Trim33 Cas9-CKO Strategy

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Project Overview

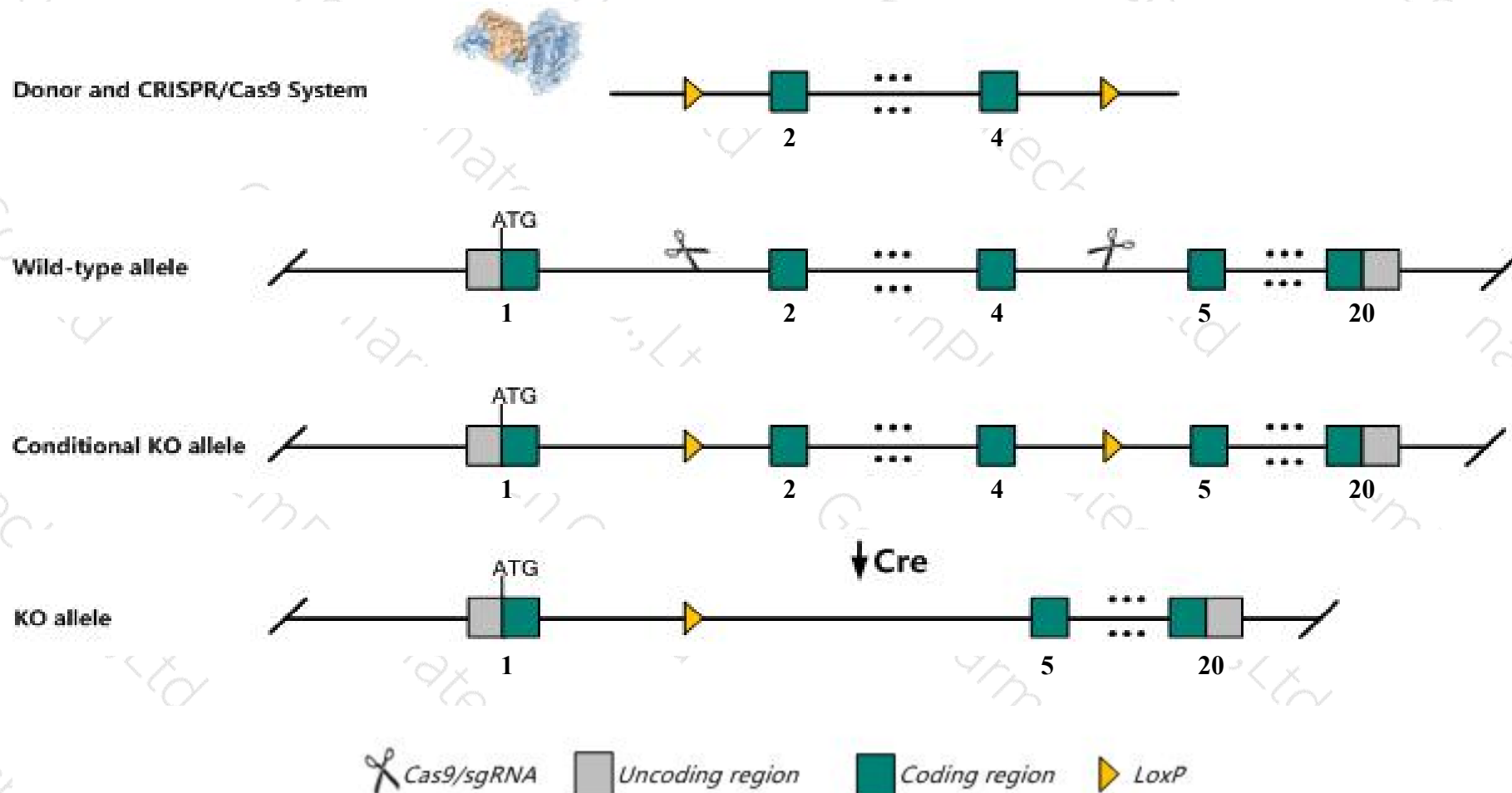| Project Name	*Trim33*
Project type	Cas9-CKO
Strain background	C57BL/6J

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Trim33* gene. The schematic diagram is as follows:

Technical routes

➢ The *Trim33* gene has 7 transcripts. According to the structure of *Trim33* gene, exon2-exon4 of *Trim33-201* (ENSMUST00000029444.12) transcript is recommended as the knockout region.The region contains 397bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Trim33* 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/6J 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/6J 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 and cell types.

Notice

➢ According to the existing MGI data,Mice homozygous for a knock-out allele exhibit embryonic lethality prior to E9.5 with abnormal embryonic development.

➢ The *Trim33* gene is located on the Chr3. 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）

Trim33 tripartite motif-containing 33 [Mus musculus (house mouse)]

Gene ID: 94093, updated on 19-Mar-2019

▲ Summary

Official Symbol	Trim33 provided by MGI
Official Full Name	tripartite motif-containing 33 provided by MGI
Primary source	MGI:MGI:2137357
See related	Ensembl:ENSMUSG00000033014
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	8030451N04Rik, AI413936, Ecto, Tif1g
Expression	Ubiquitous expression in CNS E14 (RPKM 7.3), whole brain E14.5 (RPKM 7.3) and 28 other tissuesSee more
Orthologs	human all

# Transcript information（Ensembl）

The gene has 7 transcripts,all transcripts are shown below:

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trim33-201	ENSMUST00000029444.12	8875	1140aa	Protein coding	CCDS38573	E9QP19	TSL:1 GENCODE basic APPRIS P4
Trim33-202	ENSMUST00000106860.5	5025	1123aa	Protein coding	CCDS38574	E9QME5	TSL:1 GENCODE basic APPRIS ALT2
Trim33-204	ENSMUST00000197365.1	2737	291aa	Protein coding	-	A0A0G2JFC3	CDS 5' incomplete TSL:2
Trim33-205	ENSMUST00000197779.4	577	193aa	Protein coding	-	A0A0G2JGY5	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Trim33-206	ENSMUST00000198706.1	1788	402aa	Nonsense mediated decay	-	A0A0G2JE09	CDS 5' incomplete TSL:5
Trim33-207	ENSMUST00000198969.1	795	No protein	Retained intron	-	-	TSL:3
Trim33-203	ENSMUST00000196678.1	667	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Trim33-201* transcript,The transcription is shown below
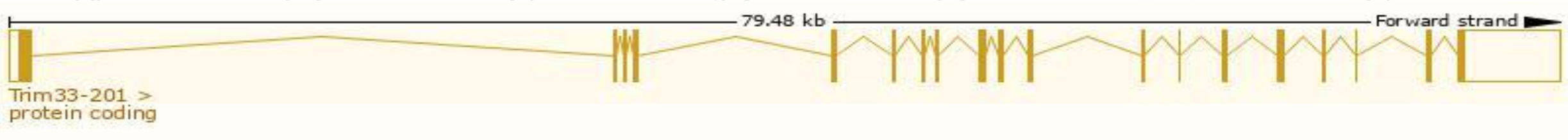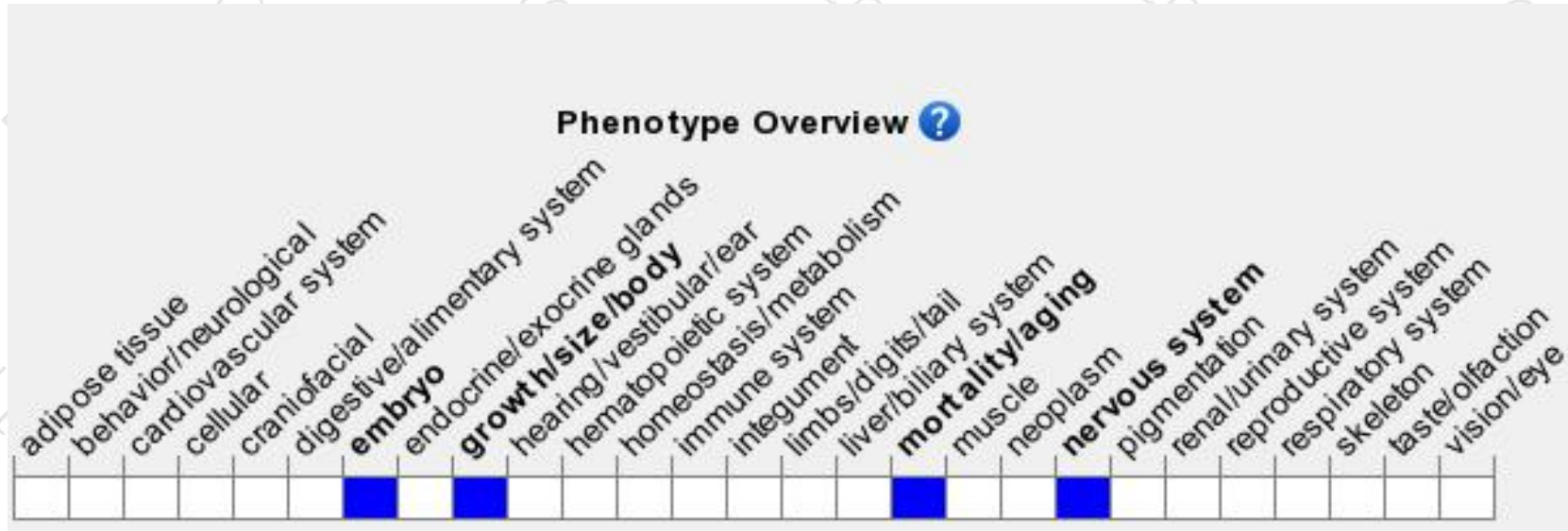
Genomic location distribution
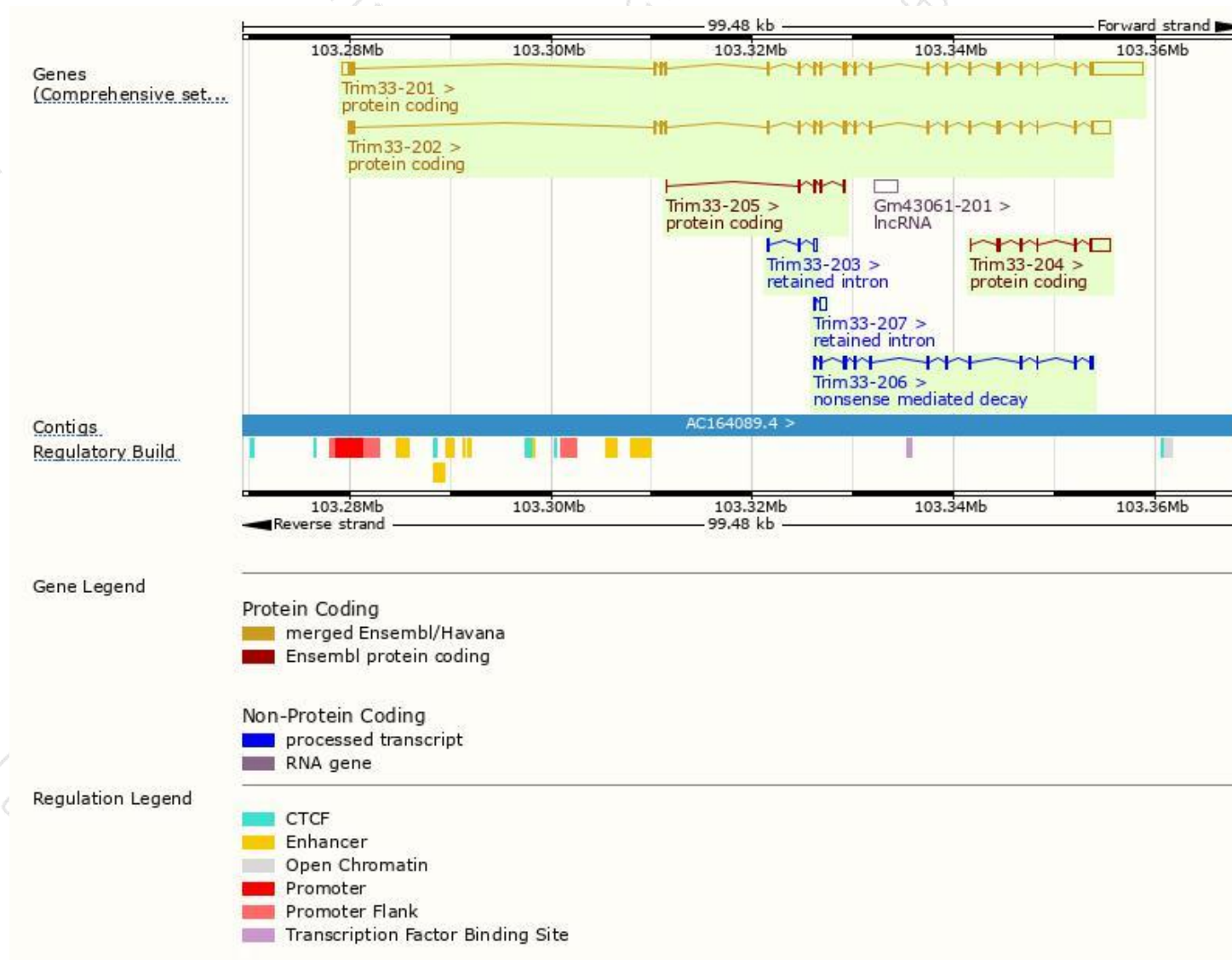
Protein domain
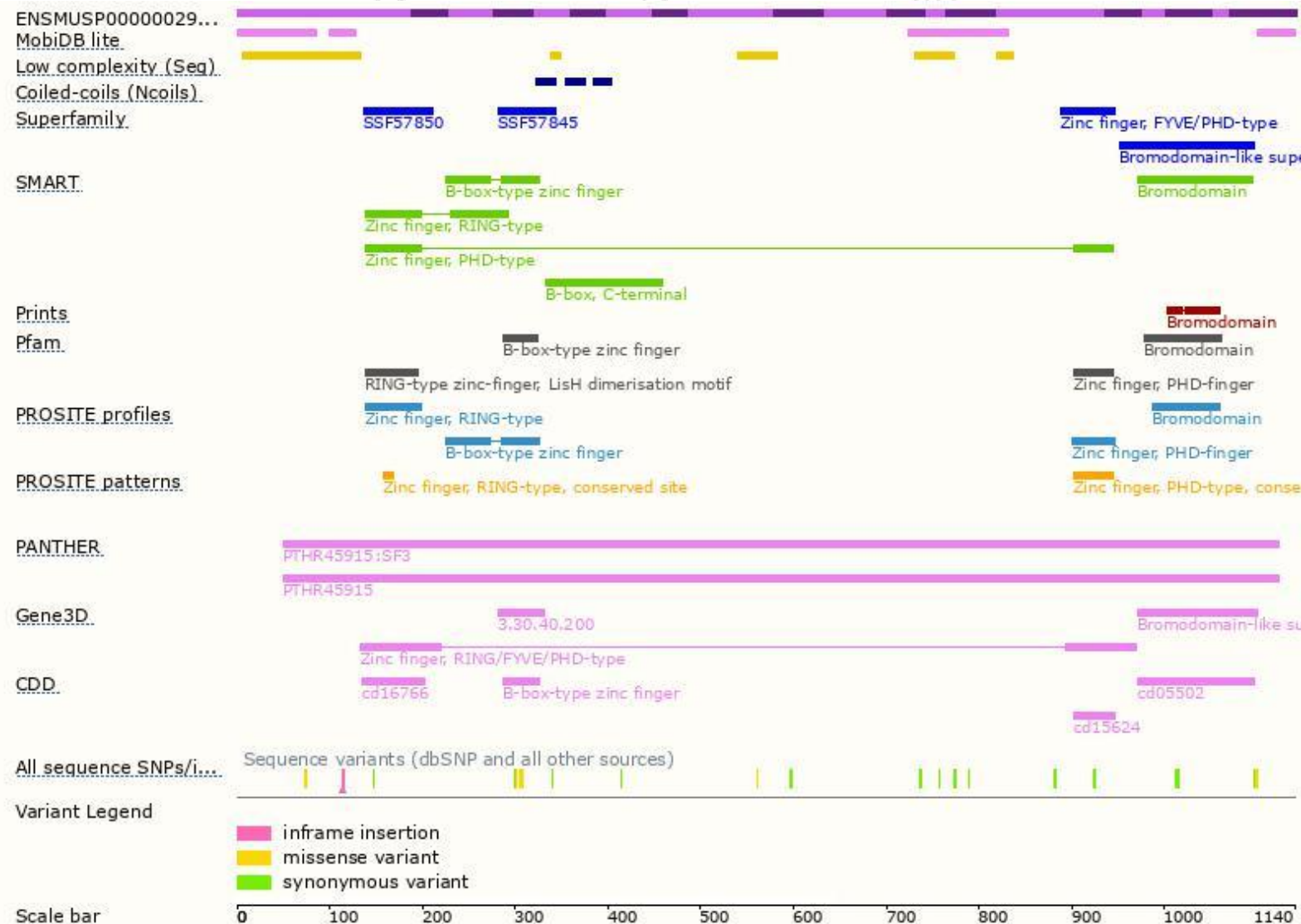
Mouse phenotype description(MGI)

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,Mice homozygous for a knock-out allele exhibit embryonic lethality prior to E9.5 with abnormal embryonic development.

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