Slc6a3-IRES-iCre Cas9-KI Strategy

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Reviewer: Jing Jin

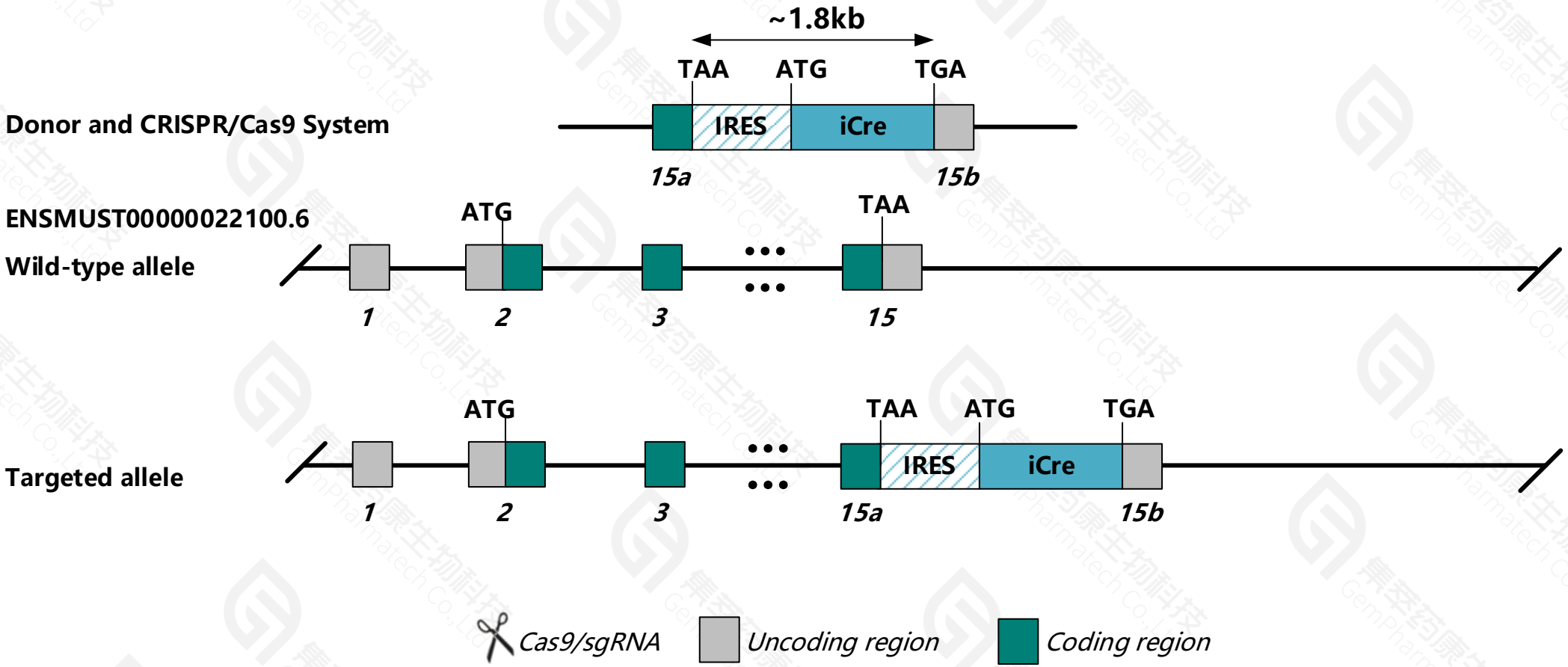
Design Date: 2021-2-8

Project Overview

Project Name	**Slc6a3-IRES-iCre**
Project type	**cas9-ki**
Strain background	**C57BL/6JGpt**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Slc6a3* gene. The schematic diagram is as follows:



Technical routes

- The *Slc6a3* gene has 1 transcript. According to the structure of *Slc6a3* gene, *Slc6a3-201*(ENSMUST00000022100.6) is selected for presentation of the recommended strategy.

- *Slc6a3-201* gene has 15 exons, with the ATG start codon in exon2 and TAA stop codon in exon15.

- We make *Slc6a3-IRES-iCre* mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be coinjected into zygotes. sgRNA direct Cas9 endonuclease cleavage near the stop codon on exon15 , and create aDSB(double-strand break). Such breaks will be repaired, and result in *IRES-iCre* inserted into exon15 of *Slc6a3* 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 null mutations exhibit dwarfism, hyperactivity (especially in a novel environment), 5-fold higher extracellular dopamine levels, impaired spatial cognitive function, anterior pituitary hypoplasia, and failure to lactate.
- According to to the existing references, Cre-mediated recombination is mainly expressed in dopaminergic neurons and little in the olfactory bulb,which can be detected in the embryonic E15.
- Insertion of iCre may affect the regulation of the 3' end of the *Slc6a3* gene.
- The IERS-linked genes will be tarnscripted together and then be translated two protein separately, but the downstream protein is lower than the upstream protein.
- There will be 1 to 2 amino acid synonymous mutation in exon15 of *Slc6a3* gene in this strategy.
- Downstream of insertion site exists polyT repeated sequence, mutations base may occur during vector construction.
- The *Slc6a3* 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)

Slc6a3 solute carrier family 6 (neurotransmitter transporter, dopamine), member 3 [*Mus musculus* (house mouse)]

Gene ID: 13162, updated on 4-Feb-2021

Download Datasets

Summary

Official Symbol	Slc6a3 provided by MGI
Official Full Name	solute carrier family 6 (neurotransmitter transporter, dopamine), member 3 provided by MGI
Primary source	MGI:MGI:94862
See related	Ensembl:ENSMUSG00000021609
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	DA; DAT; Dat1
Expression	Low expression observed in reference dataset See more
Orthologs	human all

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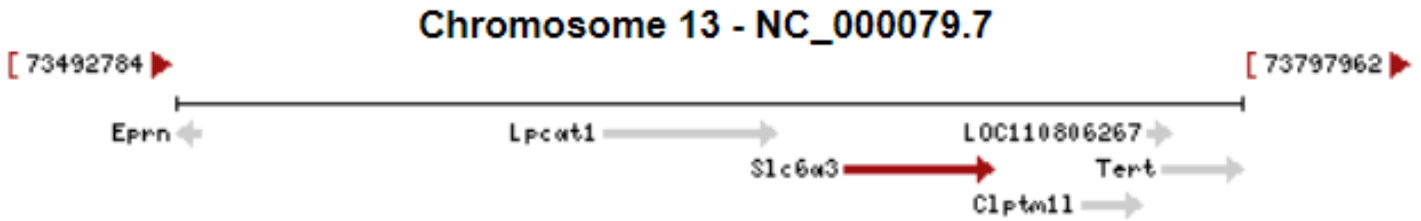
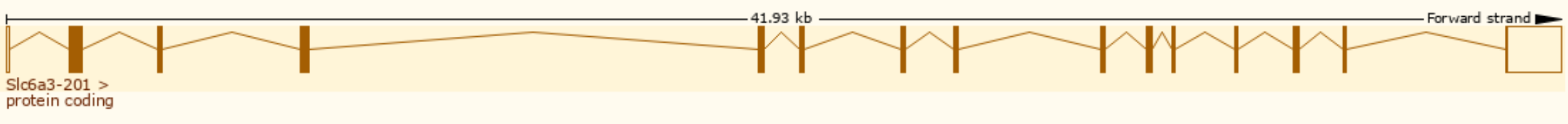


Transcript information（Ensembl）

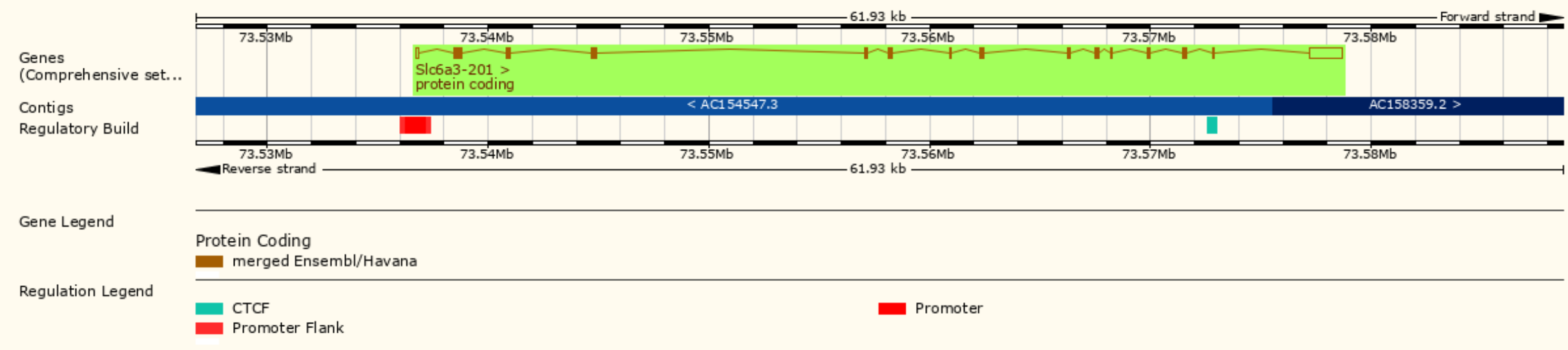
The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Slc6a3-201	ENSMUST00000022100.6	3456	619aa	Protein coding	CCDS26632	Q61327	TSL:1 GENCODE basic APPRIS P1

The strategy is based on the design of *Slc6a3-201* transcript,The transcription is shown below

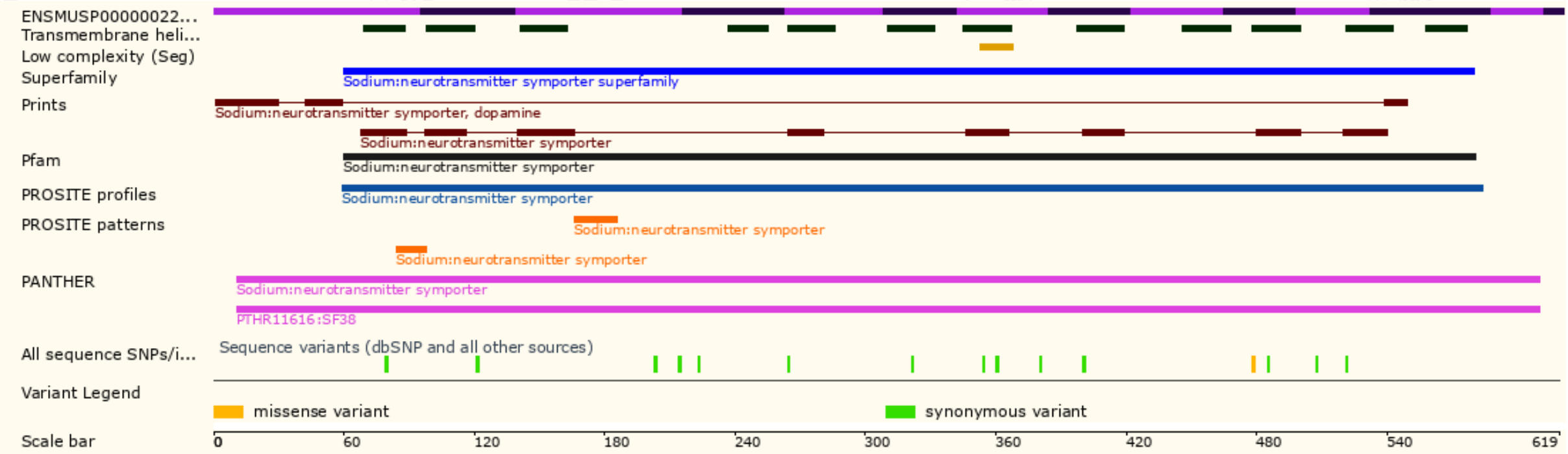


Genomic location distribution

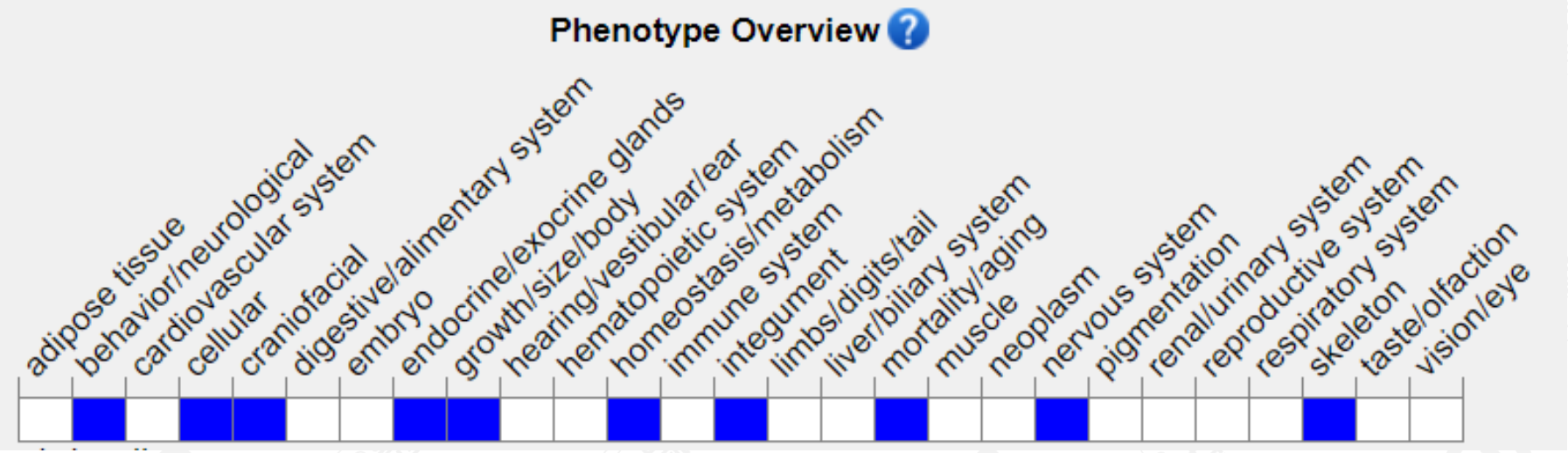


Protein domain

Protein domains for ENSMUSP00000022100.6



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:94862) .

According to the existing MGI data, Homozygotes for targeted null mutations exhibit dwarfism, hyperactivity (especially in a novel environment), 5-fold higher extracellular dopamine levels, impaired spatial cognitive function, anterior pituitary hypoplasia, and failure to lactate.

Coding Sequence of Codon-Optimized Cre Gene[1]

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGG
ATGCCACCTCTGATGAAGTCAGGAAGAACCTGATGGACATGTTCAGGGACAGGCAGGCCTTCTCTGAACACAC
CTGGAAGATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAACAACAGGAAATGGTTCCCTG
CTGAACCTGAGGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACA
GCACCTGGGCCAGCTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCC
TGGTGATGAGGAGAATCAGAAAGGAGAATGTGGATGCTGGGGAGAGAGCCAAGCAGGCCCTGGCCTTTGAAC
GCACTGACTTTGACCAAGTCAGATCCCTGATGGAGAACTCTGACAGATGCCAGGACATCAGGAACCTGGCCTTC
CTGGGCATTGCCTACAACACCCTGCTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCAC
CGATGGTGGGAGAATGCTGATCCACATTGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCC
CTGTCCCTGGGGGTTACCAAGCTGGTGGAGAGATGGATCTCTGTGTCTGGTGTGGCTGATGACCCCAACAACTA
CCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCTGCCCCTTCTGCCACCTCCCAACTGTCCACCCGGGCCCTGG
AAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGTGCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGG
TCTGGCCACTCTGCCAGAGTGGGTGCTGCCAGGGACATGGCCAGGGCTGGTGTGTCCATCCCTGAAATCATGCA
GGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCAGAAACCTGGACTCTGAGACTGGGGCCATGG
TGAGGCTGCTCGAGGATGGGGACTGA

References

[1] Shimshek DR, Kim J, Hübner MR, Spergel DJ. Codon-improved Cre recombinase (iCre) expression in the mouse. GeAlbis.2002 Jan.32(1):19-26.

[2] Bäckman CM, et al. Characterization of a mouse strain expressing Cre recombinase from the 3' untranslated region of the dopamine transporter locus. Genesis. 2006 Aug;44(8):383-90.

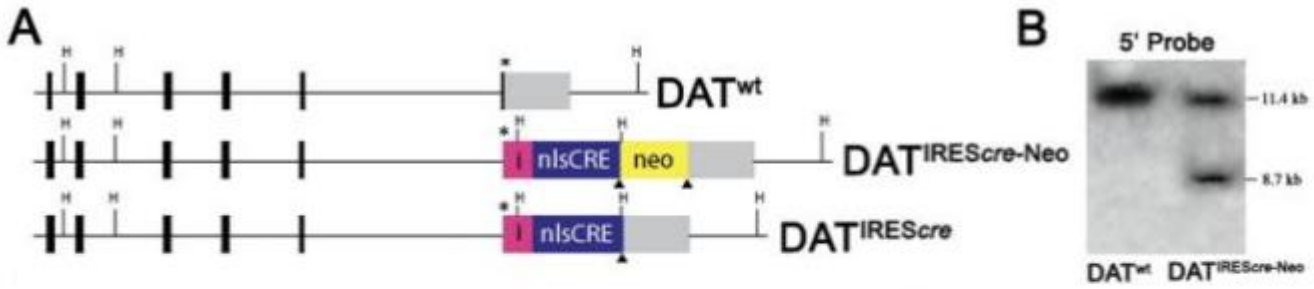


FIG. 1. Generation of *DAT*$^{IREScre\text{-}Neo}$ knockin mice by homologous recombination. (a) Maps showing the endogenous *DAT* gene (*DAT*^{wt}) and targeted variants (*DAT*$^{IREScre\text{-}Neo}$ and *DAT*^{IREScre}). Black boxes represent translated exons of the *DAT* gene and the grey box shows the 3′UTR. The stop codon has been labeled with an asterisk. FRT sites are labeled as black triangles. A targeting vector containing the *Cre recombinase* gene (blue box) proceeded by an IRES (red box), and also containing an FRT-flanked neomycin resistance gene (yellow box) was targeted by homologous recombination into the 3′UTR of the *DAT* gene (*DAT*$^{IREScre\text{-}Neo}$). The FRT-flanked neo-cassette was deleted in germline mice by crosses with an Flp-deleter line, thereby generating *DAT*^{IREScre}. Relevant restriction sites are shown (H; *Hind*III). (b) Southern blot hybridization of ES-cell DNA digested with *Hind*III and using a 5′probe. The wild-type allele gives a 11.4 kb fragment while the targeted allele gives a 8.7 kb fragment.

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

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