Cradd Cas9-CKO Strategy

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Project Overview

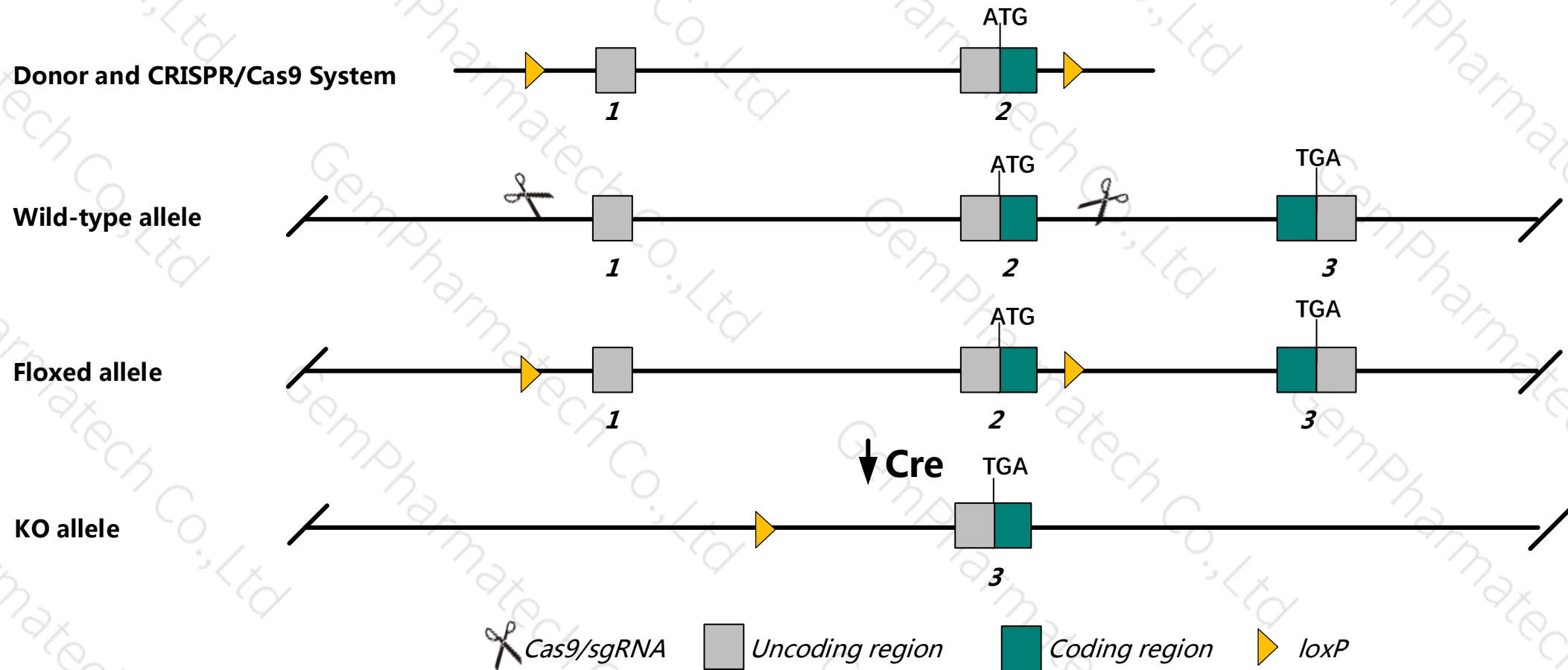|	
Project Name	*Cradd*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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

Donor and CRISPR/Cas9 System

Wild-type allele

Floxed allele

KO allele

↓ **Cre**

Cas9/sgRNA   Uncoding region   Coding region   loxP

Technical routes

➢ The *Cradd* gene has 6 transcripts. According to the structure of *Cradd* gene, exon1-exon2 of *Cradd-201* (ENSMUST00000053594.6) transcript is recommended as the knockout region.The region contains start code ATG of the coding sequence.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Cradd* 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/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 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,Homozygous mutants exhibit embryonic lethality.

➢ The KO region contains *Gm48882* and gene.Knockout the region may affect the function of *Gm48882* gene.

➢ The *Cradd* gene is located on the Chr10. 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.
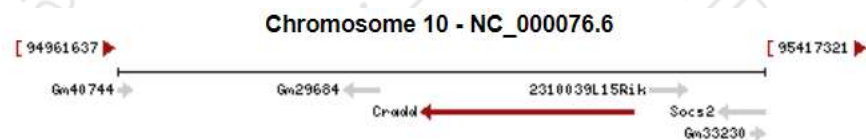
Gene information （NCBI）

Cradd   CASP2 and RIPK1 domain containing adaptor with death domain [*Mus musculus* (house mouse)]

Gene ID: 12905, updated on 12-Aug-2019

Summary

Official Symbol	Cradd provided by MGI
Official Full Name	CASP2 and RIPK1 domain containing adaptor with death domain provided by MGI
Primary source	MGI:MGI:1336168
See related	Ensembl:ENSMUSG00000045867
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	RAIDD
Expression	Ubiquitous expression in ovary adult (RPKM 6.7), adrenal adult (RPKM 6.2) and 28 other tissues See more
Orthologs	human all

Chromosome 10 - NC_000076.6

[94961637 ▶   95417321 ▶]

Gm40744   Gm29684   2310039L15Rik
                  Cradd ◀   Socs2
   Gm33230

Transcript information（Ensembl）

The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags		
Cradd-201	ENSMUST00000053594.6	1661	199aa	ENSMUSP00000050295.5	Protein coding	CCDS24135	O88843 Q549T4	TSL:1	GENCODE basic	APPRIS P1
Cradd-205	ENSMUST00000220279.1	1613	199aa	ENSMUSP00000152022.1	Protein coding	CCDS24135	O88843 Q549T4	TSL:1	GENCODE basic	APPRIS P1
Cradd-202	ENSMUST00000217809.1	1115	199aa	ENSMUSP00000151735.1	Protein coding	CCDS24135	O88843 Q549T4	TSL:1	GENCODE basic	APPRIS P1
Cradd-204	ENSMUST00000218761.1	2663	No protein	-	lncRNA	-	-	TSL:1		
Cradd-206	ENSMUST00000220446.1	717	No protein	-	lncRNA	-	-	TSL:1		
Cradd-203	ENSMUST00000218651.1	679	No protein	-	lncRNA	-	-	TSL:1		

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

< Cradd-201
protein coding

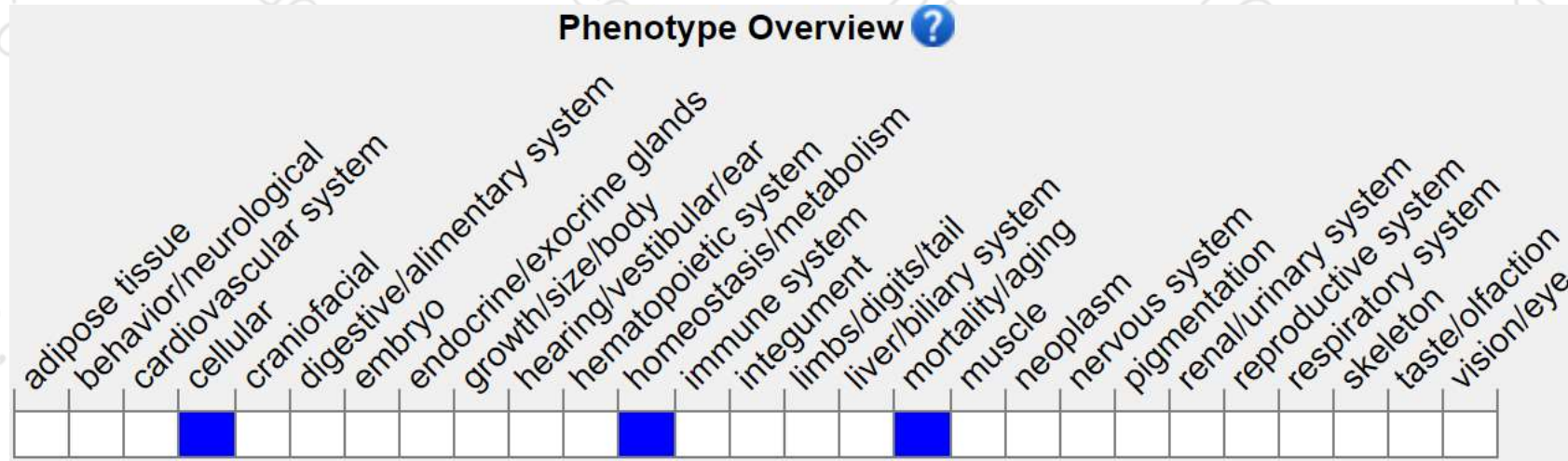◄ Reverse strand —————————— 149.36 kb ——————————
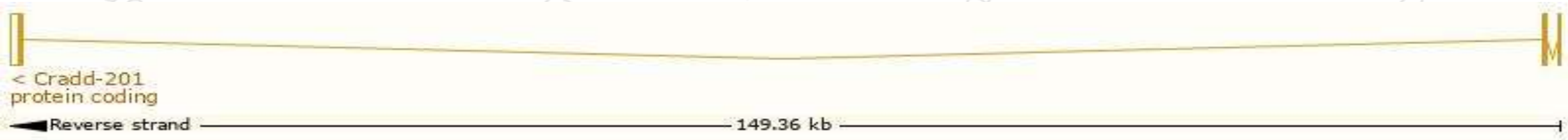
Genomic location distribution
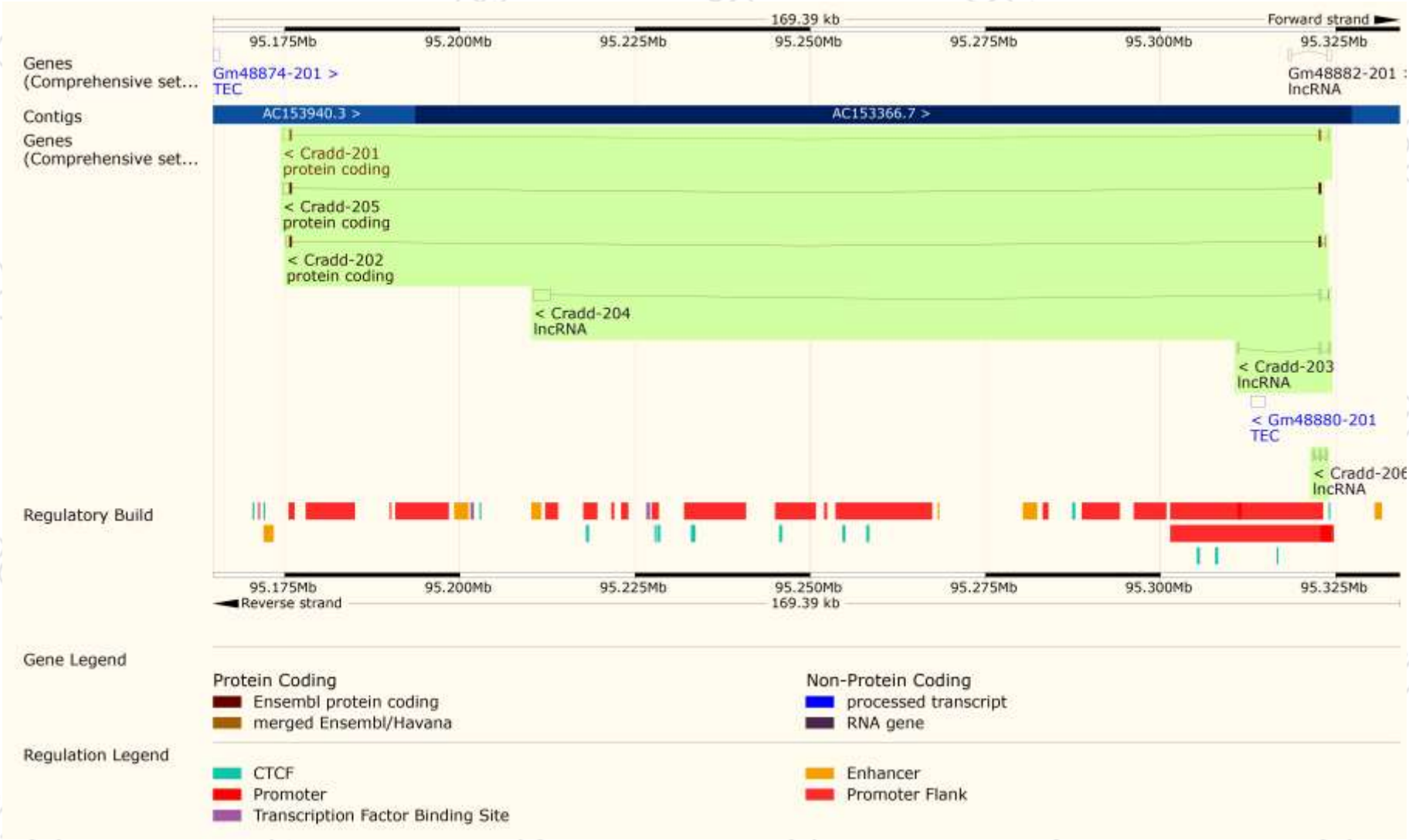
Protein domain
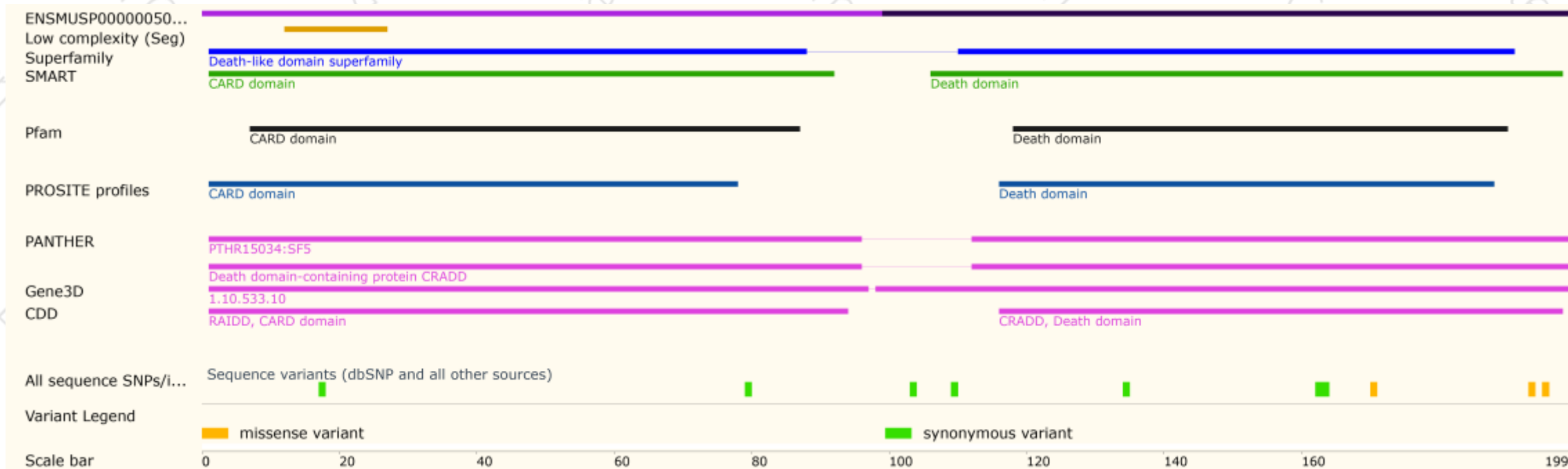
Mouse phenotype description(MGI)

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,Homozygous mutants exhibit embryonic lethality.

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