

Epor Cas9-CKO Strategy

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Reviewer: Ruirui Zhang

Design Date: 2021-4-12

Project Overview



Project Name Epor

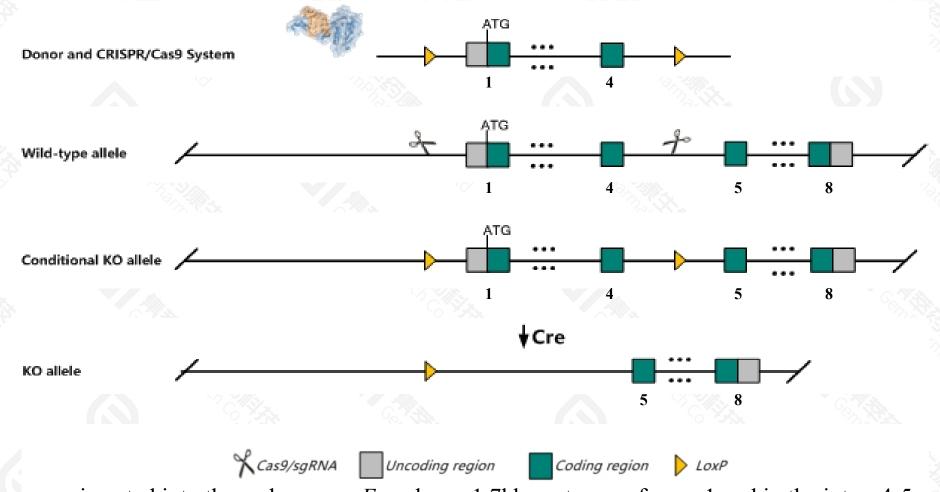
Project type Cas9-CKO

Strain background C57BL/6JGpt

Conditional Knockout strategy



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



LoxP sequences were inserted into the endogenous *Epor* locus 1.7kb upstream of exon 1 and in the intron4-5 near the EcoRV.

Technical routes



- ➤ The *Epor* gene has 2 transcripts. According to the structure of *Epor* gene, exon1-exon4 of *Epor-201*(ENSMUST00000006397.7) transcript is recommended as the knockout region. The region contains start codon ATG. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Epor* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor 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, mutations in this locus affect erythropoiesis. Targeted null mutants die at embryonic day 11-12.5 with severe anemia. Mutants with truncated alleles are viable with mild changes in erythropoiesis. A human mutation replacement allele produces polycythemia.
- > The *Epor* gene is located on the Chr9. 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.

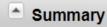
Gene information (NCBI)



Epor erythropoietin receptor [Mus musculus (house mouse)]

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Gene ID: 13857, updated on 29-Mar-2021



☆ ?

Official Symbol Epor provided by MGI

Official Full Name erythropoietin receptor provided by MGI

Primary source MGI:MGI:95408

See related Ensembl:ENSMUSG00000006235

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

Expression Biased expression in liver E14.5 (RPKM 73.4), liver E14 (RPKM 57.8) and 5 other tissues See more

Orthologs human all

NEW

Try the new Gene table

Try the new Transcript table

Transcript information (Ensembl)



The gene has 2 transcripts, all transcripts are shown below:

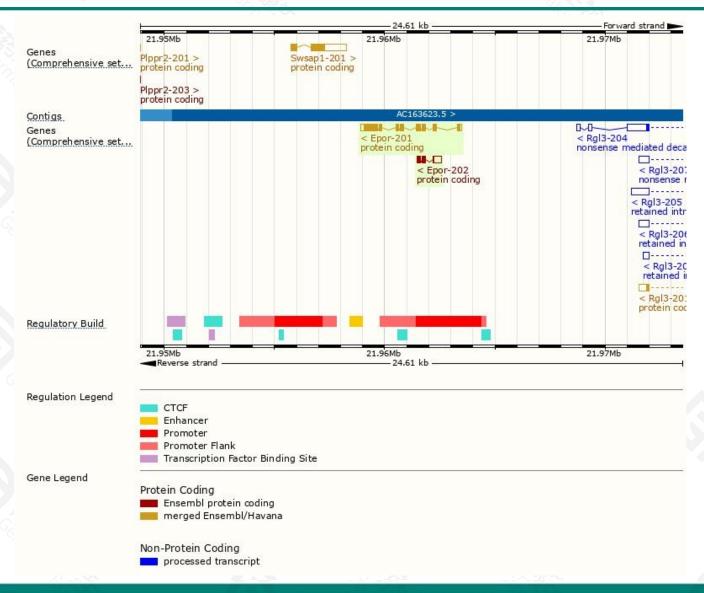
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Epor-201	ENSMUST00000006397.7	1761	<u>507aa</u>	Protein coding	CCDS22915		TSL:1 , GENCODE basic , APPRIS P1 ,
Epor-202	ENSMUST00000213181.2	659	<u>111aa</u>	Protein coding	-		CDS 3' incomplete , TSL:2 ,

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



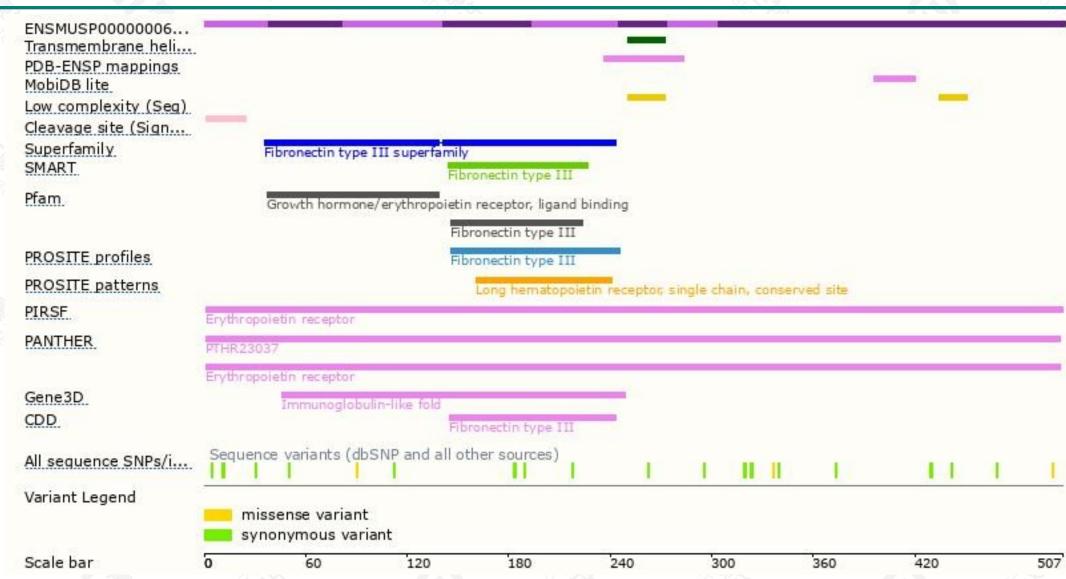
Genomic location distribution





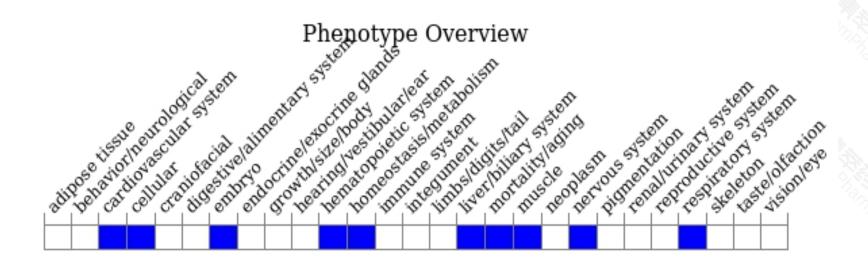
Protein domain





Mouse phenotype description(MGI)



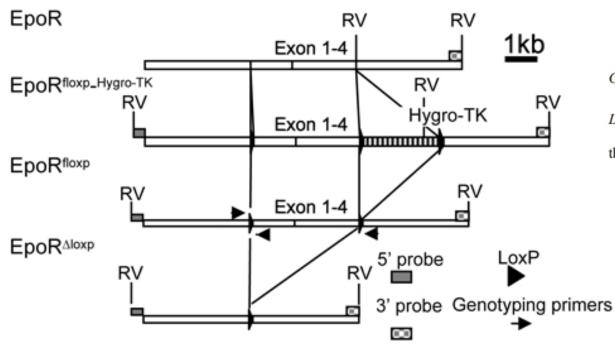


Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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Reference





Generation of hGFAP-Cre; EpoR^{floxp/floxp} and hGFAP-Cre; EpoR^{floxp/-} mice.

LoxP sequences were inserted into the endogenous EpoR locus 1.7kb upstream of exon 1 and in the intron 4 to generate pEpoR-floxHyTK targeting construct. pEpoR-floxHyTK was

Tsai PT, Ohab JJ, N Kertesz, et al. A Critical Role of Erythropoietin Receptor in Neurogenesis and Post-Stroke Recovery[J]. JOURNAL OF NEUROSCIENCE, 2006.



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

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