

Pold3 Cas9-KO Strategy

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Project Overview

Project Name

Pold3

Project type

Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pold3* gene. The schematic diagram is as follows:



- The *Pold3* gene has 10 transcripts. According to the structure of *Pold3* gene, exon4-exon7 of *Pold3-201* (ENSMUST00000032969.13) transcript is recommended as the knockout region. The region contains 508bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Pold3* 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.

- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit embryonic lethality between E4.5 and E7.5 due to failure of proliferation of the inner cell mass and trophoblast giant cells. Mice heterozygous for this allele exhibit age-related decreased male germ cells due to impaired meiosis, shortened telomeres and chromosomal instability.
- The *Pold3* gene is located on the Chr7. 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)

Pold3 polymerase (DNA-directed), delta 3, accessory subunit [Mus musculus (house mouse)]

Gene ID: 67967, updated on 10-Feb-2019

Summary



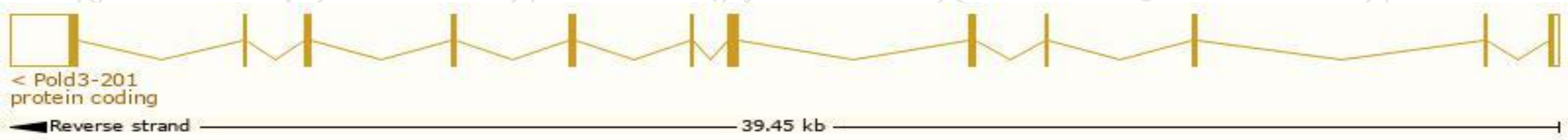
Official Symbol	Pold3 provided by MGI
Official Full Name	polymerase (DNA-directed), delta 3, accessory subunit provided by MGI
Primary source	MGI:MGI:1915217
See related	Ensembl:ENSMUSG00000030726
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2410142G14Rik, C85233, P66, P68
Expression	Ubiquitous expression in CNS E11.5 (RPKM 18.4), liver E14 (RPKM 17.3) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

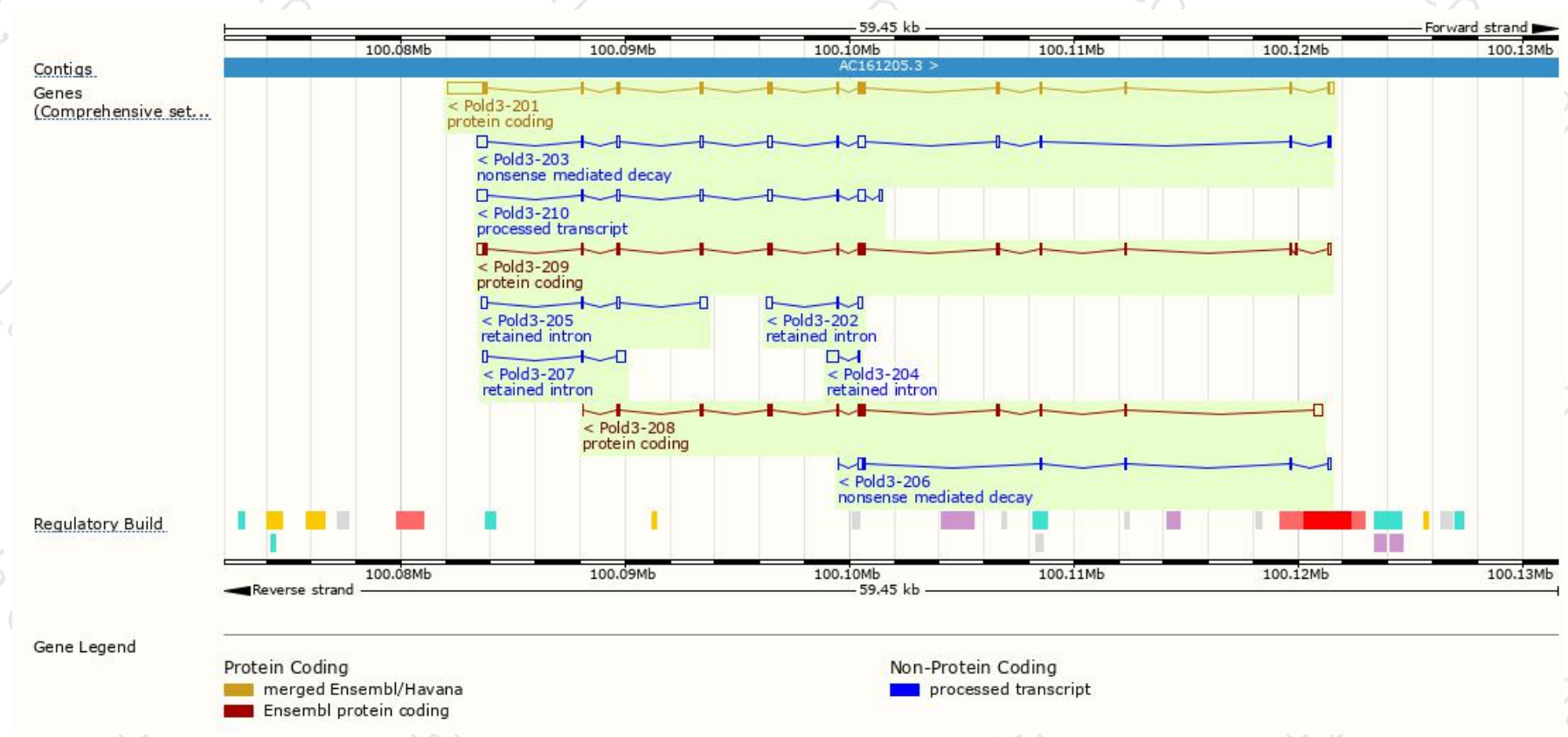
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pold3-201	ENSMUST00000032969.13	3069	461aa	Protein coding	CCDS21493	Q8BH76	TSL:1 GENCODE basic APPRIS P1
Pold3-209	ENSMUST00000208670.1	1716	422aa	Protein coding	-	A0A140LHY7	TSL:5 GENCODE basic
Pold3-208	ENSMUST00000208184.1	1329	269aa	Protein coding	-	A0A140LIW6	CDS 3' incomplete TSL:5
Pold3-203	ENSMUST00000127128.7	1574	44aa	Nonsense mediated decay	-	D6RG54	TSL:5
Pold3-206	ENSMUST00000156202.7	607	86aa	Nonsense mediated decay	-	A0A140LHJ0	TSL:5
Pold3-210	ENSMUST00000208704.1	1341	No protein	Processed transcript	-	-	TSL:5
Pold3-205	ENSMUST00000130413.7	748	No protein	Retained intron	-	-	TSL:1
Pold3-207	ENSMUST00000156518.1	658	No protein	Retained intron	-	-	TSL:2
Pold3-204	ENSMUST00000129556.1	620	No protein	Retained intron	-	-	TSL:3
Pold3-202	ENSMUST00000123675.1	487	No protein	Retained intron	-	-	TSL:2

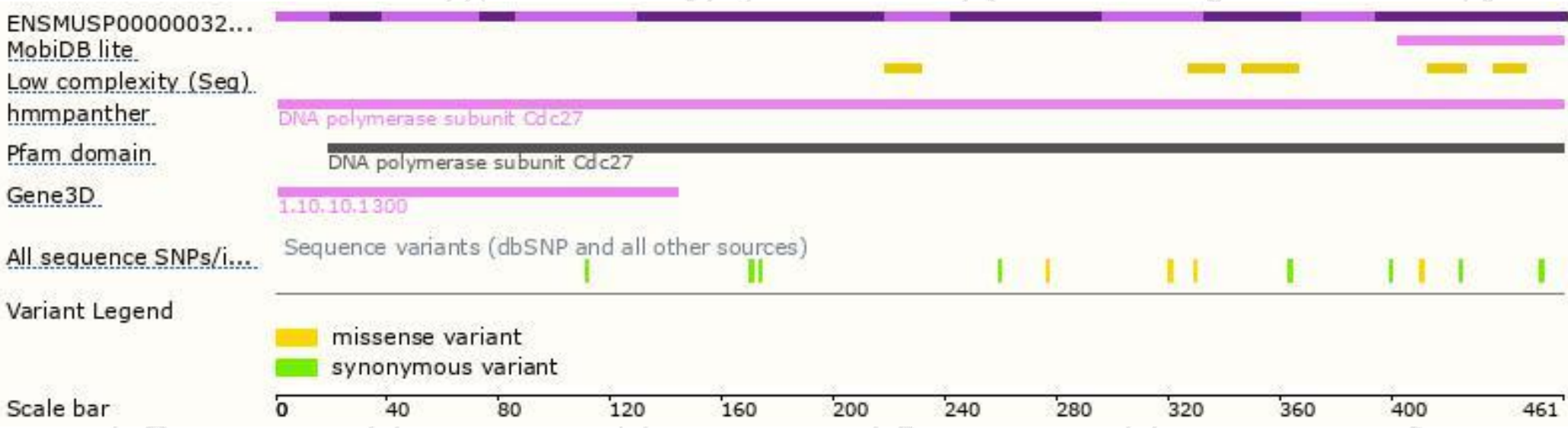
The strategy is based on the design of *Pold3-201* transcript,The transcription is shown below



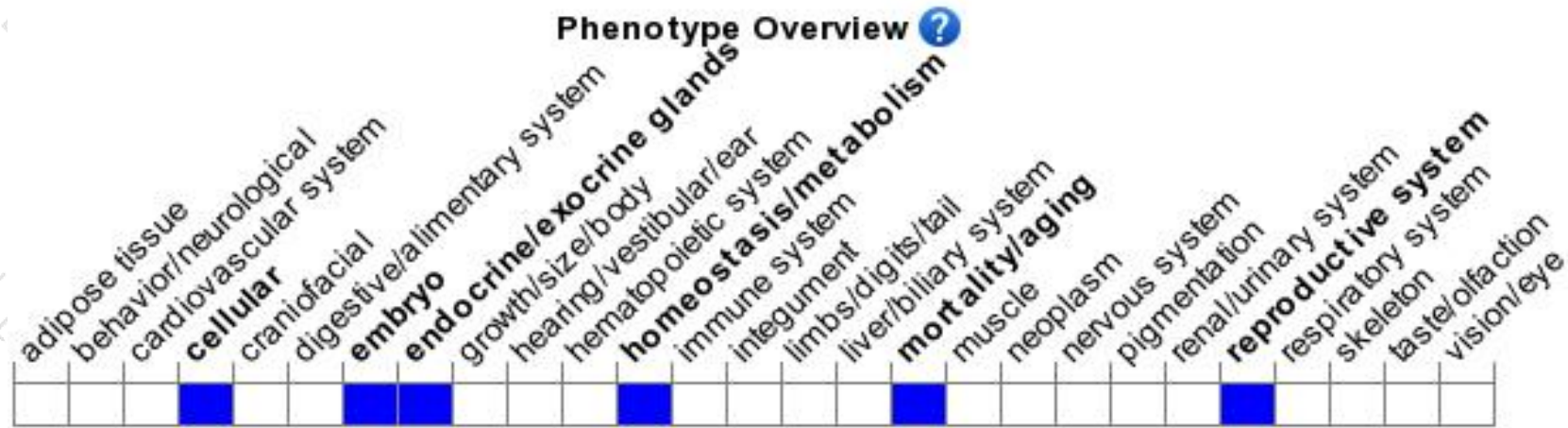
Genomic location distribution



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 between E4.5 and E7.5 due to failure of proliferation of the inner cell mass and trophoblast giant cells. Mice heterozygous for this allele exhibit age-related decreased male germ cells due to impaired meiosis, shortened telomeres and chromosomal instability.

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

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