

# Fbxw7 Cas9-CKO Strategy

Designer

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Reviewer

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**Design Date** 

2018-6-11

# **Project Overview**



**Project Name** 

Fbxw7

**Project type** 

Cas9-CKO

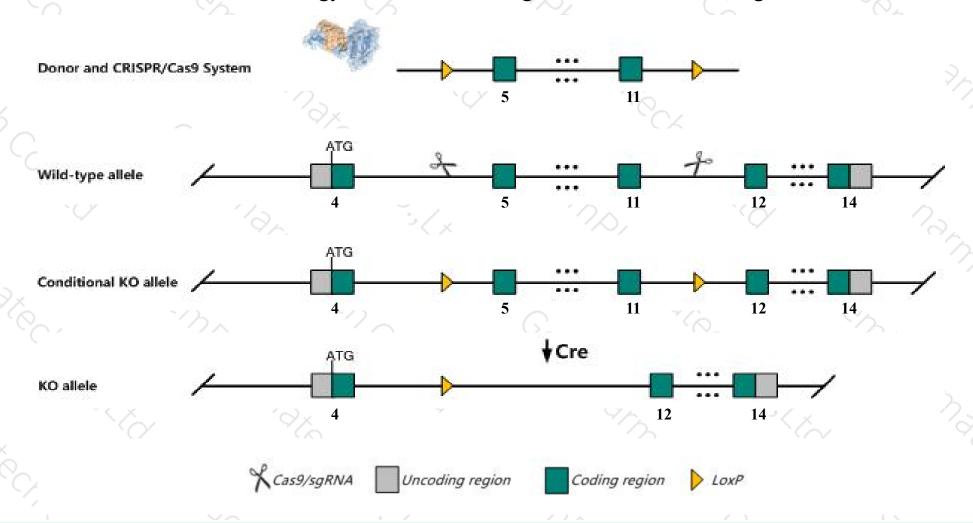
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Fbxw7* gene. The schematic diagram is as follows:



### Technical routes



- The *Fbxw7* gene has 6 transcripts. According to the structure of *Fbxw7* gene, exon5-exon11 of *Fbxw7-204*(ENSMUST00000107679.7) transcript is recommended as the knockout region. The region contains 917bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Fbxw7* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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 inactivation of this locus disrupts embryonic and extraembryonic vasculature, resulting in death by midgestation.
- ightharpoonup Transcript *Fbxw7-206* may not be affected.
- The *Fbxw7* gene is located on the Chr3. 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)



#### Fbxw7 F-box and WD-40 domain protein 7 [Mus musculus (house mouse)]

Gene ID: 50754, updated on 9-Apr-2019

#### Summary

☆ ?

Official Symbol Fbxw7 provided by MGI

Official Full Name F-box and WD-40 domain protein 7 provided by MGI

Primary source MGI:MGI:1354695

See related Ensembl:ENSMUSG00000028086

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 1110001A17Rik, AGO, Cdc4, Fbw7, Fbwd6, Fbx30, Fbxo30, Fbxw6, SEL-10

Expression Broad expression in cortex adult (RPKM 16.6), frontal lobe adult (RPKM 13.5) and 26 other tissuesSee more

Orthologs <u>human</u> all

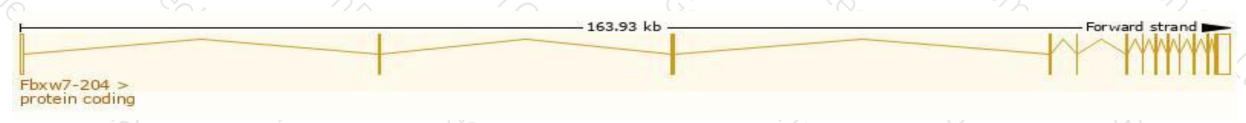
# Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

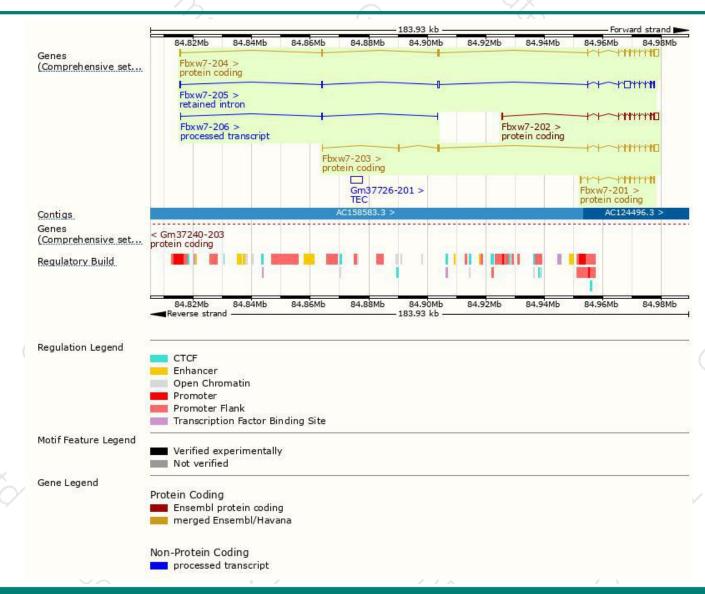
Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST00000107679.7	4473	<u>710aa</u>	Protein coding	CCDS50940	Q8VBV4	TSL:1 GENCODE basic
ENSMUST00000107678.7	4073	<u>710aa</u>	Protein coding	CCDS50940	Q8VBV4	TSL:5 GENCODE basic
ENSMUST00000029727.7	2221	<u>629aa</u>	Protein coding	CCDS17440	Q8VBV4	TSL:1 GENCODE basic APPRIS P2
ENSMUST00000107675.7	3599	<u>589aa</u>	Protein coding	120	D3YUA8	TSL:5 GENCODE basic APPRIS ALT1
ENSMUST00000212597.1	56	No protein	Processed transcript	153	1.5	TSL:5
ENSMUST00000151410.1	4348	No protein	Retained intron	691		TSL:2
	ENSMUST00000107679.7 ENSMUST00000107678.7 ENSMUST00000029727.7 ENSMUST00000107675.7 ENSMUST00000212597.1	ENSMUST00000107679.7 4473 ENSMUST00000107678.7 4073 ENSMUST00000029727.7 2221 ENSMUST00000107675.7 3599 ENSMUST00000212597.1 56	ENSMUST00000107679.7 4473 710aa  ENSMUST00000107678.7 4073 710aa  ENSMUST00000029727.7 2221 629aa  ENSMUST00000107675.7 3599 589aa  ENSMUST00000212597.1 56 No protein	ENSMUST00000107679.7         4473         710aa         Protein coding           ENSMUST00000107678.7         4073         710aa         Protein coding           ENSMUST00000029727.7         2221         629aa         Protein coding           ENSMUST00000107675.7         3599         589aa         Protein coding           ENSMUST00000212597.1         56         No protein         Processed transcript	ENSMUST00000107679.7         4473         710aa         Protein coding         CCDS50940           ENSMUST00000107678.7         4073         710aa         Protein coding         CCDS50940           ENSMUST00000029727.7         2221         629aa         Protein coding         CCDS17440           ENSMUST00000107675.7         3599         589aa         Protein coding         -           ENSMUST00000212597.1         56         No protein         Processed transcript         -	ENSMUST00000107679.7         4473         710aa         Protein coding         CCDS50940         Q8VBV4           ENSMUST00000107678.7         4073         710aa         Protein coding         CCDS50940         Q8VBV4           ENSMUST00000029727.7         2221         629aa         Protein coding         CCDS17440         Q8VBV4           ENSMUST00000107675.7         3599         589aa         Protein coding         -         D3YUA8           ENSMUST000000212597.1         56         No protein         Processed transcript         -         -

The strategy is based on the design of Fbxw7-204 transcript, The transcription is shown below



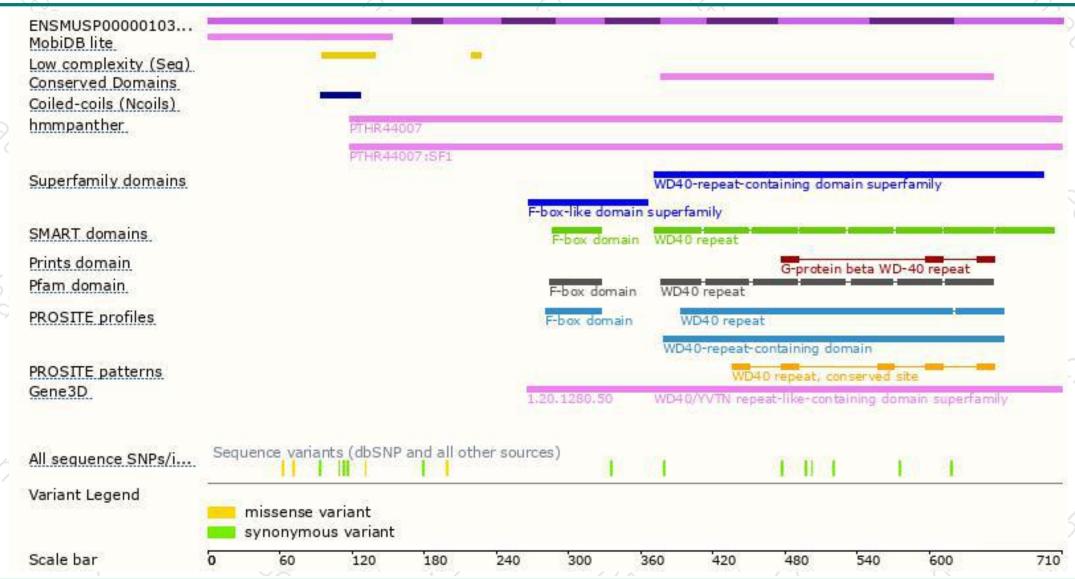
### Genomic location distribution





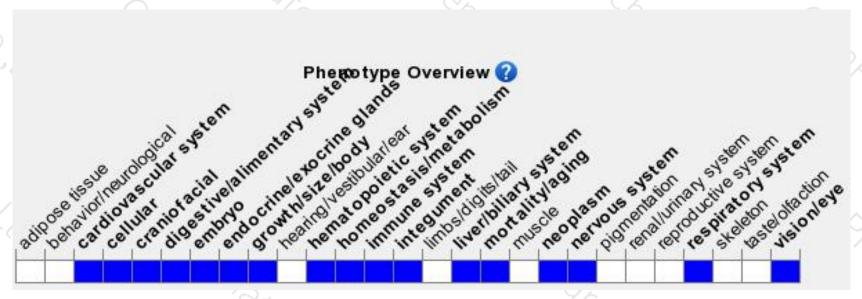
### Protein domain





# 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 inactivation of this locus disrupts embryonic and extraembryonic vasculature, resulting in death by midgestation.



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

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