

# Epb4113 Cas9-CKO Strategy

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**Reviewer:** 

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## **Project Overview**



**Project Name** 

**Epb4113** 

**Project type** 

Cas9-CKO

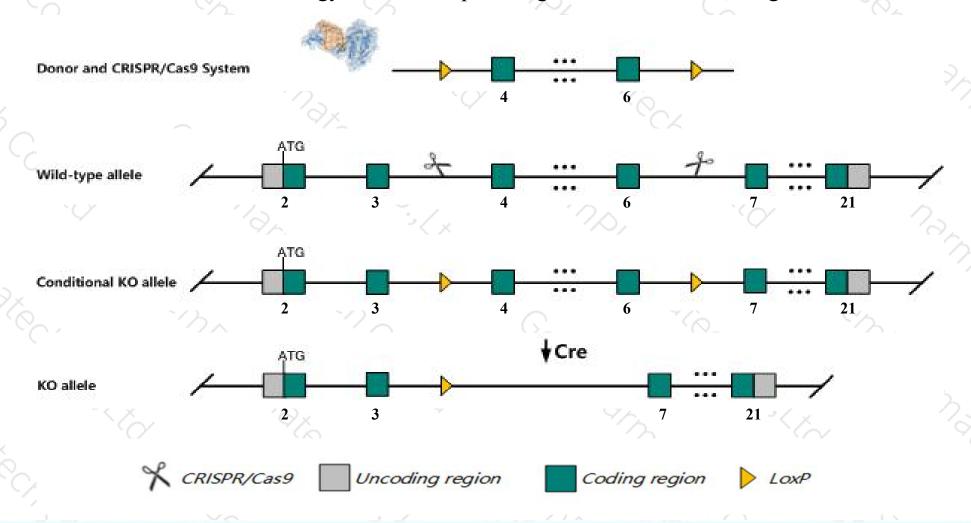
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Epb4113* gene. The schematic diagram is as follows:



### Technical routes



- The *Epb4113* gene has 18 transcripts. According to the structure of *Epb4113* gene, exon4-exon6 of *Epb4113-201* (ENSMUST00000080208.6) transcript is recommended as the knockout region. The region contains 224bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Epb41l3* 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, Mice homozygous for one knock-out allele display a normal phenotype.

  Mice homozygous for a different knock-out allele exhibit ataxia, gait abnormalities, clasping, hypermyelination, abnormal axon morphology, and decreased internode length.
- > The *Epb4113* gene is located on the Chr17. 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)



#### Epb41I3 erythrocyte membrane protein band 4.1 like 3 [ Mus musculus (house mouse) ]

Gene ID: 13823, updated on 14-Aug-2019

#### Summary



Official Symbol Epb41I3 provided by MGI

Official Full Name erythrocyte membrane protein band 4.1 like 3 provided by MGI

Primary source MGI:MGI:103008

See related Ensembl: ENSMUSG00000024044

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 4.1B; Dal1; NBL3; DAL1P; Epb4.1l3

Expression Broad expression in cerebellum adult (RPKM 14.3), frontal lobe adult (RPKM 11.1) and 15 other tissues See more

Orthologs <u>human</u> all

## Transcript information (Ensembl)



The gene has 18 transcripts, all transcripts are shown below:

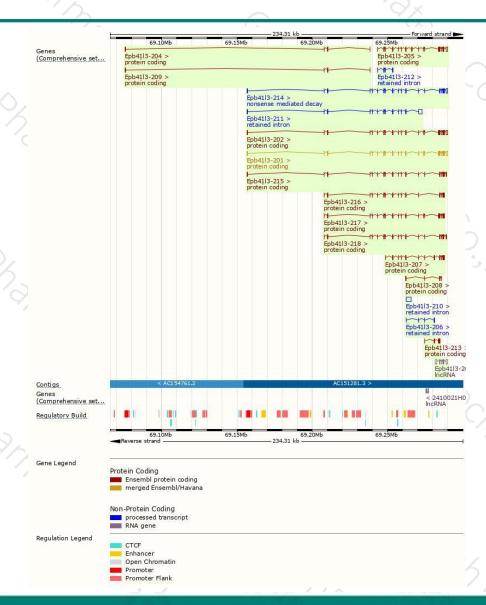
Name	Transcript ID	bp 🛊	Protein	Biotype	CCDS	UniProt	Flags
Epb41I3-201	ENSMUST00000080208.6	4047	929aa	Protein coding	CCDS28952@	Q9WV92 €	TSL:1 GENCODE basic APPRIS P2
Epb41I3-202	ENSMUST00000112680.7	4073	939aa	Protein coding	170	Q9WV92 €	TSL:5 GENCODE basic APPRIS ALT2
Epb4113-205	ENSMUST00000223703.1	3687	852aa	Protein coding	929	A0A286YDY4@	CDS 5' incomplete
Epb4113-215	ENSMUST00000225977.1	3188	876aa	Protein coding	878	<u>A7YY80</u> ₽	GENCODE basic APPRIS ALT2
Epb41I3-217	ENSMUST00000233509.1	2736	<u>911aa</u>	Protein coding	928	D0VYV6@	GENCODE basic   APPRIS ALT2
Epb4113-218	ENSMUST00000233964.1	2703	819aa	Protein coding	878	A0A3B2WBE1₽	CDS 5' incomplete
Epb4113-216	ENSMUST00000232706.1	2439	812aa	Protein coding	929	A0A3B2WD96₽	GENCODE basic APPRIS ALT2
Epb4113-207	ENSMUST00000224523.1	1817	605aa	Protein coding	17.1	A0A286YD84 ₪	CDS 5' incomplete
Epb4113-208	ENSMUST00000224951.1	786	262aa	Protein coding	\$ <b>2</b> 8	A0A286YCY1@	CDS 5' and 3' incomplete
Epb4113-213	ENSMUST00000225695.1	670	223aa	Protein coding	878	A0A286YCT6₽	CDS 5' and 3' incomplete
Epb4113-209	ENSMUST00000225062.1	666	29aa	Protein coding	228	A0A286YD94 €	CDS 3' incomplete
Epb4113-204	ENSMUST00000223610.1	559	<u>53aa</u>	Protein coding	878	A0A286YDD0 €	CDS 3' incomplete
Epb4113-214	ENSMUST00000225740.1	3952	287aa	Nonsense mediated decay	928	A0A286YCY8₽	
Epb4113-211	ENSMUST00000225316.1	3966	No protein	Retained intron	878	<del>-</del>	<u> </u>
Epb41I3-210	ENSMUST00000225209.1	3219	No protein	Retained intron	928	20	- E
Epb41I3-212	ENSMUST00000225604.1	647	No protein	Retained intron	878		8
Epb41I3-206	ENSMUST00000223816.1	471	No protein	Retained intron	948	2	· 20
Epb4113-203	ENSMUST00000223565.1	3026	No protein	IncRNA	17.0	-	5

The strategy is based on the design of *Epb4113-201* transcript, The transcription is shown below



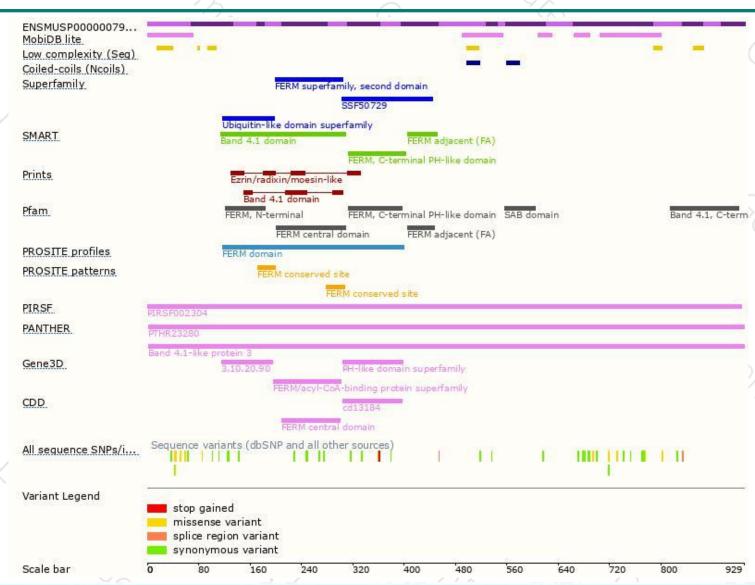
## Genomic location distribution





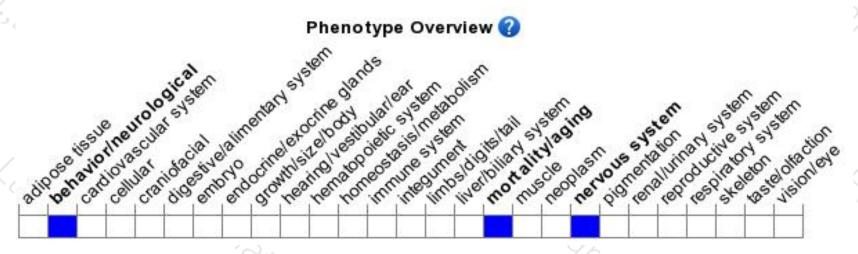
### Protein domain





## 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, Mice homozygous for one knock-out allele display a normal phenotype. Mice homozygou for a different knock-out allele exhibit ataxia, gait abnormalities, clasping, hypermyelination, abnormal axon morphology, and decreased internode length.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





