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Fbxl3 Cas9-CKO Strategy

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Design Date: 2023-11-6

Overview

Target Gene Name

- Fbxl3

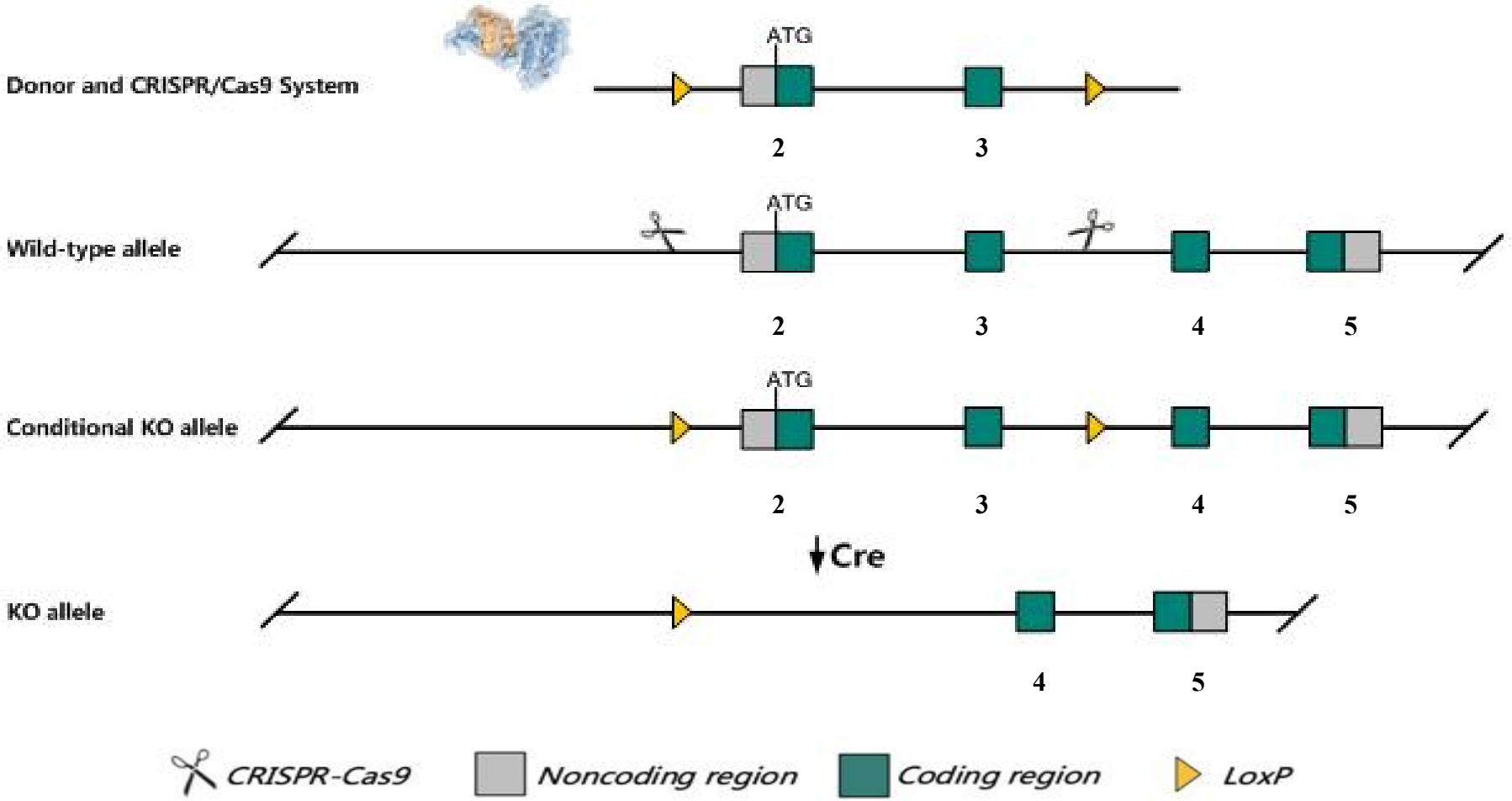
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Fbxl3* gene.

Technical Information

- The *Fbxl3* gene has 7 transcripts. According to the structure of *Fbxl3* gene, exon2-exon3 of *Fbxl3*-201 (ENSMUST00000022720.15) transcript is recommended as the knockout region. The region contains start codon ATG. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Fbxl3* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

Gene Information

Fbxl3 F-box and leucine-rich repeat protein 3 [Mus musculus (house mouse)]

Gene ID: 50789, updated on 19-Mar-2019

Summary

Official Symbol	Fbxl3 provided by MGI
Official Full Name	F-box and leucine-rich repeat protein 3 provided by MGI
Primary source	MGI:MGI:1354702
See related	Ensembl:ENSMUSG00000022124
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	AU041772, AW212966, FBK, Fbl3a, Fbxl3a, Ovtm, Play68
Expression	Ubiquitous expression in cerebellum adult (RPKM 14.1), bladder adult (RPKM 13.9) and 28 other tissues See more
Orthologs	human all

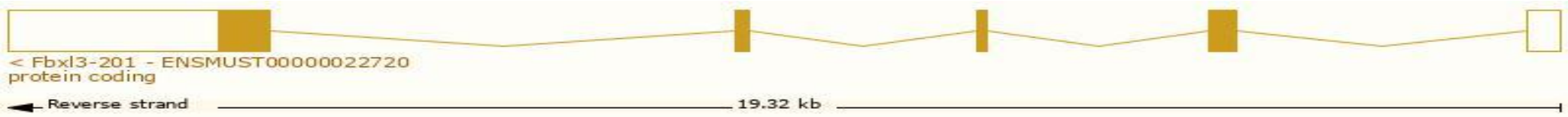
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 7 transcripts,all transcripts are shown below:

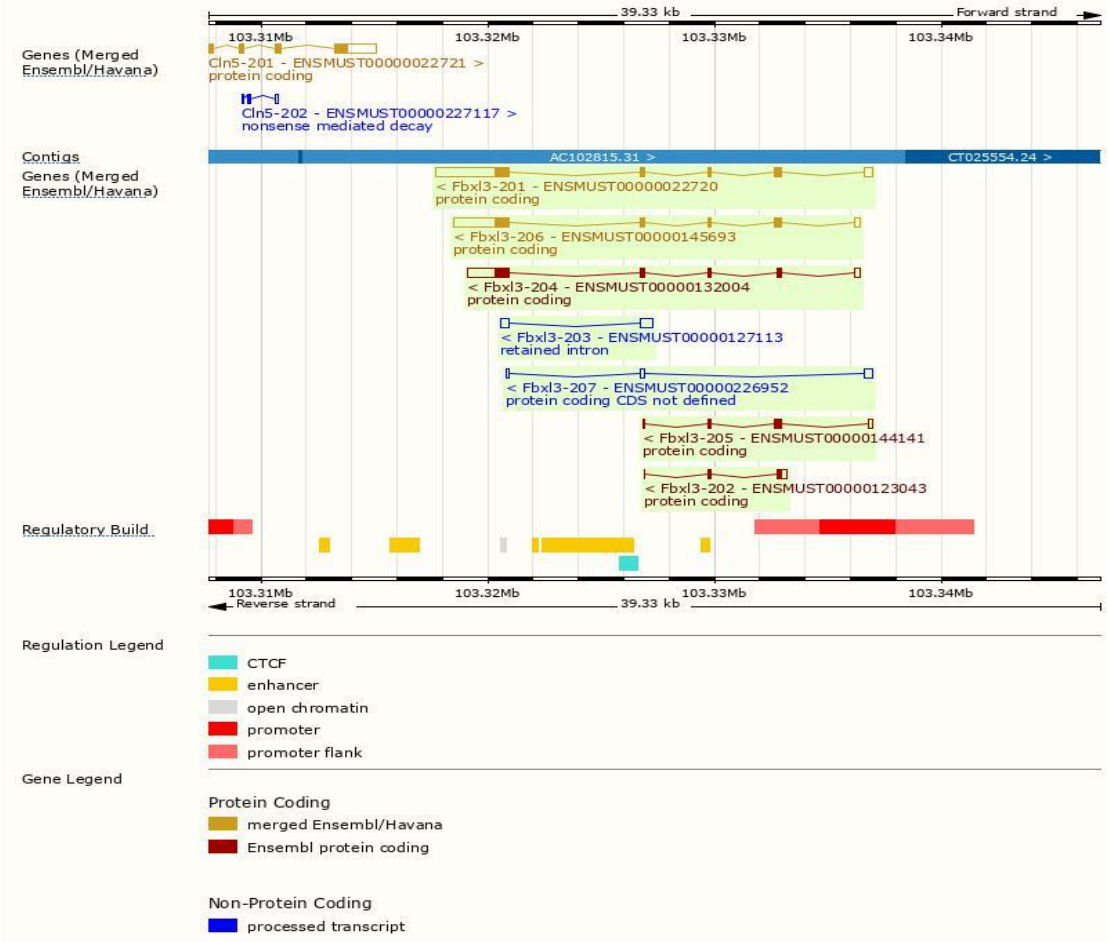
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Fbxl3-201	ENSMUST00000022720.14	4309	428aa	Protein coding	CCDS27315	Q5PRF6 Q8C4V4	TSL:1 GENCODE basic APPRIS P1
Fbxl3-206	ENSMUST00000145693.7	3352	428aa	Protein coding	CCDS27315	Q5PRF6 Q8C4V4	TSL:1 GENCODE basic APPRIS P1
Fbxl3-204	ENSMUST00000132004.7	2534	380aa	Protein coding	CCDS84159	Q8C4V4	TSL:1 GENCODE basic
Fbxl3-205	ENSMUST00000144141.7	743	177aa	Protein coding	-	D3YWZ0	CDS 3' incomplete TSL:3
Fbxl3-202	ENSMUST00000123043.1	557	116aa	Protein coding	-	D3Z388	CDS 3' incomplete TSL:5
Fbxl3-203	ENSMUST00000127113.1	922	No protein	Retained intron	-	-	TSL:2
Fbxl3-207	ENSMUST00000226952.1	668	No protein	lncRNA	-	-	

The strategy is based on the design of *Fbxl3*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org

Genomic Information



Source: : https://www.ensembl.org

Protein Information

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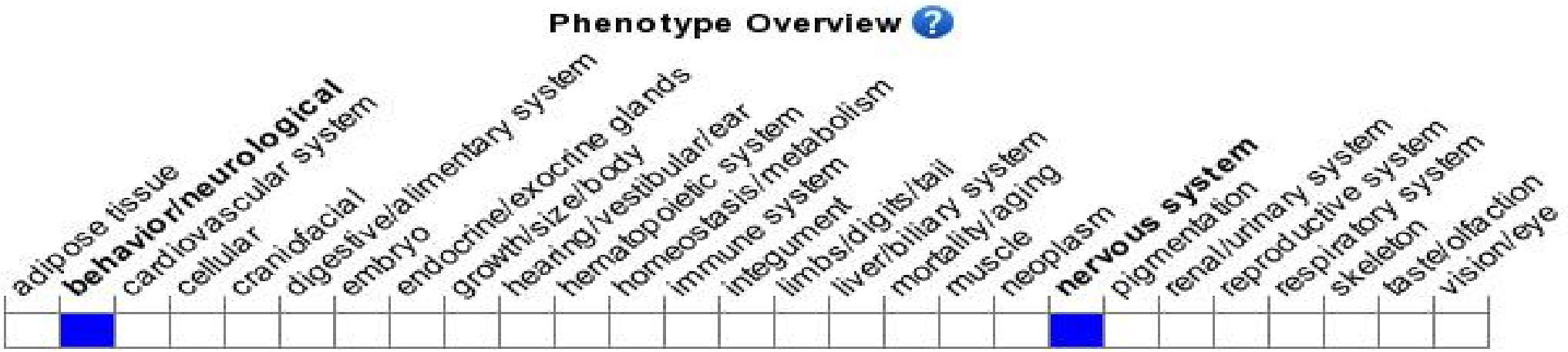
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Both heterozygous and homozygous mutant mice display a longer free running period than that of wild-type mice.

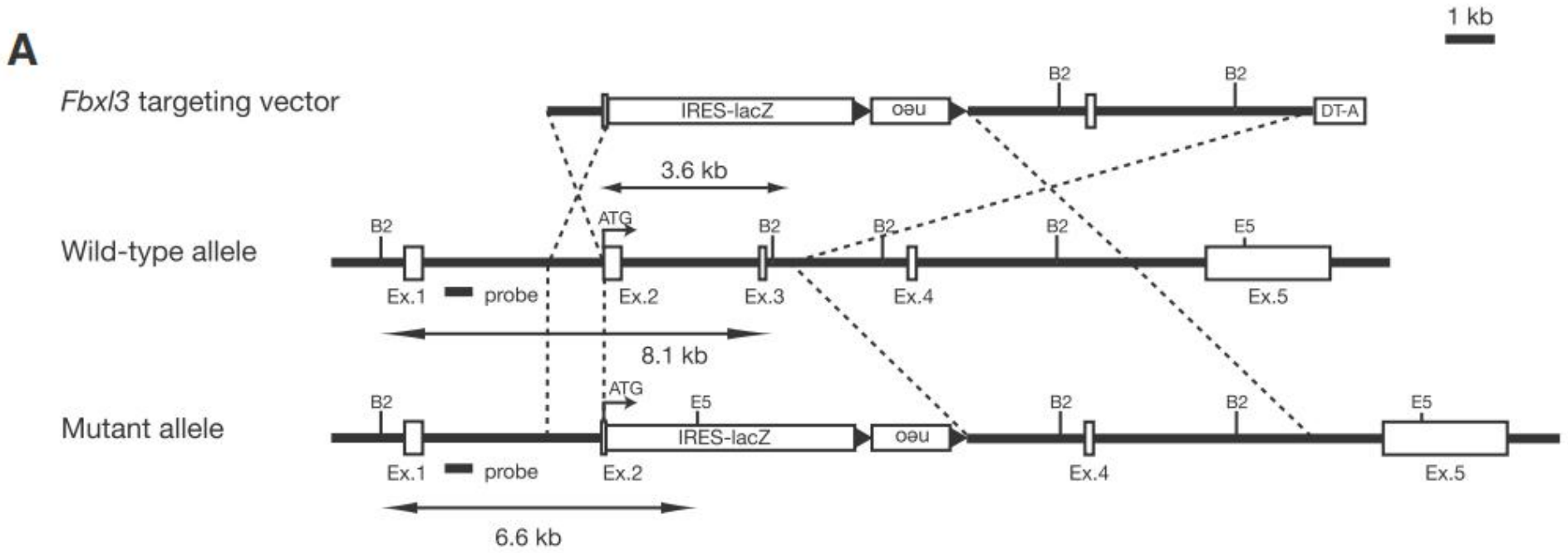
Source: https://www.informatics.jax.org

Important Information

- According to the existing MGI data,both heterozygous and homozygous mutant mice display a longer free running period than that of wild-type mice.
- The effect of the transcript *Fbxl3-202&205* is unknown.
- The floxed region contains start codon ATG of *Fbxl3* gene,and there is a risk of identifying new ATG sites to restart translation after Cre recombination.
- *Fbxl3* is located on Chr14. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.

Reference

Hirano A, et al., FBXL21 regulates oscillation of the circadian clock through ubiquitination and stabilization of cryptochromes. Cell. 2013 Feb 28;152(5):1106-18



Generation of *Fbxl3*$^{-/-}$ and *Fbxl21*$^{-/-}$ Mice

Cloned DNA corresponding to the *Fbxl3* and *Fbxl21* locus was amplified from the genome of E14 mouse ES cells with the use of LA-Taq polymerase (TaKaRa). The *Fbxl3* targeting vector was constructed by replacing the 3.6-kb fragment of genomic DNA containing exon 2 and 3 of *Fbxl3* with IRES-*lacZ* and PGK-*neo*-poly(A)-loxP cassettes. The vector thus contained 1.2- and 6.8-kb regions of homology located 5′ and 3′, respectively, relative to IRES-*lacZ* and the neomycin resistance gene (*neo*). The *Fbxl21* targeting vector