Mettl3 Cas9-CKO Strategy

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Project Overview

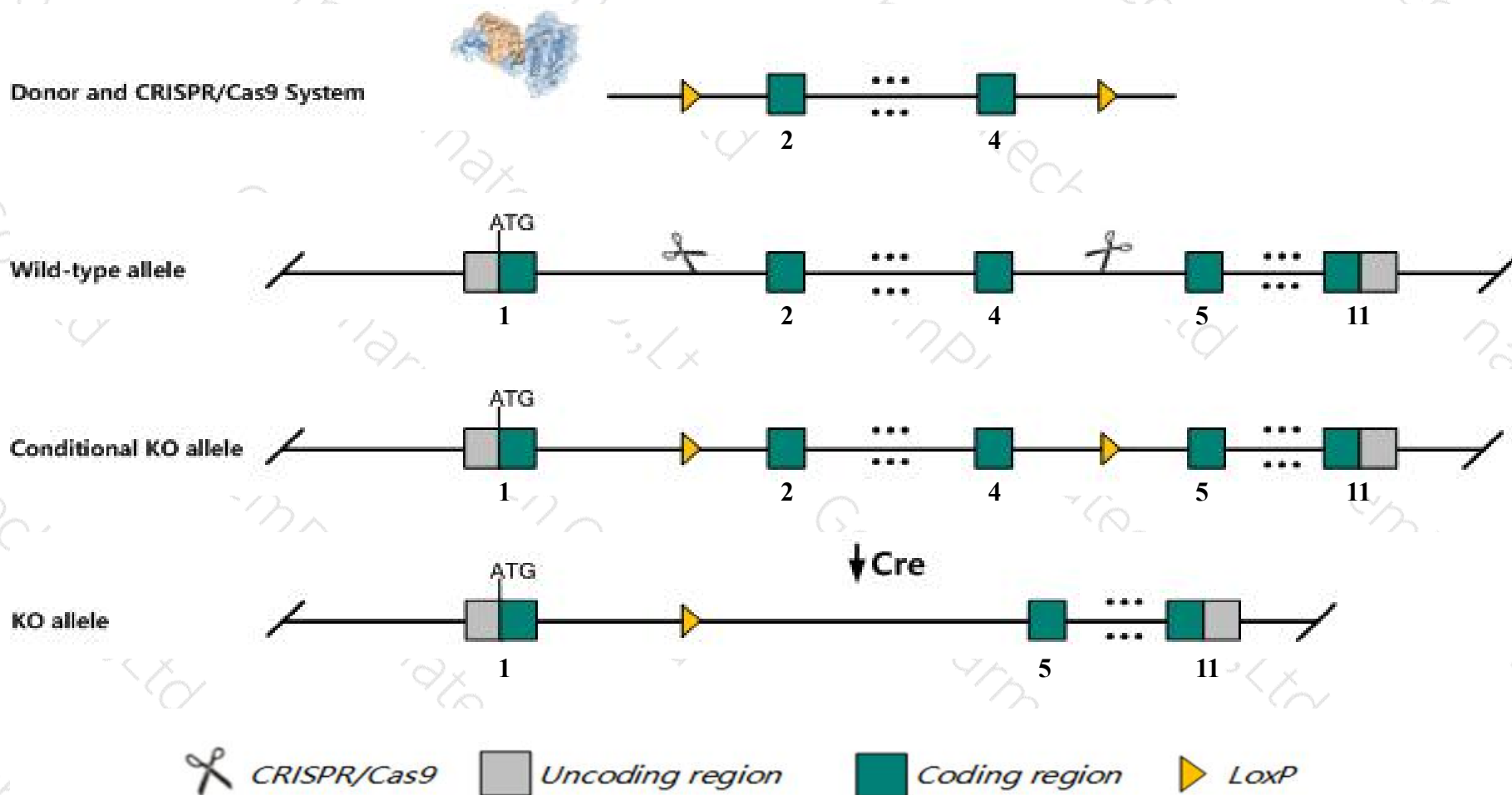|	
Project Name	*Mettl3*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Mettl3* gene. The schematic diagram is as follows:

Technical routes

➢ The *Mettl3* gene has 15 transcripts. According to the structure of *Mettl3* gene, exon2-exon4 of *Mettl3-201* (ENSMUST00000022767.15) transcript is recommended as the knockout region.The region contains 799bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Mettl3* 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 exhibit embryonic lethality between E3.5 and E8.5 with a deficiency in adopting the epiblast egg cylinder.

➢ The *Mettl3* 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）

Mettl3 methyltransferase like 3 [Mus musculus (house mouse)]

Gene ID: 56335, updated on 3-Feb-2019

▲Summary

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Official Symbol	Mettl3 provided by MGI
Official Full Name	methyltransferase like 3 provided byMGI
Primary source	MGI:MGI:1927165
See related	Ensembl:ENSMUSG00000022160
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	2310024F18Rik, M6A, Spo8
Expression	Ubiquitous expression in CNS E11.5 (RPKM 8.6), CNS E14 (RPKM 7.3) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 15 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mettl3-201	ENSMUST00000022767.15	2025	580aa	Protein coding	CCDS27053	A0A0R4J041	TSL:1 GENCODE basic APPRIS P1
Mettl3-214	ENSMUST00000174853.1	857	245aa	Protein coding	-	G3UXX7	CDS 3' incomplete TSL:3
Mettl3-213	ENSMUST00000174360.1	438	104aa	Protein coding	-	G3UZP3	CDS 5' incomplete TSL:3
Mettl3-210	ENSMUST00000173656.1	392	73aa	Protein coding	-	G3UXN3	CDS 5' incomplete TSL:3
Mettl3-206	ENSMUST00000147768.7	2159	245aa	Nonsense mediated decay	-	G3UZP2	TSL:1
Mettl3-202	ENSMUST00000122962.2	1708	36aa	Nonsense mediated decay	-	G3UY98	TSL:1
Mettl3-212	ENSMUST00000174351.7	1469	335aa	Nonsense mediated decay	-	G5E922	CDS 5' incomplete TSL:5
Mettl3-211	ENSMUST00000173896.7	1091	153aa	Nonsense mediated decay	-	G3UYB5	TSL:5
Mettl3-208	ENSMUST00000173138.1	667	153aa	Nonsense mediated decay	-	G3UYB5	TSL:5
Mettl3-205	ENSMUST00000145875.1	330	37aa	Nonsense mediated decay	-	G3UX68	TSL:3
Mettl3-215	ENSMUST00000197394.1	1504	No protein	Retained intron	-	-	TSL:NA
Mettl3-203	ENSMUST00000127797.8	963	No protein	Retained intron	-	-	TSL:2
Mettl3-207	ENSMUST00000156611.1	927	No protein	Retained intron	-	-	TSL:2
Mettl3-204	ENSMUST00000130550.2	727	No protein	Retained intron	-	-	TSL:2
Mettl3-209	ENSMUST00000173546.1	504	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Mettl3-201* transcript,The transcription is shown below

< Mettl3-201
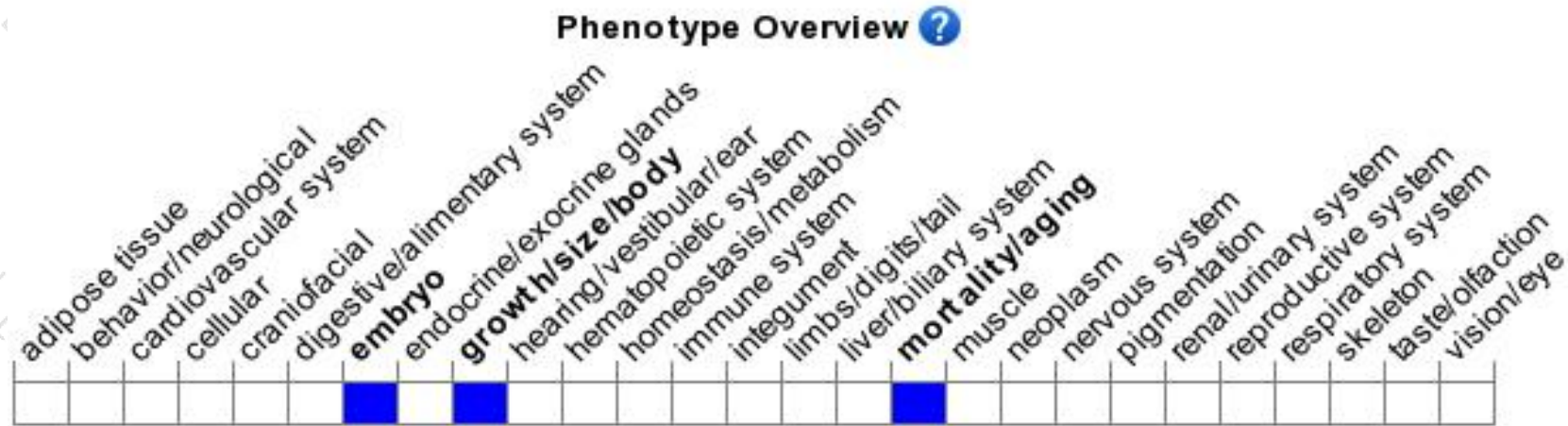protein coding

◀ Reverse strand

10.29 kb
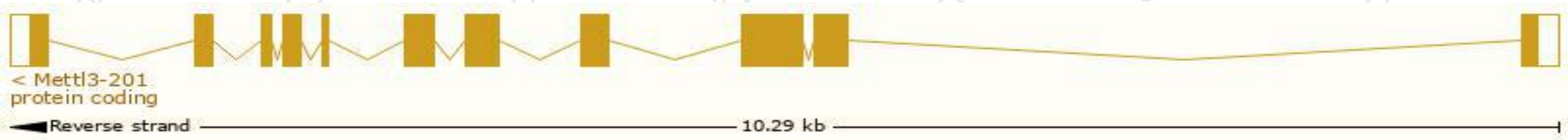
Genomic location distribution
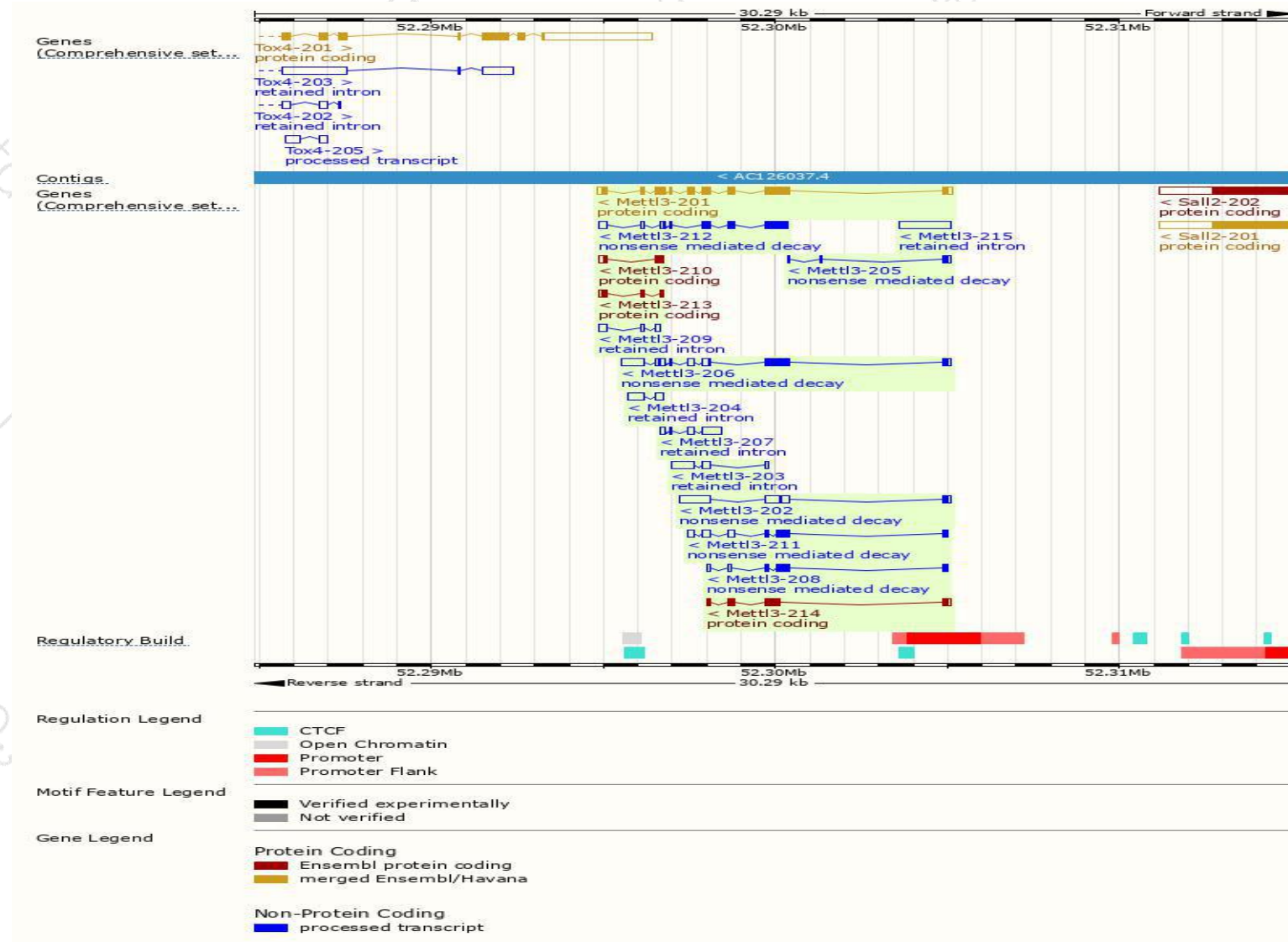
Protein domain
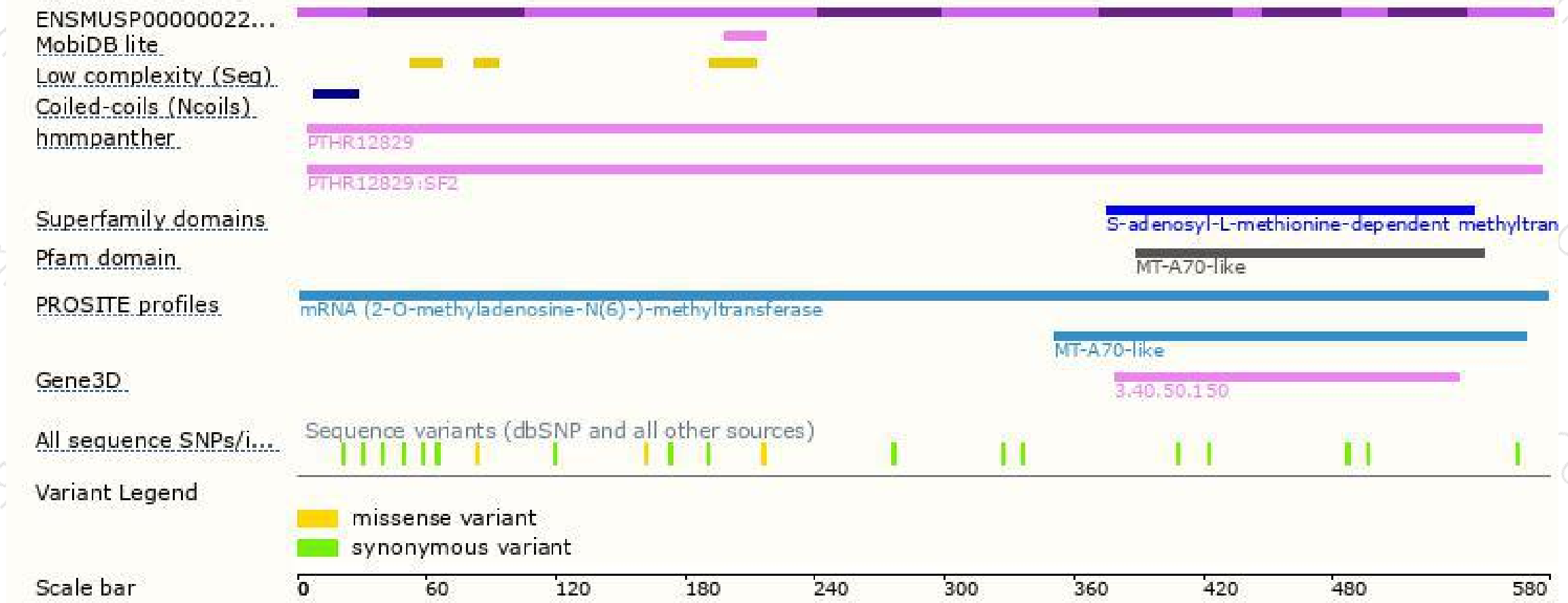
Mouse phenotype description(MGI)

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 between E3.5 and E8.5 with a deficiency in adopting the epiblast egg cylinder.

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