Pde4d Cas9-CKO Strategy

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Project Overview

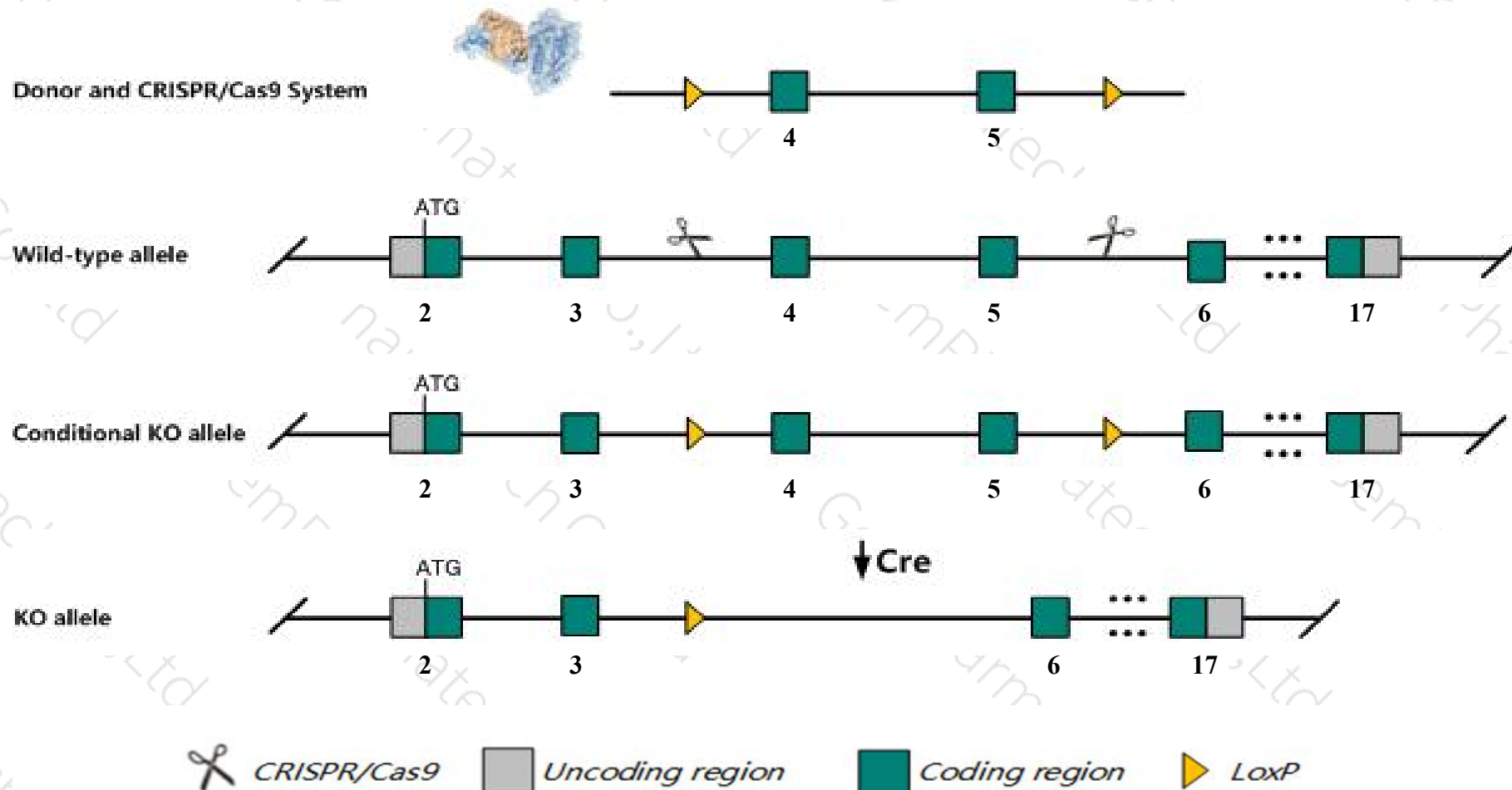|	
Project Name	*Pde4d*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pde4d* gene. The schematic diagram is as follows:

Technical routes

➤ The *Pde4d* gene has 20 transcripts. According to the structure of *Pde4d* gene, exon4-exon5 of *Pde4d-209* (ENSMUST00000122041.7) transcript is recommended as the knockout region.The region contains Ens 229bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Pde4d* 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,homozygotes for targeted null mutations exhibit delayed growth, female infertility associated with impaired ovulation, and reduced postnatal viability.

➢ Transcript *Pde4d*-206&208&210&211&215 will be destroyed.

➢ The effect on transcript *Pde4d*-203&213&218&219 is unknown.

➢ The *Pde4d* gene is located on the Chr13. 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）

Pde4d phosphodiesterase 4D, cAMP specific [Mus musculus (house mouse)]

Gene ID: 238871, updated on 13-Mar-2020

Summary

|	
Official Symbol	Pde4d provided by MGI
Official Full Name	phosphodiesterase 4D, cAMP specific provided by MGI
Primary source	MGI:MGI:99555
See related	Ensembl:ENSMUSG00000021699
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	9630011N22Rik, Dpde3
Expression	Ubiquitous expression in frontal lobe adult (RPKM 2.7), thymus adult (RPKM 2.4) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 20 transcripts,all transcripts are shown below:

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pde4d-209	ENSMUST00000122041.7	2554	747aa	Protein coding	CCDS49359	A2RSH1 Q01063	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
Pde4d-220	ENSMUST00000177907.7	2244	747aa	Protein coding	CCDS49359	A2RSH1 Q01063	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
Pde4d-207	ENSMUST00000120664.7	4207	584aa	Protein coding	-	A6XDA6 Q01063	TSL:1 GENCODE basic
Pde4d-208	ENSMUST00000120671.7	2455	803aa	Protein coding	-	E9PVU7	TSL:5 GENCODE basic
Pde4d-218	ENSMUST00000153234.7	2284	754aa	Protein coding	-	F6QFD1	CDS 5' incomplete TSL:5
Pde4d-202	ENSMUST00000079975.10	2280	698aa	Protein coding	-	B1PSD9	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 ALT1
Pde4d-201	ENSMUST00000074103.11	2167	678aa	Protein coding	-	Q01063	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 ALT1
Pde4d-205	ENSMUST00000119507.7	2163	703aa	Protein coding	-	B2KF84	TSL:5 GENCODE basic
Pde4d-213	ENSMUST00000135275.7	2123	700aa	Protein coding	-	B2KF57	CDS 5' incomplete TSL:5
Pde4d-203	ENSMUST00000117420.7	1955	517aa	Protein coding	-	A6XDB0	TSL:1 GENCODE basic
Pde4d-204	ENSMUST00000117879.7	1724	504aa	Protein coding	-	A6XDB1	TSL:1 GENCODE basic
Pde4d-219	ENSMUST00000155459.1	714	207aa	Protein coding	-	F6YIS2	CDS 5' incomplete TSL:3
Pde4d-206	ENSMUST00000119672.7	2051	48aa	Nonsense mediated decay	-	F7BK43	TSL:1
Pde4d-210	ENSMUST00000129374.1	2397	No protein	Processed transcript	-	-	TSL:1
Pde4d-211	ENSMUST00000133929.7	1530	No protein	Processed transcript	-	-	TSL:1
Pde4d-217	ENSMUST00000152630.1	524	No protein	Processed transcript	-	-	TSL:2
Pde4d-215	ENSMUST00000151111.1	481	No protein	Processed transcript	-	-	TSL:3
Pde4d-214	ENSMUST00000138938.7	1864	No protein	Retained intron	-	-	TSL:1
Pde4d-212	ENSMUST00000134973.7	1156	No protein	Retained intron	-	-	TSL:1
Pde4d-216	ENSMUST00000151429.1	757	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Pde4d-209* transcript,the transcription is shown below:
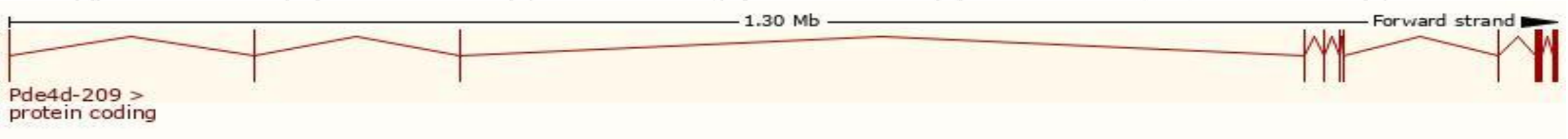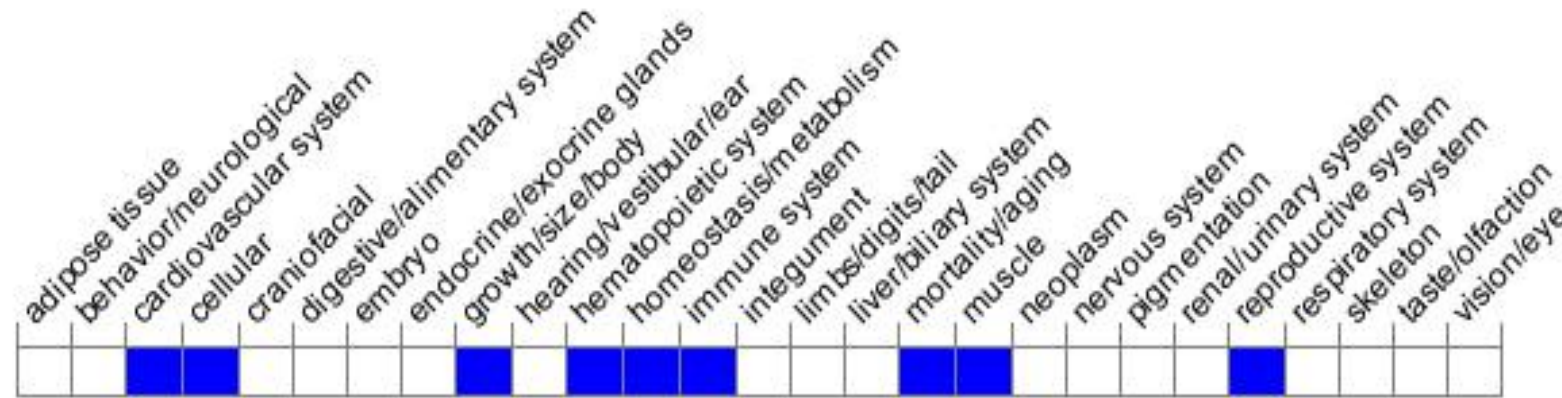
Genomic location distribution
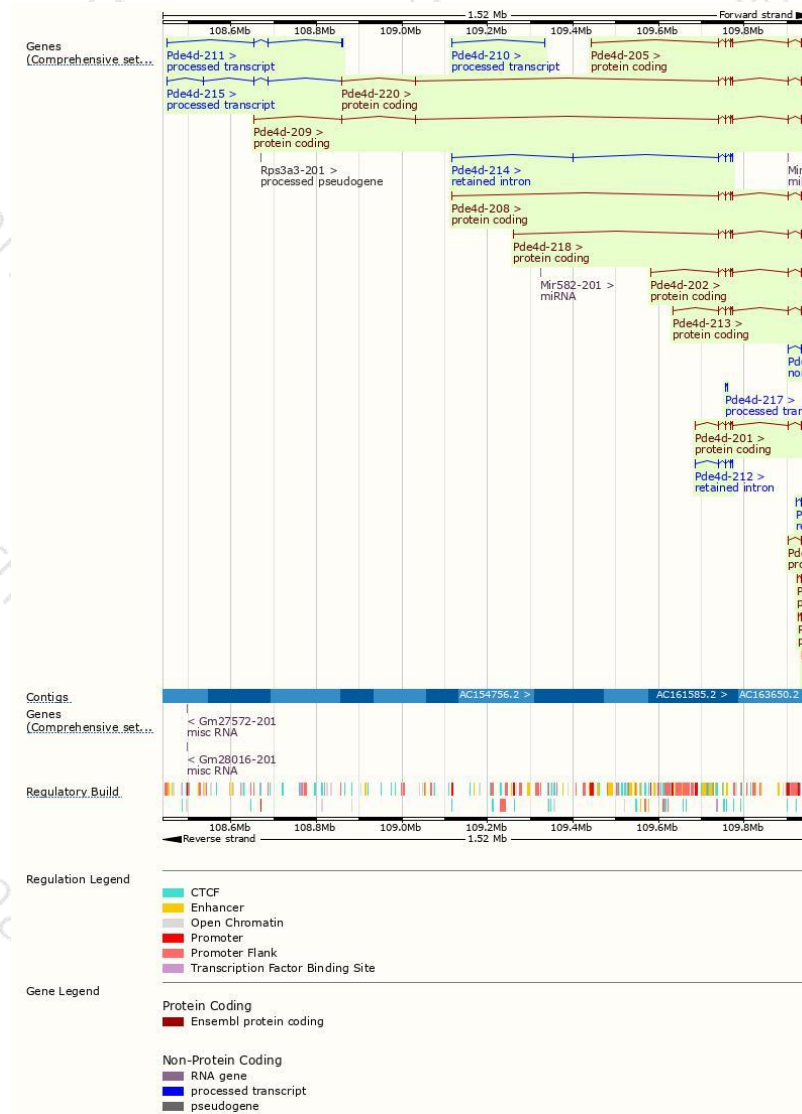
Protein domain
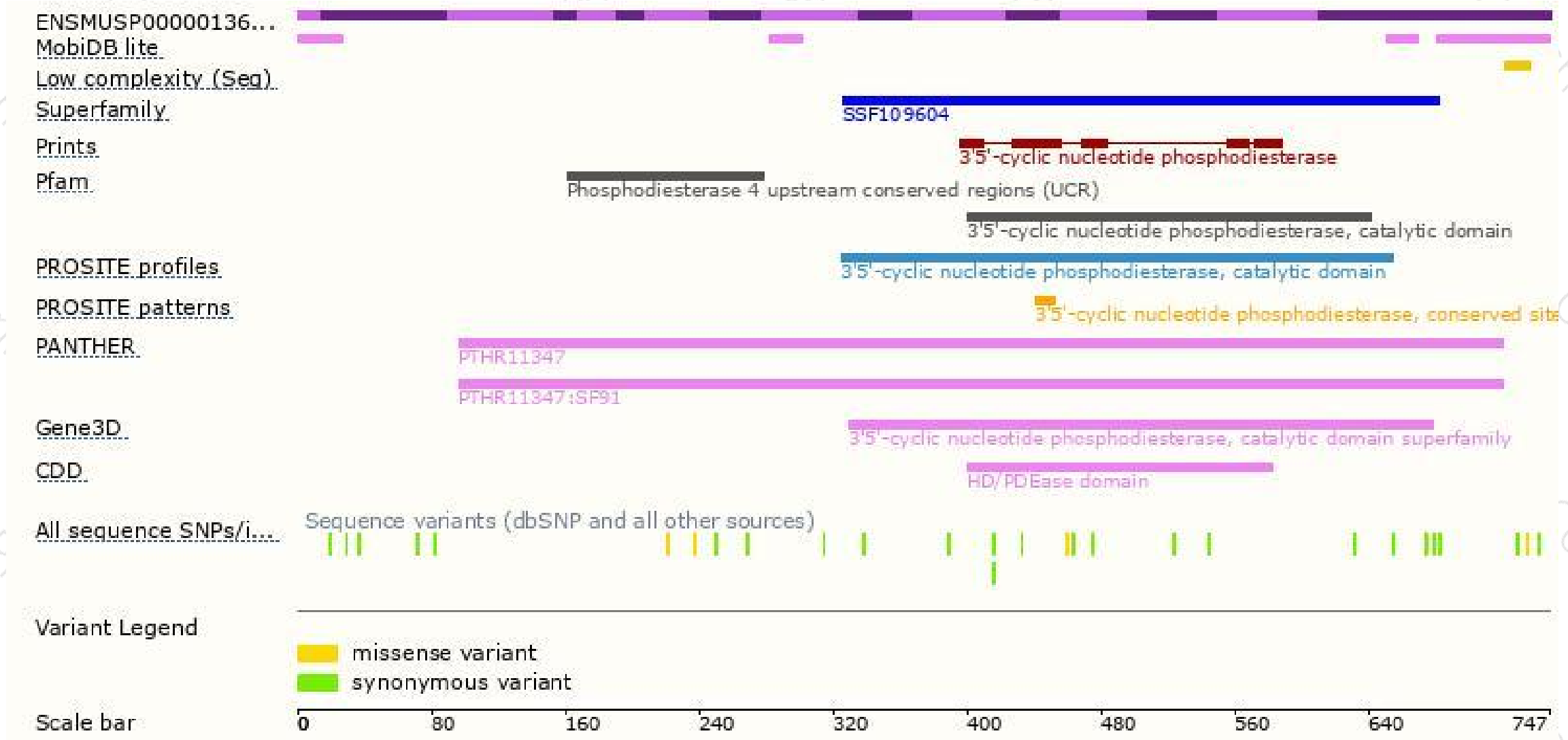
Mouse phenotype description(MGI)

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 targeted null mutations exhibit delayed growth, female infertility associated with impaired ovulation, and reduced postnatal viability.

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