Drd1(Drd1a)-P2A-iCre Cas9-KI Strategy

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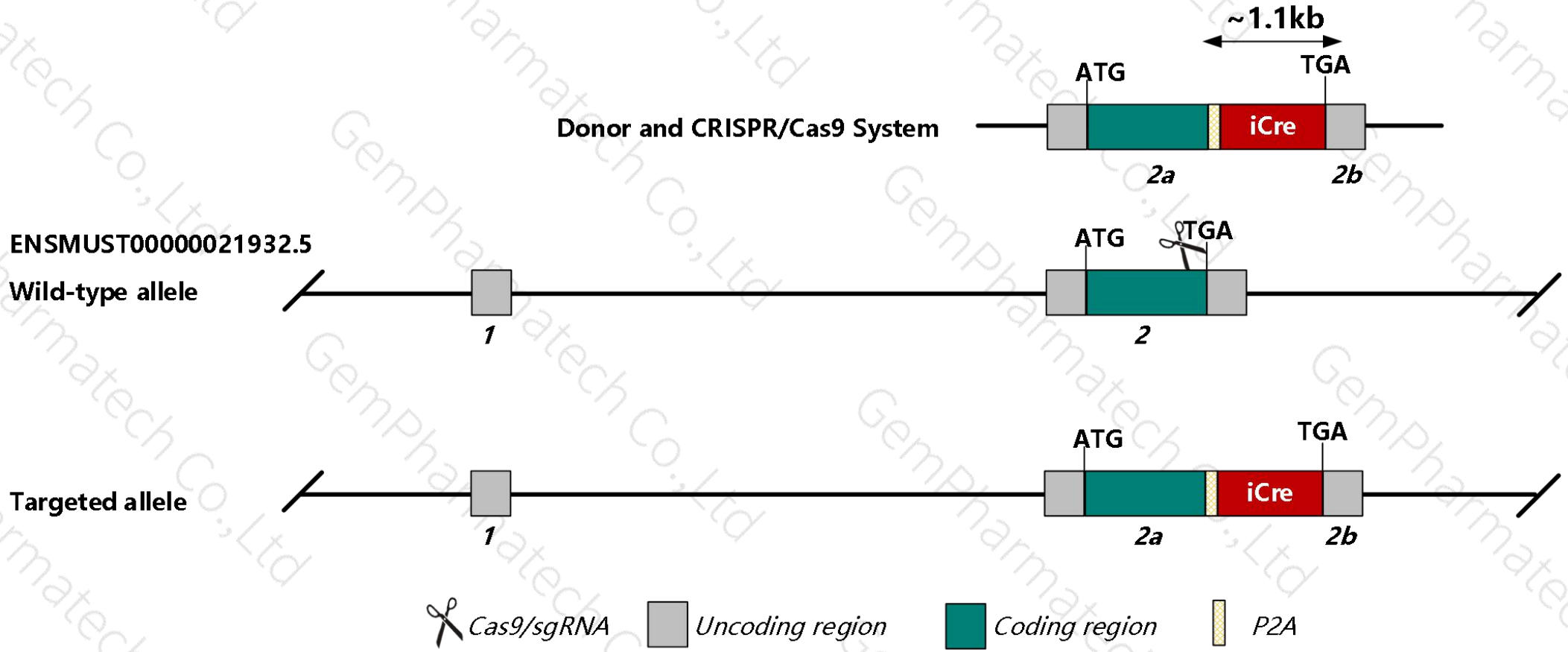
Reviewer:	**Jia Yu**
Design Date:	**2019-08-09**

Project Overview

Project Name	***Drd1(Drd1a)-P2A-iCre***
Project type	**Cas9-KI**
Strain background	**C57BL/6J**

Knockin 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, *Drd1-201*(ENSMUST00000021932.5) is selected for presentation of the recommended strategy.

- *Drd1-201* gene has 2 exons, with the ATG start codon in exon2 and TGA stop codon in exon2.

- We make *Drd1-P2A-iCre* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near stop coding(TGA) of *Drd1* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in *P2A-iCre* before stop coding(TGA) of *Drd1* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

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.
- According to the existing JAX data, Cre is expressed in dopaminoceptive neurons.
- Insertion of iCre may affect the regulation of the 3' end of the *Drd1* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon2 of *Drd1* gene in this strategy.
- The P2A-linked gene drives expression in the same promoter and is cleaved at the translational level. The gene expression levels are consistent, and the before of P2A expressing gene carries the P2A-translated polypeptide.
- The *Drd1* gene is located on the Chr13. If the knockin 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information （NCBI)

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

Gene ID: 13488, updated on 6-Aug-2019

Summary

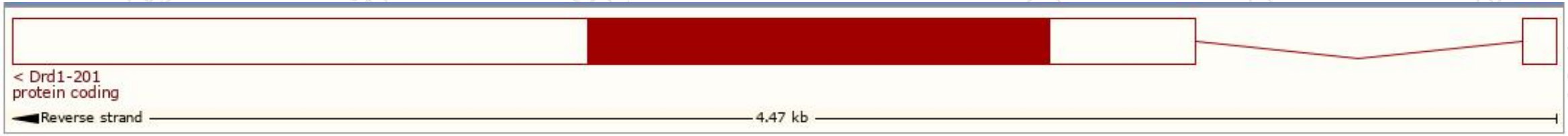
Official Symbol	Drd1 provided by MGI
Official Full Name	dopamine receptor D1 provided by MGI
Primary source	MGI:MGI:99578
See related	Ensembl:ENSMUSG00000021478
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Drd-1; Drd1a; Gpcr15; C030036C15Rik
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）

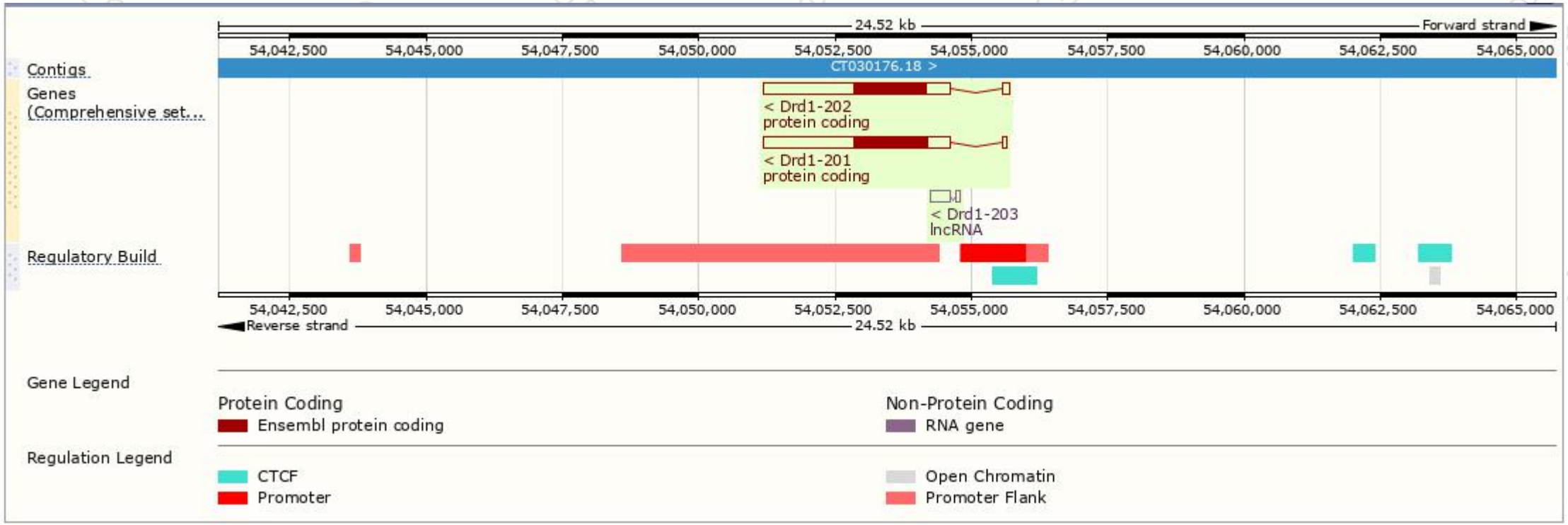
The gene has 3 transcripts, and the transcript is shown below：

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	lncRNA	-	-	TSL:3

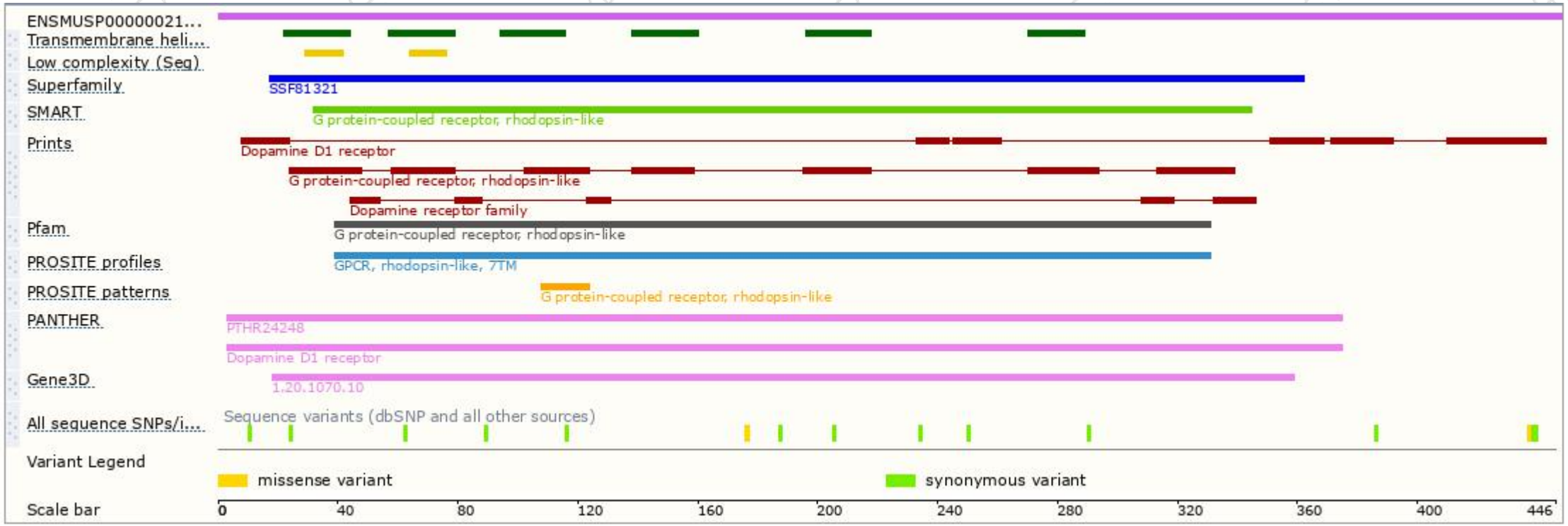
The strategy is based on the design of *Drd1-201* transcript,The transcription is shown below



Genomic location distribution



Protein domain



Targeted Progress (from JAX)

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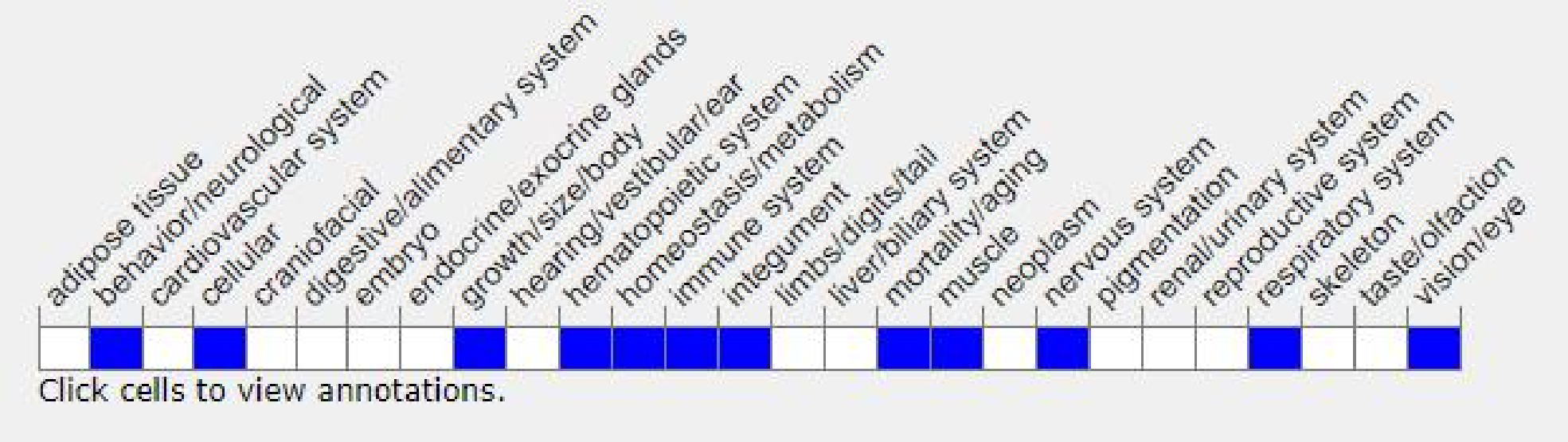
Allele Symbol: Tg(Drd1a-cre)AGsc MGI

Allele Name	transgene insertion A, Gunther Schutz
Allele Type	Transgenic (Recombinase-expressing)
Allele Synonym(s)	D1Cre; Tg(Drd1a-cre)2Gsc; dr-1 Cre
Gene Symbol and Name	Tg(Drd1a-cre)AGsc MGI, transgene insertion A, Gunther Schutz
Gene Synonym(s)	D1Cre; Tg(Drd1a-cre)2Gsc; Tg(Drd1a-cre)2Gsc; dr-1 Cre; transgene insertion 2, Gunter Schutz
Promoter	*Drd1*, dopamine receptor D1, mouse, laboratory
Expressed Gene	*cre*, cre recombinase, bacteriophage P1
Site of Expression	Cre is expressed in dopaminoceptive neurons.
Strain of Origin	FVB/N
Chromosome	UN
General Note	% transgenic mouse lines were create (A, R, S, T and U). Lines T and U do not contain complete transgenes while the other three lines exhibit virtually identical expression patterns. Line A was choosen as the representative line.
Molecular Note	A YAC containing the cre open reading frame with a nuclear localization signal and under the control of a Drd1a promoter was used to create a transgene. Expression was confirmed through crosses with three reporter lines and localized to the major dopaminoceptive regions. Line A contains 3 copies of the transgene.
Mutations Made By	Guenther Schuetz (Gnther Schtz), German Cancer Research Center (DKFZ)

https://www.jax.org/strain/030329

Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/) .

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.

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

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