

# Bend6 Cas9-CKO Strategy

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Reviewer: Daohua Xu

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## **Project Overview**



**Project Name** 

Bend6

**Project type** 

Cas9-CKO

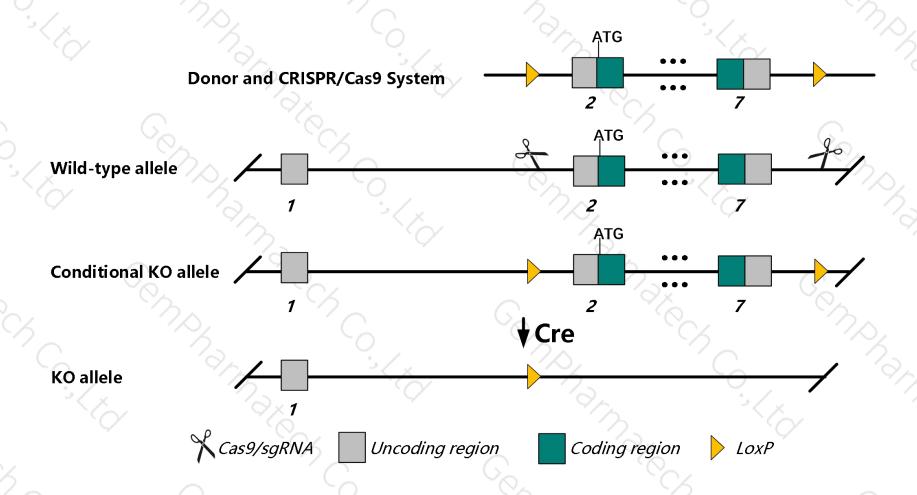
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Bend6* gene. The schematic diagram is as follows:



### Technical routes



- > The *Bend6* gene has 6 transcripts. According to the structure of *Bend6* gene, exon2-exon7 of *Bend6-201*(ENSMUST00000062289.10) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Bend6* 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**



- > The *Bend6* gene is located on the Chr1. 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)



#### Bend6 BEN domain containing 6 [Mus musculus (house mouse)]

Gene ID: 320705, updated on 13-Mar-2020

#### Summary

☆ ?

Official Symbol Bend6 provided by MGI

Official Full Name BEN domain containing 6 provided by MGI

Primary source MGI:MGI:2444572

See related Ensembl: ENSMUSG00000042182

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 B230209C24Rik

Expression Biased expression in cerebellum adult (RPKM 60.3), cortex adult (RPKM 30.9) and 4 other tissuesSee more

Orthologs <u>human all</u>

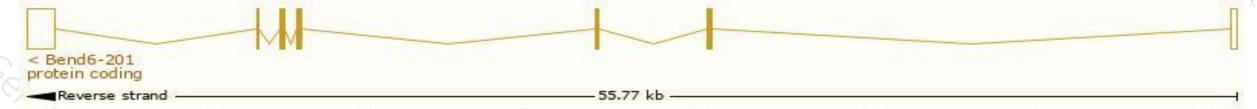
## Transcript information (Ensembl)



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

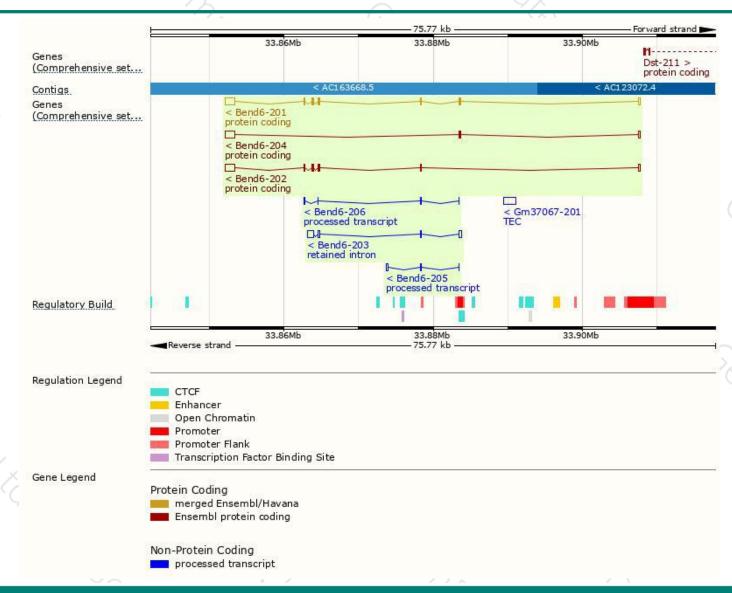
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Bend6-201	ENSMUST00000062289.10	2487	281aa	Protein coding	CCDS35533	Q6PFX2	TSL:1 GENCODE basic APPRIS P1
Bend6-202	ENSMUST00000115161.7	2231	219aa	Protein coding	CCDS78557	Q6PFX2	TSL:1 GENCODE basic
Bend6-204	ENSMUST00000129464.7	1750	<u>42aa</u>	Protein coding		A0A087WSH8	TSL:1 GENCODE basic
Bend6-205	ENSMUST00000139700.2	382	No protein	Processed transcript	-		TSL:3
Bend6-206	ENSMUST00000195350.5	349	No protein	Processed transcript	2	12	TSL:3
Bend6-203	ENSMUST00000126724.7	1580	No protein	Retained intron	-	103/	TSL:1

The strategy is based on the design of *Bend6-201* transcript, the transcription is shown below:



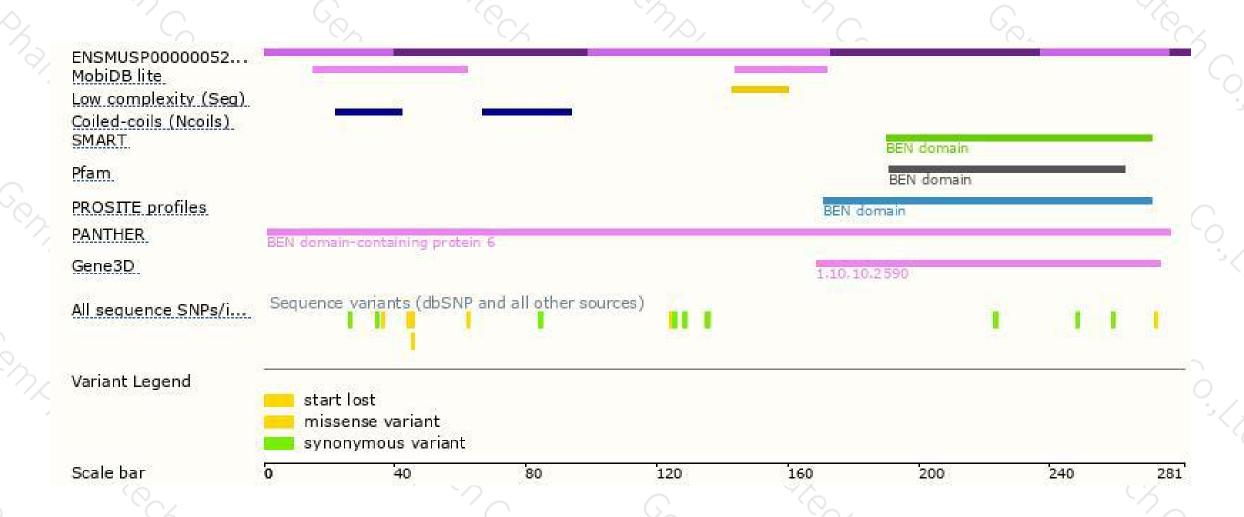
## Genomic location distribution





### Protein domain







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

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