Pth1r Cas9-CKO Strategy

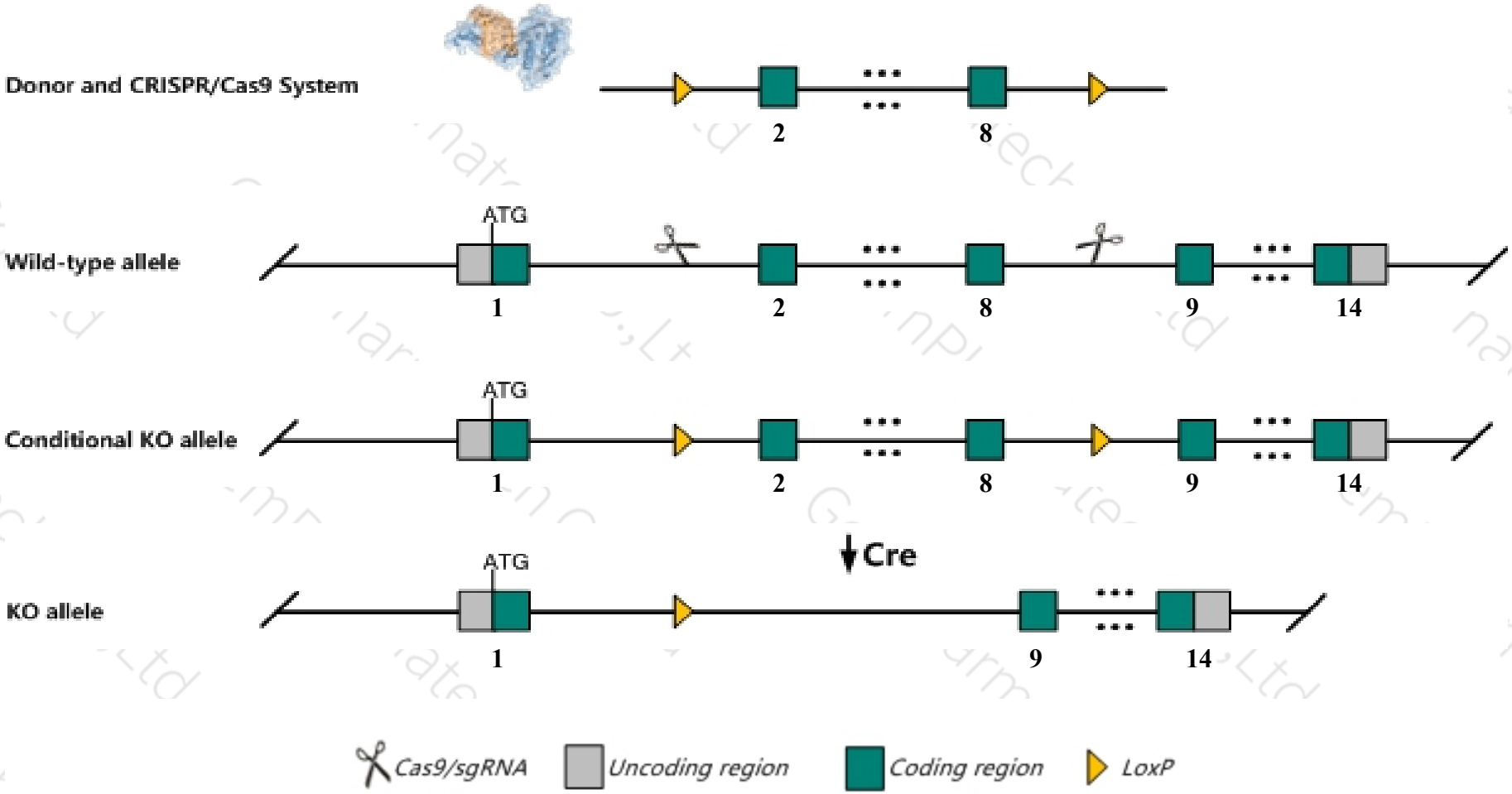
Designer: JiaYu

Project Overview

Project Name	***Pth1r***
Project type	**Cas9-CKO**
Strain background	**C57BL/6J**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pth1r* gene. The schematic diagram is as follows:



Technical routes

- The *Pth1r* gene has 6 transcripts. According to the structure of *Pth1r* gene, exon2-exon8 of *Pth1r-201* (ENSMUST00000006005.11) transcript is recommended as the knockout region.The region contains 913bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Pth1r* 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/6J 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/6J 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,Homozygous mutant mice die in mid-gestation or shortly after birth depending on genetic background, are small in size, have short limbs, and accelerated differentiation of chondrocytes resulting in accelerated bone mineralization.

- The *Pth1r* 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）

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Pth1r parathyroid hormone 1 receptor [Mus musculus (house mouse)]

Gene ID: 19228, updated on 19-Mar-2019

Summary

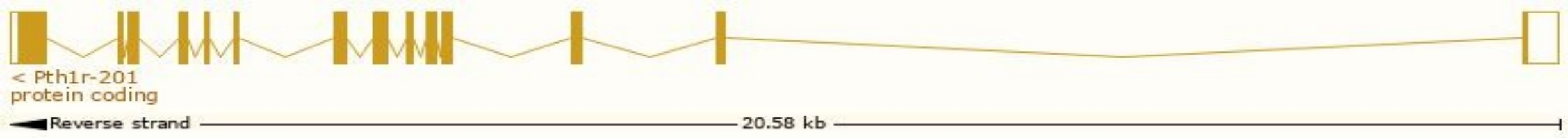
Official Symbol	Pth1r provided by MGI
Official Full Name	parathyroid hormone 1 receptor provided by MGI
Primary source	MGI:MGI:97801
See related	Ensembl:ENSMUSG00000032492
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	PPR, Pthr, Pthr1
Expression	Biased expression in kidney adult (RPKM 207.3), limb E14.5 (RPKM 43.7) and 4 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

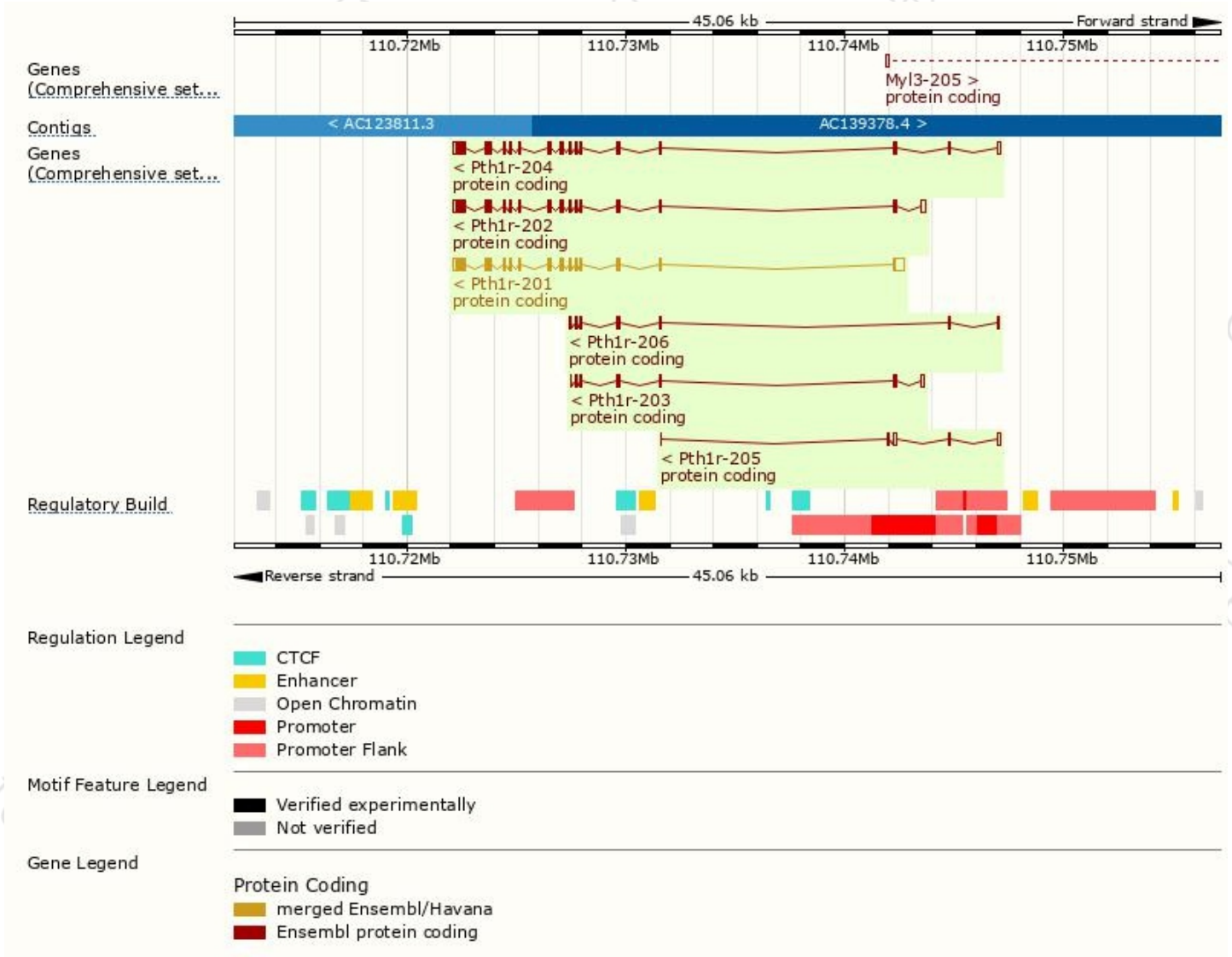
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pth1r-201	ENSMUST00000006005.11	2307	591aa	Protein coding	CCDS52940	P41593	TSL:1 GENCODE basic APPRIS P1
Pth1r-202	ENSMUST00000166716.7	2219	591aa	Protein coding	CCDS52940	P41593	TSL:1 GENCODE basic APPRIS P1
Pth1r-204	ENSMUST00000198865.4	2201	591aa	Protein coding	CCDS52940	P41593	TSL:1 GENCODE basic APPRIS P1
Pth1r-203	ENSMUST00000196057.1	830	197aa	Protein coding	-	A0A0G2JG89	CDS 3' incomplete TSL:5
Pth1r-206	ENSMUST00000199862.4	682	198aa	Protein coding	-	A0A0G2JE85	CDS 3' incomplete TSL:5
Pth1r-205	ENSMUST00000199791.1	508	54aa	Protein coding	-	A0A0G2JEZ1	CDS 3' incomplete TSL:5

The strategy is based on the design of *Pth1r-201* transcript,The transcription is shown below

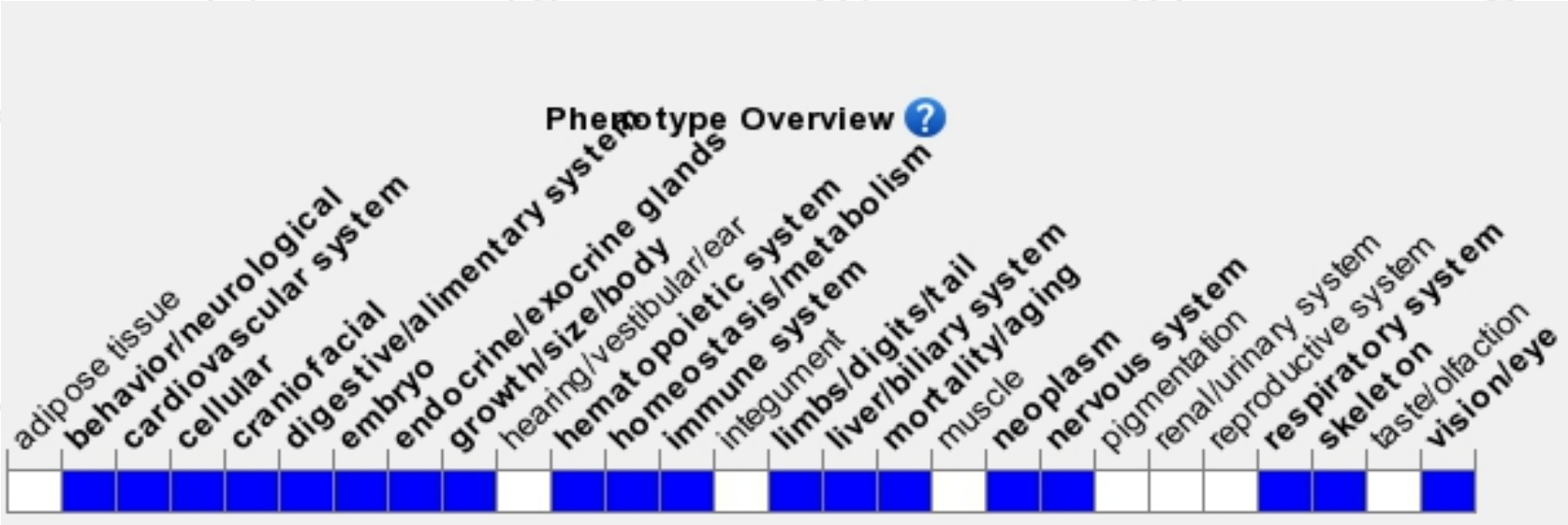


Genomic location distribution



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.

Tel: 025-5864 1534

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GemPharmatech Co.,Ltd