# *Slc6a3-iCre-P2A* Cas9-KI Strategy

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**Reviewer:** Yang Zeng
**Design Date:** 2019-8-18

# Project Overview

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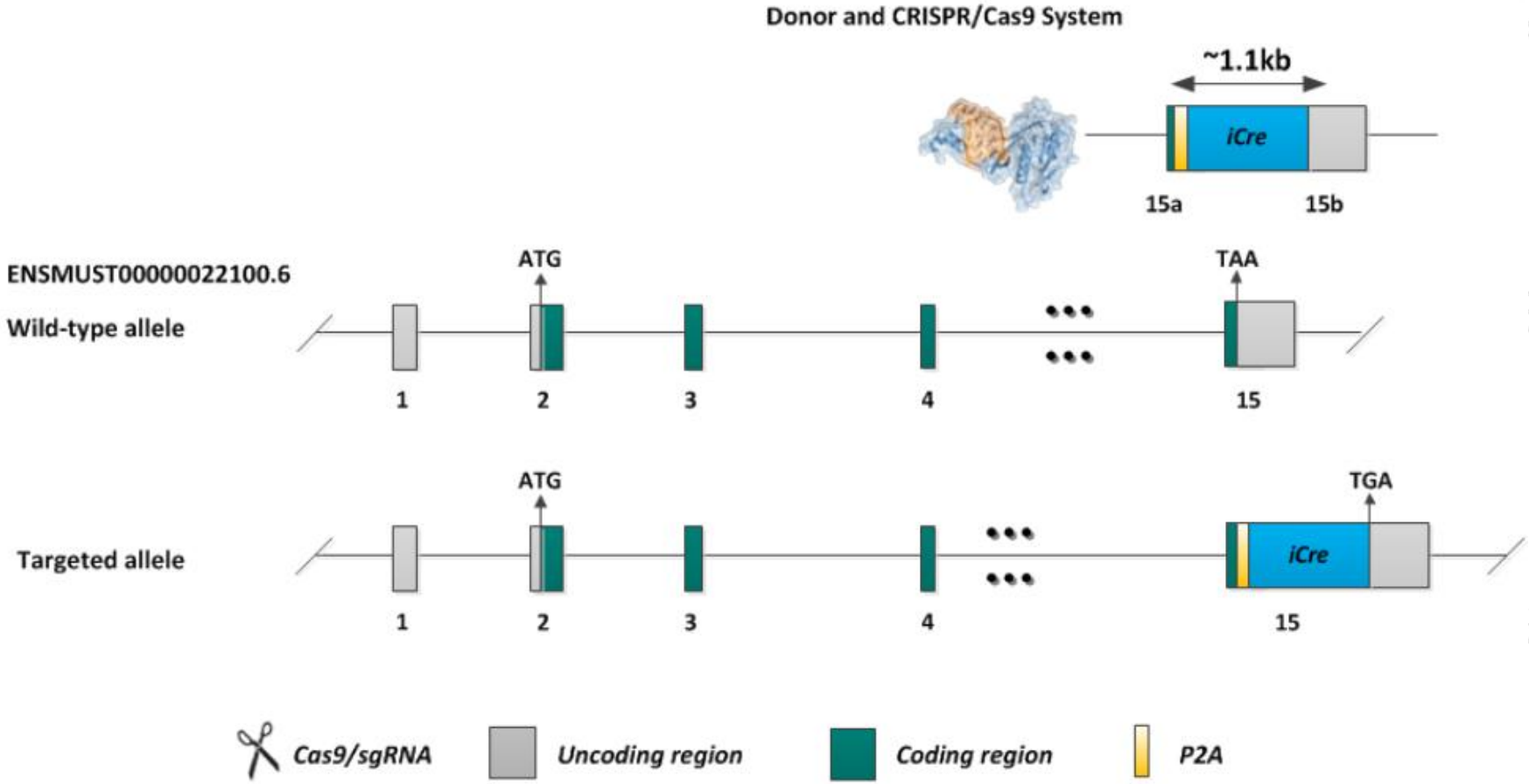
|---|---|
| **Project Name** | ***Slc6a3-P2A-iCre*** |
| **Project type** | **Cas9-KI** |
| **Strain background** | **C57BL/6J** |

# 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*-P2A-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 P2A-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 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.
- 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)

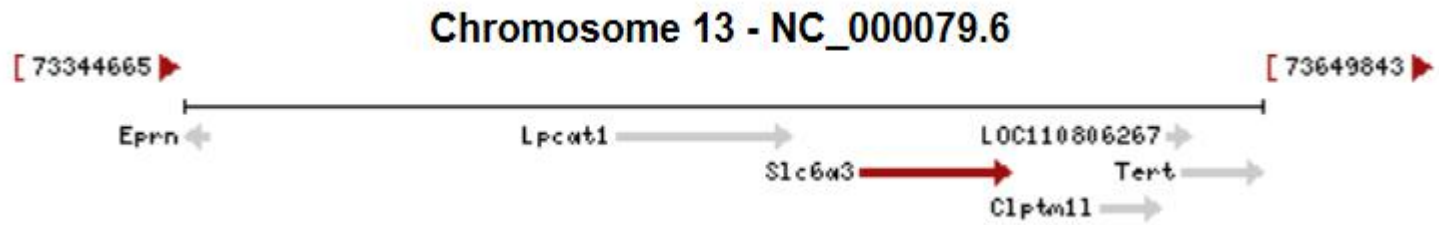
集萃药康 GemPharmatech

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

Gene ID: 13162, updated on 13-Aug-2019

## 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** | DAT; Dat1 |
| **Expression** | Low expression observed in reference dataset See more |
| **Orthologs** | human all |

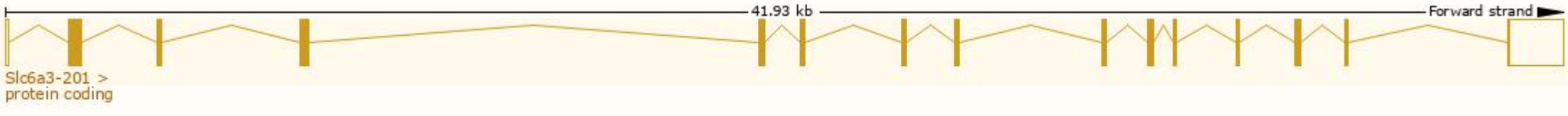


# Transcript information （Ensembl）

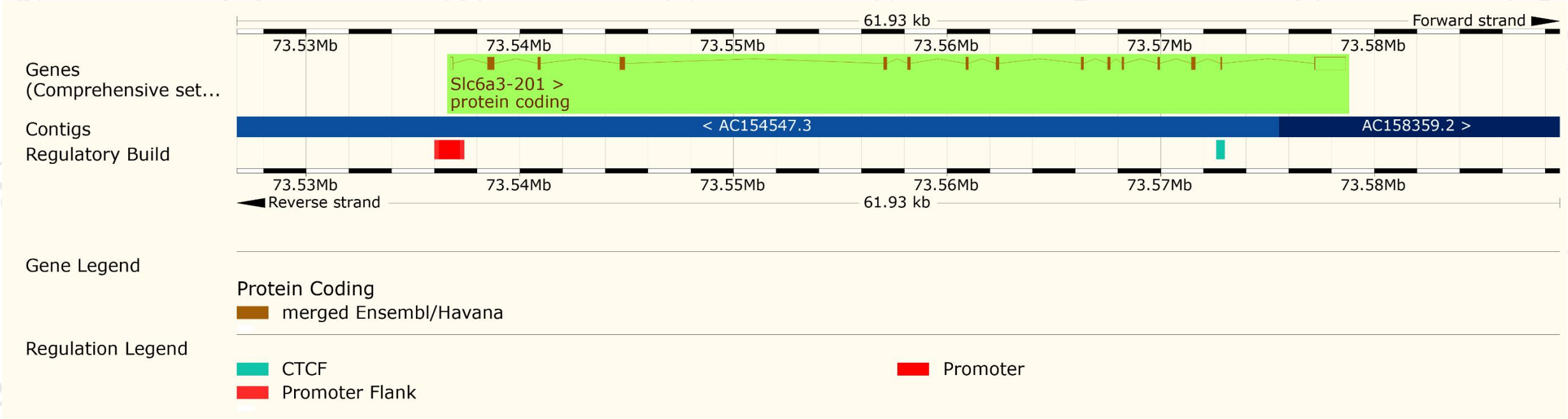
The gene has 1 transcript, and the transcript is shown below:

| Name | Transcript ID | bp | Protein | Translation ID | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|---|
| Slc6a3-201 | ENSMUST00000022100.6 | 3456 | 619aa | ENSMUSP00000022100.6 | Protein coding | CCDS26632 | Q61327 | TSL:1 GENCODE basic APPRIS P1 |

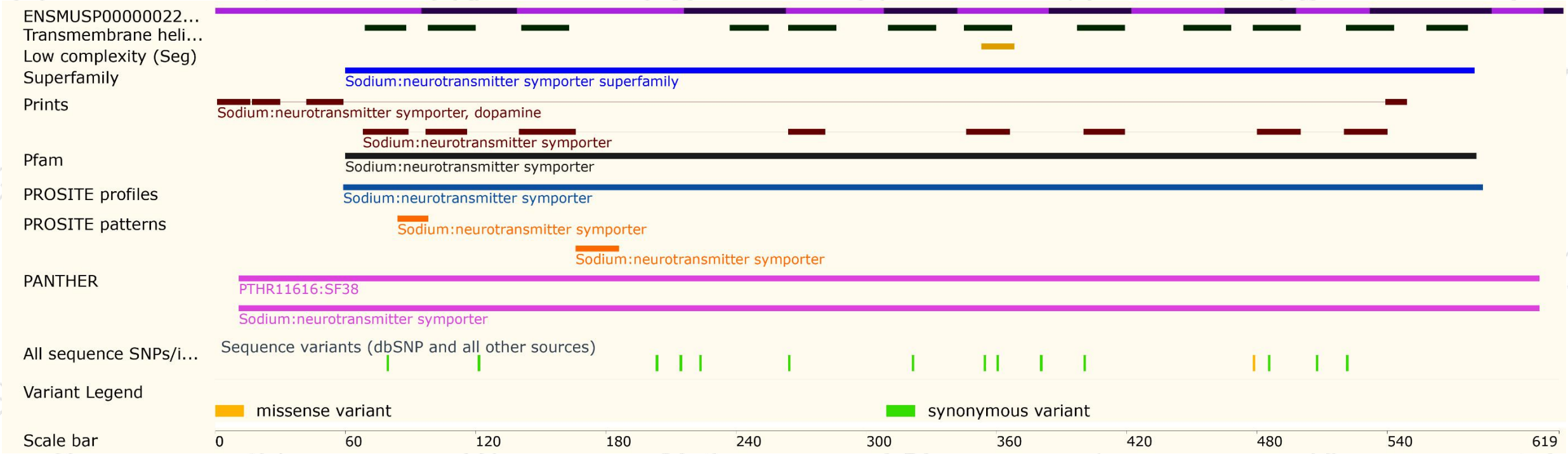
The strategy is based on the design of *Slc6a3-201* transcript,The transcription is shown below



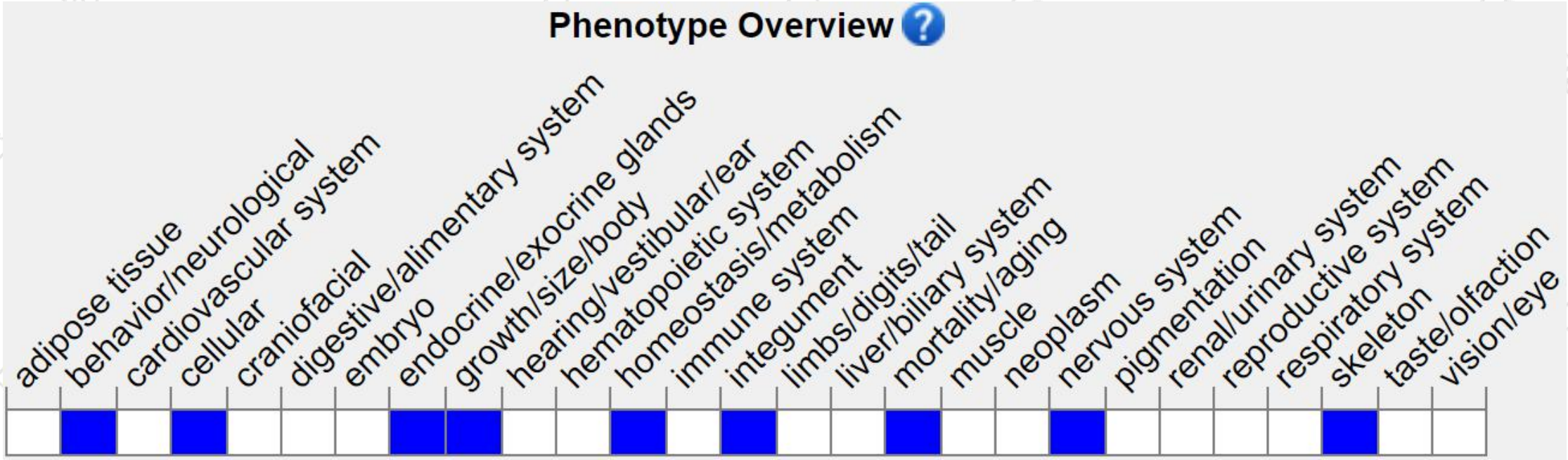
# Genomic location distribution



# Protein domain



# 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]

ATGGTGCCCAAGAAGAAGAGGAAAGTCTCCAACCTGCTGACTGTGCACCAAAACCTGCCTGCCCTCCCTGTGGATGCCACCTCTGATGAAGTCAGGAAGA
ACCTGATGGACATGTTCAGGGACAGGCAGGCCTTCTCTGAACACACCTGGAAGATGCTCCTGTCTGTGTGCAGATCCTGGGCTGCCTGGTGCAAGCTGAA
CAACAGGAAATGGTTCCCTGCTGAACCTGAGGATGTGAGGGACTACCTCCTGTACCTGCAAGCCAGAGGCCTGGCTGTGAAGACCATCCAACAGCACCTG
GGCCAGCTCAACATGCTGCACAGGAGATCTGGCCTGCCTCGCCCTTCTGACTCCAATGCTGTGTCCCTGGTGATGAGGAGAATCAGAAAGGAGAATGTGG
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GAACCTGGCCTTCCTGGGCATTGCCTACAACACCCTGCTGCGCATTGCCGAAATTGCCAGAATCAGAGTGAAGGACATCTCCCGCACCGATGGTGGGAGA
ATGCTGATCCACATTGGCAGGACCAAGACCCTGGTGTCCACAGCTGGTGTGGAGAAGGCCCTGTCCCTGGGGGTTACCAAGCTGGTGGAGAGATGGATCT
CTGTGTCTGGTGTGGCTGATGACCCCAACAACTACCTGTTCTGCCGGGTCAGAAAGAATGGTGTGGCTGCCCCTTCTGCCACCTCCCAACTGTCCACCCG
GGCCCTGGAAGGGATCTTTGAGGCCACCCACCGCCTGATCTATGGTGCCAAGGATGACTCTGGGCAGAGATACCTGGCCTGGTCTGGCCACTCTGCCAGA
GTGGGTGCTGCCAGGGACATGGCCAGGGCTGGTGTGTCCATCCCTGAAATCATGCAGGCTGGTGGCTGGACCAATGTGAACATTGTGATGAACTACATCA
GAAACCTGGACTCTGAGACTGGGGCCATGGTGAGGCTGCTCGAGGATGGGGACTGA

# 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.

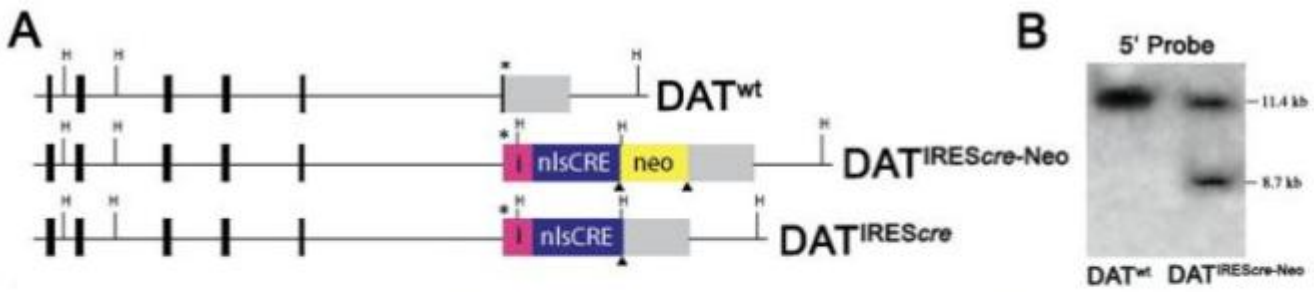


**FIG. 1.** Generation of *DAT*$^{IREScre\text{-}Neo}$ knockin mice by homologous recombination. (a) Maps showing the endogenous *DAT* gene (*DAT*$^{wt}$) and targeted variants (*DAT*$^{IREScre\text{-}Neo}$ and *DAT*$^{IREScre}$). Black boxes represent translated exons of the *DAT* gene and the grey 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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