Drd1 Cas9-CKO Strategy

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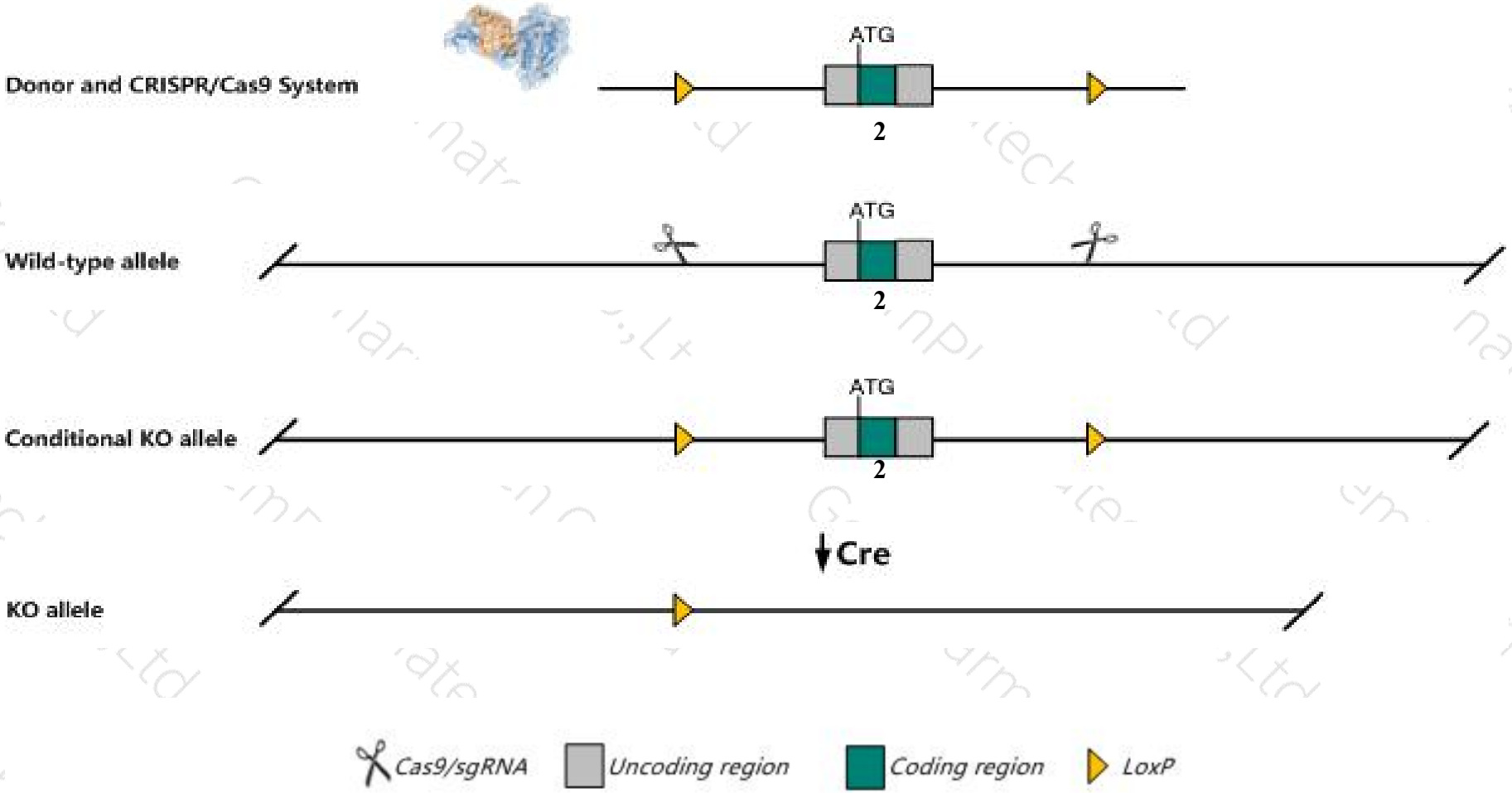
Project Overview

Project Name	***Drd1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Drd1* gene. The schematic diagram is as follows:



Technical routes

- The *Drd1* gene has 3 transcripts. According to the structure of *Drd1* gene, exon2 of *Drd1-201* (ENSMUST00000021932.5) transcript is recommended as the knockout region.The region contains all 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 *Drd1* 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,Homozygotes for targeted mutations show variably abnormalities that may include growth retardation, death after weaning unless given hydrated food, nonresponsiveness to dopamine D1 receptor agonists and antagonists, and normal to hyperactive locomotor activity.

➢ The *Drd1* 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）

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Drd1 dopamine receptor D1 [Mus musculus (house mouse)]

Gene ID: 13488, updated on 9-Apr-2019

Summary

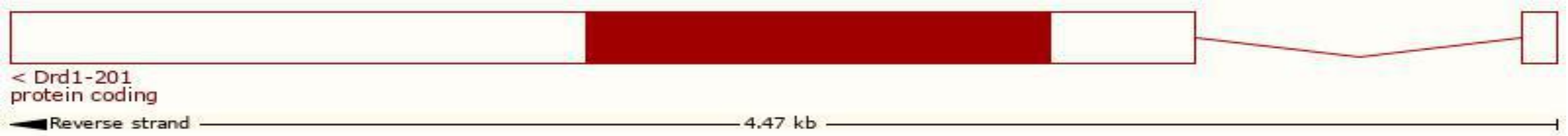
Official Symbol	Drd1 provided by MGI
Official Full Name	dopamine receptor D1 provided by MGI
Primary source	MGI:MGI:99578
See related	Ensembl:ENSMUSG00000021478
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	C030036C15Rik, Drd-1, Drd1a, Gpcr15
Expression	Biased expression in cortex adult (RPKM 2.4), CNS E18 (RPKM 1.8) and 5 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

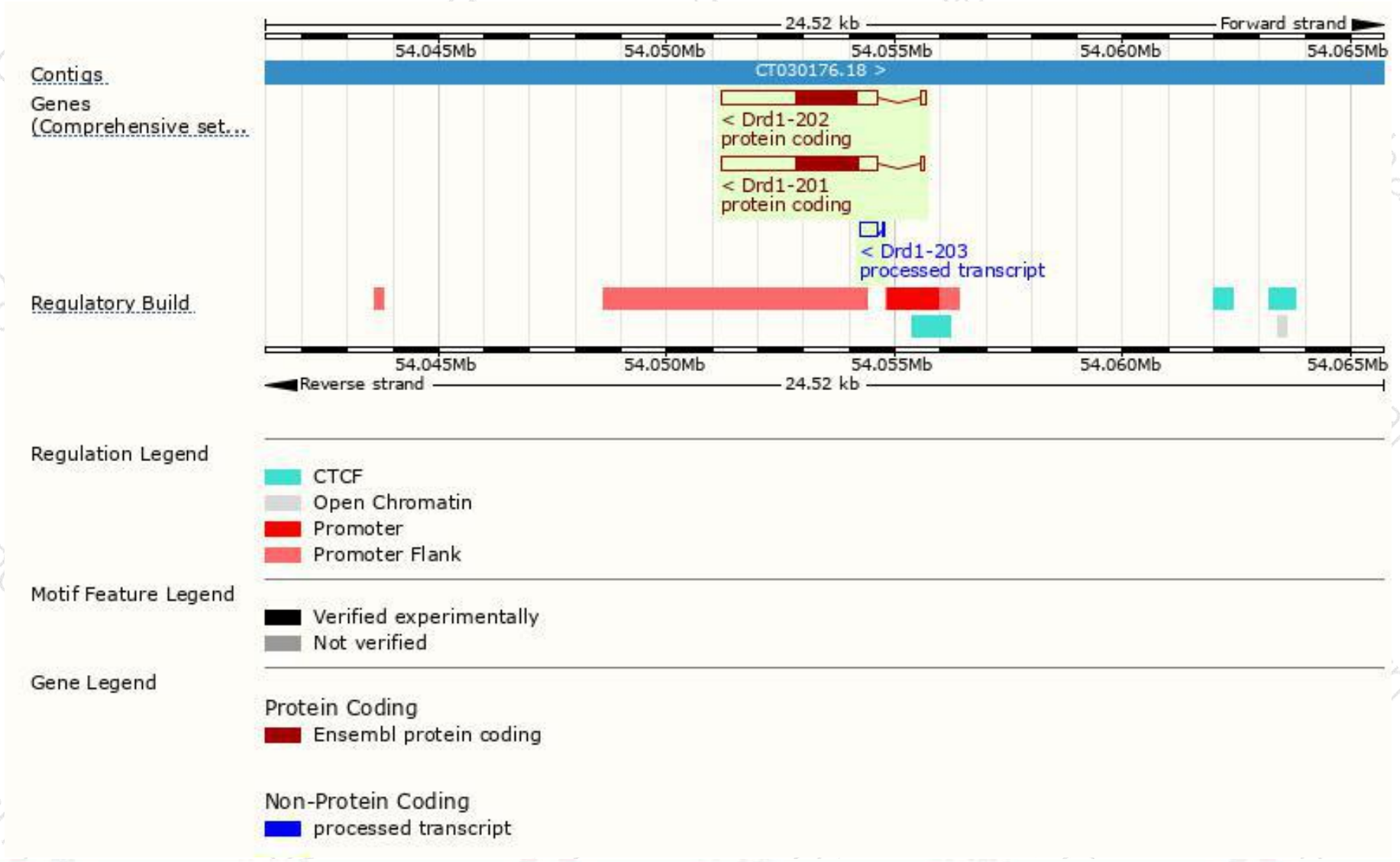
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Drd1-201	ENSMUST00000021932.5	3526	446aa	Protein coding	CCDS26524	Q61616	TSL:1 GENCODE basic APPRIS P2
Drd1-202	ENSMUST00000221470.1	3576	439aa	Protein coding	-	A0A1Y7VK92	TSL:1 GENCODE basic APPRIS ALT2
Drd1-203	ENSMUST00000222706.1	405	No protein	Processed transcript	-	-	TSL:3

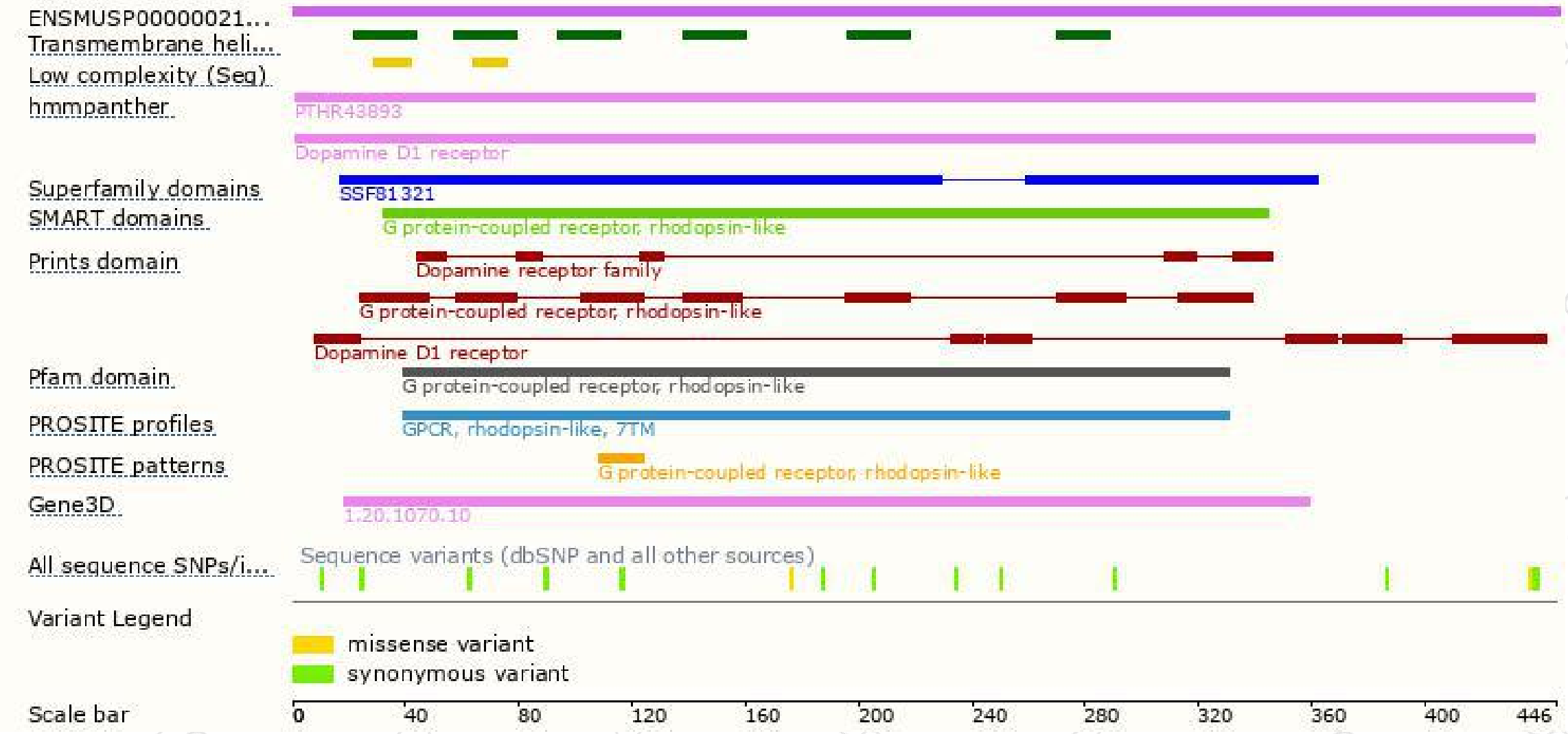
The strategy is based on the design of *Drd1-201* transcript,The transcription is shown below



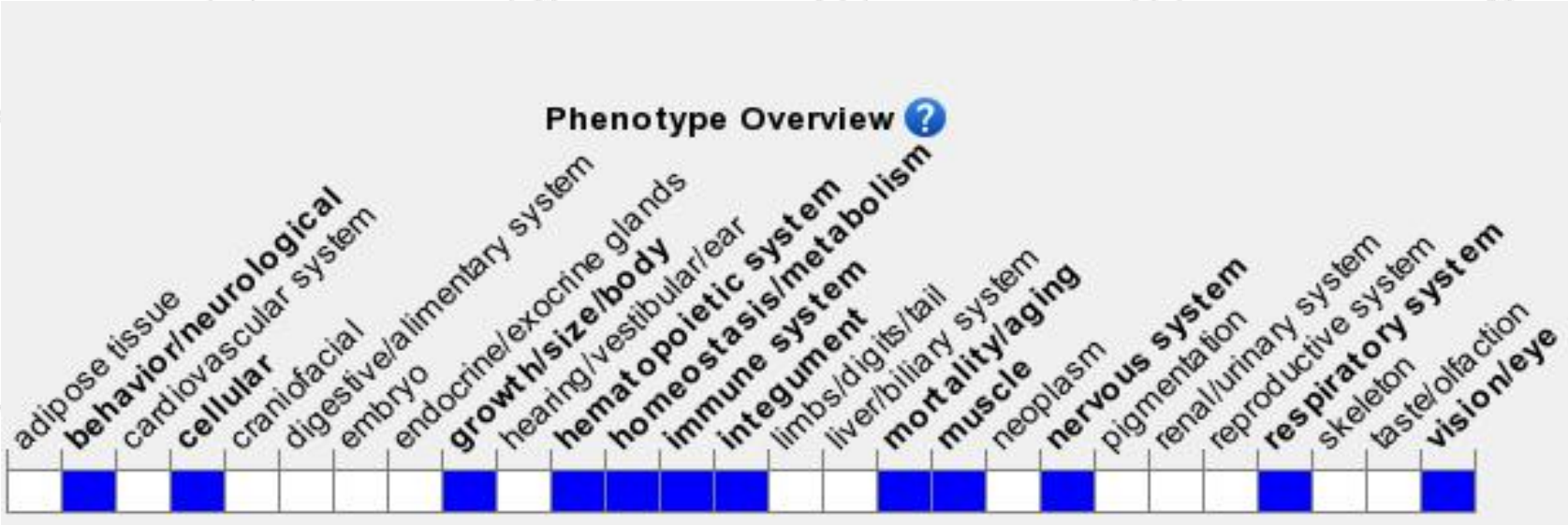
Genomic location distribution



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/).

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

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