Dido1 Cas9-KO Strategy

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Design Date: 2020-7-24

Project Overview

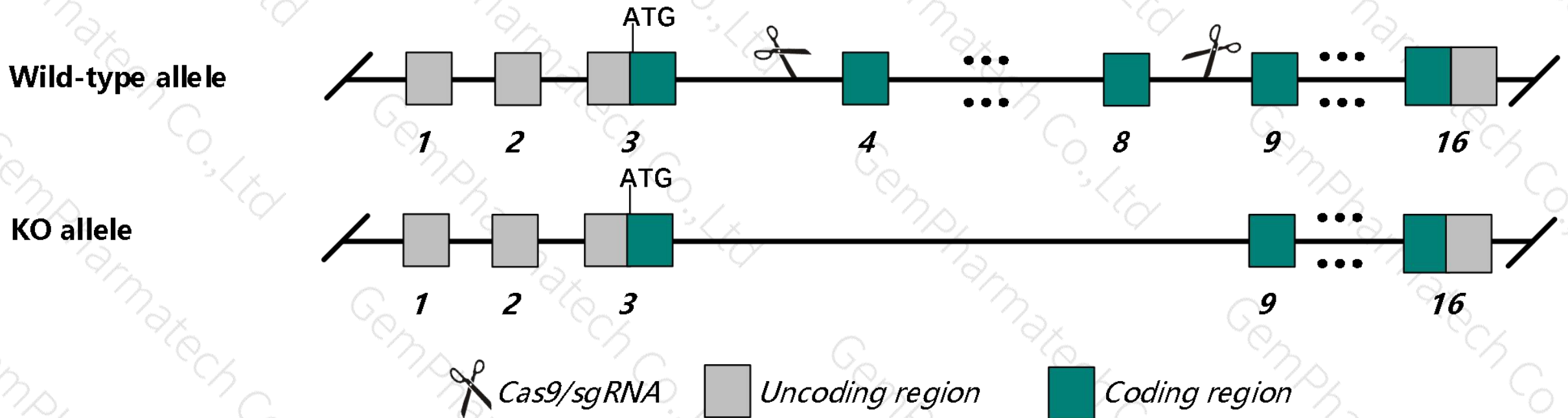|	
Project Name	*Dido1*
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Dido1* gene. The schematic diagram is as follows:

Technical routes

➢ The *Dido1* gene has 7 transcripts. According to the structure of *Dido1* gene, exon4-exon8 of *Dido1-202*(ENSMUST00000087517.9) transcript is recommended as the knockout region.The region contains 1375bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Dido1* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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.

Notice

➢ According to the existing MGI data,mice homozygous for a knock-out allele exhibit severely reduced fertility; about one-half develop a transplantable disease characterized by anomalies in spleen, bone marrow, and peripheral blood and including anemia and various symptoms typical of myeloid dysplasia or myeloid proliferation.

➢ The *Dido1* gene is located on the Chr2. 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）

Dido1 death inducer-obliterator 1 [Mus musculus (house mouse)]

Gene ID: 23856, updated on 13-Mar-2020

Summary

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Official Symbol	Dido1 provided by MGI
Official Full Name	death inducer-obliterator 1 provided by MGI
Primary source	MGI:MGI:1344352
See related	Ensembl:ENSMUSG00000038914
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	6720461J16Rik, D130048F08Rik, DATF-1, DIO-1, Datf1, dido
Summary	This gene encodes a transcription factor involved in apoptosis. The encoded protein functions in cell cycle progression and plays a role in chromosomal stability. This protein regulates the self-renewal of embryonic stem cells. Disruption of this gene in mice causes symptoms similar to myelodysplastic/myeloproliferative diseases in humans. Mice lacking this gene show severely reduced fertility. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Apr 2014]
Expression	Ubiquitous expression in CNS E11.5 (RPKM 4.5), thymus adult (RPKM 4.0) 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
Dido1-202	ENSMUST00000087517.9	8723	2256aa	Protein coding	CCDS17182	Q8C9B9	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 P4
Dido1-206	ENSMUST00000103057.7	4906	1183aa	Protein coding	CCDS17183	Q8C9B9	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 ALT2
Dido1-207	ENSMUST00000130986.7	4898	614aa	Protein coding	CCDS17184	Q8C9B9	TSL:1 GENCODE basic
Dido1-204	ENSMUST00000103055.7	4613	614aa	Protein coding	CCDS17184	Q8C9B9	TSL:1 GENCODE basic
Dido1-205	ENSMUST00000103056.9	4281	1183aa	Protein coding	CCDS17183	Q8C9B9	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 ALT2
Dido1-201	ENSMUST00000037764.11	3570	643aa	Protein coding	-	A0A0R4J0L7	TSL:1
Dido1-203	ENSMUST00000103054.3	1636	No protein	Processed transcript	-	-	TSL:1

The strategy is based on the design of *Dido1-202* transcript,the transcription is shown below:
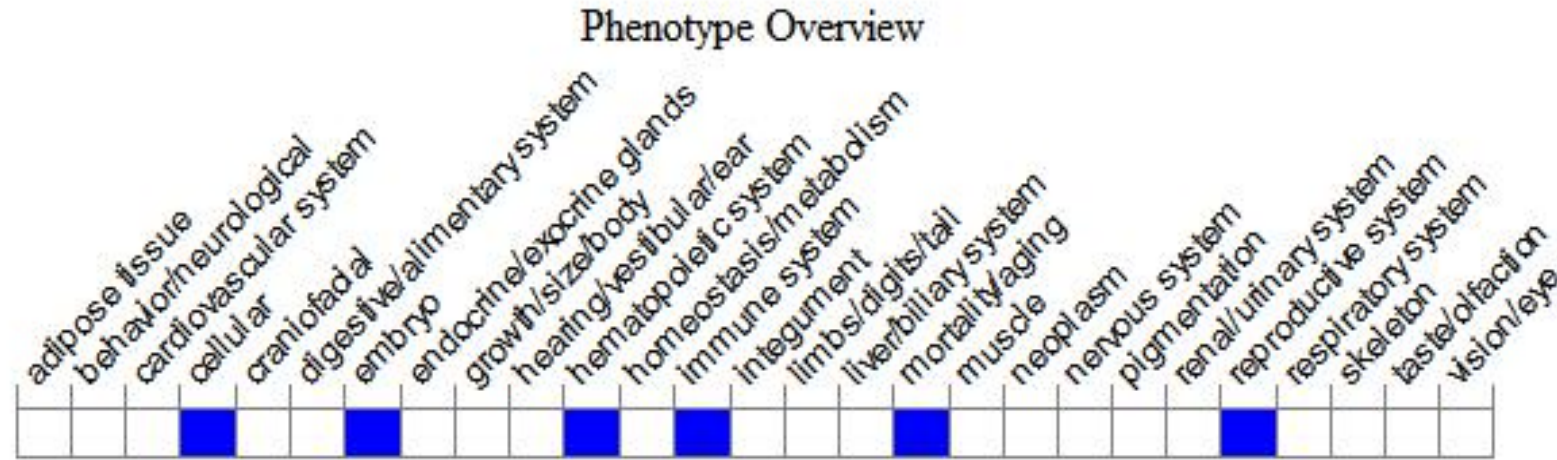

< Dido1-202
protein coding

Reverse strand ———————————— 44.39 kb ————————————
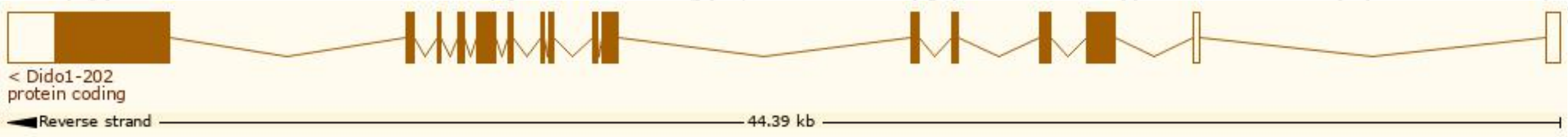
Genomic location distribution
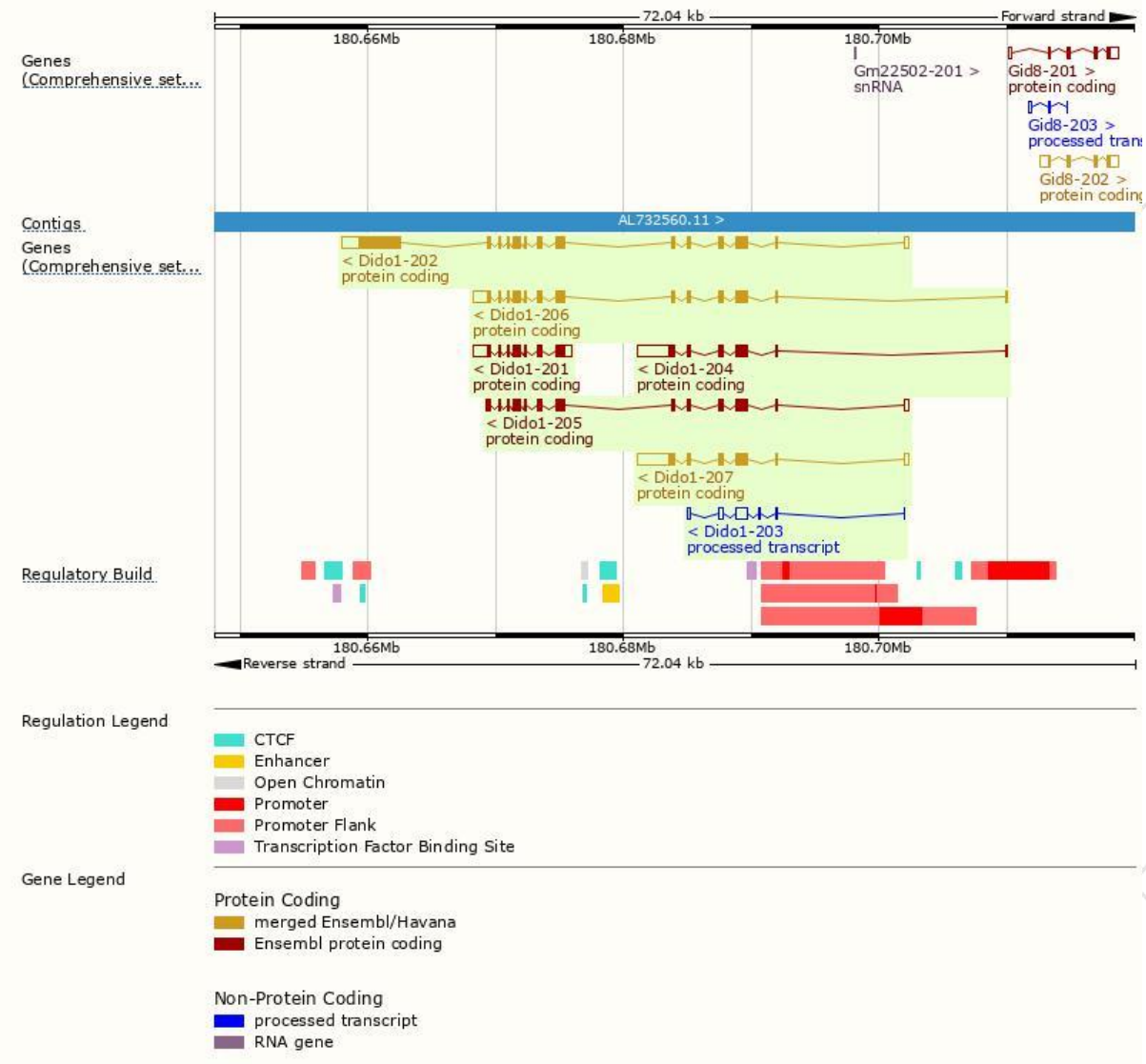
Protein domain
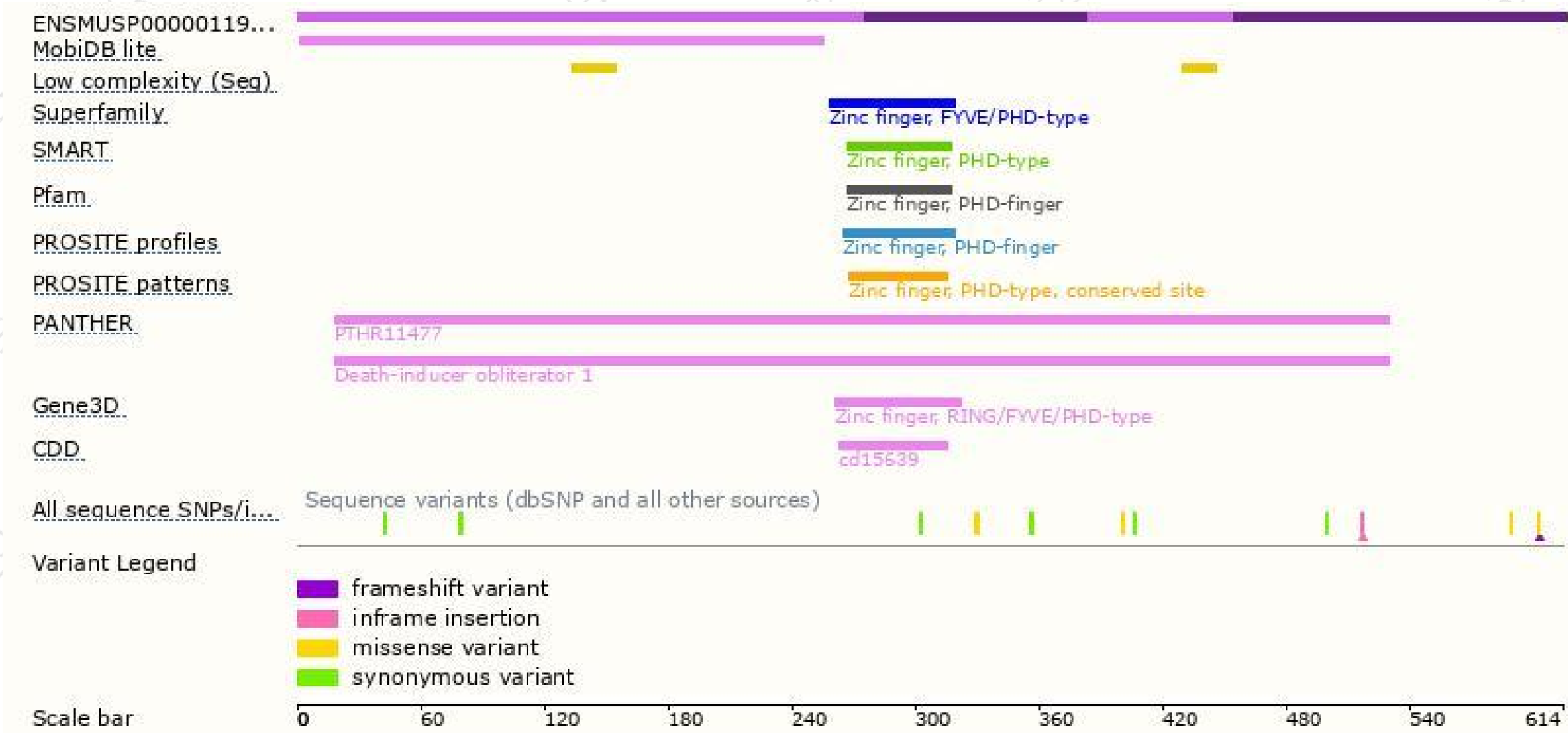
Mouse phenotype description(MGI)

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,mice homozygous for a knock-out allele exhibit severely reduced fertility; about one-half develop a transplantable disease characterized by anomalies in spleen, bone marrow, and peripheral blood and including anemia and various symptoms typical of myeloid dysplasia or myeloid proliferation.

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