Ihh Cas9-CKO Strategy

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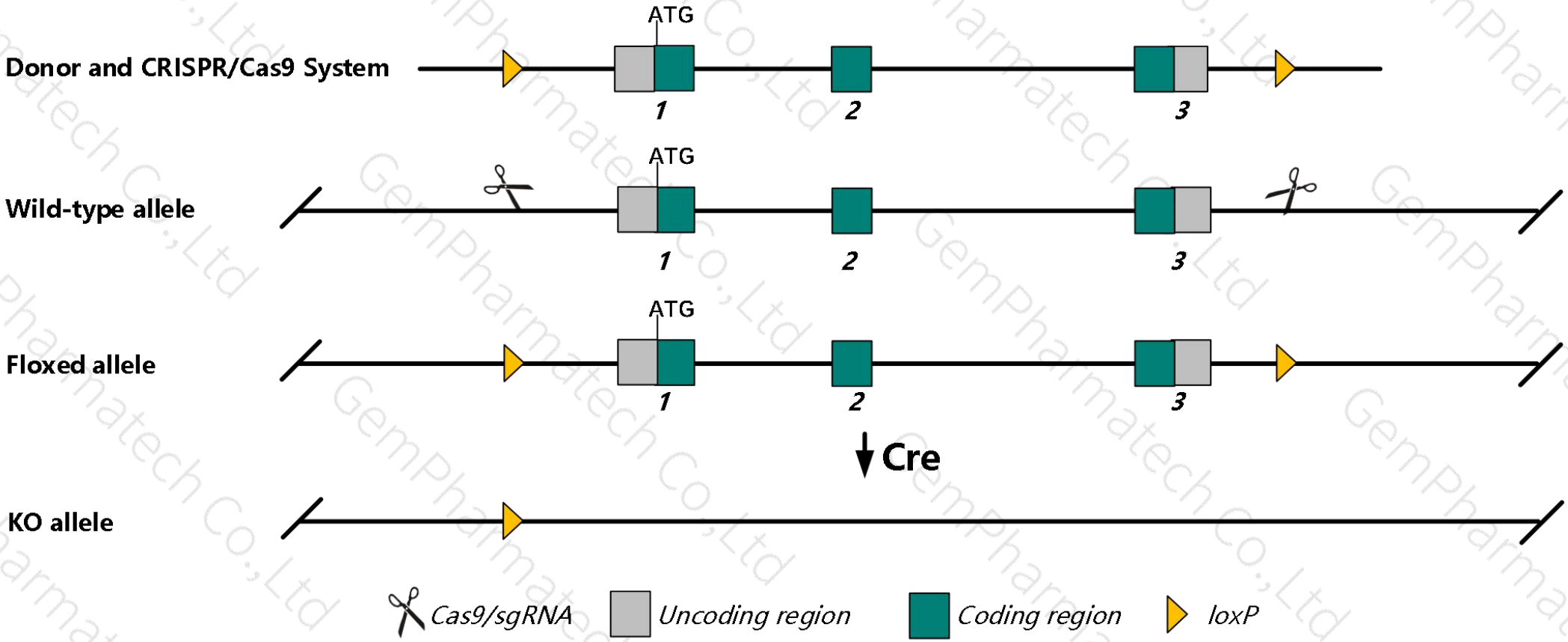
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Ihh* gene has 2 transcripts. According to the structure of *Ihh* gene, exon1-exon3 of *Ihh-201* (ENSMUST00000164097.3) transcript is recommended as the knockout region.The region contains all of the coding sequence.Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ihh* 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,Homozygotes die before or immediately after birth due to respiratory failure, exhibiting limb dwarfism associated with reduced chondrocyte proliferation, ectopic maturation of chondrocytes, and a failure of osteoblast development in endochondral bones.

➢*Gm37744-201* gene may be destroyed.

➢The *Ihh* 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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Ihh Indian hedgehog [*Mus musculus* (house mouse)]

Gene ID: 16147, updated on 11-Feb-2020

Summary

Official Symbol	Ihh provided by MGI
Official Full Name	Indian hedgehog provided by MGI
Primary source	MGI:MGI:96533
See related	Ensembl:ENSMUSG00000006538
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	HHG-2
Expression	Biased expression in colon adult (RPKM 64.9), duodenum adult (RPKM 59.3) and 8 other tissues See more
Orthologs	human all

Genomic context

Location: 1 C4; 1 38.55 cM

See Ihh in Genome Data Viewer

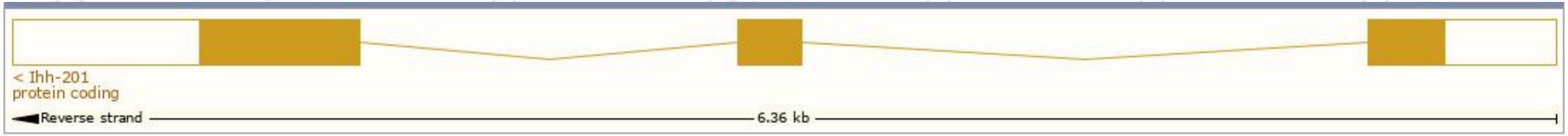
Exon count: 3

Transcript information（Ensembl）

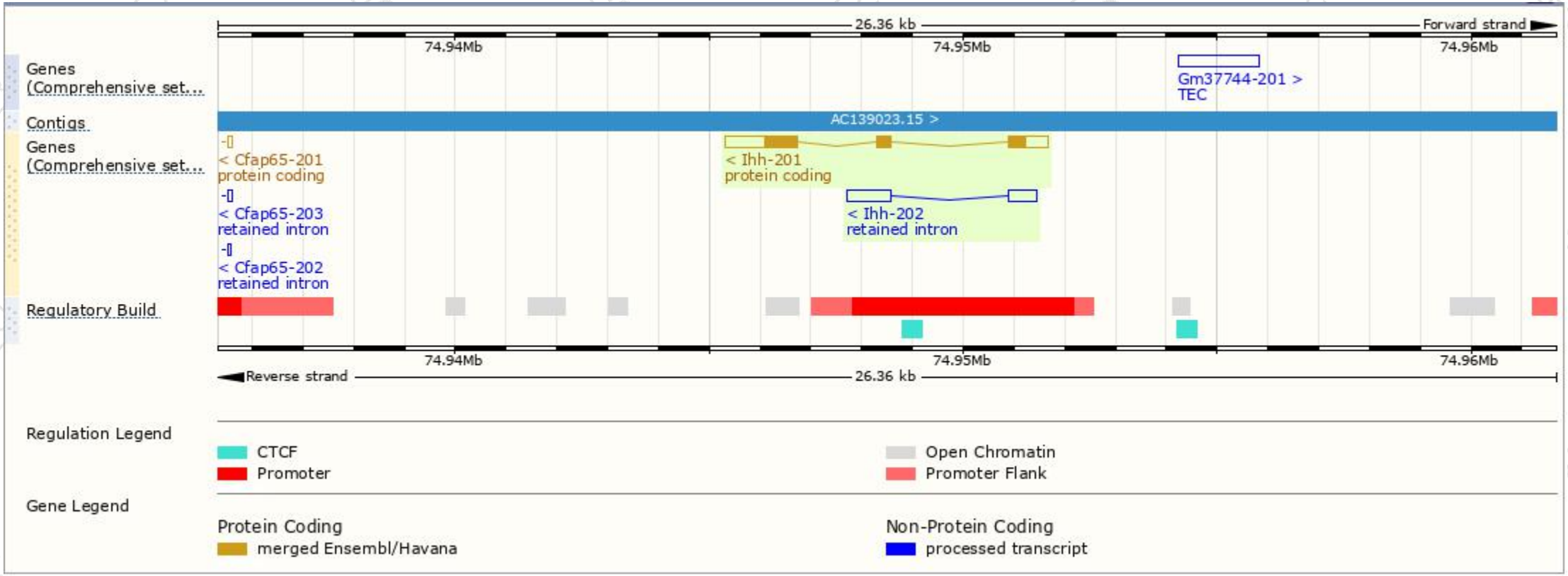
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ihh-201	ENSMUST00000164097.3	2468	411aa	Protein coding	-	F6RLD8 P97812	TSL:1 GENCODE basic APPRIS P1
Ihh-202	ENSMUST00000189364.1	1385	No protein	Retained intron	-	-	TSL:1

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

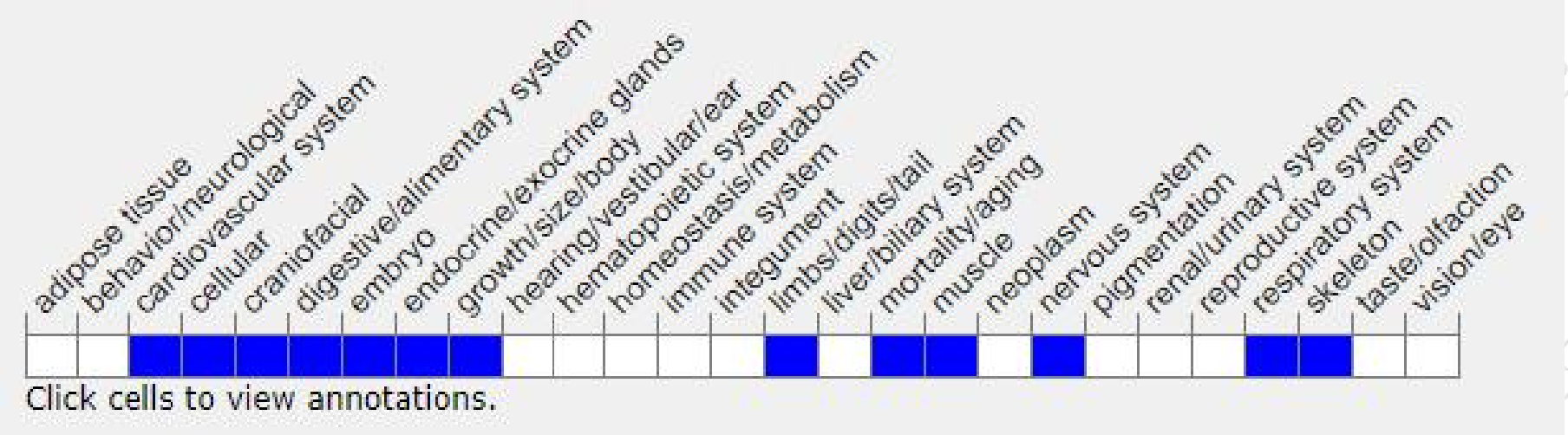


Genomic location distribution



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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