Lap3 Cas9-CKO Strategy

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Project Overview

Project Name	***Lap3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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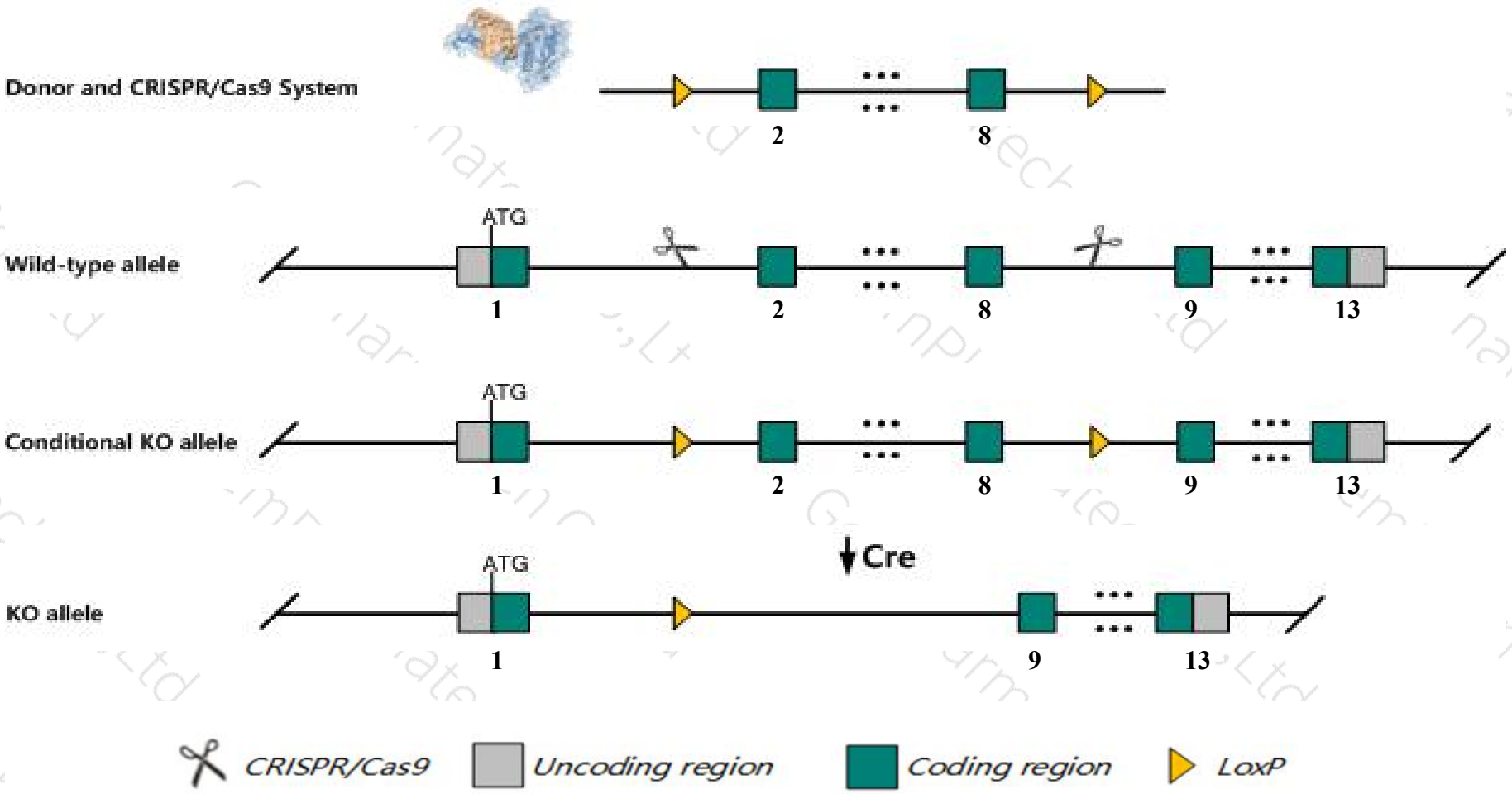
This model will use CRISPR/Cas9 technology to edit the *Lap3* gene. The schematic diagram is as follows:



Technical routes

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- The *Lap3* gene has 6 transcripts. According to the structure of *Lap3* gene, exon2-exon8 of *Lap3-201* (ENSMUST00000046122.10) transcript is recommended as the knockout region.The region contains 886bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Lap3* 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 a knock-out allele are viable and developmentally normal with no detectable abnormalities in generating peptides presented by MHC class I under constitutive conditions or after stimulation with IFN.
- Transcript *Lap3*-205 may not be affected.
- The *Lap3* gene is located on the Chr5. 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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Lap3 leucine aminopeptidase 3 [*Mus musculus* (house mouse)]

Gene ID: 66988, updated on 9-Feb-2020

Summary

Official Symbol	Lap3 provided by MGI
Official Full Name	leucine aminopeptidase 3 provided by MGI
Primary source	MGI:MGI:1914238
See related	Ensembl:ENSMUSG00000039682
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	Lap; Pep7; Peps; LAP-3; Lapep; Pep-7; Pep-S; AA410100; 2410015L10Rik
Expression	Broad expression in genital fat pad adult (RPKM 73.0), large intestine adult (RPKM 53.4) and 25 other tissues See more
Orthologs	human all

Genomic context

Location: 5 B3; 5 24.98 cM

See Lap3 in Genome Data Viewer

Exon count: 13

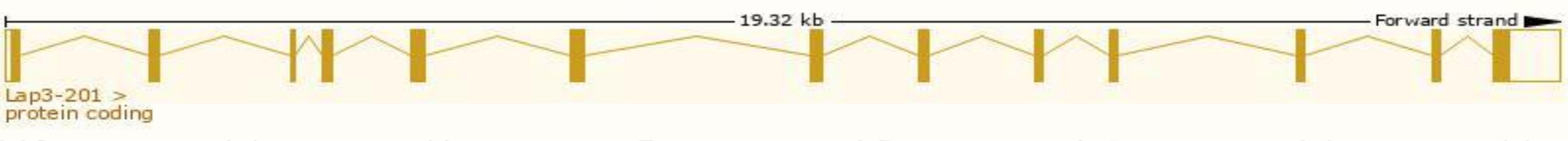
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	5	NC_000071.6 (45493374..45512691)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	5	NC_000071.5 (45884613..45903930)

Transcript information（Ensembl）

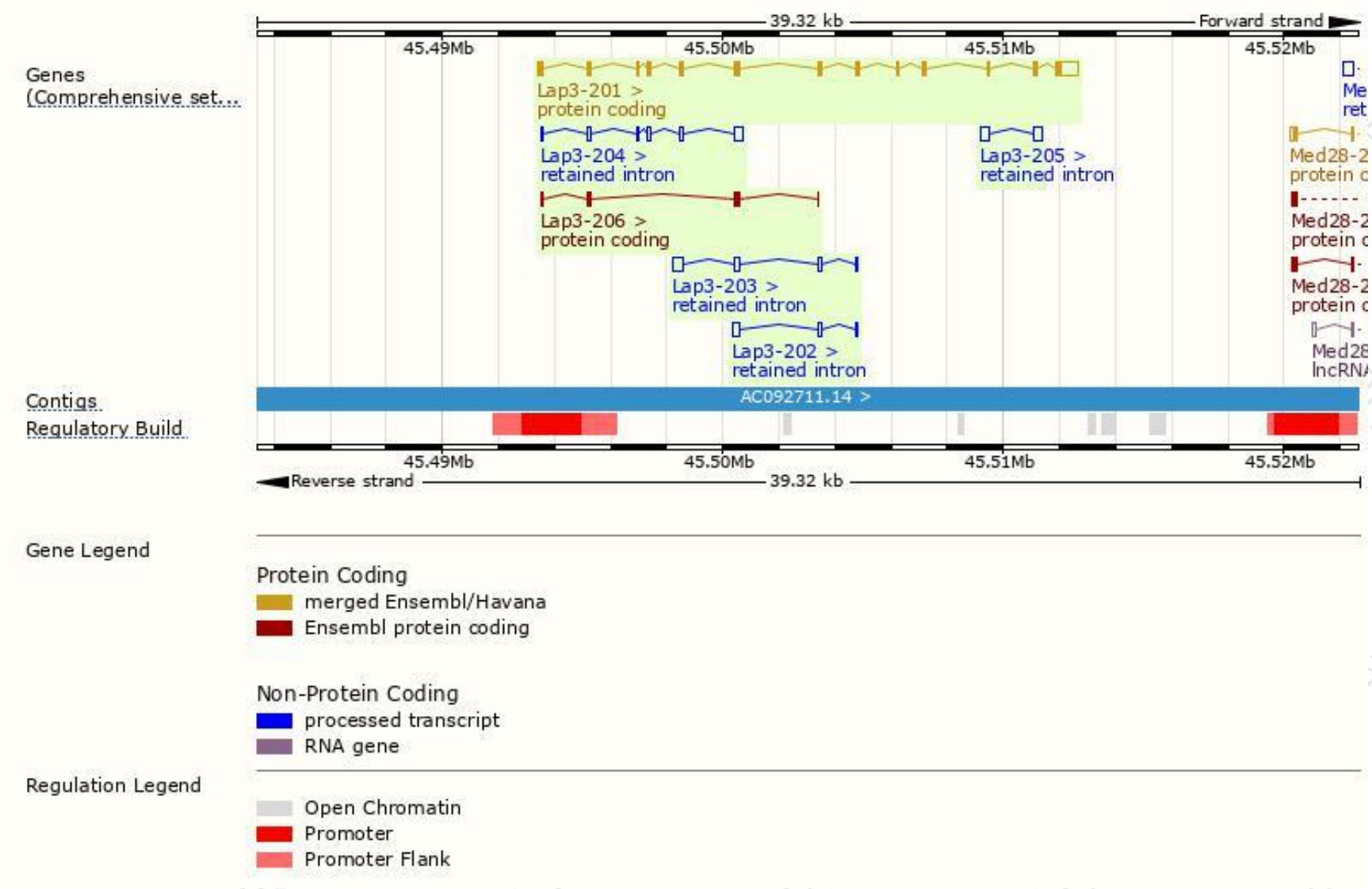
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lap3-201	ENSMUST00000046122.10	2267	519aa	Protein coding	CCDS19274	Q9CPY7	TSL:1 GENCODE basic APPRIS P1
Lap3-206	ENSMUST00000198534.1	322	107aa	Protein coding	-	A0A0G2JEM7	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Lap3-204	ENSMUST00000137699.2	838	No protein	Retained intron	-	-	TSL:2
Lap3-203	ENSMUST00000135413.7	795	No protein	Retained intron	-	-	TSL:3
Lap3-205	ENSMUST00000146811.1	575	No protein	Retained intron	-	-	TSL:3
Lap3-202	ENSMUST00000131289.1	466	No protein	Retained intron	-	-	TSL:1

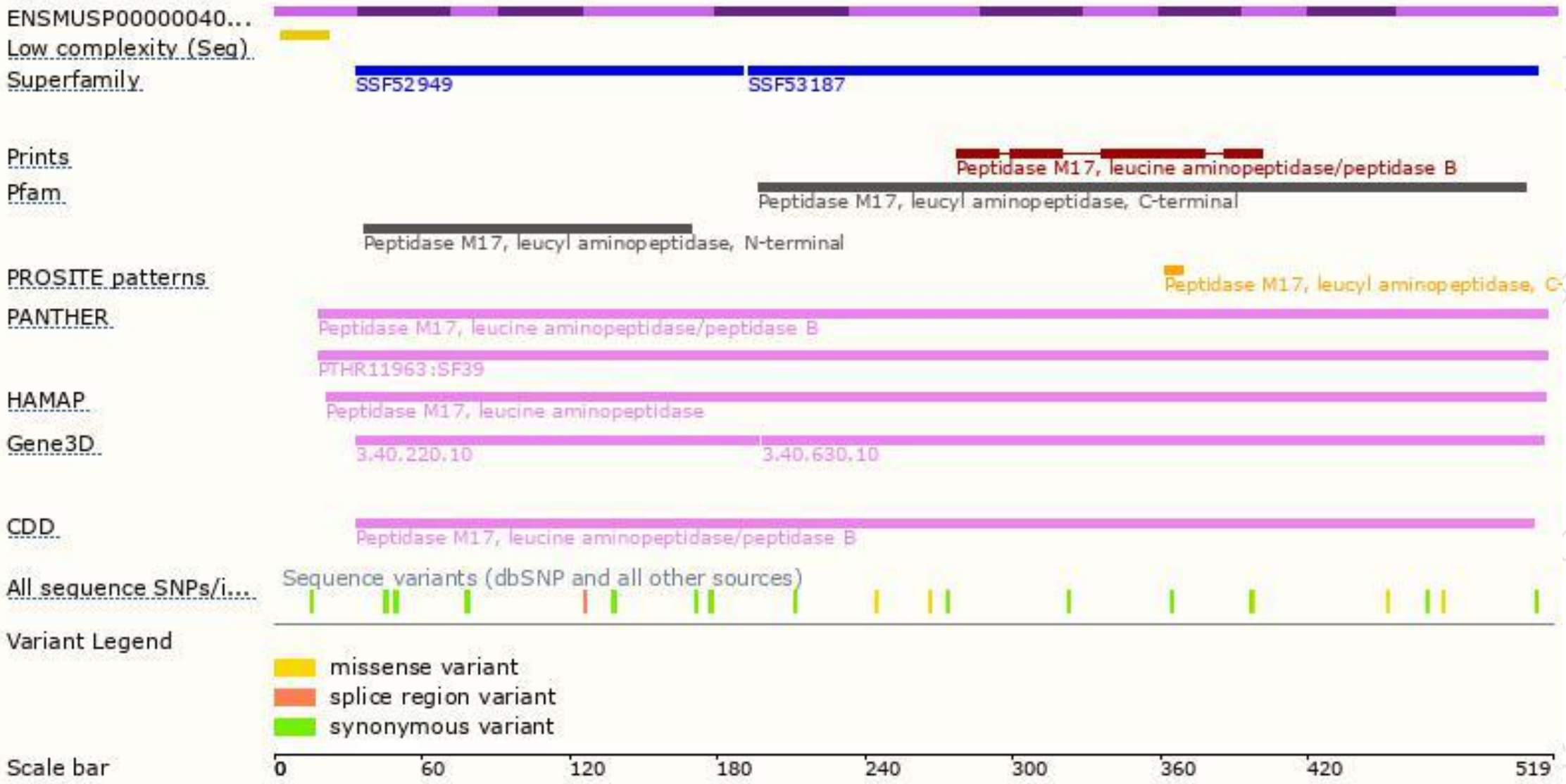
The strategy is based on the design of *Lap3-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



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