

***Drd2-P2A-iCre* Cas9-KI Strategy**

Designer:

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Reviewer

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Project Overview

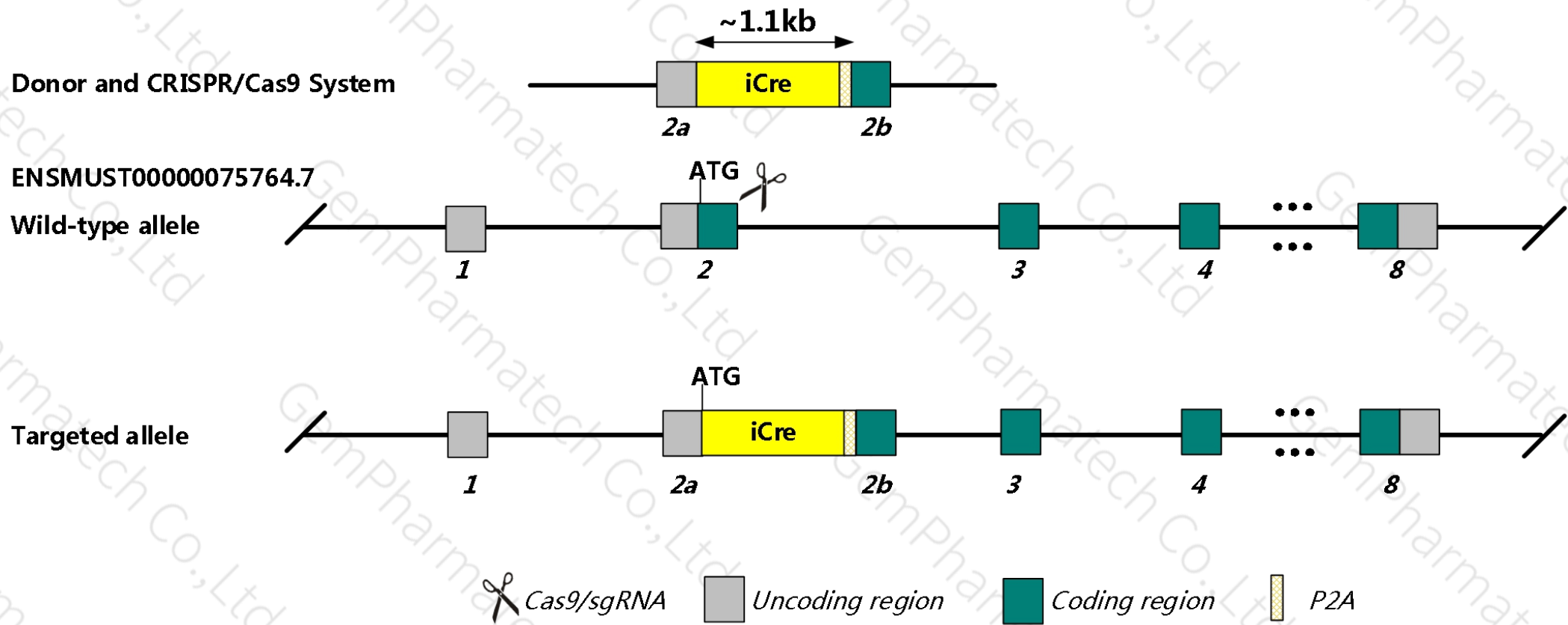
Project Name	<i>Drd2-P2A-iCre</i>
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Project type	Cas9-KI
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Strain background	C57BL/6J
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Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Drd2* gene. The schematic diagram is as follows:



- The *Drd2* gene has 1 transcript. According to the structure of *Drd2* gene, *Drd2-201*(ENSMUST00000075764.7) is selected for presentation of the recommended strategy.
- *Drd2-201* gene has 8 exons, with the ATG start codon in exon2 and TGA stop codon in exon8.
- We make *Drd2-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 start coding(ATG) of *Drd2* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in P2A-iCre after start coding(ATG) of *Drd2* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

- According to the existing MGI data, Homozygous null mice show Parkinson's disease like symptoms, including akinetic and bradykinetic behavior. Mice lacking only the long isoform are hypoactive and exhibit increased stereotypic behavior in response to dopamine agonists.
- According to the existing JAX data(030255 : B6;FVB-Tg(Drd2-EGFP/Rpl10a)CP101Htz/J), EGFP is expressed primarily in Drd2 positive medium spiny neurons.
- 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.
- Insertion of iCre may affect the regulation of the 5' end of the *Drd2* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon2 of *Drd2* gene in this strategy.
- The *Drd2* gene is located on the Chr9. 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)

Drd2 dopamine receptor D2 [*Mus musculus* (house mouse)]

Gene ID: 13489, updated on 13-Aug-2019

Summary

- Official Symbol
- Drd2 provided by [MGI](#)
- Official Full Name
- dopamine receptor D2 provided by [MGI](#)
- Primary source
- [MGI:MGI:94924](#)
- See related
- [Ensembl:ENSMUSG00000032259](#)
- 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
- D2R; Drd-2
- Expression
- Biased expression in CNS E18 (RPKM 3.9), cortex adult (RPKM 2.9) and 8 other tissues [See more](#)
- Orthologs
- [human](#) [all](#)

Genomic context

Location: 9 A5.3; 9 26.72 cM

Exon count: 8

See Drd2 in [Genome Data Viewer](#)

Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	9	NC_000075.6 (49340360..49408177)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	9	NC_000075.5 (49148767..49215319)

Transcript information (Ensembl)

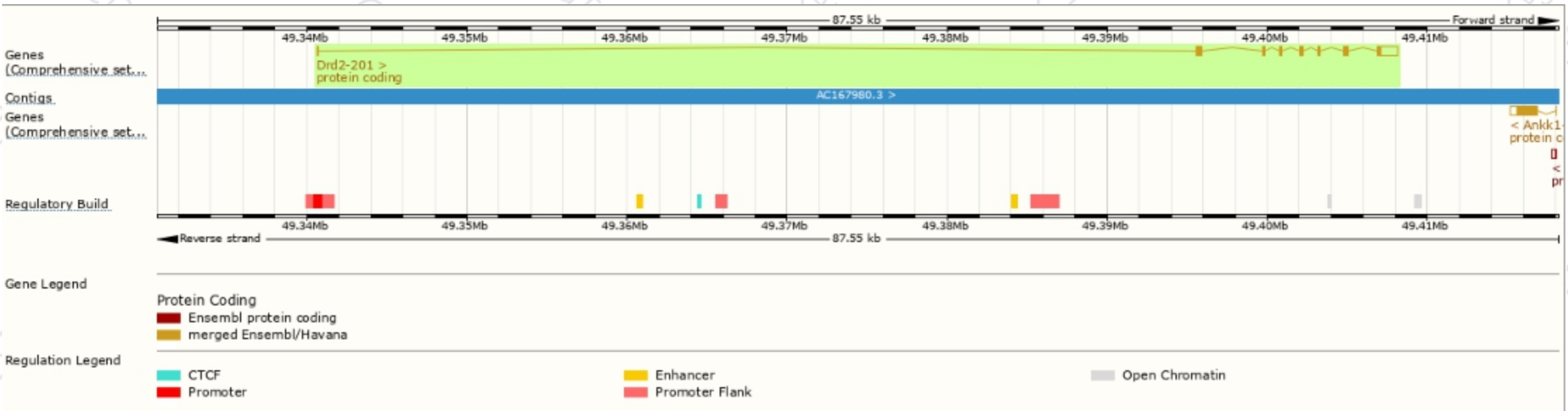
The gene has 1 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Drd2-201	ENSMUST00000075764.7	2547	444aa	Protein coding	CCDS40615	P61168	TSL:1 Gencode basic APPRIS P1

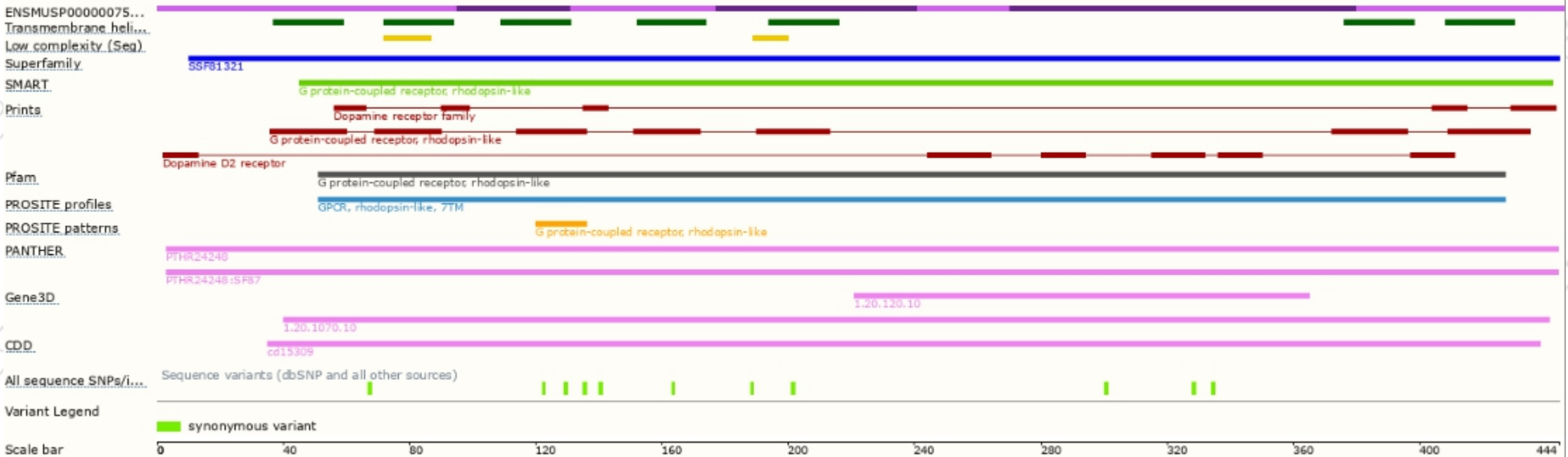
The strategy is based on the design of *Drd2-201* transcript, The transcription is shown below



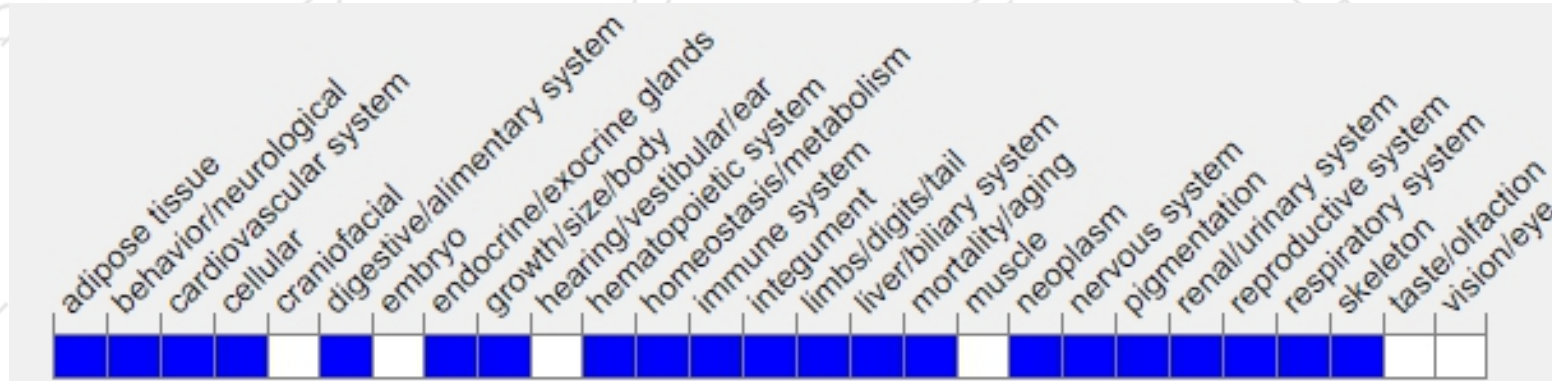
Genomic location distribution



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/marker/MGI:94924>) .

Homozygous null mice show Parkinson's disease like symptoms, including akinetic and bradykinetic behavior. Mice lacking only the long isoform are hypoactive and exhibit increased stereotypic behavior in response to dopamine agonists.

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