Mapt Cas9-CKO Strategy

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Project Overview

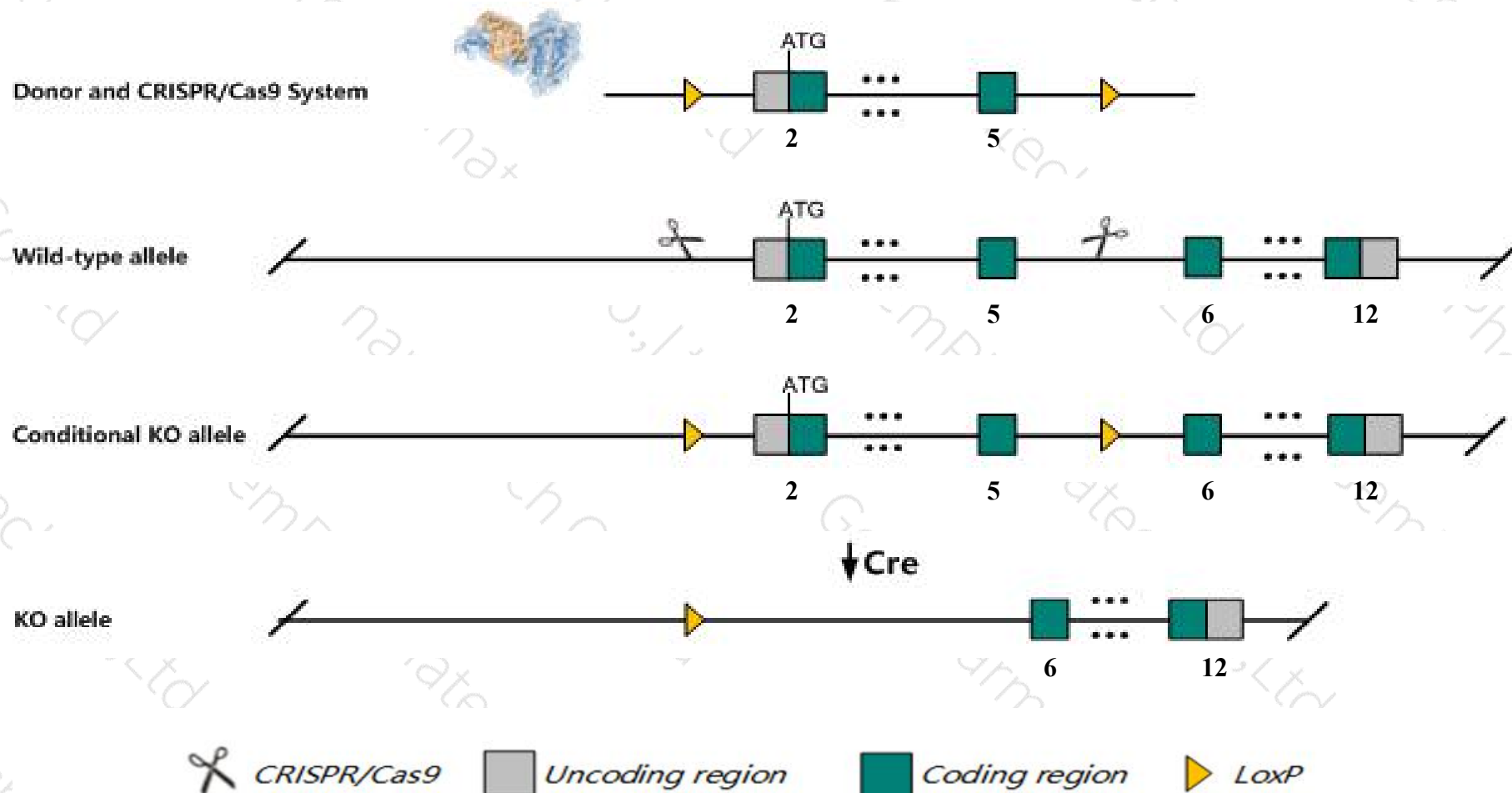|	
Project Name	*Mapt*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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

CRISPR/Cas9   Uncoding region   Coding region   LoxP

Technical routes

➤ The *Mapt* gene has 12 transcripts. According to the structure of *Mapt* gene, exon2-exon5 of *Mapt-201*(ENSMUST00000100347.10) transcript is recommended as the knockout region.The region contains start codon ATG.Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Mapt* 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,homozygous mutants exhibit altered performance in behavioral tests and show mircotubule changes in small-calibre axons. Embryonic hippocampal cultures from mutants exhibit delayed axonal and neuritic maturation.

➢ The *Mapt* 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）

Mapt microtubule-associated protein tau [Mus musculus (house mouse)]

Gene ID: 17762, updated on 13-Mar-2020

Summary

|	
Official Symbol	Mapt provided by MGI
Official Full Name	microtubule-associated protein tau provided byMGI
Primary source	MGI:MGI:97180
See related	Ensembl:ENSMUSG00000018411
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	AI413597, AW045860, Mtapt, Tau
Expression	Biased expression in CNS E18 (RPKM 123.0), whole brain E14.5 (RPKM 80.2) and 9 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 12 transcripts,all transcripts are shown below:

| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Mapt-204	ENSMUST00000106992.9	5253	372aa	Protein coding	CCDS25528	P10637 Q547J4	TSL:1 GENCODE basic APPRIS ALT2
Mapt-201	ENSMUST00000100347.10	1362	430aa	Protein coding	CCDS25527	P10637	TSL:1 GENCODE basic APPRIS P4
Mapt-203	ENSMUST00000106989.2	2250	749aa	Protein coding	-	A2A5Y6	TSL:5 GENCODE basic APPRIS ALT2
Mapt-202	ENSMUST00000106988.7	2202	733aa	Protein coding	-	A0A0A0MQC7	TSL:5 GENCODE basic APPRIS ALT2
Mapt-205	ENSMUST00000106993.9	1527	390aa	Protein coding	-	B1AQW2	TSL:5 APPRIS ALT2
Mapt-211	ENSMUST00000145227.7	600	154aa	Protein coding	-	B1AQW4	CDS 3' incomplete TSL:3
Mapt-208	ENSMUST00000132977.7	418	123aa	Protein coding	-	B1AQW5	CDS 3' incomplete TSL:2
Mapt-207	ENSMUST00000132245.7	293	84aa	Protein coding	-	B1AQW6	CDS 3' incomplete TSL:3
Mapt-206	ENSMUST00000126820.1	831	No protein	Processed transcript	-	-	TSL:2
Mapt-210	ENSMUST00000144836.1	779	No protein	Processed transcript	-	-	TSL:3
Mapt-212	ENSMUST00000146353.1	464	No protein	Processed transcript	-	-	TSL:2
Mapt-209	ENSMUST00000138384.7	701	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Mapt-201* transcript,the transcription is shown below:
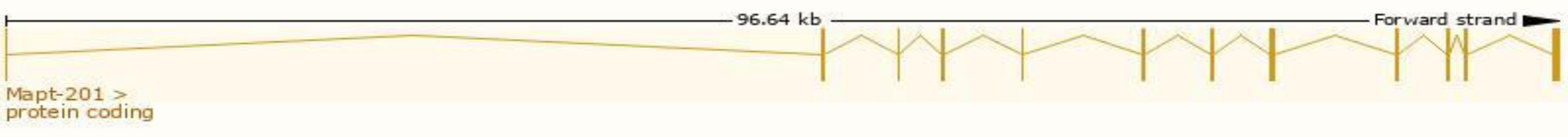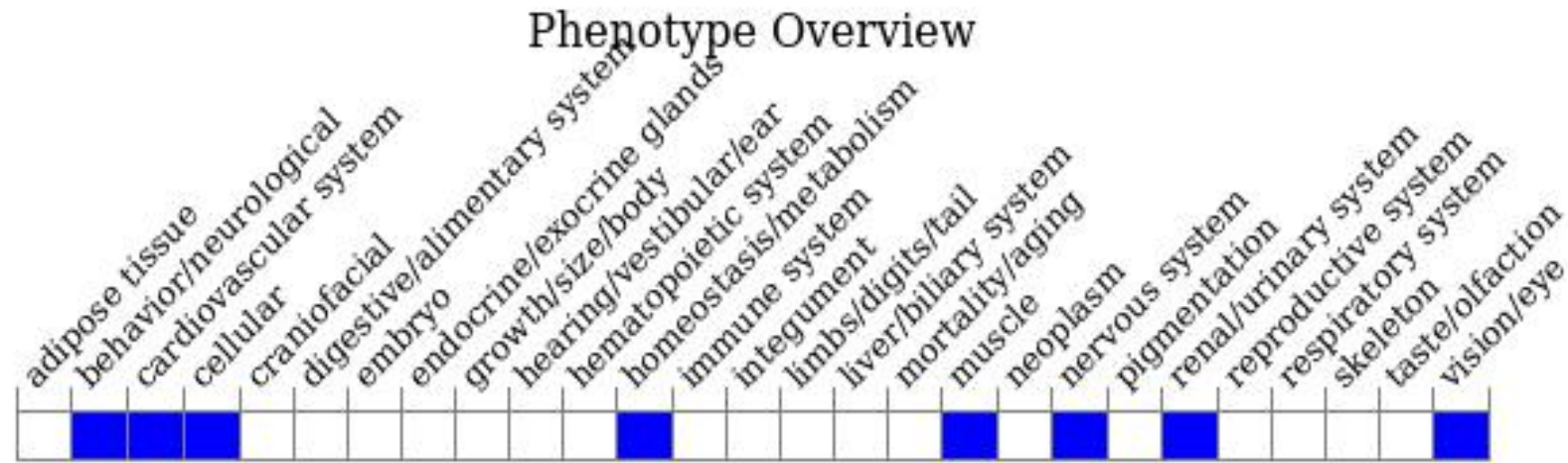
Genomic location distribution
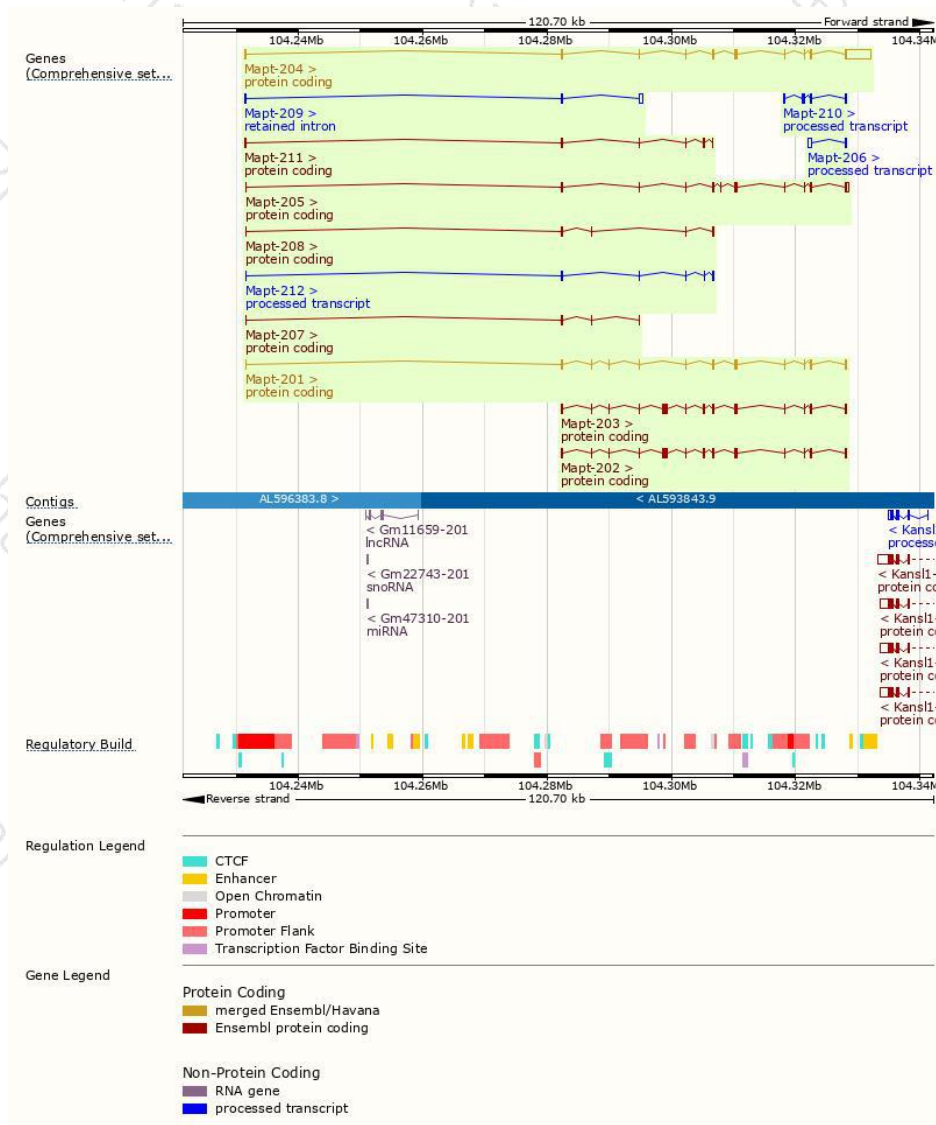
Protein domain
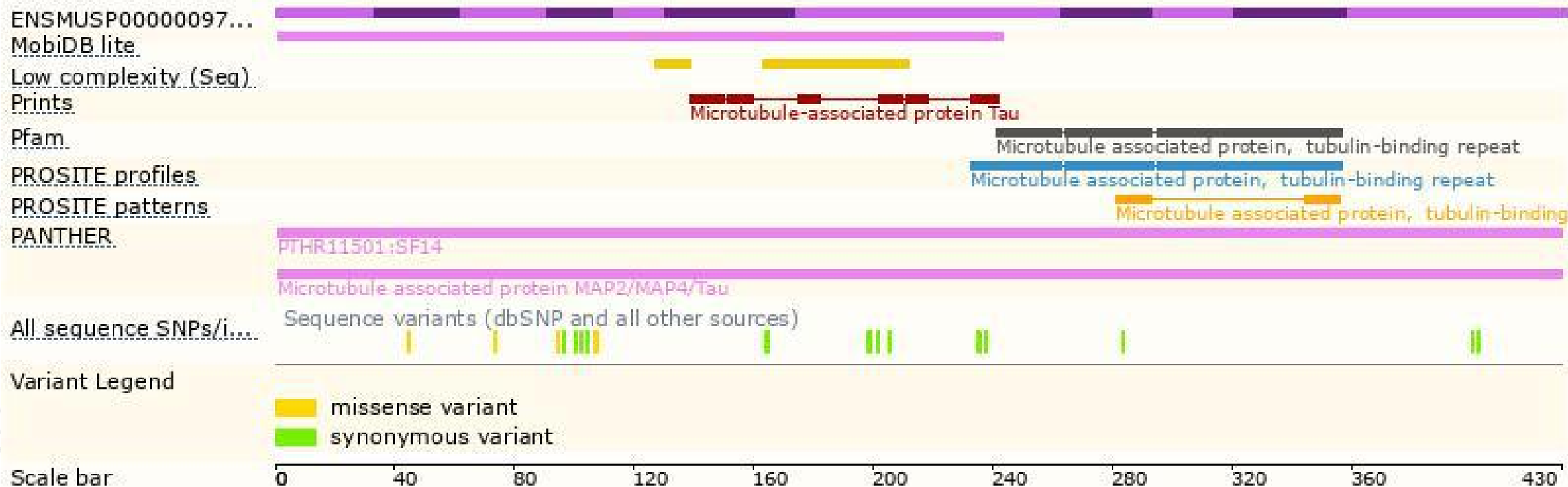
Mouse phenotype description(MGI)

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

According to the existing MGI data,homozygous mutants exhibit altered performance in behavioral tests and show mircotubule changes in small-calibre axons. Embryonic hippocampal cultures from mutants exhibit delayed axonal and neuritic maturation.

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