Ddx3x Cas9-CKO Strategy

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Project Overview

Project Name	***Ddx3x***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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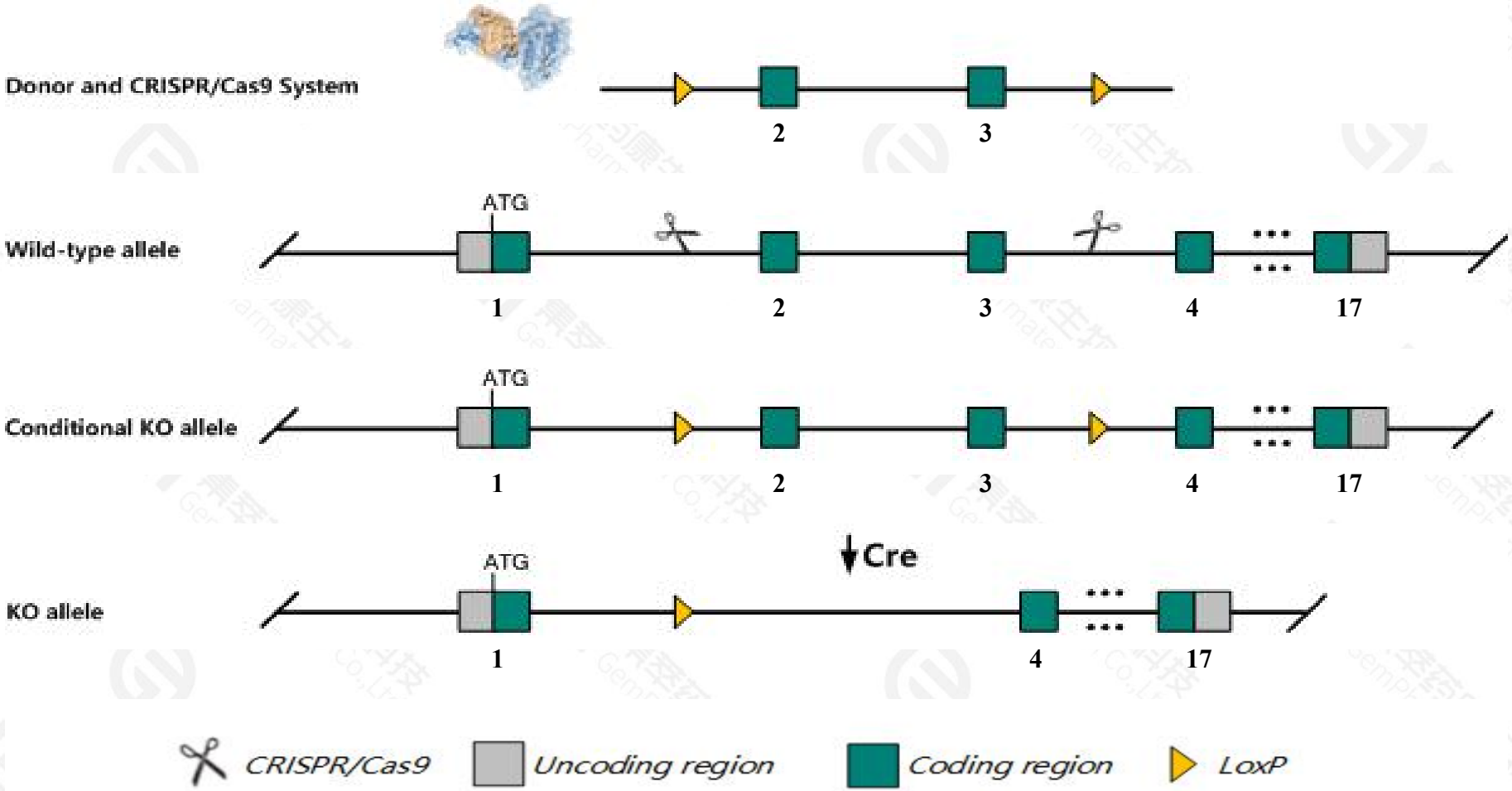
This model will use CRISPR/Cas9 technology to edit the *Ddx3x* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ddx3x* gene has 5 transcripts. According to the structure of *Ddx3x* gene, exon2-exon3 of *Ddx3x-201*(ENSMUST00000000804.7) transcript is recommended as the knockout region.The region contains 106bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ddx3x* 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,hemizygous males and heterozygous females with a maternally inherited null allele show early embryonic and fetal lethality, respectively. Both males and females show defects in trophoblast giant cells. Females show defects in placental formation.

➢ The *Ddx3x* gene is located on the ChrX. 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）

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Ddx3x DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3, X-linked [Mus musculus (house mouse)]

Gene ID: 13205, updated on 13-Mar-2020

Summary

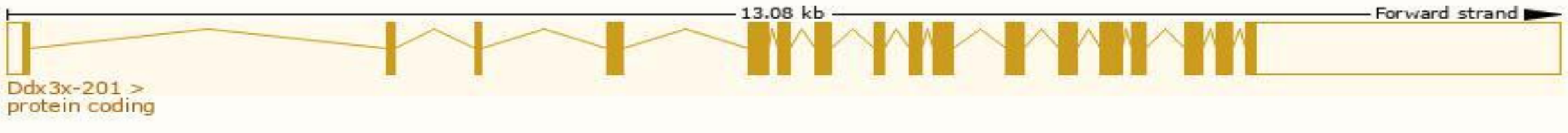
Official Symbol	Ddx3x provided by MGI
Official Full Name	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3, X-linked provided by MGI
Primary source	MGI:MGI:103064
See related	Ensembl:ENSMUSG00000000787
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	D1Pas1-rs2, Ddx3, Fin14
Expression	Broad expression in CNS E11.5 (RPKM 64.5), placenta adult (RPKM 53.4) and 23 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

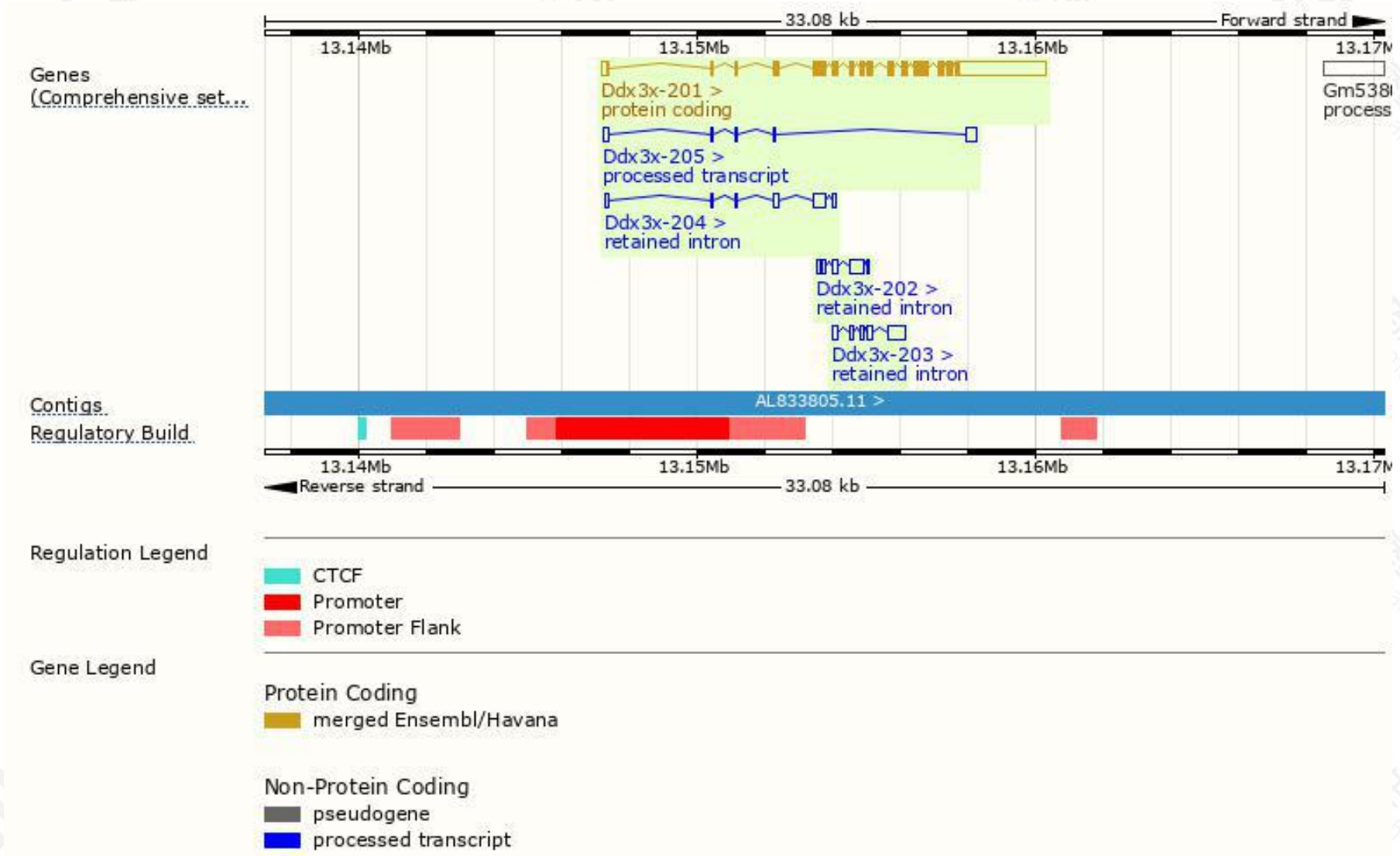
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ddx3x-201	ENSMUST00000000804.6	4692	662aa	Protein coding	CCDS30027	Q3TQX5 Q62167	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 P1
Ddx3x-205	ENSMUST00000153851.7	587	No protein	Processed transcript	-	-	TSL:5
Ddx3x-203	ENSMUST00000149639.1	1003	No protein	Retained intron	-	-	TSL:2
Ddx3x-202	ENSMUST00000123096.7	802	No protein	Retained intron	-	-	TSL:5
Ddx3x-204	ENSMUST00000153611.1	792	No protein	Retained intron	-	-	TSL:5

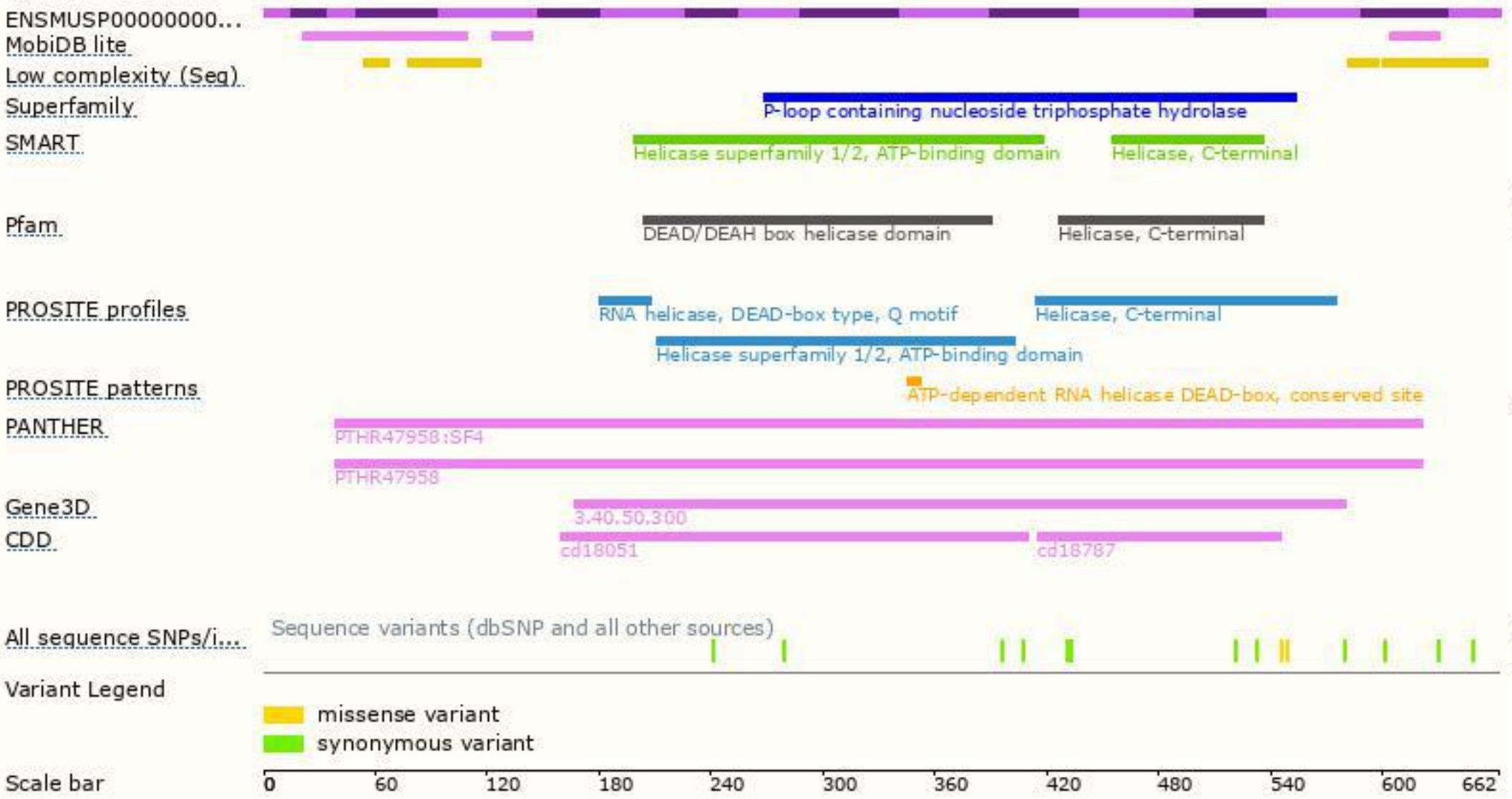
The strategy is based on the design of *Ddx3x-201* transcript,the transcription is shown below:



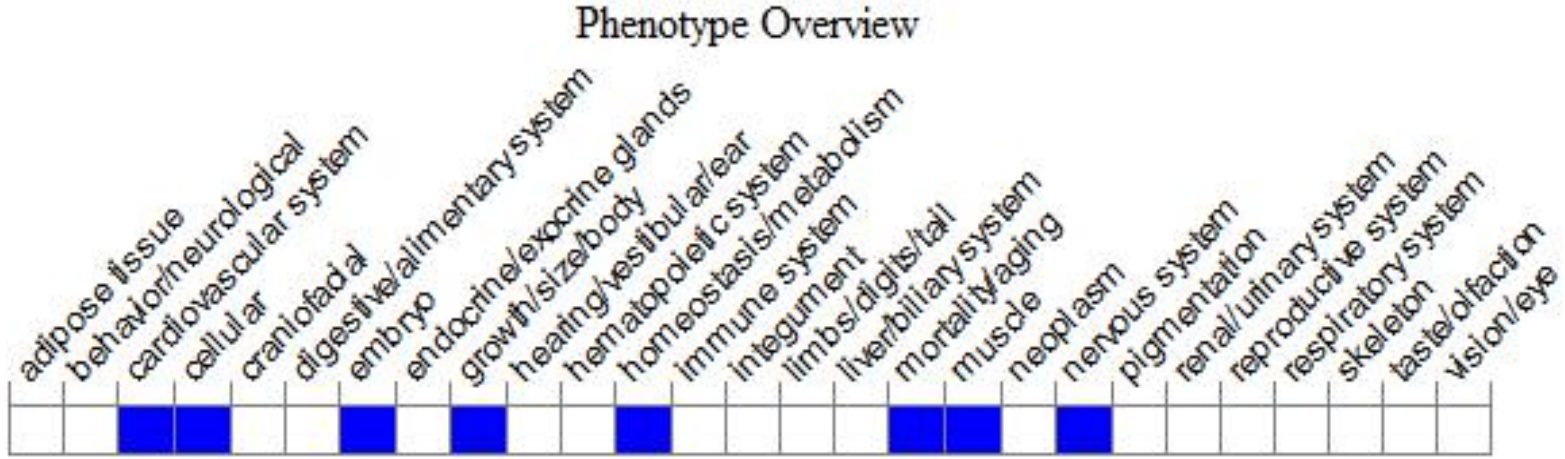
Genomic location distribution



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,hemizygous males and heterozygous females with a maternally inherited null allele show early embryonic and fetal lethality, respectively. Both males and females show defects in trophoblast giant cells. Females show defects in placental formation.

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