Epha4 Cas9-CKO Strategy

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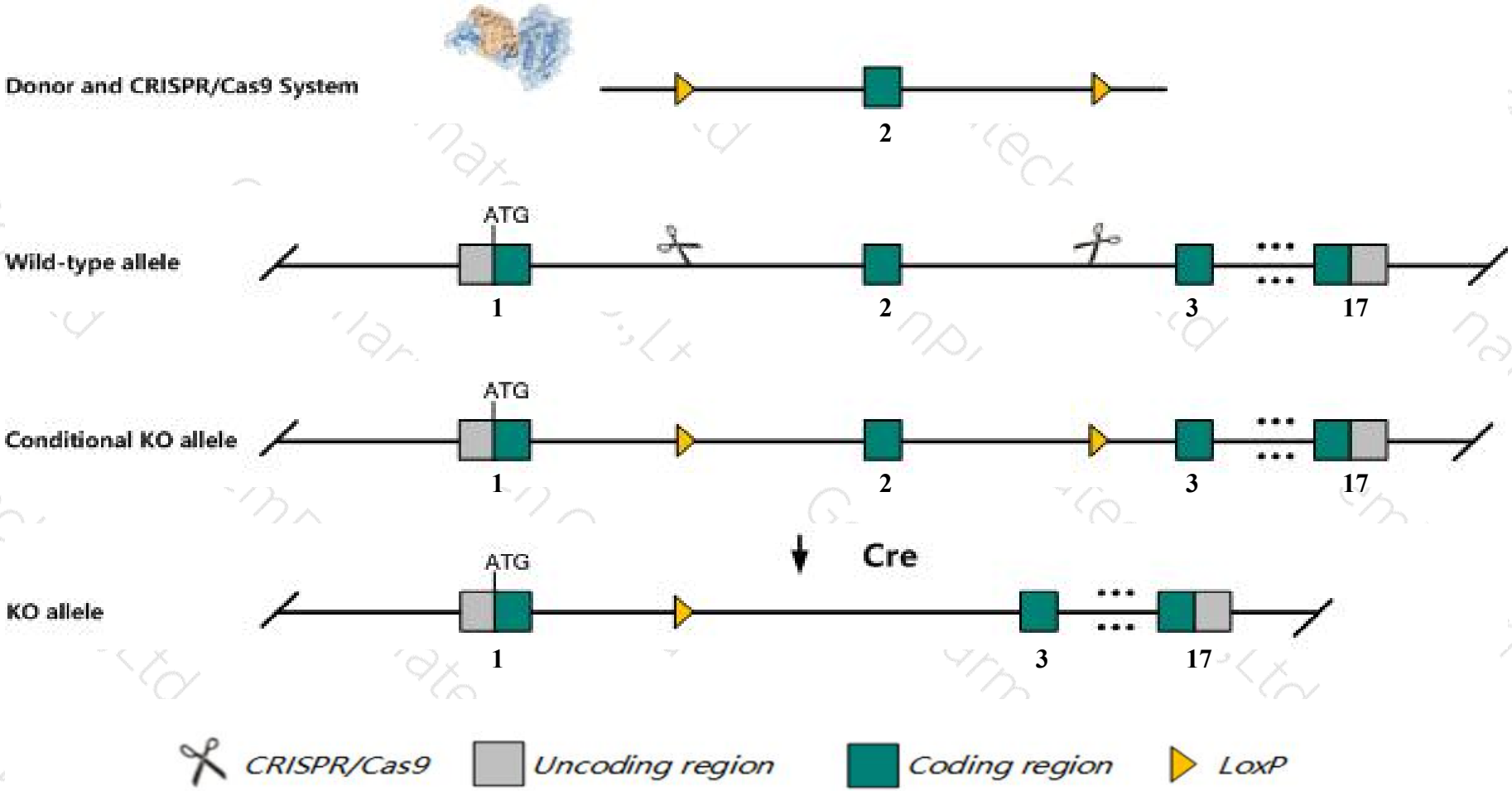
Project Overview

Project Name	***Epha4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Epha4* gene. The schematic diagram is as follows:



Technical routes

- The *Epha4* gene has 7 transcripts. According to the structure of *Epha4* gene, exon2 of *Epha4-201* (ENSMUST00000027451.12) transcript is recommended as the knockout region.The region contains 68bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Epha4* 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,Mutants are known for their "hopping gait". Homozygotes for targeted null mutations show loss of limb alternation in locomotion and axon guidance defects of the corticospinal tract within medulla and spinal cord, resulting in aberrant midline projections. Heterozygotes show less severe phenotype.
- Transcript *Epha4-202，203* may not be affected.
- The *Epha4* 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）

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Epha4 Eph receptor A4 [Mus musculus (house mouse)]

Gene ID: 13838, updated on 12-Mar-2019

Summary

Official Symbol	Epha4 provided by MGI
Official Full Name	Eph receptor A4 provided byMGI
Primary source	MGI:MGI:98277
See related	Ensembl:ENSMUSG00000026235
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	2900005C20Rik, AI385584, Cek8, Hek8, Sek, Sek1, Tyro1, rb
Expression	Biased expression in cortex adult (RPKM 17.3), whole brain E14.5 (RPKM 14.7) and 13 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

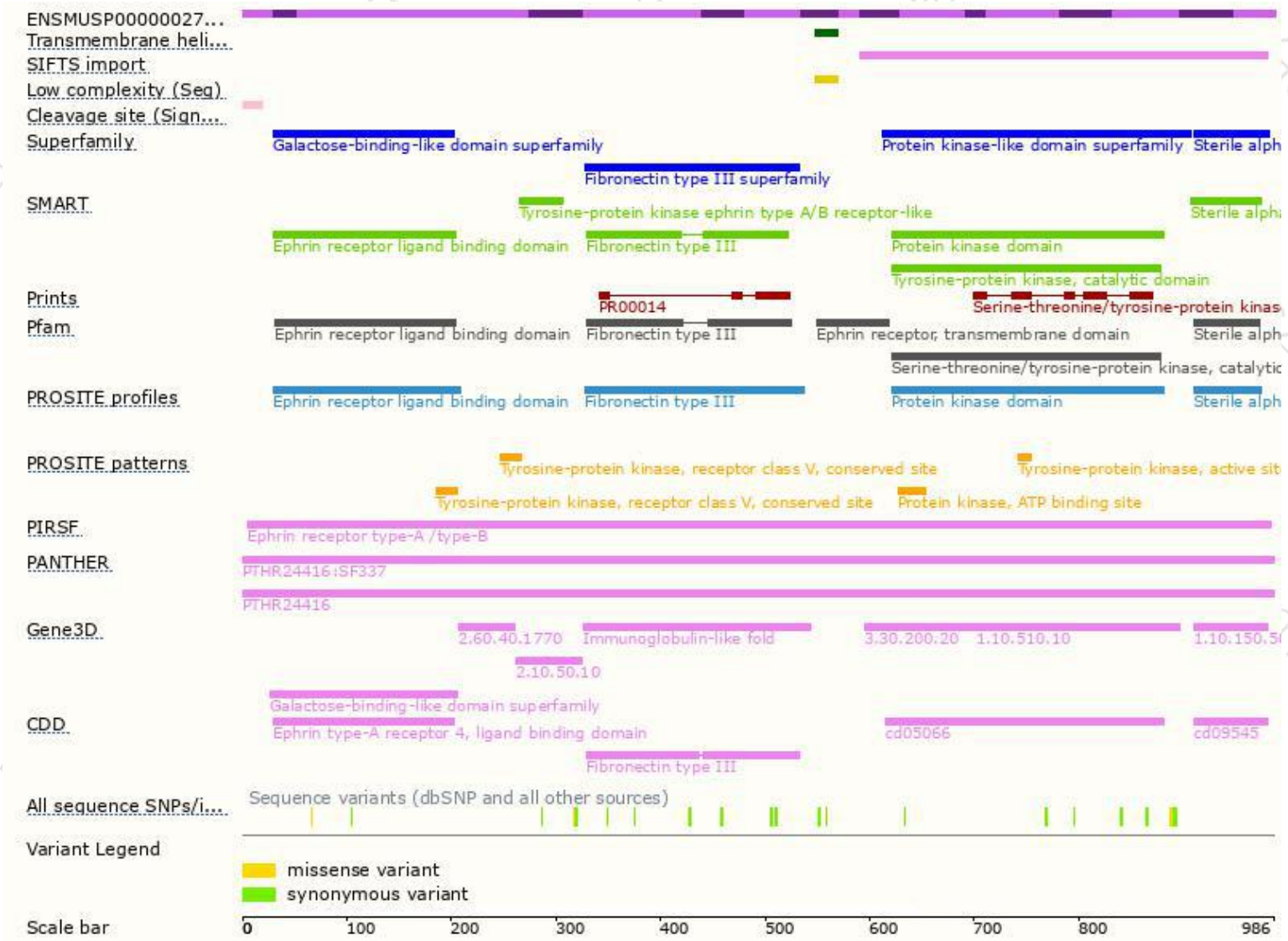
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Epha4-201	ENSMUST00000027451.12	6328	986aa	Protein coding	CCDS35627	Q03137	TSL:1 GENCODE basic APPRIS P1
Epha4-204	ENSMUST00000188797.6	3612	986aa	Protein coding	CCDS35627	Q03137	TSL:1 GENCODE basic APPRIS P1
Epha4-203	ENSMUST00000187346.1	614	117aa	Protein coding	-	A0A087WRH4	CDS 5' incomplete TSL:3
Epha4-202	ENSMUST00000186930.1	584	177aa	Protein coding	-	A0A087WQW6	CDS 3' incomplete TSL:5
Epha4-205	ENSMUST00000188952.6	6315	986aa	Nonsense mediated decay	CCDS35627	Q03137	TSL:1
Epha4-207	ENSMUST00000190149.6	3043	38aa	Nonsense mediated decay	-	A0A087WQZ6	TSL:5
Epha4-206	ENSMUST00000189934.1	3466	No protein	Retained intron	-	-	TSL:1

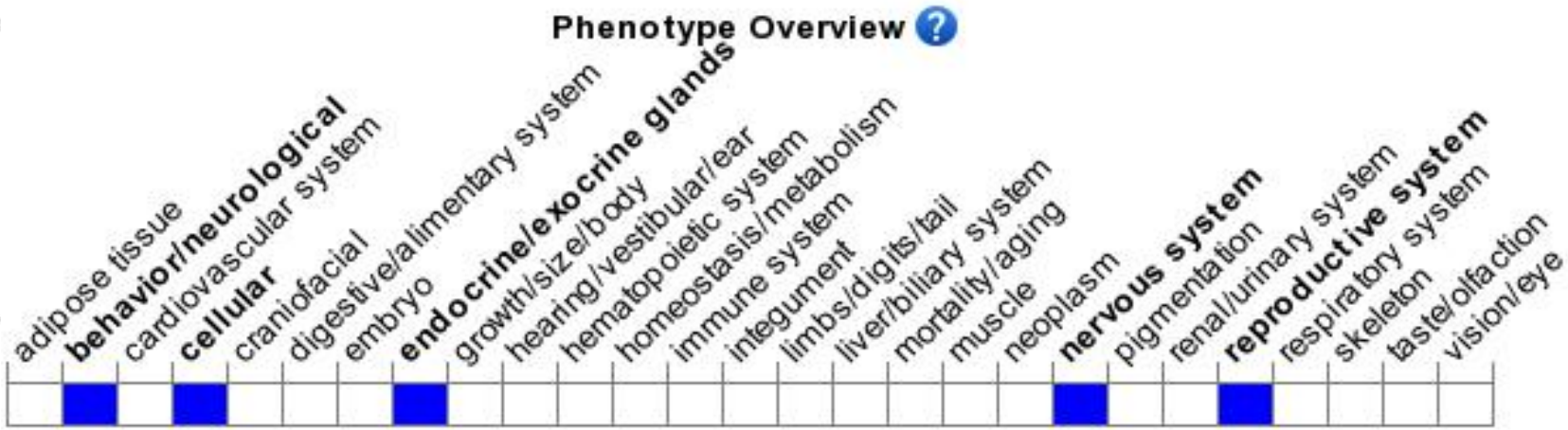
The strategy is based on the design of *Epha4-201* transcript,The transcription is shown below

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

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