Phactr4 Cas9-KO Strategy

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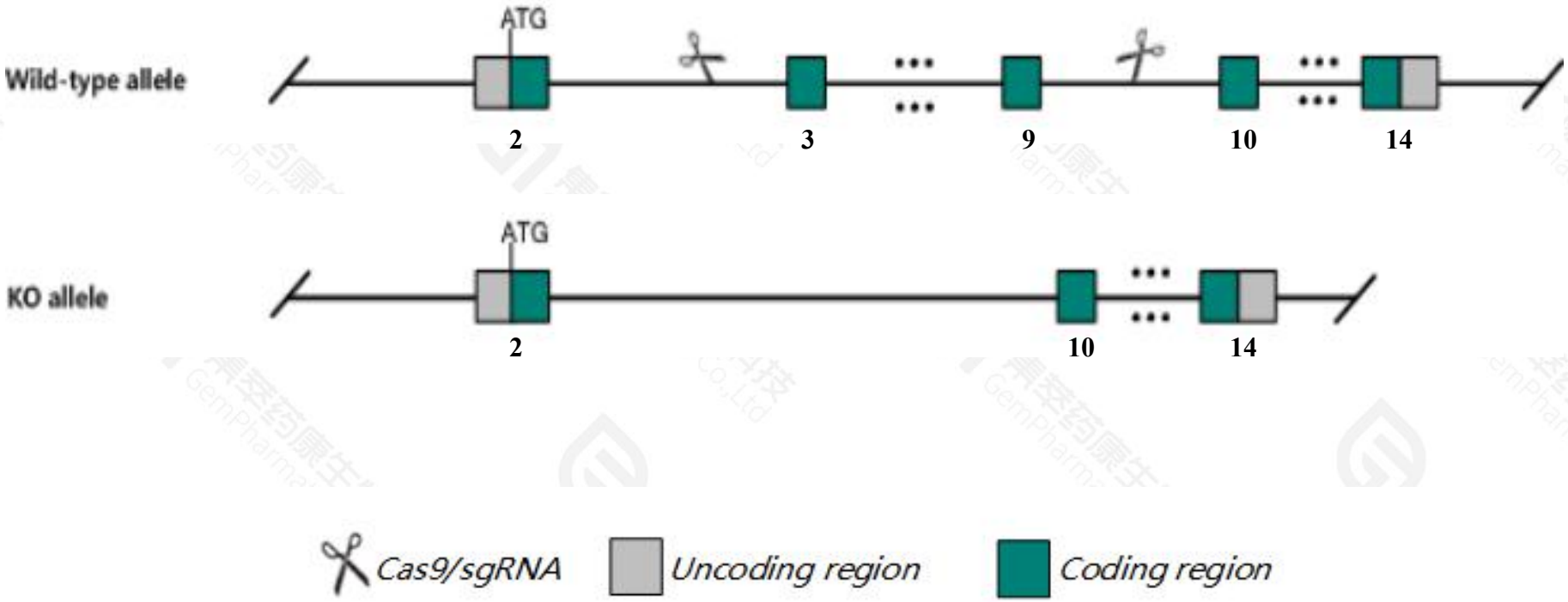
Project Overview

Project Name	***Phactr4***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Phactr4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Phactr4* gene has 6 transcripts. According to the structure of *Phactr4* gene, exon3-exon9 of *Phactr4-203*(ENSMUST00000102568.9) transcript is recommended as the knockout region.The region contains 1720bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Phactr4* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

➢ According to the existing MGI data,mice homozygous for a null allele exhibit embryonic and neonatal lethality, exencephaly, neural tube defects, coloboma, and altered cell cycles.

➢ The *Phactr4* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

集萃药康 GemPharmatech

Phactr4 phosphatase and actin regulator 4 [Mus musculus (house mouse)]

Gene ID: 100169, updated on 13-Mar-2020

Summary

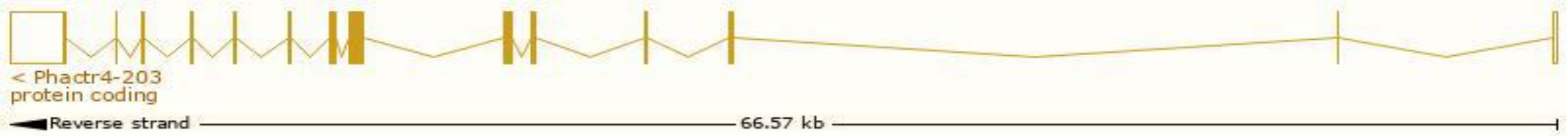
Official Symbol	Phactr4 provided by MGI
Official Full Name	phosphatase and actin regulator 4 provided by MGI
Primary source	MGI:MGI:2140327
See related	Ensembl:ENSMUSG00000066043
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	3110001B12Rik, AI527228, AW495572, C330013F19Rik, N28169, mKIAA4120
Expression	Ubiquitous expression in CNS E11.5 (RPKM 6.0), limb E14.5 (RPKM 5.9) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

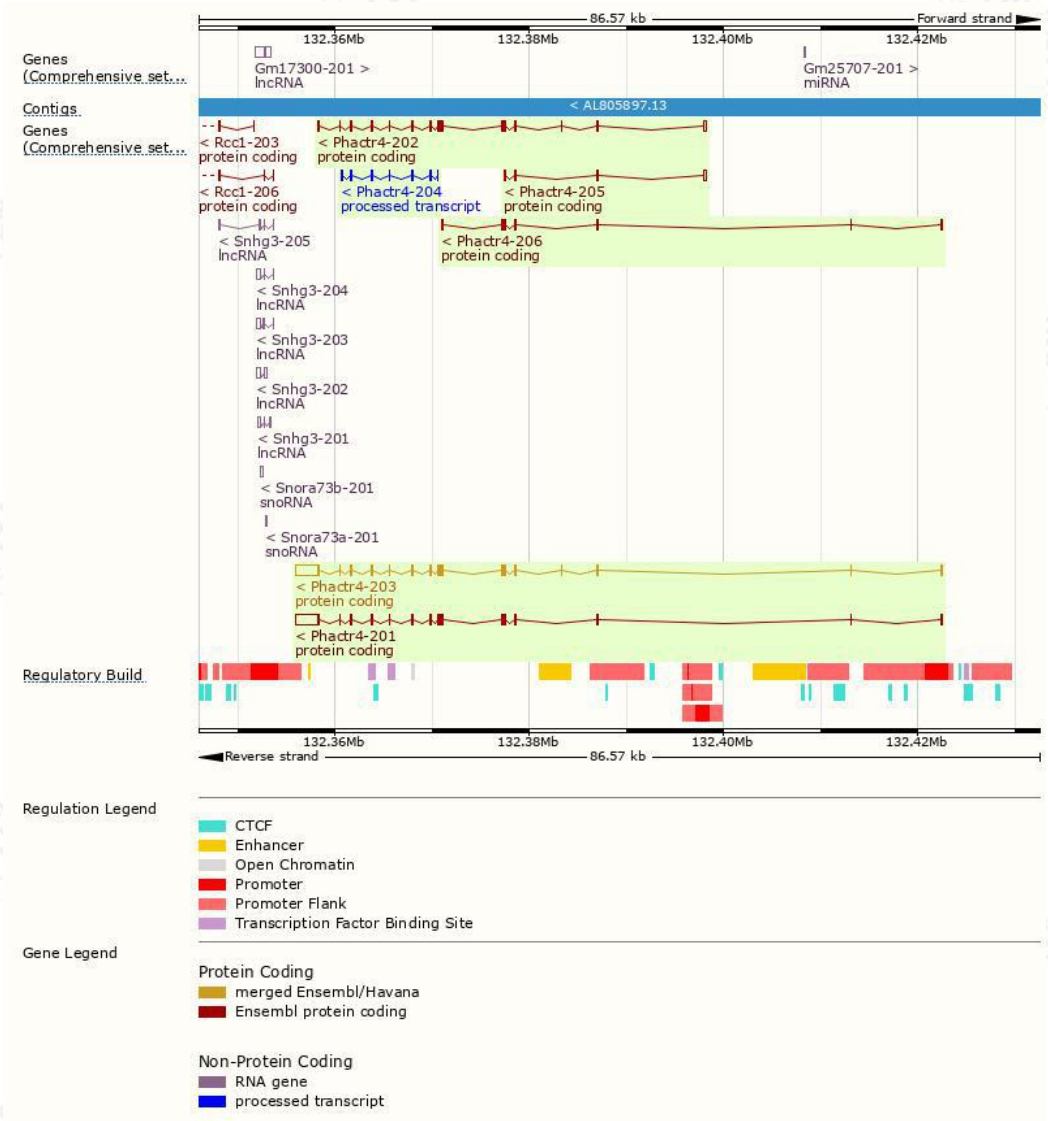
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Phactr4-203	ENSMUST00000102568.9	4614	694aa	Protein coding	CCDS18724	Q501J7	TSL:1 GENCODE basic APPRIS P2
Phactr4-201	ENSMUST00000084170.11	4483	667aa	Protein coding	CCDS51318	Q501J7	TSL:1 GENCODE basic
Phactr4-202	ENSMUST00000084249.10	2349	704aa	Protein coding	-	Q501J7	TSL:5 GENCODE basic APPRIS ALT2
Phactr4-206	ENSMUST00000152271.7	1029	298aa	Protein coding	-	A0A0A0MQJ4	CDS 3' incomplete TSL:5
Phactr4-205	ENSMUST00000136711.1	675	157aa	Protein coding	-	A0A0A0MQL5	CDS 3' incomplete TSL:3
Phactr4-204	ENSMUST00000127516.1	684	No protein	Processed transcript	-	-	TSL:5

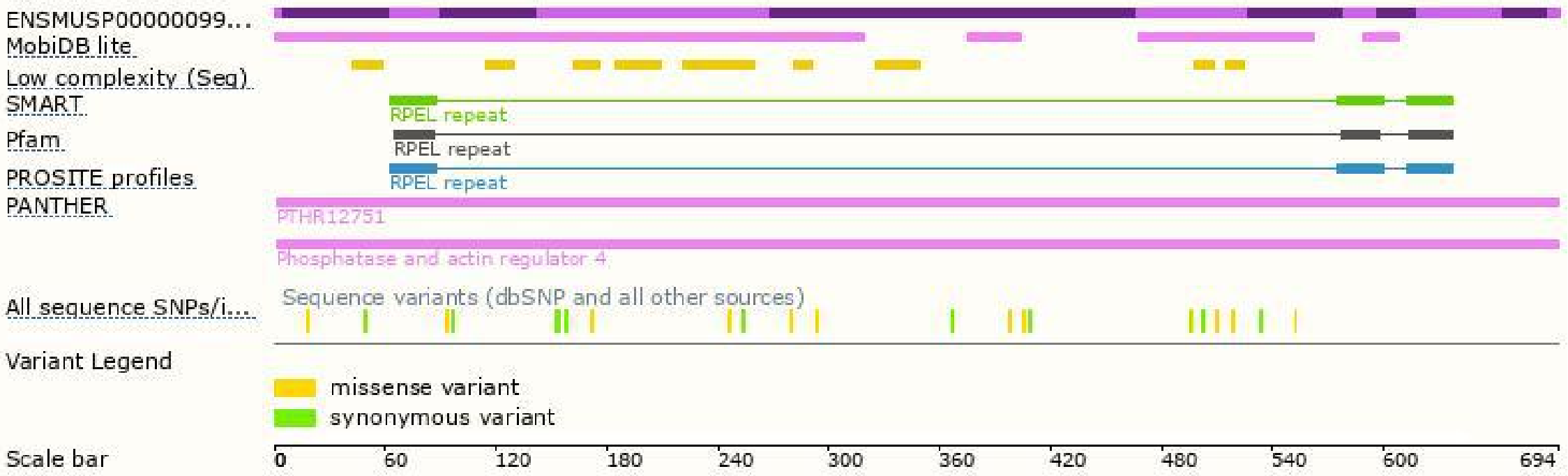
The strategy is based on the design of *Phactr4-203* transcript,the transcription is shown below:



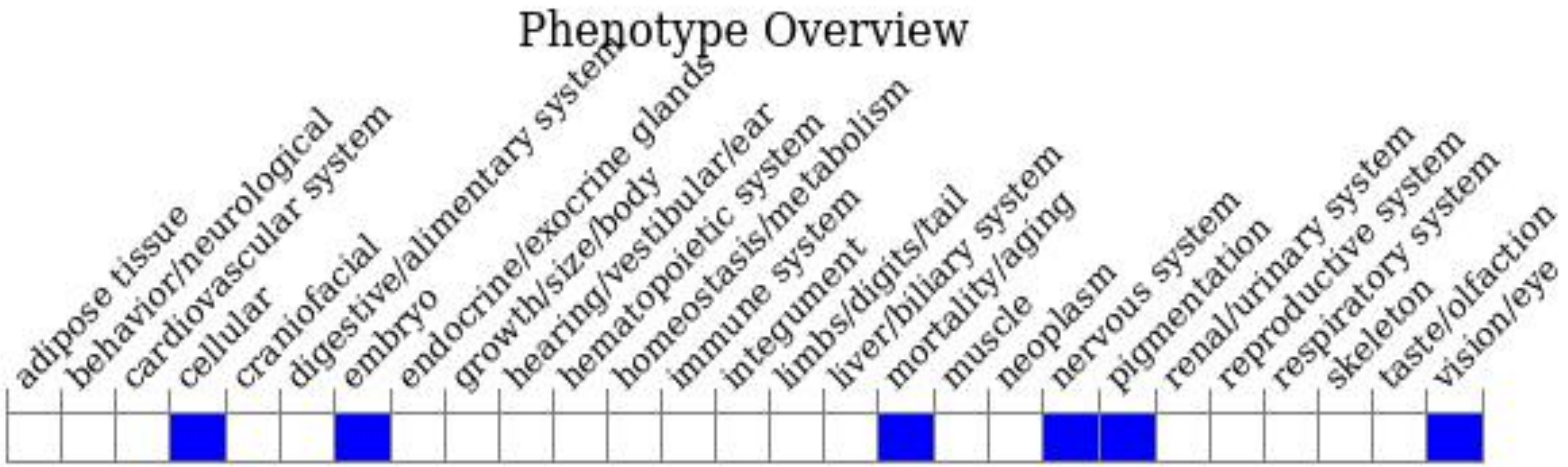
Genomic location distribution



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 embryonic and neonatal lethality, exencephaly, neural tube defects, coloboma, and altered cell cycles.

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

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