Cyfip1 Cas9-CKO Strategy

Designer: Qiong Zhou

Project Overview

Project Name	***Cyfip1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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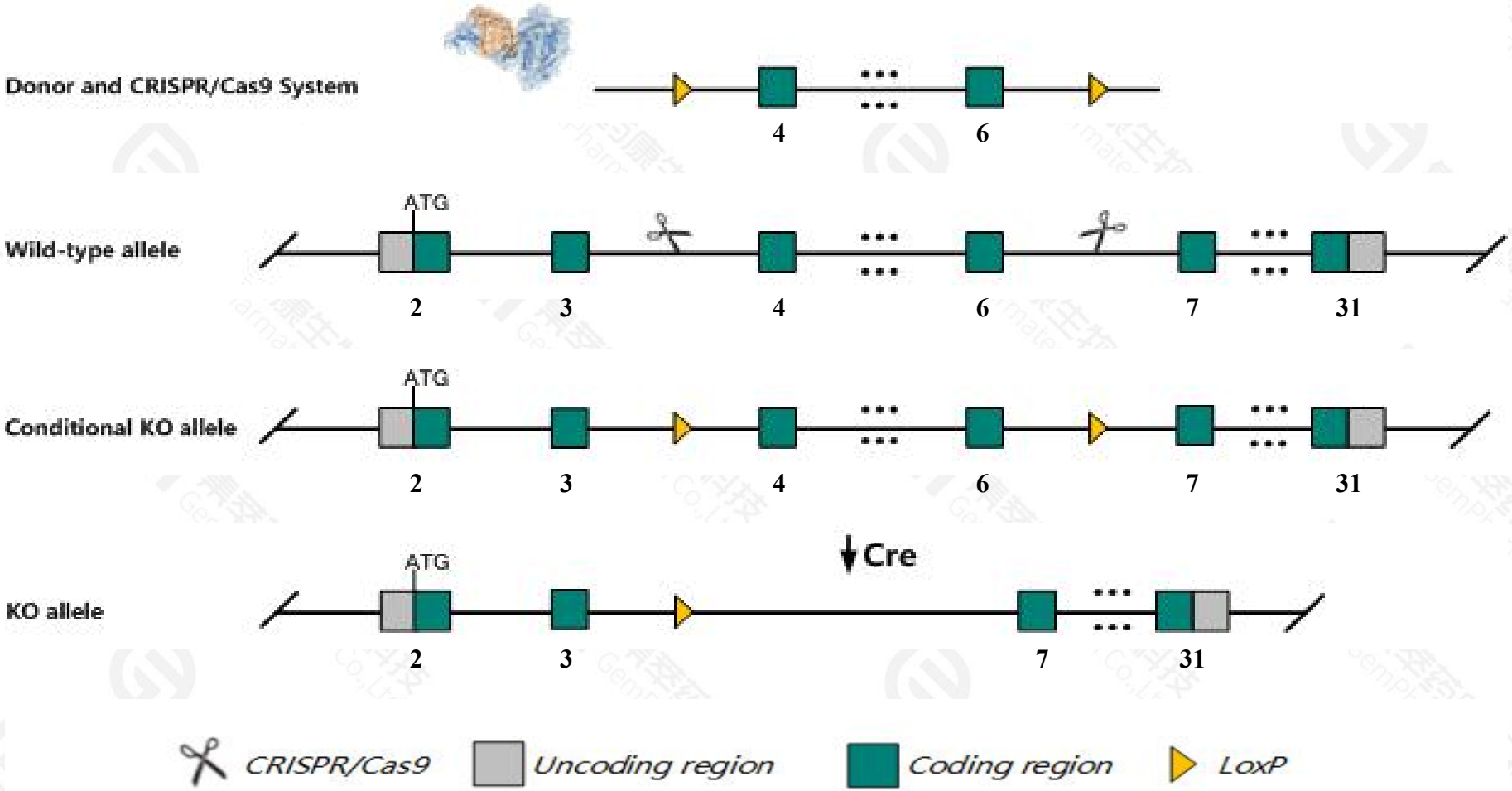
This model will use CRISPR/Cas9 technology to edit the *Cyfip1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Cyfip1* gene has 12 transcripts. According to the structure of *Cyfip1* gene, exon4-exon6 of *Cyfip1-201*(ENSMUST00000032629.16) transcript is recommended as the knockout region.The region contains 362bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Cyfip1* 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,mutations at this locus result in embryonic lethality before the turning stage in homozygotes. Heterozygotes exhibit abnormal synaptic transmission. Parental origin of the mutant allele in heterozygotes has an effect on long term depression, cued fear conditioning, anxiety, and activity.

➢ Transcript *Cyfip1-205, Cyfip1-206, Cyfip1-209, Cyfip1-210, Cyfip1-211* may not be affected.

➢ The *Cyfip1* gene is located on the Chr7. 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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Cyfip1 cytoplasmic FMR1 interacting protein 1 [Mus musculus (house mouse)]

Gene ID: 20430, updated on 13-Mar-2020

Summary

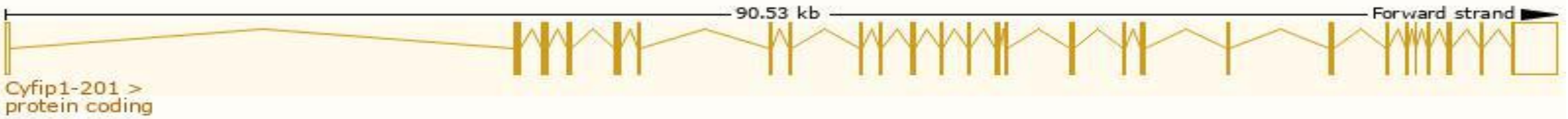
Official Symbol	Cyfip1 provided by MGI
Official Full Name	cytoplasmic FMR1 interacting protein 1 provided by MGI
Primary source	MGI:MGI:1338801
See related	Ensembl:ENSMUSG00000030447
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	E030028J09Rik, P140SRA-1, P140sra1, Shyc, Sra-1, Sra1, I(7)1RI, I71RI, I7RI1, mKIAA0068, pl-1
Expression	Ubiquitous expression in bladder adult (RPKM 10.5), limb E14.5 (RPKM 10.3) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

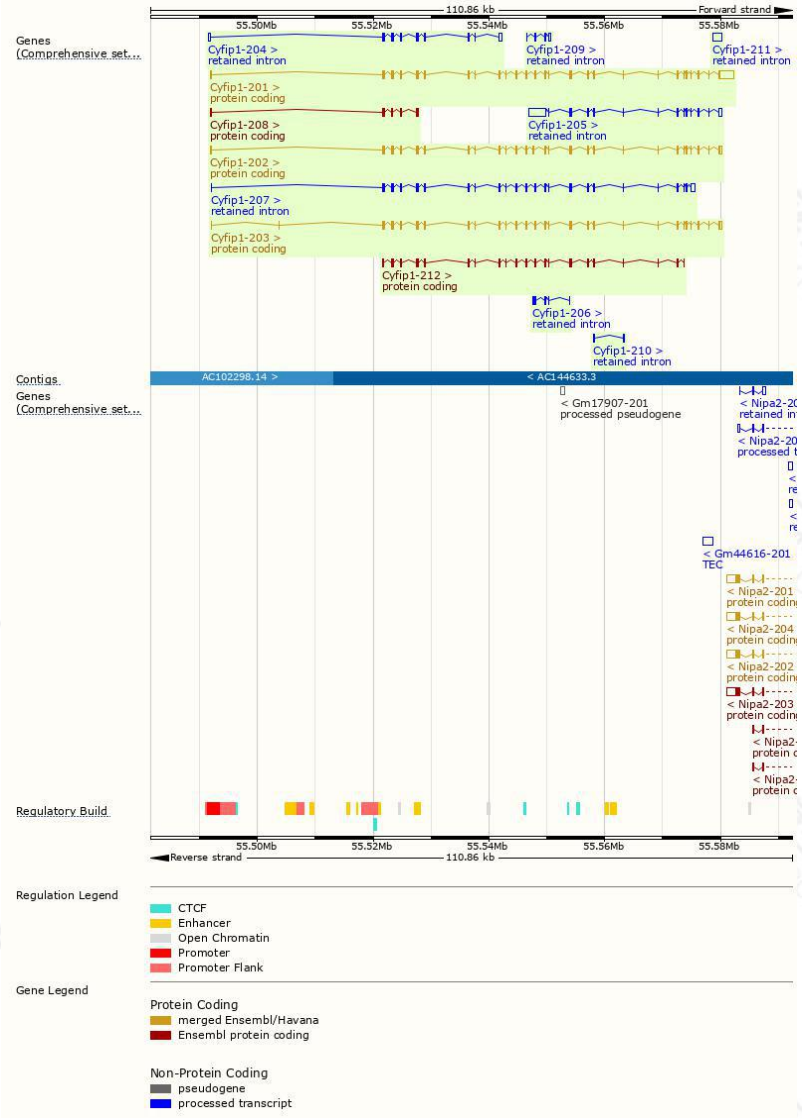
The gene has 12 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cyfip1-201	ENSMUST00000032629.15	6440	1253aa	Protein coding	CCDS21315	Q7TMB8	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
Cyfip1-202	ENSMUST00000085255.10	4195	1251aa	Protein coding	CCDS52262	A0A0R4J119	TSL:1 GENCODE basic
Cyfip1-203	ENSMUST00000163845.3	4178	1253aa	Protein coding	CCDS21315	Q7TMB8	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
Cyfip1-212	ENSMUST00000206862.1	2908	969aa	Protein coding	-	A0A0U1RQ05	CDS 3' incomplete TSL:5
Cyfip1-208	ENSMUST00000173783.7	926	229aa	Protein coding	-	G3UZI5	CDS 3' incomplete TSL:3
Cyfip1-205	ENSMUST00000173267.7	5111	No protein	Retained intron	-	-	TSL:1
Cyfip1-207	ENSMUST00000173497.7	3849	No protein	Retained intron	-	-	TSL:1
Cyfip1-204	ENSMUST00000168271.8	1876	No protein	Retained intron	-	-	TSL:1
Cyfip1-211	ENSMUST00000205656.1	1588	No protein	Retained intron	-	-	TSL:NA
Cyfip1-209	ENSMUST00000174660.7	719	No protein	Retained intron	-	-	TSL:2
Cyfip1-206	ENSMUST00000173384.1	629	No protein	Retained intron	-	-	TSL:3
Cyfip1-210	ENSMUST00000174793.1	466	No protein	Retained intron	-	-	TSL:2

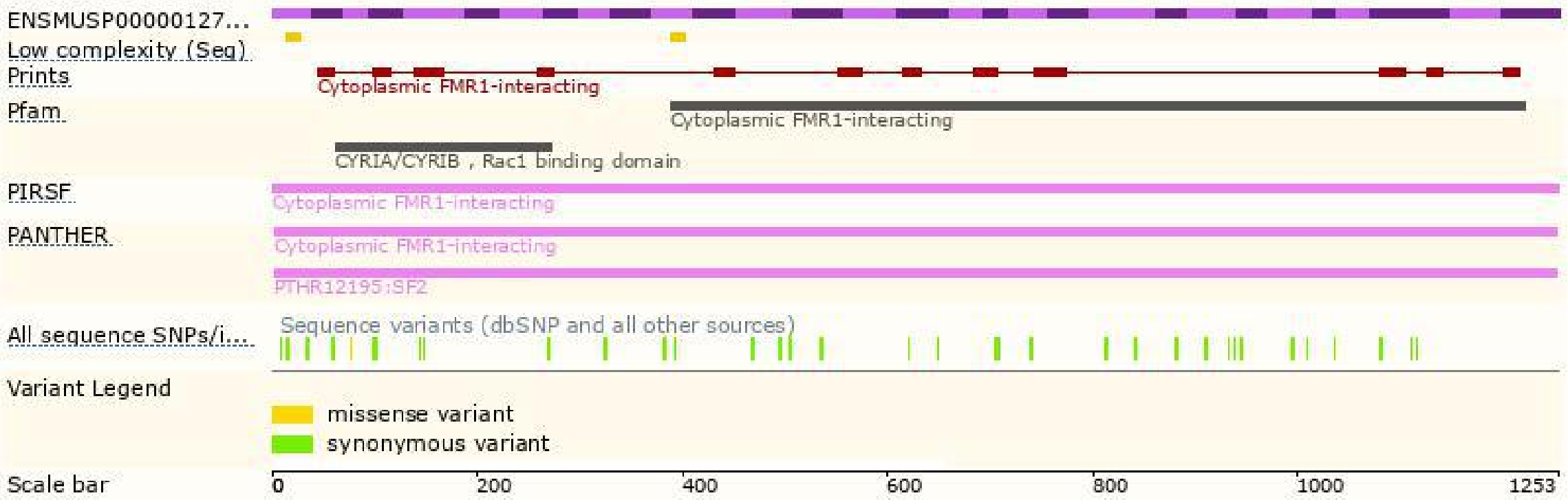
The strategy is based on the design of *Cyfip1-201* transcript,the transcription is shown below:



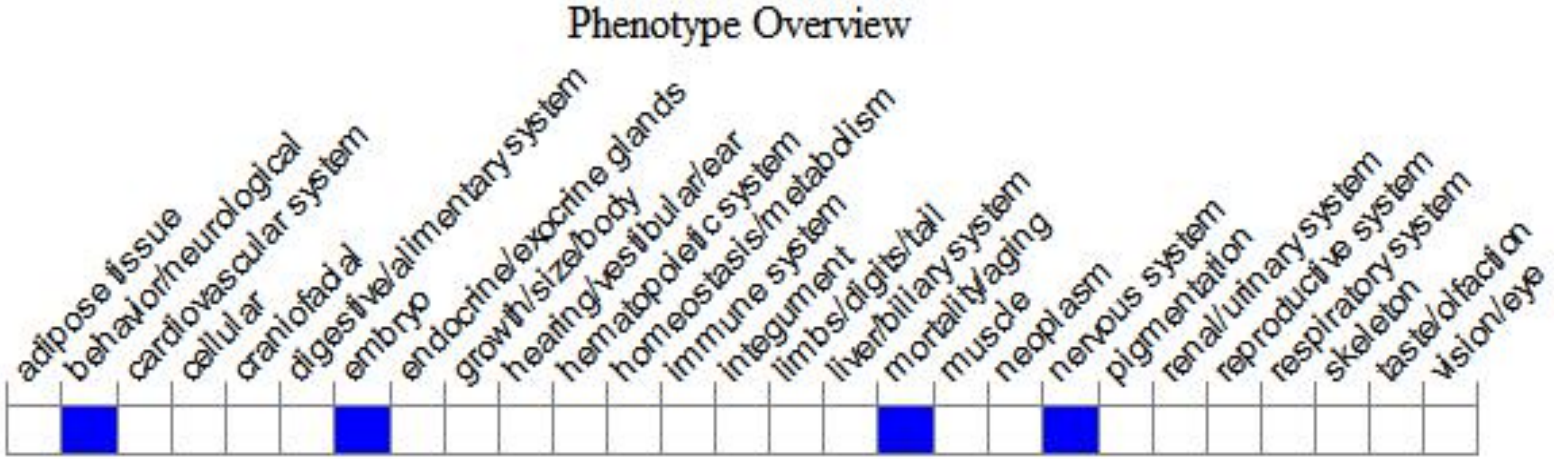
Genomic location distribution



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/).

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