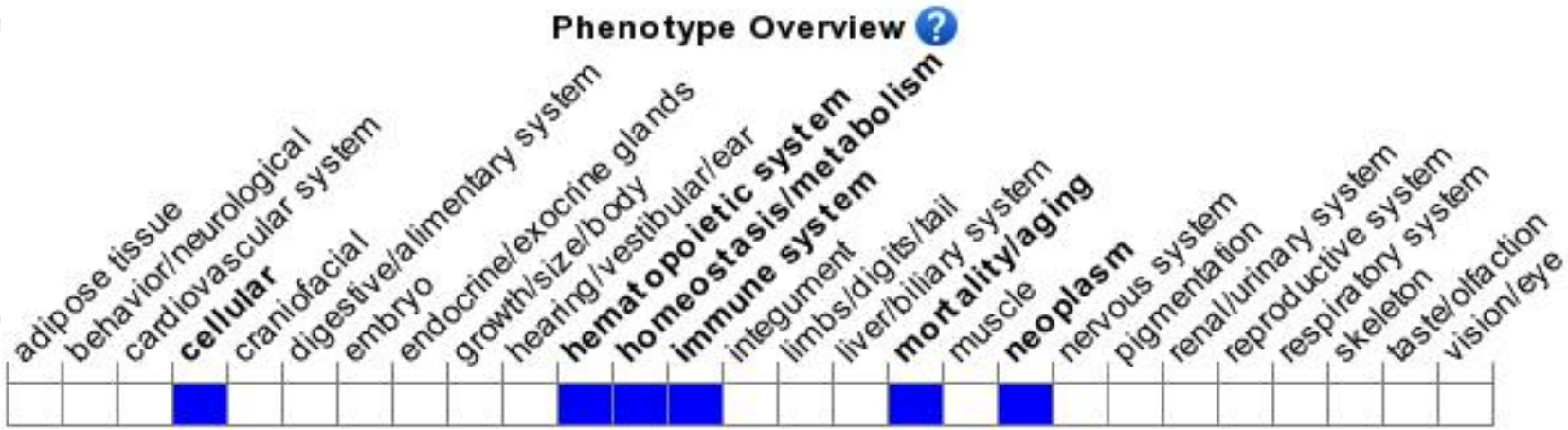
Genomic location distribution



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/).

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

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