Brdt Cas9-CKO Strategy

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Project Overview

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

Conditional Knockout strategy

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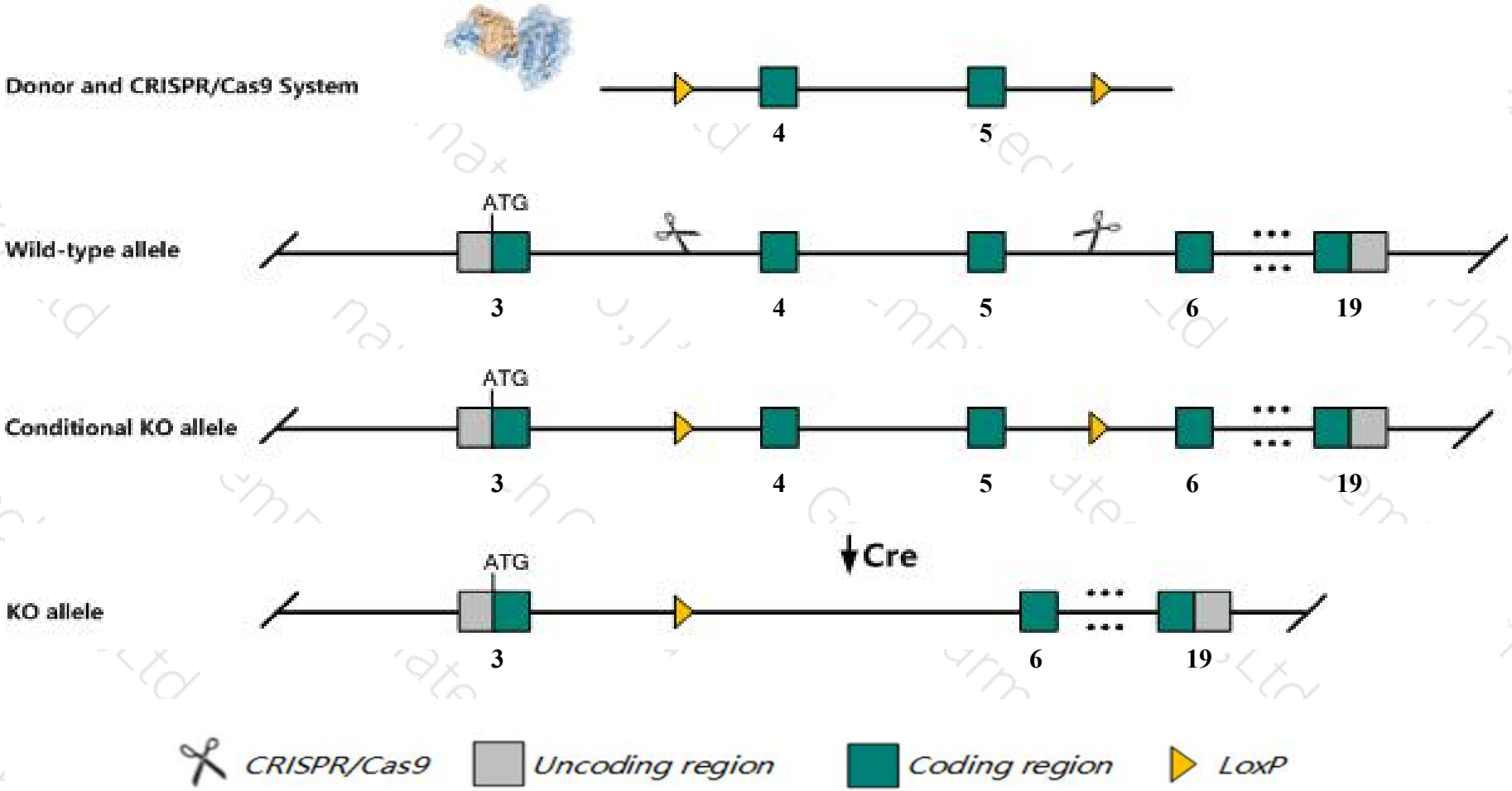
This model will use CRISPR/Cas9 technology to edit the *Brdt* gene. The schematic diagram is as follows:



Technical routes

➢ The *Brdt* gene has 4 transcripts. According to the structure of *Brdt* gene, exon4-exon5 of *Brdt-201*(ENSMUST00000031215.14) transcript is recommended as the knockout region.The region contains 253bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Brdt* 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,homozygous inactivation of this genes leads to arrest of spermatogenesis and male infertility.

➢ The *Brdt* gene is located on the Chr5. 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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Brdt bromodomain, testis-specific [*Mus musculus* (house mouse)]

Gene ID: 114642, updated on 26-Jun-2020

Summary

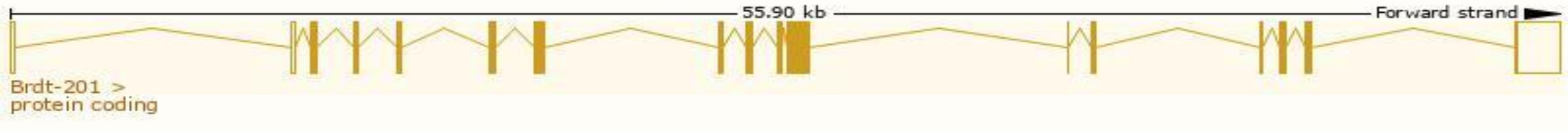
Official Symbol	Brdt provided by MGI
Official Full Name	bromodomain, testis-specific provided by MGI
Primary source	MGI:MGI:1891374
See related	Ensembl:ENSMUSG00000029279
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Brd6; Fsrg3; 7420412D09Rik
Summary	The protein encoded by this gene belongs to the BET protein family. BET proteins have two N-terminal bromodomains and one C-terminal extraterminal domain (ET domain). BET proteins regulate chromatin reorganization via binding to acetylated histones. This gene is thought to play a role in the transcriptional regulation of spermatogenesis. Although referred to as testis-specific bromodomain (Brdt) protein, RT-PCR indicates that this gene is expressed in both mouse oocytes and testes. Alternative splicing results in multiple transcript variants encoding different proteins. [provided by RefSeq, Jul 2008]
Expression	Biased expression in testis adult (RPKM 12.3), cortex adult (RPKM 1.6) and 6 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

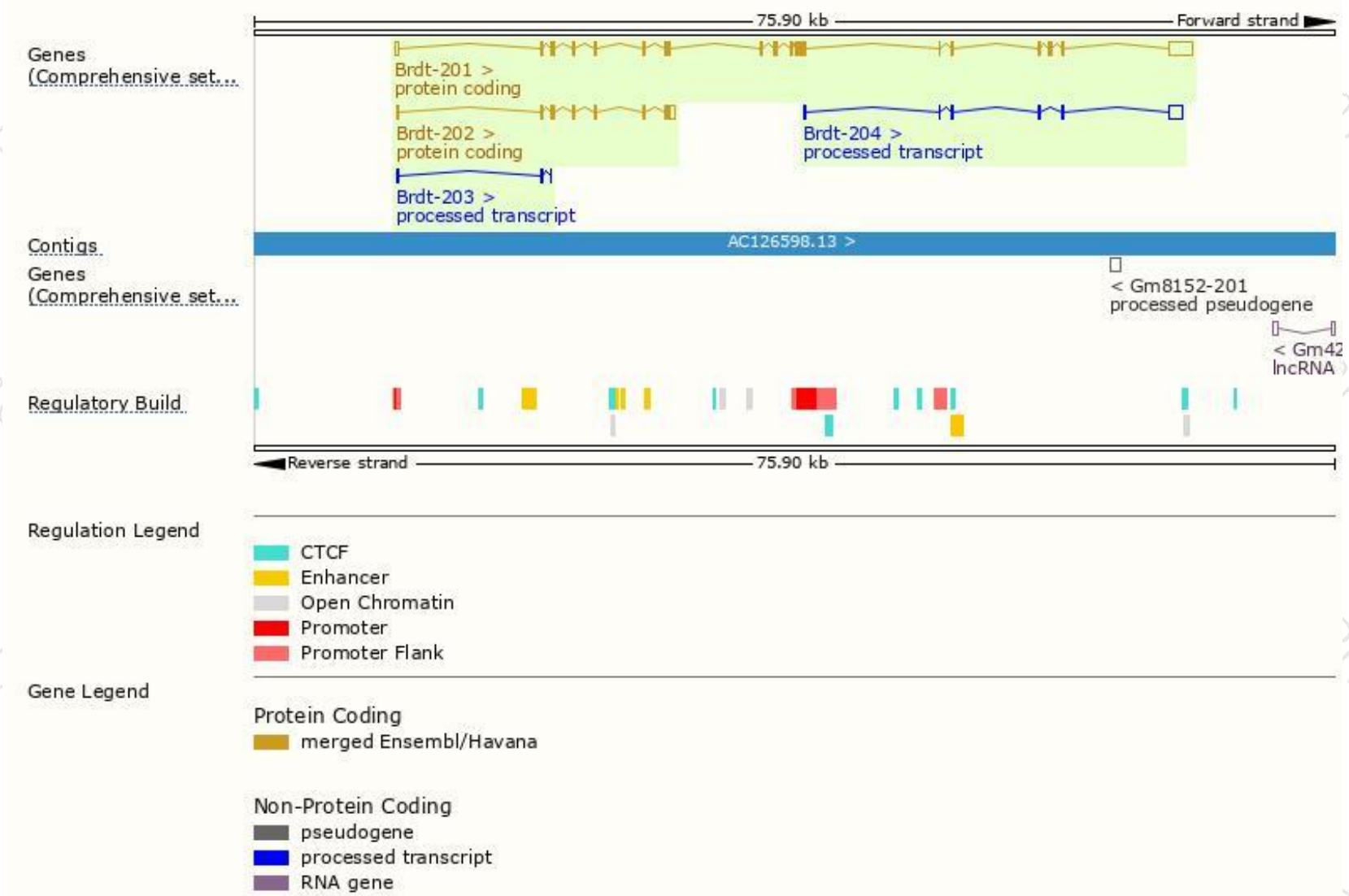
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Brdt-201	ENSMUST00000031215.14	4745	956aa	Protein coding	CCDS19500	Q91Y44	TSL:1 GENCODE basic APPRIS P1
Brdt-202	ENSMUST00000112677.9	1607	326aa	Protein coding	CCDS39197	Q91Y44	TSL:1 GENCODE basic
Brdt-204	ENSMUST00000162804.1	1568	No protein	Processed transcript	-	-	TSL:1
Brdt-203	ENSMUST00000161377.1	369	No protein	Processed transcript	-	-	TSL:3

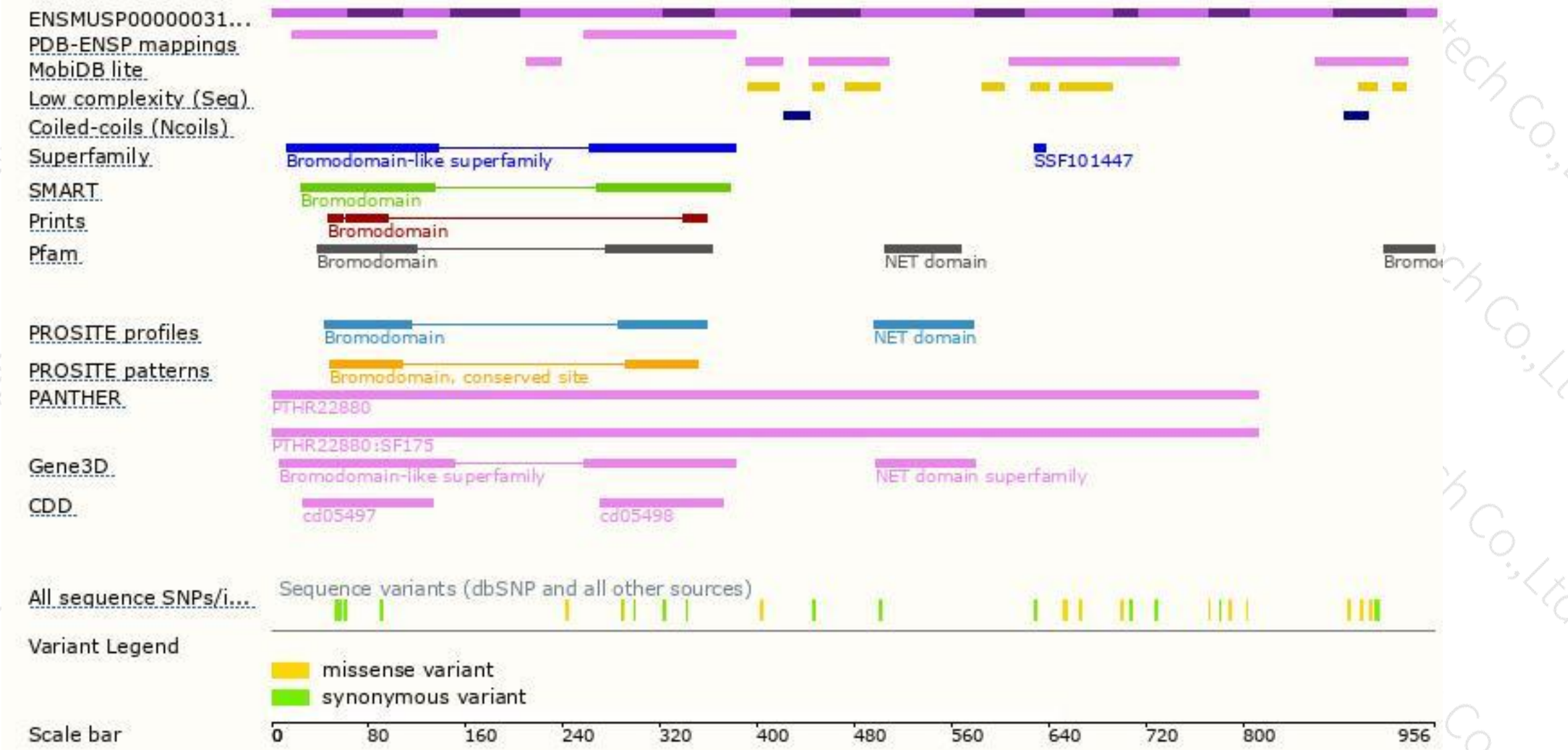
The strategy is based on the design of *Brdt-201* transcript,the transcription is shown below:



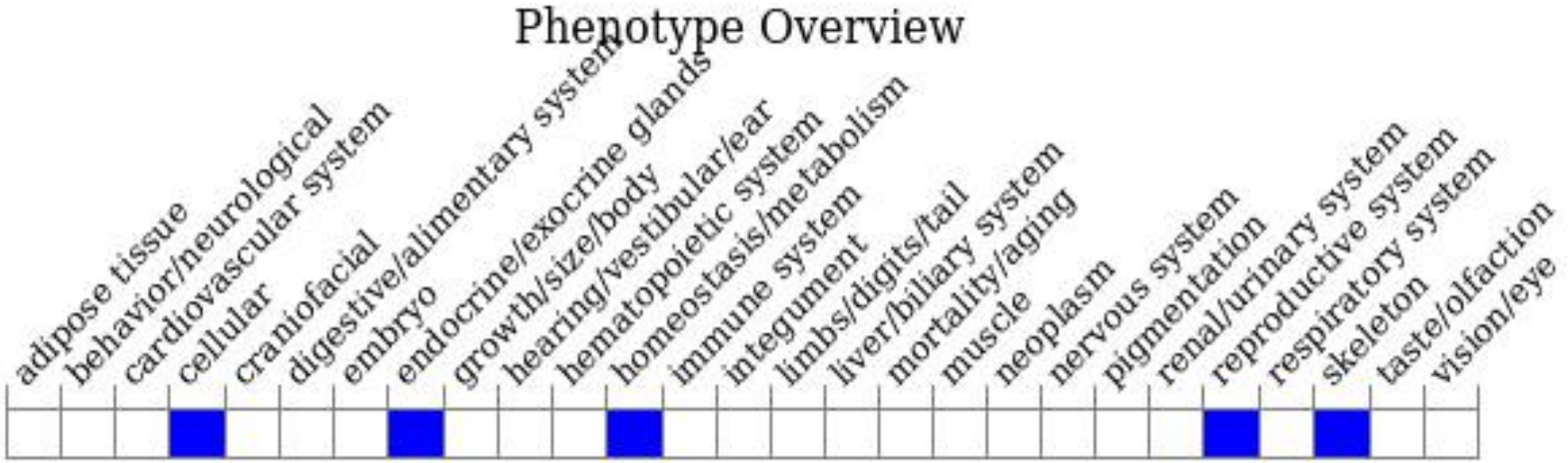
Genomic location distribution



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,homozygous inactivation of this genes leads to arrest of spermatogenesis and male infertility.

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

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