Eps15 Cas9-KO Strategy

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Project Overview

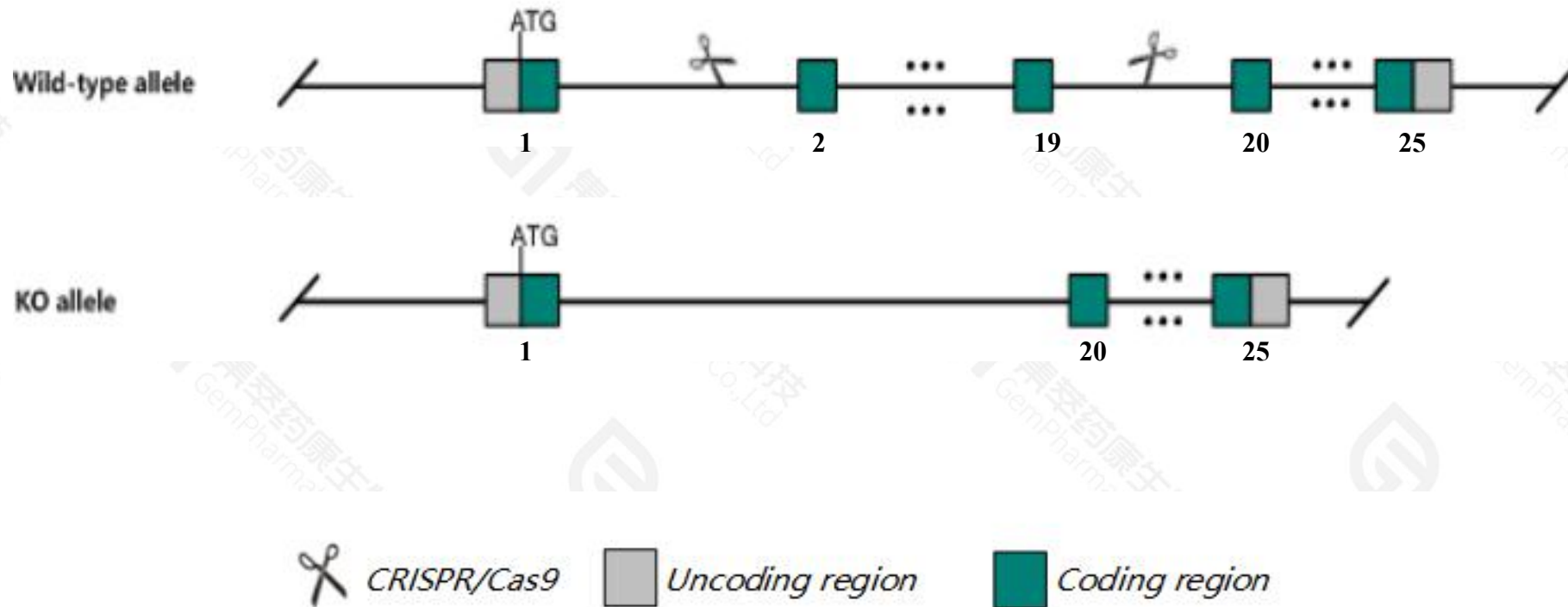|	
Project Name	*Eps15*
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Eps15* gene. The schematic diagram is as follows:

Technical routes

➢ The *Eps15* gene has 11 transcripts. According to the structure of *Eps15* gene, exon2-exon19 of *Eps15-202*(ENSMUST00000102729.10) transcript is recommended as the knockout region.The region contains 1885bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Eps15* gene. The brief process is as follows: CRISPR/Cas9 system 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,homozygotes for a null allele show increased marginal zone B cell number with no changes in precursor cells, proliferation, apoptosis, migration or B cell responses. Homozygotes for a different null allele show decreased mean corpuscular hemoglobin (MCH), decreased MCH concentration, and dermatitis.

➤ The *Eps15* gene is located on the Chr4. 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）

Eps15 epidermal growth factor receptor pathway substrate 15 [Mus musculus (house mouse)]

Gene ID: 13858, updated on 13-Mar-2020

Summary

|	
Official Symbol	Eps15 provided by MGI
Official Full Name	epidermal growth factor receptor pathway substrate 15 provided by MGI
Primary source	MGI:MGI:104583
See related	Ensembl:ENSMUSG00000028552
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	2410112D09Rik
Expression	Broad expression in cortex adult (RPKM 26.3), cerebellum adult (RPKM 24.7) and 21 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

The gene has 11 transcripts,all transcripts are shown below:

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Eps15-202	ENSMUST00000102729.9	5190	897aa	Protein coding	CCDS18462	P42567 Q80ZL3	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P2
Eps15-201	ENSMUST00000030281.11	2980	583aa	Protein coding	CCDS51259	A0A0R4J0A0	TSL:1 GENCODE basic
Eps15-207	ENSMUST00000175776.7	2802	933aa	Protein coding	-	H3BK65	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Eps15-204	ENSMUST00000132165.8	2295	764aa	Protein coding	-	F6W2Q5	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Eps15-211	ENSMUST00000177192.1	906	150aa	Protein coding	-	H3BLE4	CDS 5' incomplete TSL:3
Eps15-209	ENSMUST00000177089.7	346	103aa	Protein coding	-	H3BJB8	CDS 3' incomplete TSL:3
Eps15-208	ENSMUST00000176251.7	2963	793aa	Nonsense mediated decay	-	Q5JC28	TSL:1
Eps15-210	ENSMUST00000177140.1	308	No protein	Processed transcript	-	-	TSL:3
Eps15-203	ENSMUST00000126015.2	1584	No protein	Retained intron	-	-	TSL:1
Eps15-206	ENSMUST00000150755.1	627	No protein	Retained intron	-	-	TSL:3
Eps15-205	ENSMUST00000141751.1	470	No protein	Retained intron	-	-	TSL:2

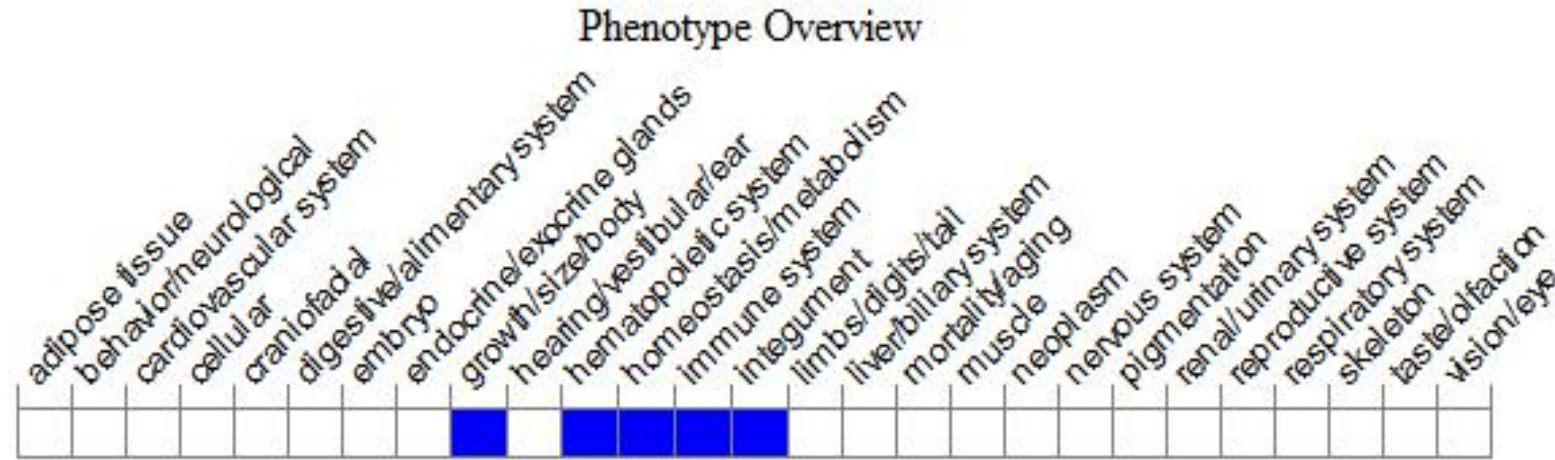The strategy is based on the design of *Eps15-202* transcript,the transcription is shown below:
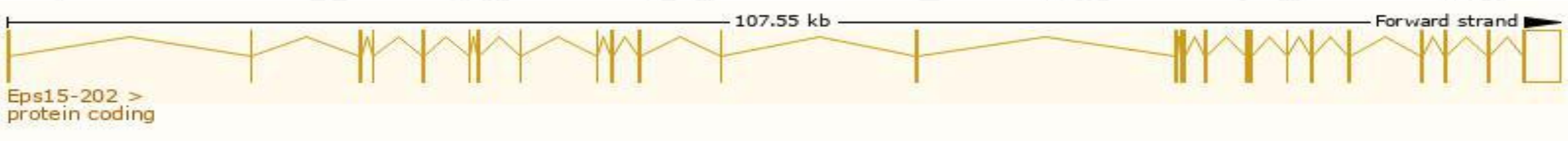
Genomic location distribution
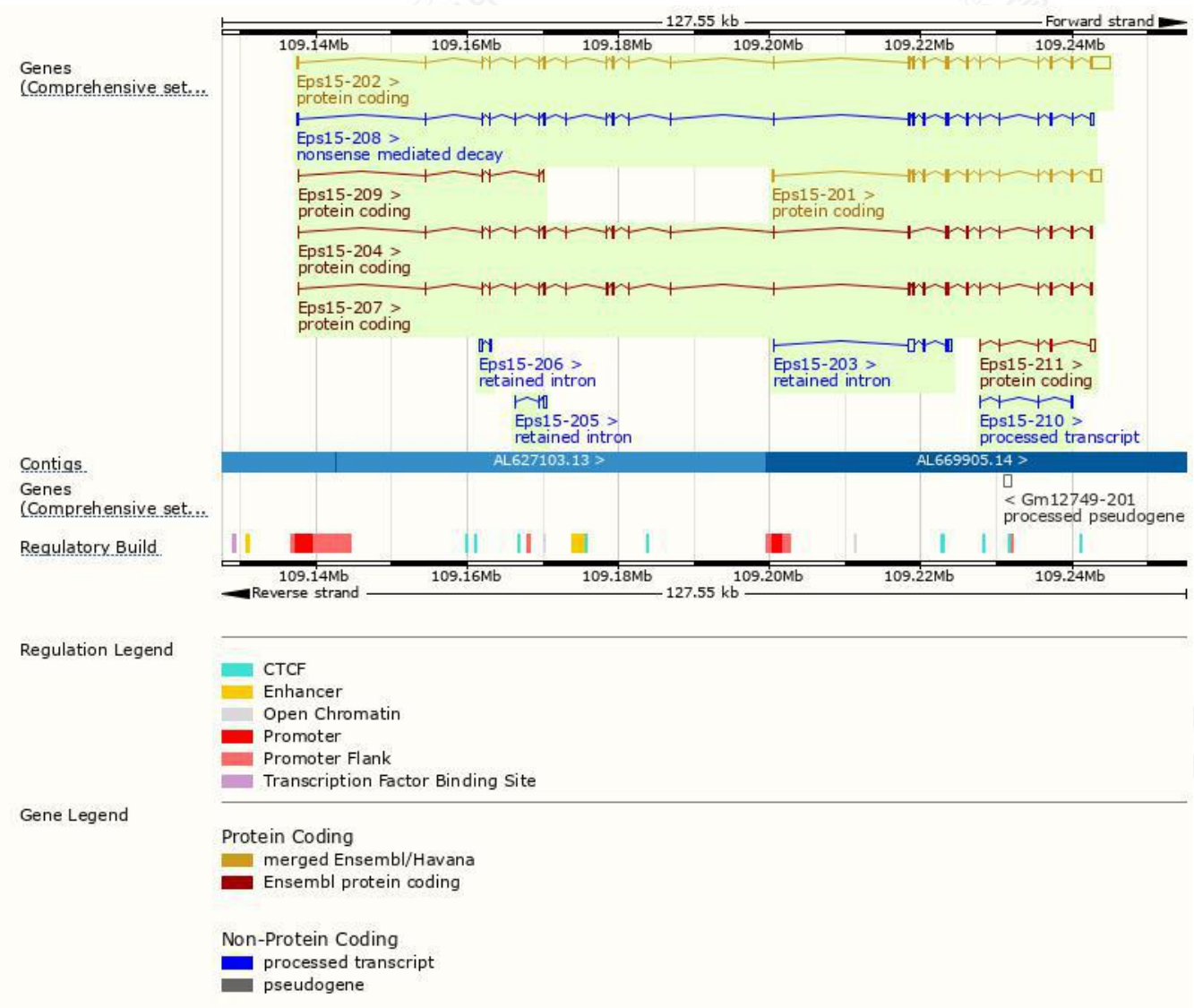
Protein domain
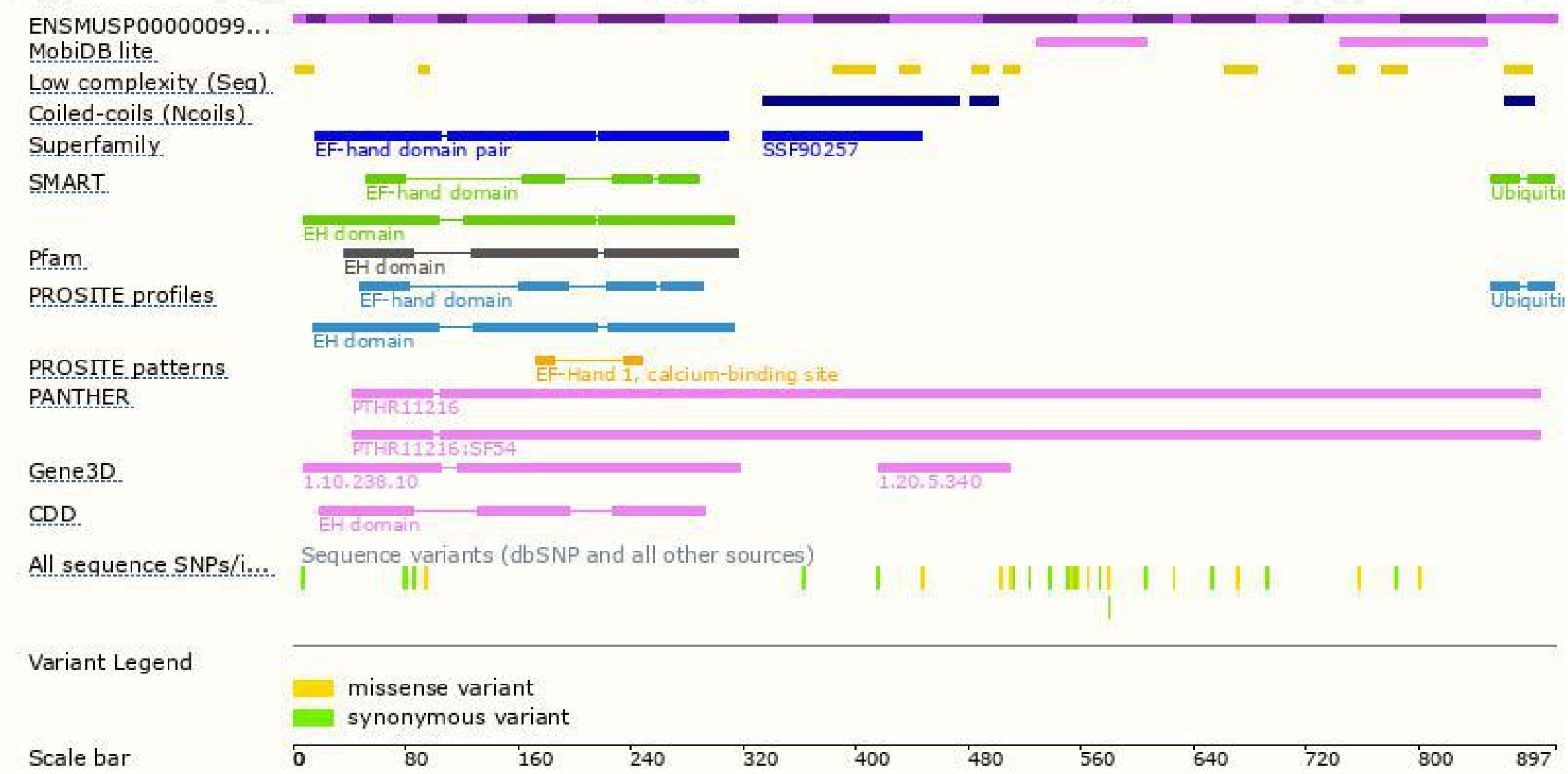
Mouse phenotype description(MGI)

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,homozygotes for a null allele show increased marginal zone B cell number with no changes in precursor cells, proliferation, apoptosis, migration or B cell responses. Homozygotes for a different null allele show decreased mean corpuscular hemoglobin (MCH), decreased MCH concentration, and dermatitis.

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