Blm Cas9-CKO Strategy

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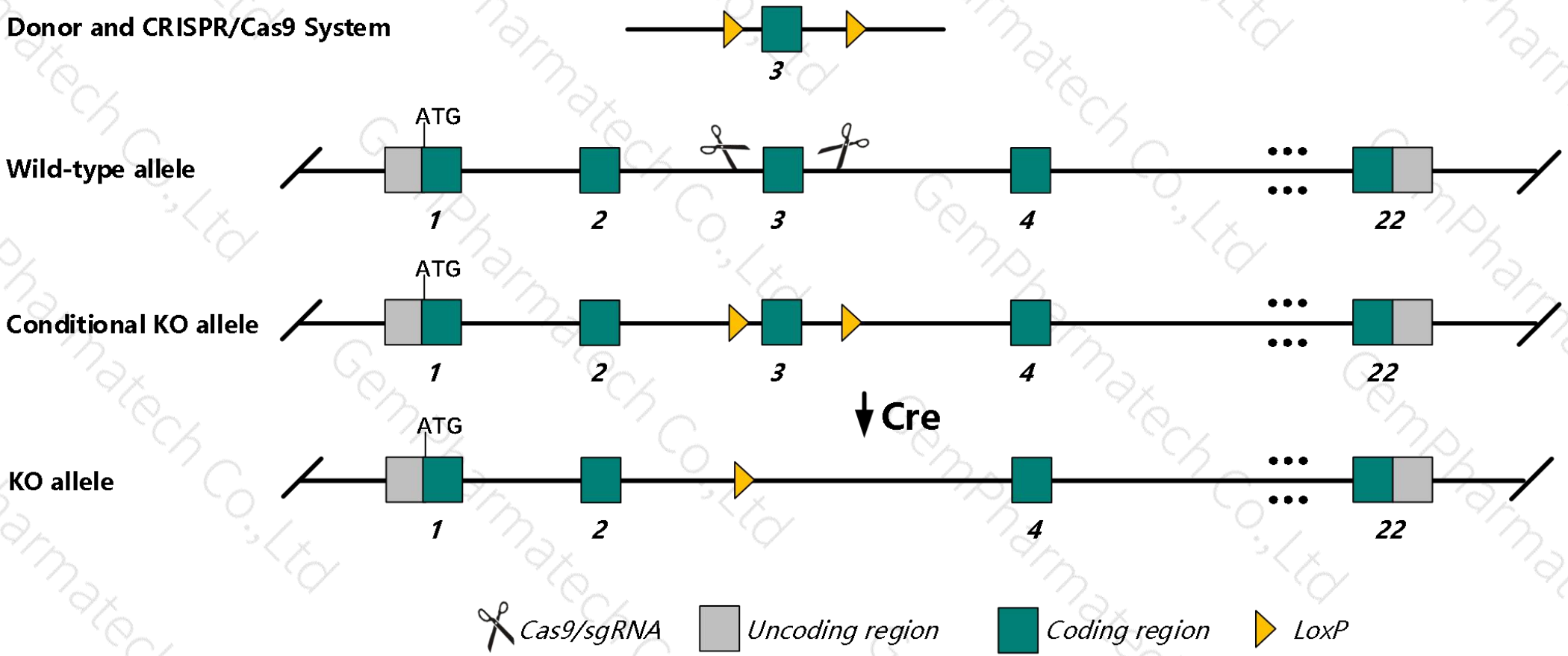
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Blm* gene has 10 transcripts. According to the structure of *Blm* gene, exon3 of *Blm-202* (ENSMUST00000170315.2) transcript is recommended as the knockout region.The region contains 716bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Blm* 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,homozygous null mutants are developmentally delayed, with increased apopotosis in the epiblast and severe anemia, dying at embyronic day 13.5; but homozygotes for a cre mediated recombinant allele are viable Bloom syndrome-like mice prone to a wide variety of cancers and showing increased rates of LOH.
- Transcripts 204,205,206,207,209,210 may not be affected. The effect of transcript 208 is unknown.
- The *Blm* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Blm Bloom syndrome, RecQ like helicase [*Mus musculus* (house mouse)]

Gene ID: 12144, updated on 20-Mar-2020

Summary

Official Symbol	Blm provided by MGI
Official Full Name	Bloom syndrome, RecQ like helicase provided by MGI
Primary source	MGI:MGI:1328362
See related	Ensembl:ENSMUSG00000030528
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
Expression	Biased expression in liver E14 (RPKM 7.3), liver E14.5 (RPKM 5.7) and 12 other tissues See more
Orthologs	human all

Genomic context

Location: 7 D2; 7 45.65 cM

See Blm in Genome Data Viewer

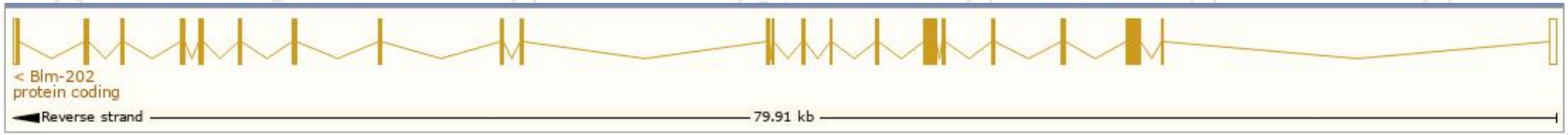
Exon count: 24

Transcript information（Ensembl）

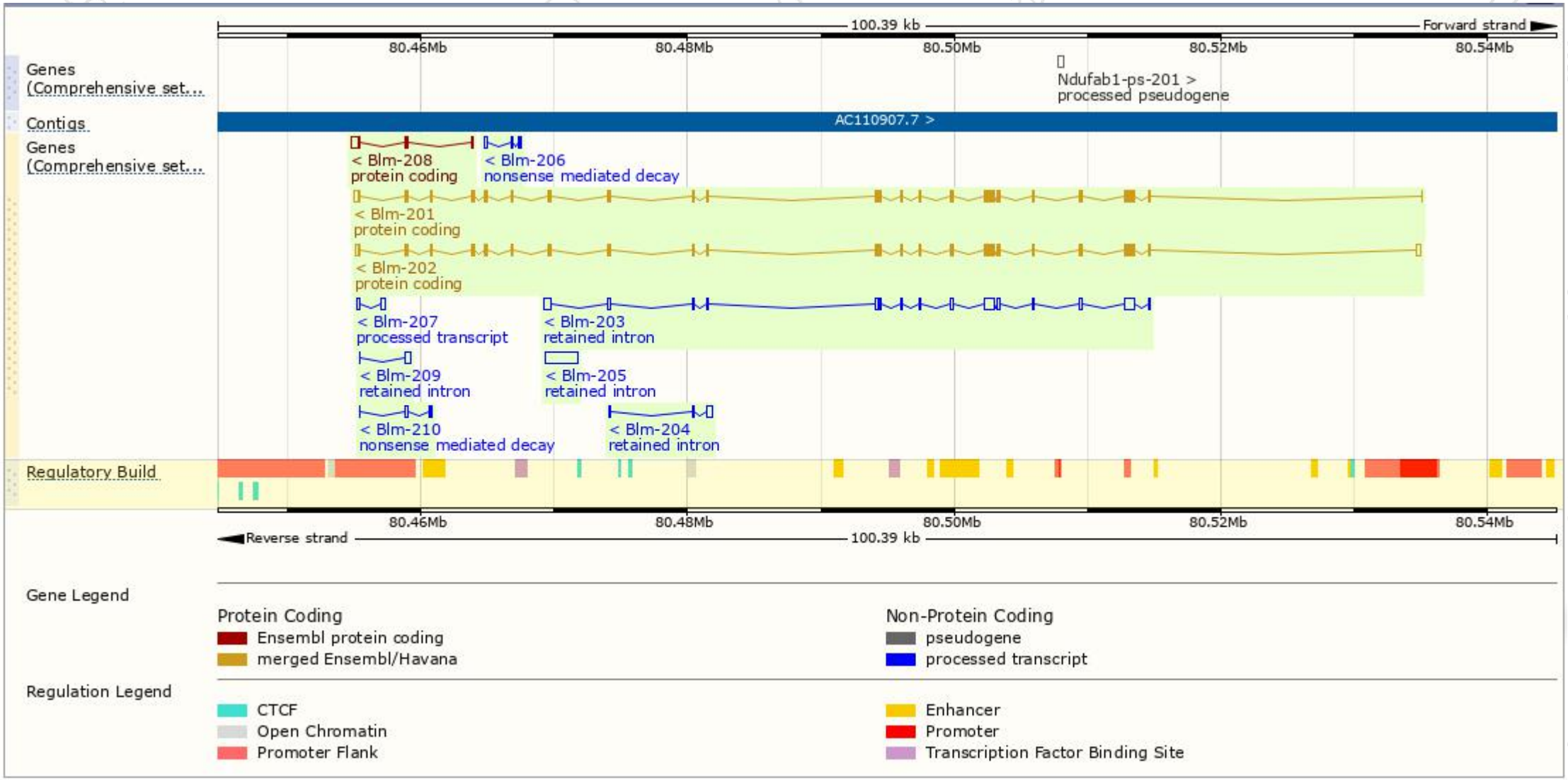
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID ▲	bp	Protein	Biotype	CCDS	UniProt	Flags
Blm-201	ENSMUST00000081314.10	4598	1416aa	Protein coding	CCDS40000	O88700	TSL:1 GENCODE basic APPRIS P3
Blm-202	ENSMUST00000170315.2	4770	1419aa	Protein coding	CCDS52281	E9PZ97	TSL:1 GENCODE basic APPRIS ALT2
Blm-203	ENSMUST00000205263.1	3581	No protein	Retained intron	-	-	TSL:5
Blm-204	ENSMUST00000205584.1	749	No protein	Retained intron	-	-	TSL:3
Blm-205	ENSMUST00000205713.1	2507	No protein	Retained intron	-	-	TSL:NA
Blm-206	ENSMUST00000205730.1	465	51aa	Nonsense mediated decay	-	A0A0U1RNI0	CDS 5' incomplete TSL:5
Blm-207	ENSMUST00000206518.1	540	No protein	Processed transcript	-	-	TSL:3
Blm-208	ENSMUST00000206901.1	993	155aa	Protein coding	-	A0A0U1RPP0	CDS 5' incomplete TSL:5
Blm-209	ENSMUST00000206948.1	485	No protein	Retained intron	-	-	TSL:2
Blm-210	ENSMUST00000206989.1	383	42aa	Nonsense mediated decay	-	A0A0U1RPS3	CDS 5' incomplete TSL:5

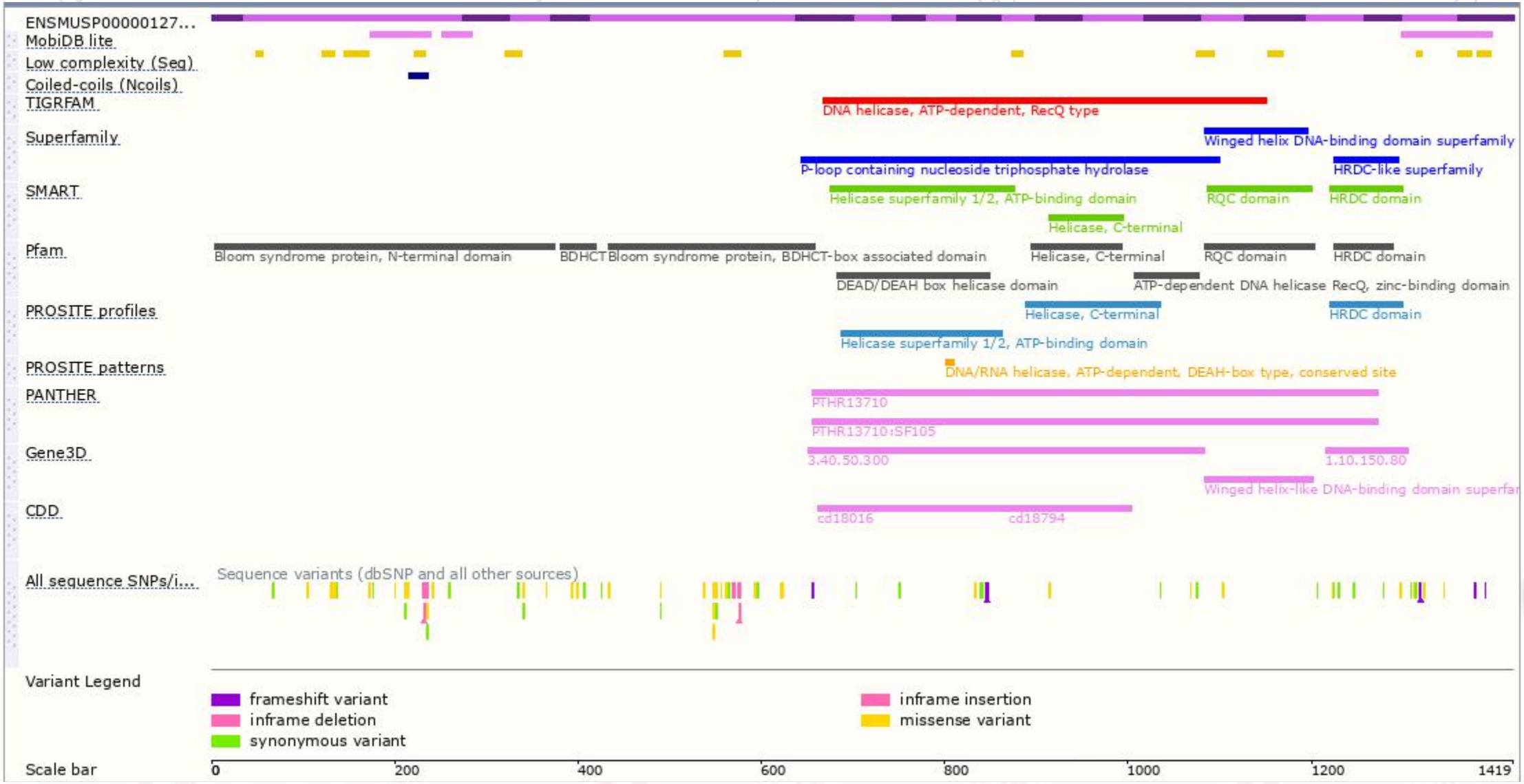
The strategy is based on the design of *Blm-202* transcript,the transcription is shown below:



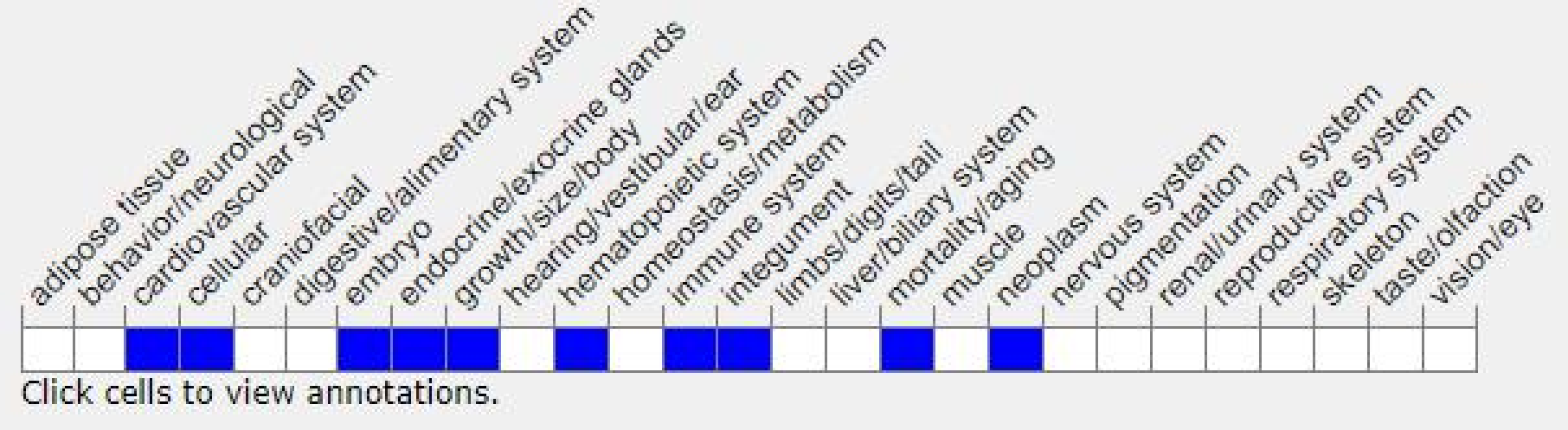
Genomic location distribution



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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