Ephb2 Cas9-CKO Strategy

Designer: Jinling Wang

Reviewer: Rui Xiong

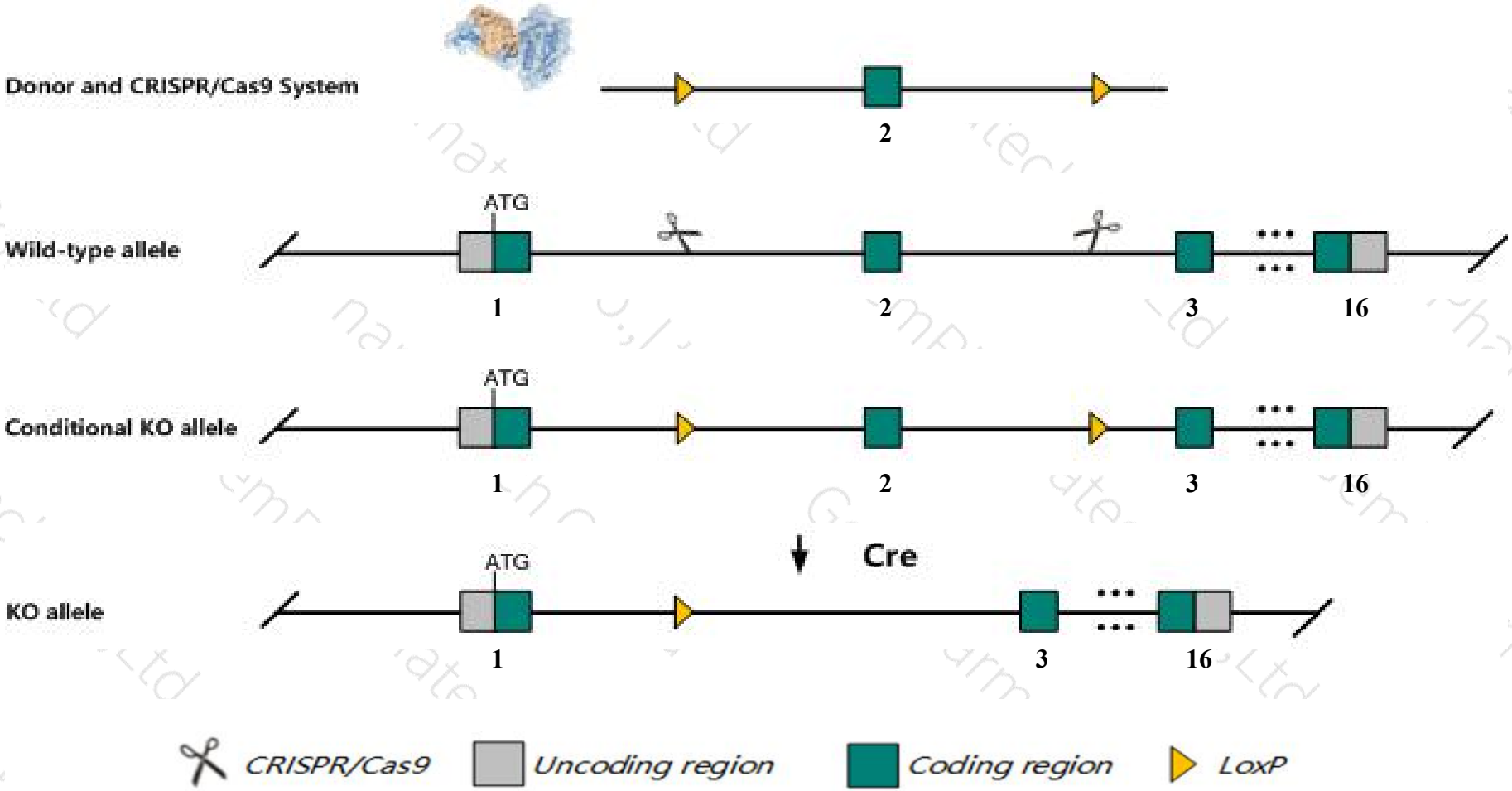
Design Date: 2018-11-16

Project Overview

Project Name	***Ephb2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ephb2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ephb2* gene has 6 transcripts. According to the structure of *Ephb2* gene, exon2 of *Ephb2-201*(ENSMUST00000059287.13) transcript is recommended as the knockout region.The region contains 65bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ephb2* 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 a null allele exhibit abnormal axon guidance, circling, head bobbing, and hyperactivity.

➢ The *Ephb2* gene is located on the Chr4. 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）

集萃药康 GemPharmatech

Ephb2 Eph receptor B2 [Mus musculus (house mouse)]

Gene ID: 13844, updated on 13-Mar-2020

Summary

Official Symbol	Ephb2 provided by MGI
Official Full Name	Eph receptor B2 provided byMGI
Primary source	MGI:MGI:99611
See related	Ensembl:ENSMUSG00000028664
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Cek5, Drt, ETECK, Erk, Hek5, Nuk, Prkm5, Qek5, Sek3, Tyro5
Summary	This gene encodes a member of the Eph receptor family of receptor tyrosine kinase transmembrane glycoproteins. These receptors consist of an N-terminal glycosylated ligand-binding domain, a transmembrane region and an intracellular kinase domain. The encoded receptor preferentially binds membrane-bound ephrin-B ligands and is involved in nervous system and vascular development. This gene is used as a marker of intestinal stem cells. Homozygous knockout mice for this gene exhibit impaired axon guidance and vestibular function. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Aug 2015]
Expression	Broad expression in whole brain E14.5 (RPKM 18.7), CNS E14 (RPKM 13.6) and 18 other tissuesSee more
Orthologs	human all

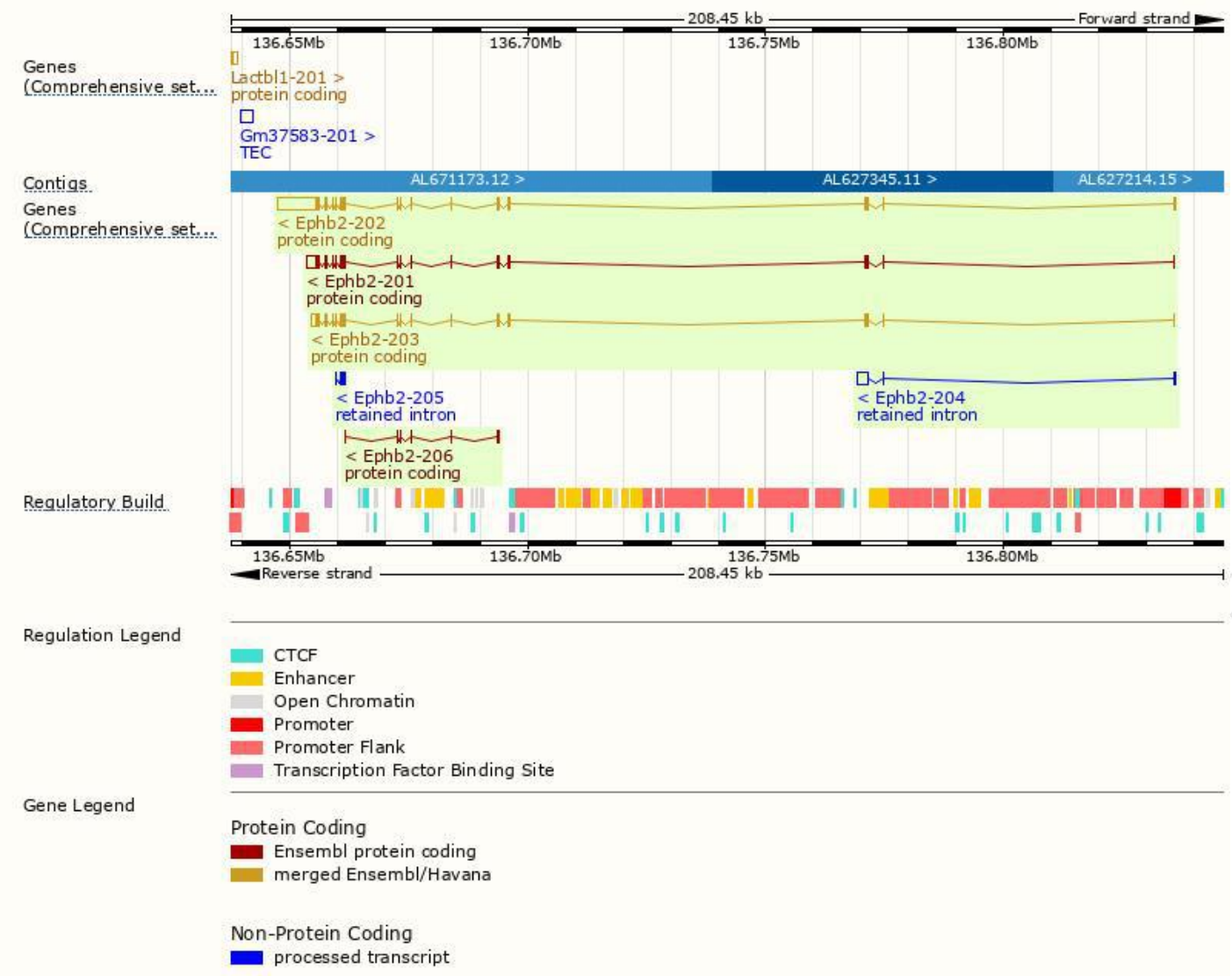
Transcript information（Ensembl）

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

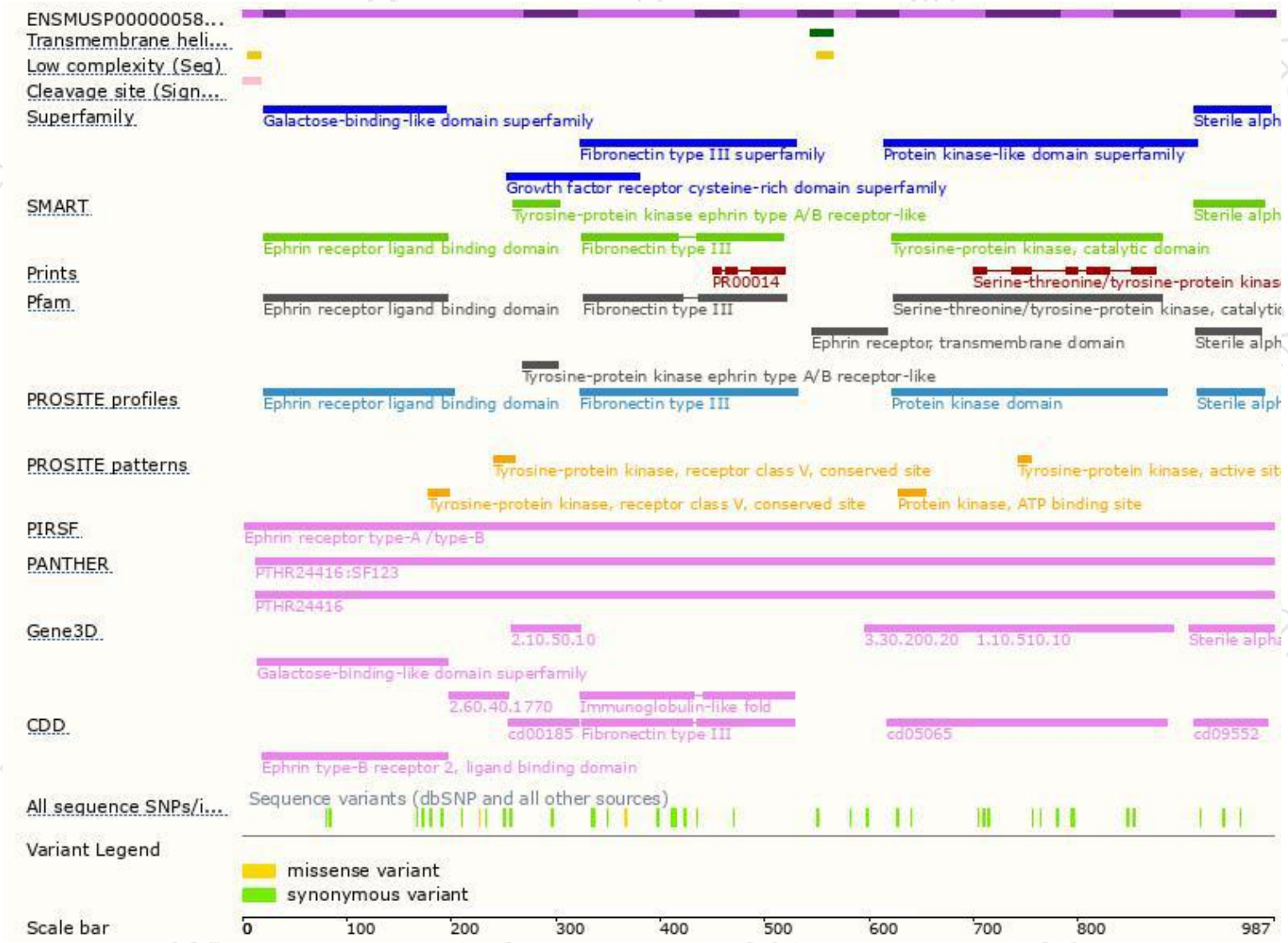
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ephb2-202	ENSMUST00000105845.8	10902	986aa	Protein coding	CCDS18809	P54763	TSL:1 GENCODE basic APPRIS P2
Ephb2-201	ENSMUST00000059287.13	4804	987aa	Protein coding	CCDS71498	P54763	TSL:2 GENCODE basic
Ephb2-203	ENSMUST00000105846.8	3653	987aa	Protein coding	-	P54763	TSL:5 GENCODE basic APPRIS ALT1
Ephb2-206	ENSMUST00000156558.1	828	276aa	Protein coding	-	F6Q4H2	CDS 5' and 3' incomplete TSL:3
Ephb2-204	ENSMUST00000144573.1	2519	No protein	Retained intron	-	-	TSL:1
Ephb2-205	ENSMUST00000151502.1	599	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Ephb2-201* transcript,the transcription is shown below:

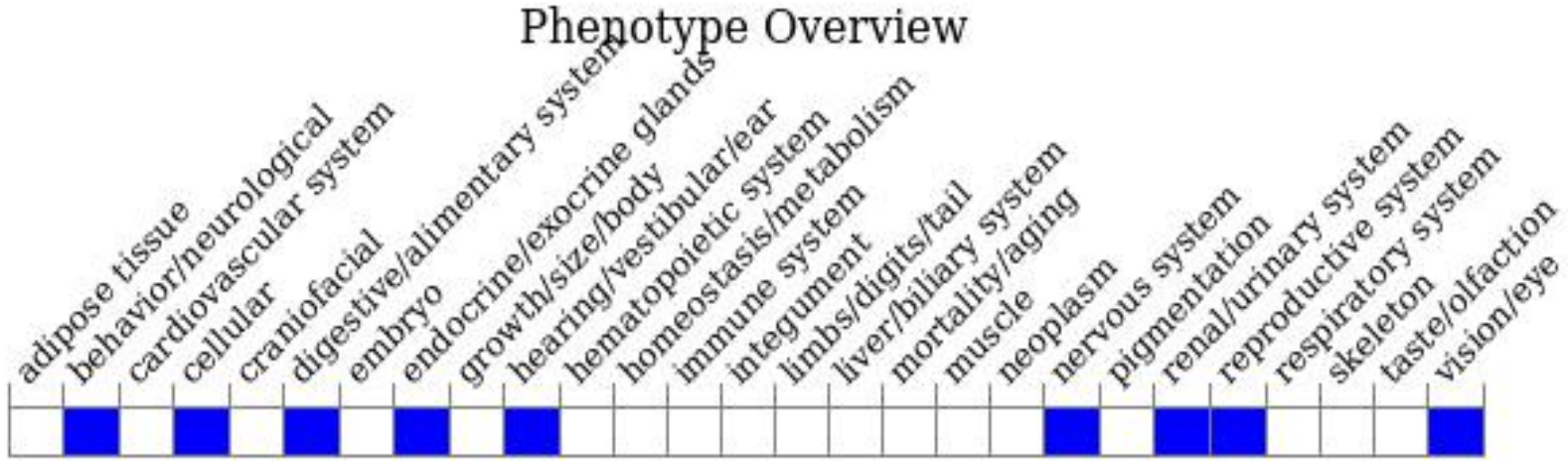
Genomic location distribution



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 a null allele exhibit abnormal axon guidance, circling, head bobbing, and hyperactivity.

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd