Slc6a4 Cas9-CKO Strategy

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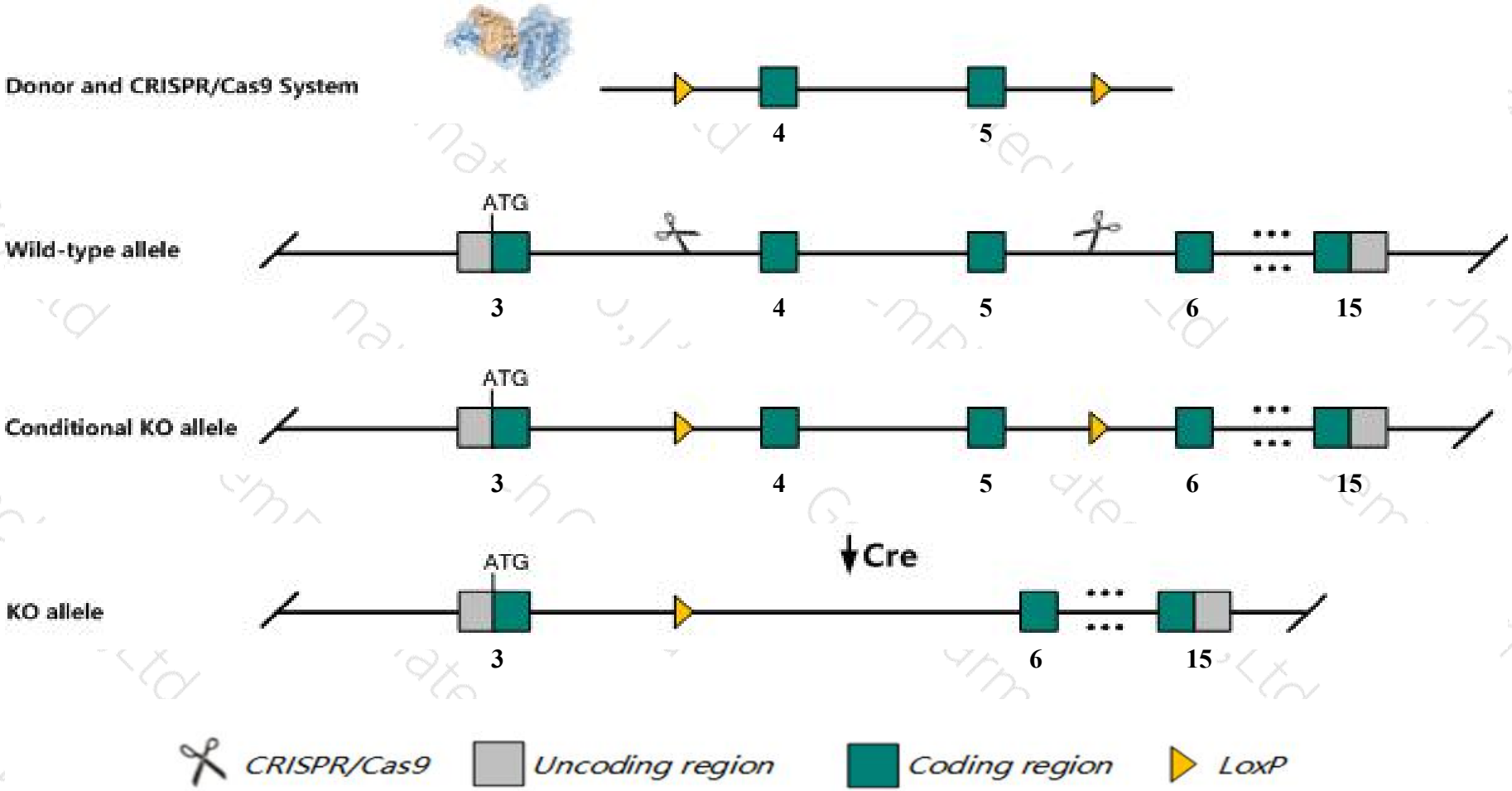
Project Overview

Project Name	***Slc6a4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Slc6a4* gene. The schematic diagram is as follows:



Technical routes

- The *Slc6a4* gene has 4 transcripts. According to the structure of *Slc6a4* gene, exon4-exon5 of *Slc6a4-202* (ENSMUST00000108402.8) transcript is recommended as the knockout region.The region contains 355bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Slc6a4* gene. The brief process is as follows:CRISPR/Cas9 system 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 will be 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 a targeted null mutation exhibit greatly diminished brain serotonin levels and lack cortical barrel patterns. also, mutants lack the locomotor enhancing response to the drug (+)-3,4-methylenedioxymethamphetamine.
- The *Slc6a4* gene is located on the Chr11. 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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Slc6a4 solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 [Mus musculus (house mouse)]

Gene ID: 15567, updated on 13-Mar-2020

Summary

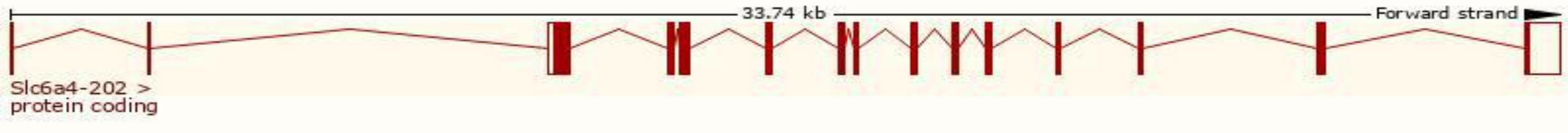
Official Symbol	Slc6a4 provided by MGI
Official Full Name	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 provided by MGI
Primary source	MGI:MGI:96285
See related	Ensembl:ENSMUSG00000020838
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	5-HTT, AI323329, Htt, Sert
Expression	Broad expression in large intestine adult (RPKM 24.9), small intestine adult (RPKM 15.4) and 16 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

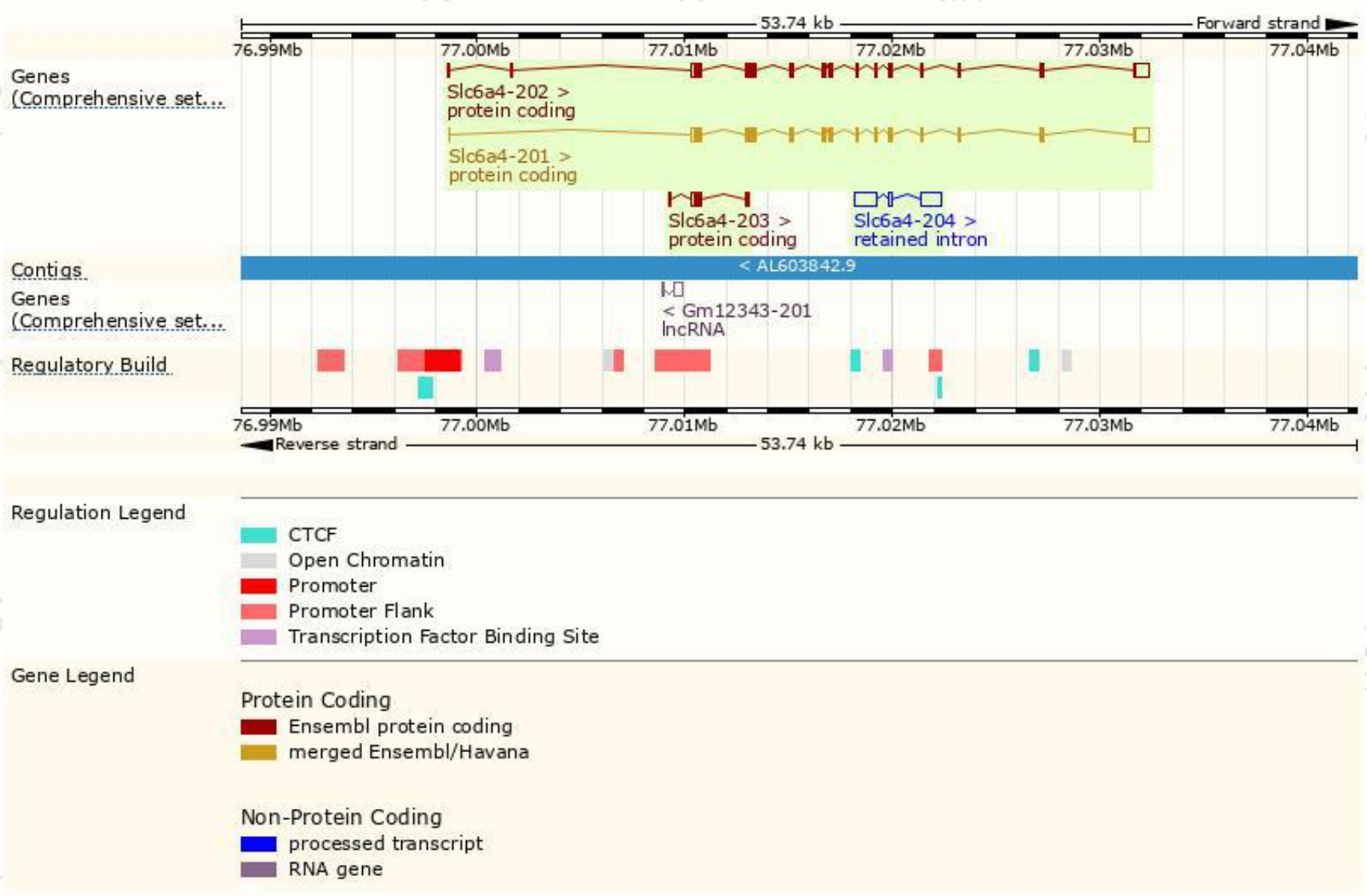
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc6a4-202	ENSMUST00000108402.8	2796	630aa	Protein coding	CCDS25074	Q60857	TSL:5 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Slc6a4-201	ENSMUST00000021195.10	2725	630aa	Protein coding	CCDS25074	Q60857	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Slc6a4-203	ENSMUST00000129572.2	643	158aa	Protein coding	-	Q5NCR5	CDS 3' incomplete TSL:5
Slc6a4-204	ENSMUST00000137819.1	2149	No protein	Retained intron	-	-	TSL:1

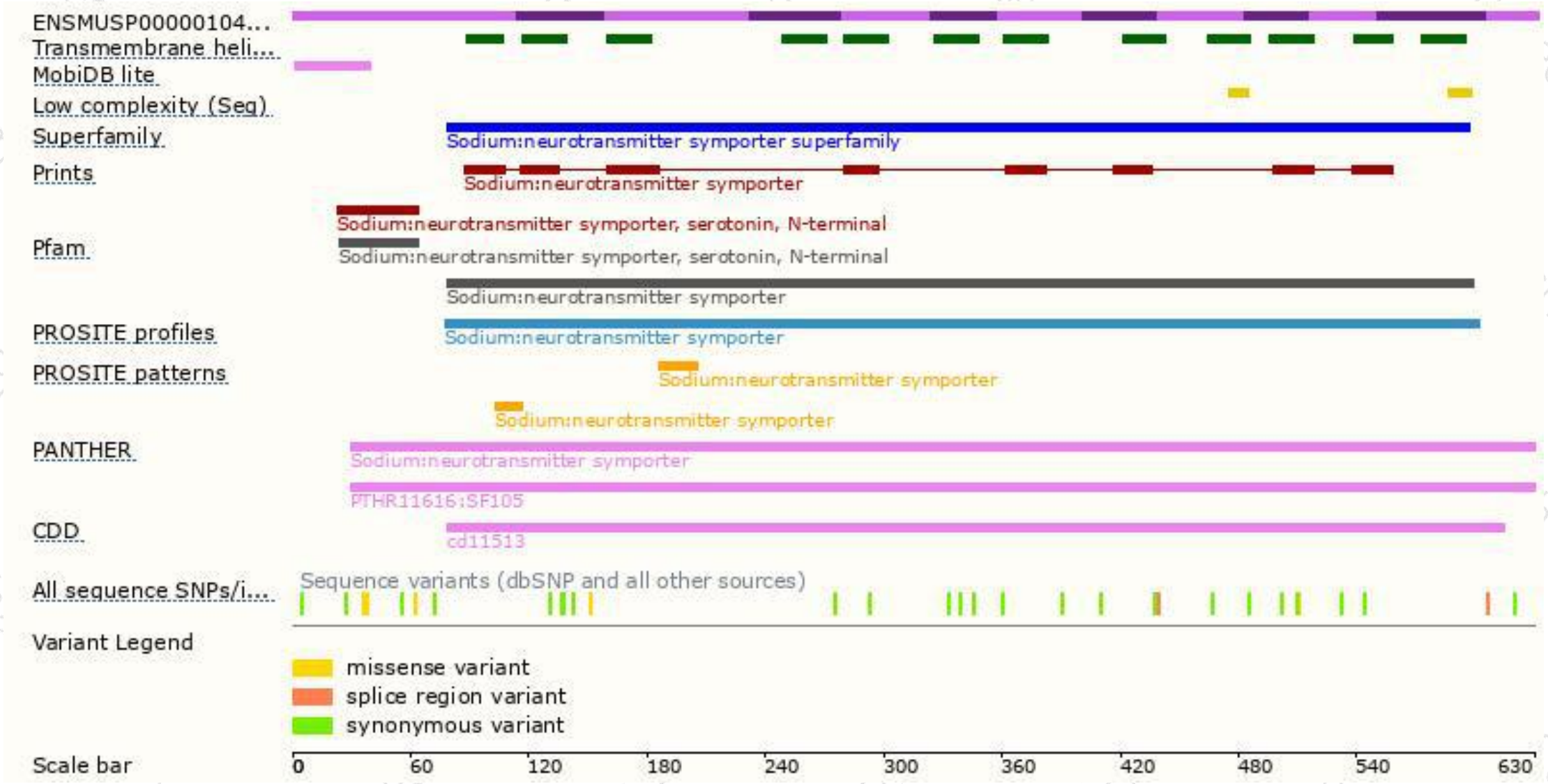
The strategy is based on the design of *Slc6a4-202* transcript,the transcription is shown below



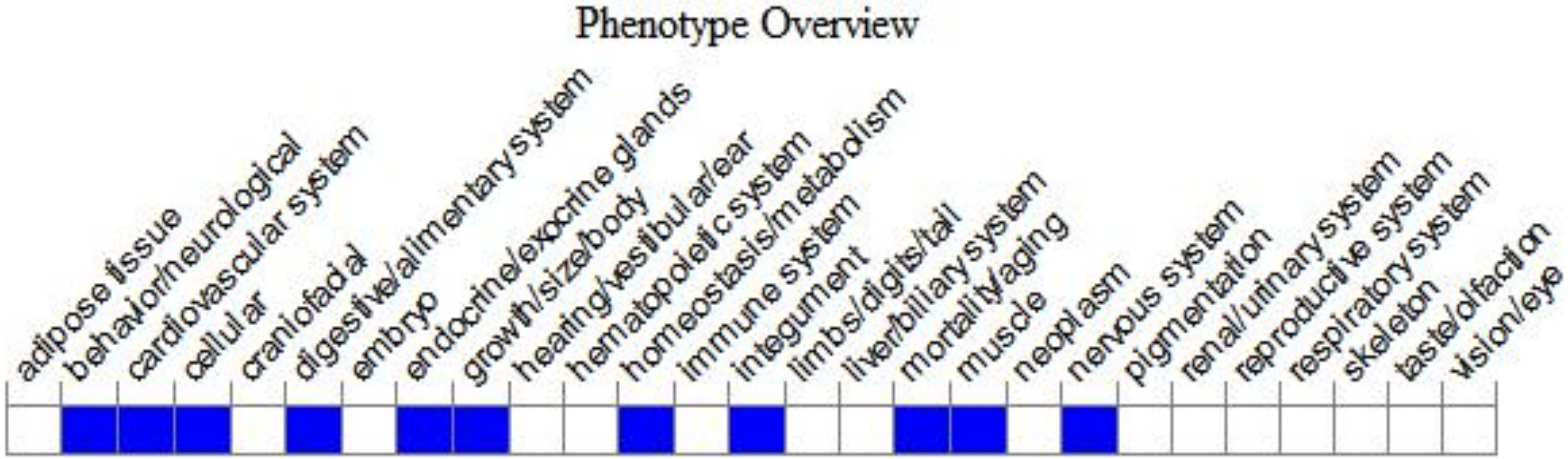
Genomic location distribution



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/).

According to the existing MGI data,homozygotes for a targeted null mutation exhibit greatly diminished brain serotonin levels and lack cortical barrel patterns. Also, mutants lack the locomotor enhancing response to the drug (+)-3,4-methylenedioxymethamphetamine.

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

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