



Epb4ll4a Cas9-CKO Strategy

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Reviewer: Daohua Xu

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Project Overview

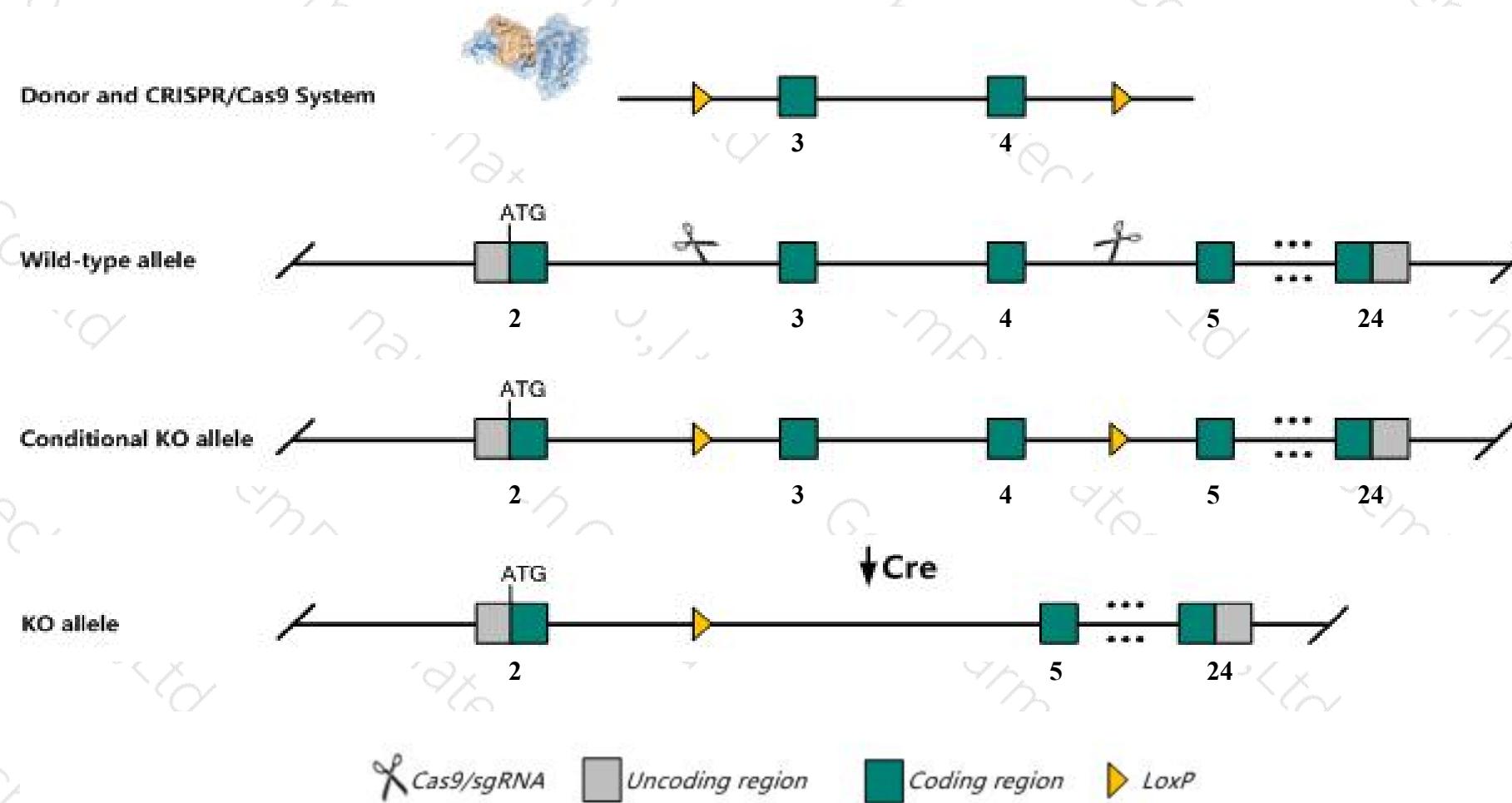
Project Name***Epb41l4a***

Project type**Cas9-CKO**

Strain background**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Epb41l4a* gene. The schematic diagram is as follows:



Technical routes

- The *Epb41l4a* gene has 7 transcripts. According to the structure of *Epb41l4a* gene, exon3-exon4 of *Epb41l4a-201*(ENSMUST00000025234.6) transcript is recommended as the knockout region. The region contains 157bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Epb41l4a* 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.



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Notice

- The *Epb4ll4a* gene is located on the Chr18. 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.
- Transcript *Epb4ll4a-203&205&206&207* may not be affected.
- 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)

Epb41l4a erythrocyte membrane protein band 4.1 like 4a [Mus musculus (house mouse)]

Gene ID: 13824, updated on 13-Mar-2020

Summary



Official Symbol Epb41l4a provided by [MGI](#)

Official Full Name erythrocyte membrane protein band 4.1 like 4a provided by [MGI](#)

Primary source [MGI:MGI:103007](#)

See related [Ensembl:ENSMUSG00000024376](#)

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 Epb4.1l4, Epb4.1l4a, NBL4

Expression Broad expression in CNS E11.5 (RPKM 2.9), whole brain E14.5 (RPKM 2.8) and 23 other tissues [See more](#)

Orthologs [human](#) [all](#)

Transcript information (Ensembl)

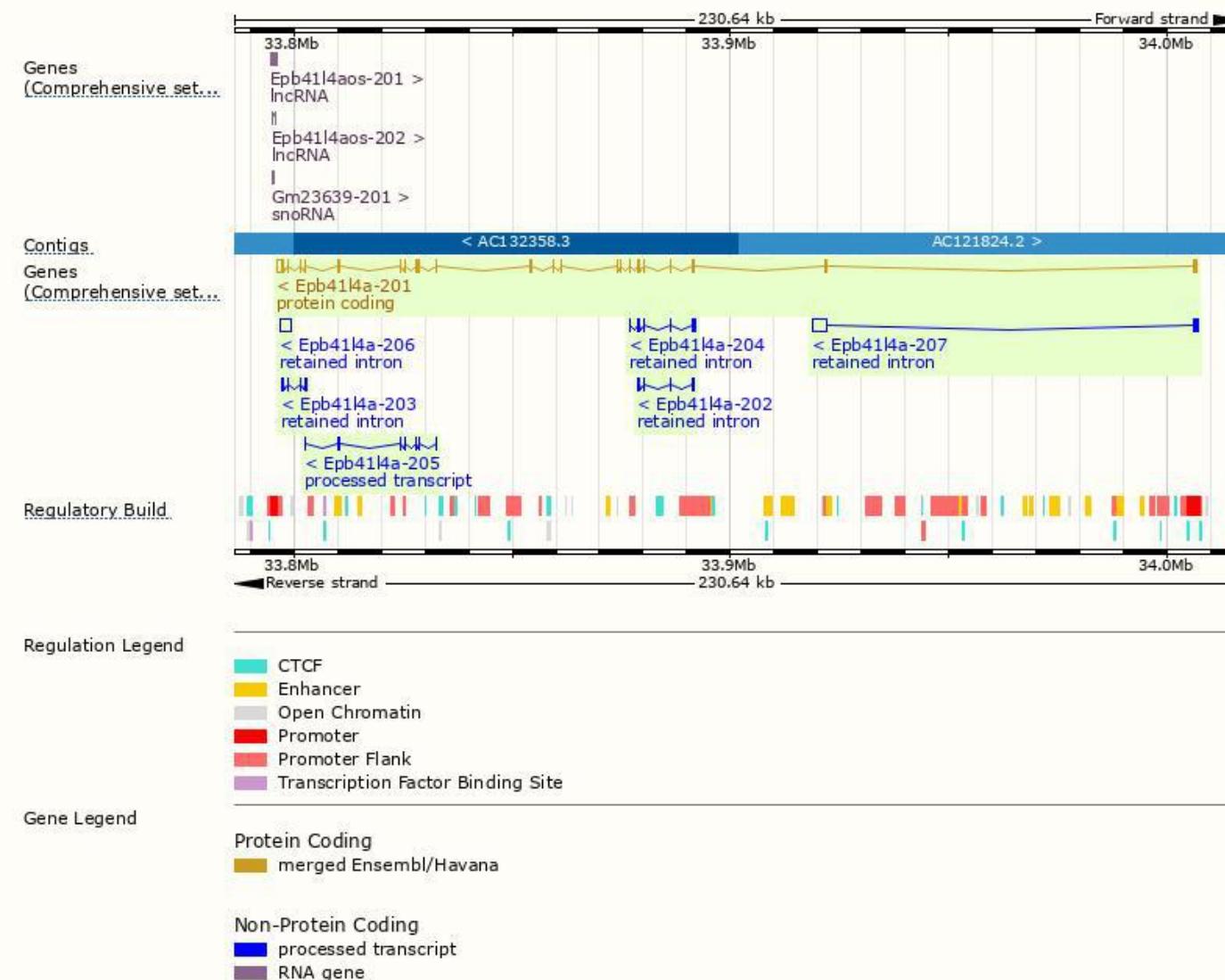
The gene has 7 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Epb41l4a-201	ENSMUST0000025234_6	3566	686aa	Protein coding	CCDS29124	P52963	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
Epb41l4a-205	ENSMUST00000236514_1	529	No protein	Processed transcript	-	-	
Epb41l4a-207	ENSMUST00000237971_1	3777	No protein	Retained intron	-	-	
Epb41l4a-206	ENSMUST00000236716_1	2286	No protein	Retained intron	-	-	
Epb41l4a-203	ENSMUST00000235393_1	928	No protein	Retained intron	-	-	
Epb41l4a-204	ENSMUST00000235876_1	540	No protein	Retained intron	-	-	
Epb41l4a-202	ENSMUST00000235242_1	487	No protein	Retained intron	-	-	

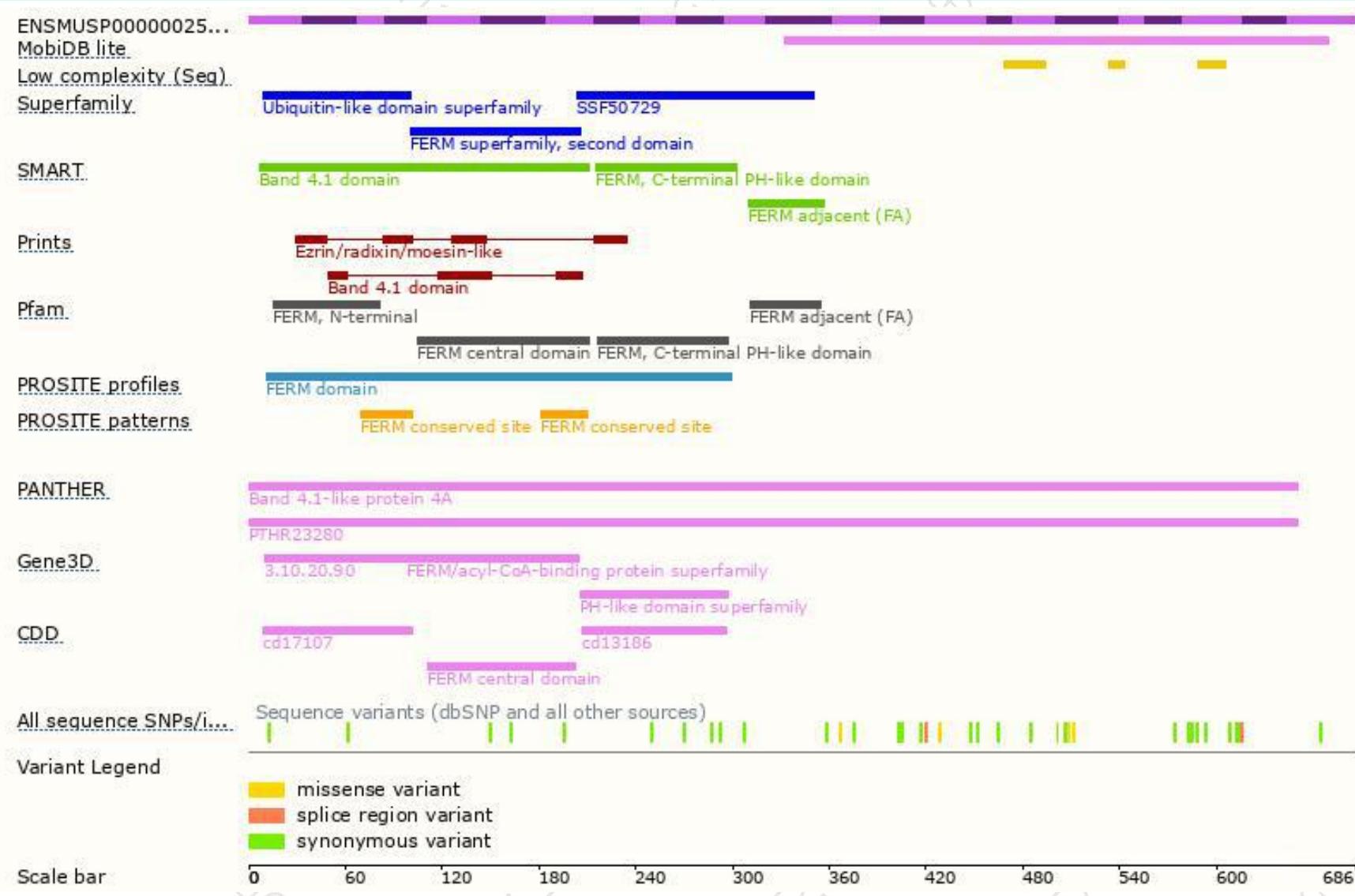
The strategy is based on the design of *Epb41l4a-201* transcript, the transcription is shown below:



Genomic location distribution



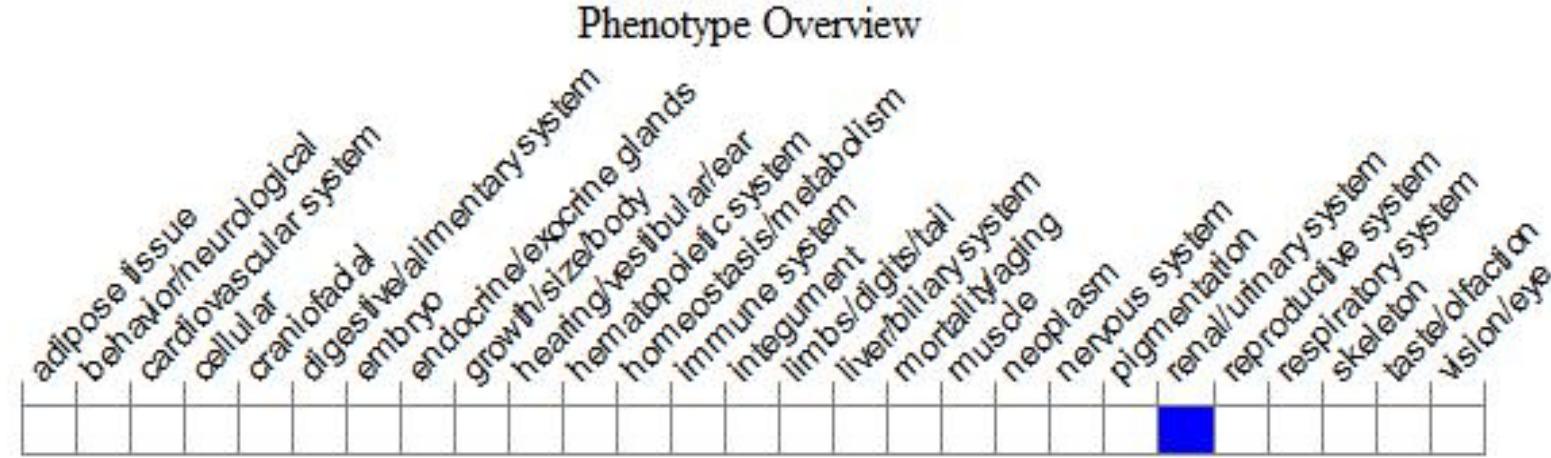
Protein domain





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Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).



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

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