Hpd Cas9-KO Strategy

Designer: Xueting Zhang

Reviewer: Yanhua Shen

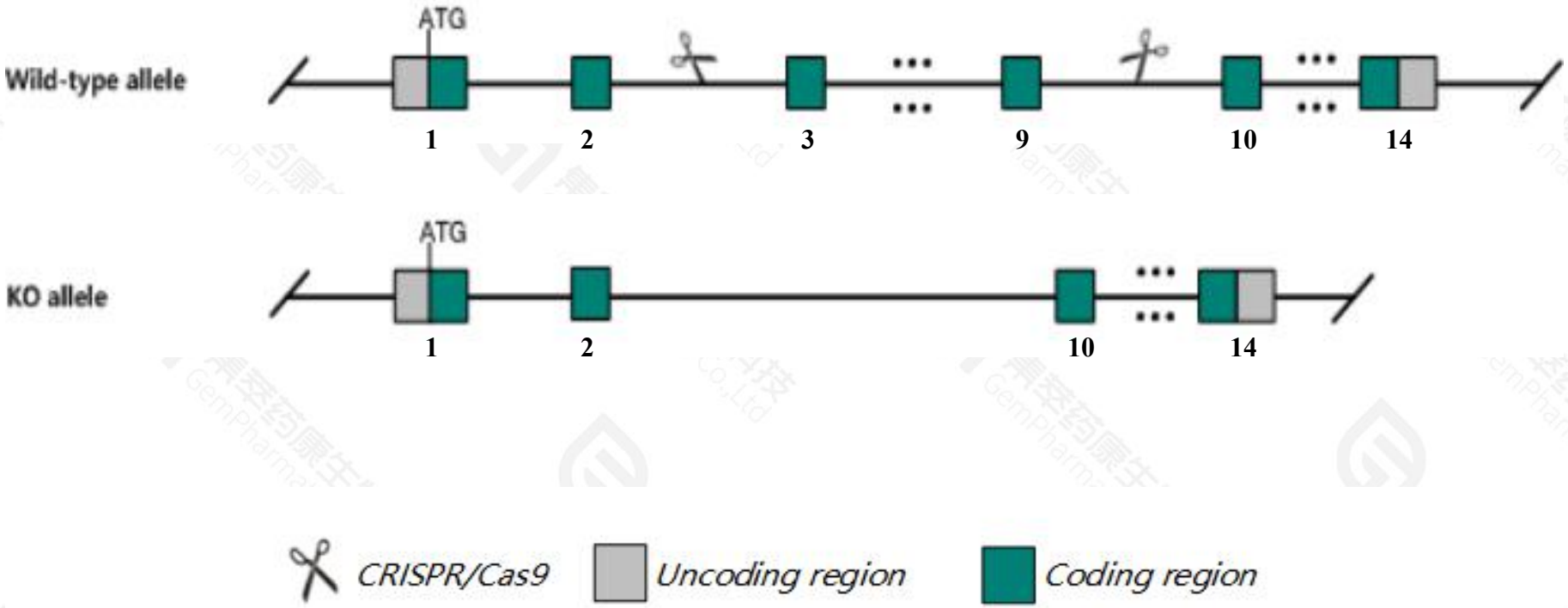
Design Date: 2021-9-23

Project Overview

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

Knockout strategy

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



Technical routes

➢ The *Hpd* gene has 6 transcripts. According to the structure of *Hpd* gene, exon3-exon9 of *Hpd-201*(ENSMUST00000031398.14) transcript is recommended as the knockout region.The region contains 566bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Hpd* gene. The brief process is as follows: CRISPR/Cas9 system 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,cBA, C3H, DBA/2, SM and AKR have the F.1 form of this soluble liver antigen; A/J, A2G, BALB/c and C57BL/10 the F.2 form. F.2 antigen induces precipitating antibodies in F.1 but not F.2 strains and vice versa. F antigen immune response requires H2 Kk or Ak alleles.

➢ Transcript *Hpd*-205 may not be affected .And the effect on transcript *Hpd*-203 is unknown.

➢ The partial intron of *Psmd9*-206 will be deleted.

➢ The *Hpd* gene is located on the Chr5. 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

Hpd 4-hydroxyphenylpyruvic acid dioxygenase [Mus musculus (house mouse)]

Gene ID: 15445, updated on 13-Mar-2020

Summary

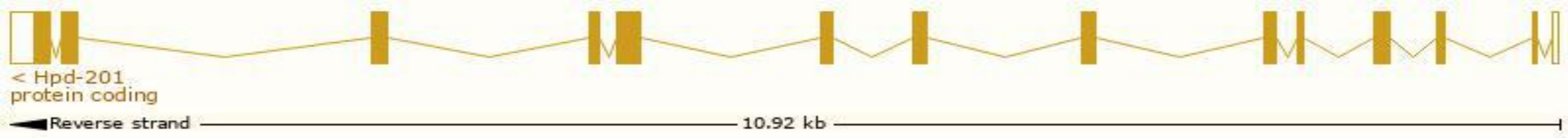
Official Symbol	Hpd provided by MGI
Official Full Name	4-hydroxyphenylpyruvic acid dioxygenase provided by MGI
Primary source	MGI:MGI:96213
See related	Ensembl:ENSMUSG00000029445
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4HPPD, Fla, Flp, Hppd, Laf
Expression	Biased expression in liver adult (RPKM 1170.9), liver E18 (RPKM 236.0) and 1 other tissue See more
Orthologs	human all

Transcript information（Ensembl）

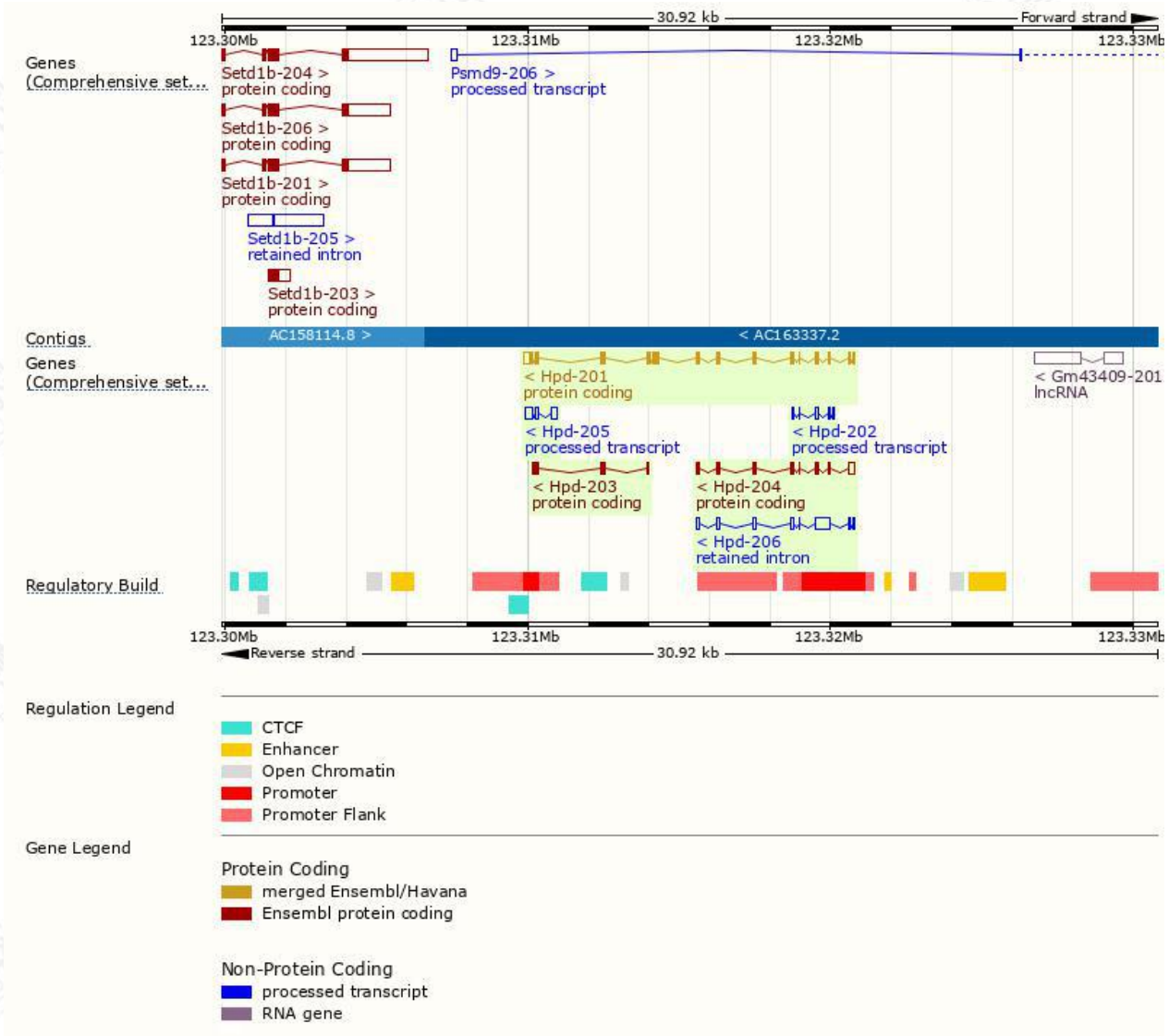
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hpd-201	ENSMUST00000031398.13	1404	393aa	Protein coding	CCDS39265	P49429	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Hpd-204	ENSMUST00000154713.7	744	159aa	Protein coding	-	D3Z1U3	CDS 3' incomplete TSL:3
Hpd-203	ENSMUST00000144679.1	334	96aa	Protein coding	-	F6X9Z6	CDS 5' incomplete TSL:3
Hpd-205	ENSMUST00000155092.1	511	No protein	Processed transcript	-	-	TSL:5
Hpd-202	ENSMUST00000124110.1	326	No protein	Processed transcript	-	-	TSL:3
Hpd-206	ENSMUST00000156539.7	975	No protein	Retained intron	-	-	TSL:5

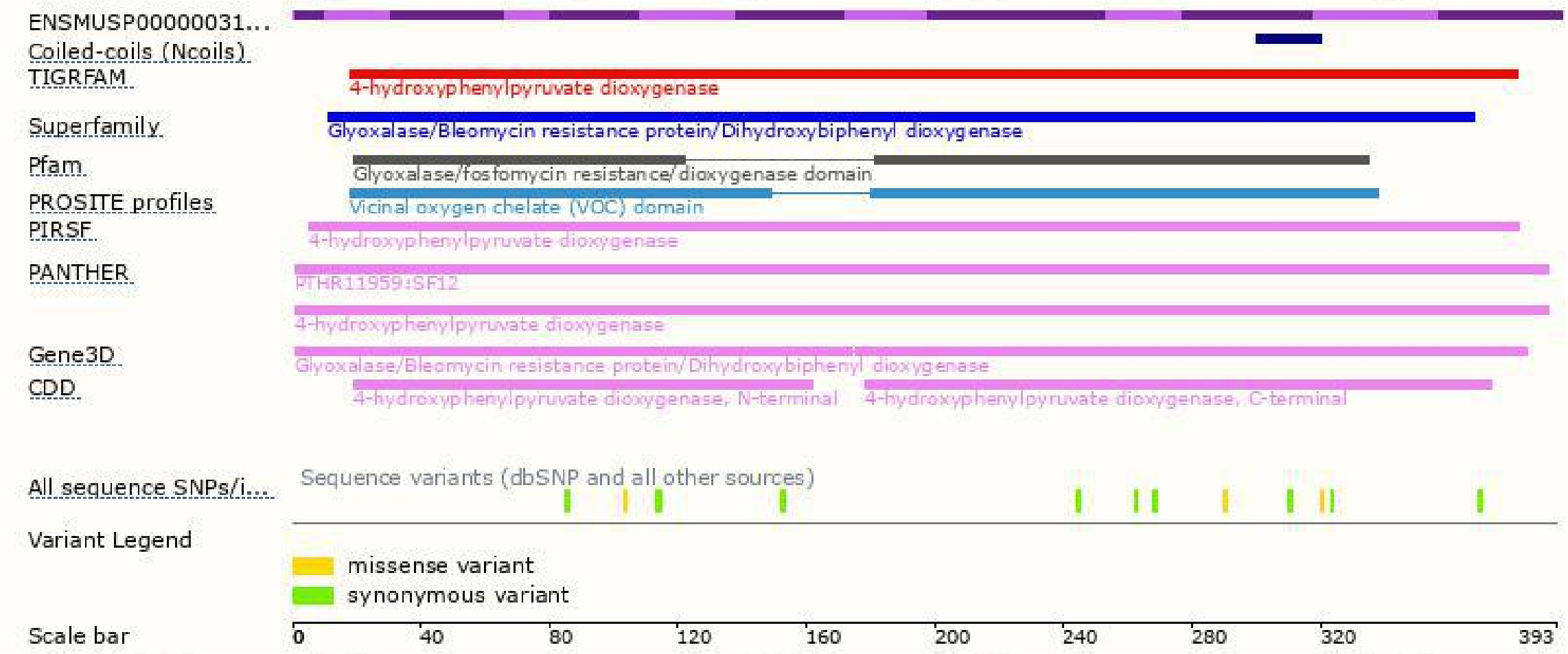
The strategy is based on the design of *Hpd-201* transcript,the transcription is shown below:



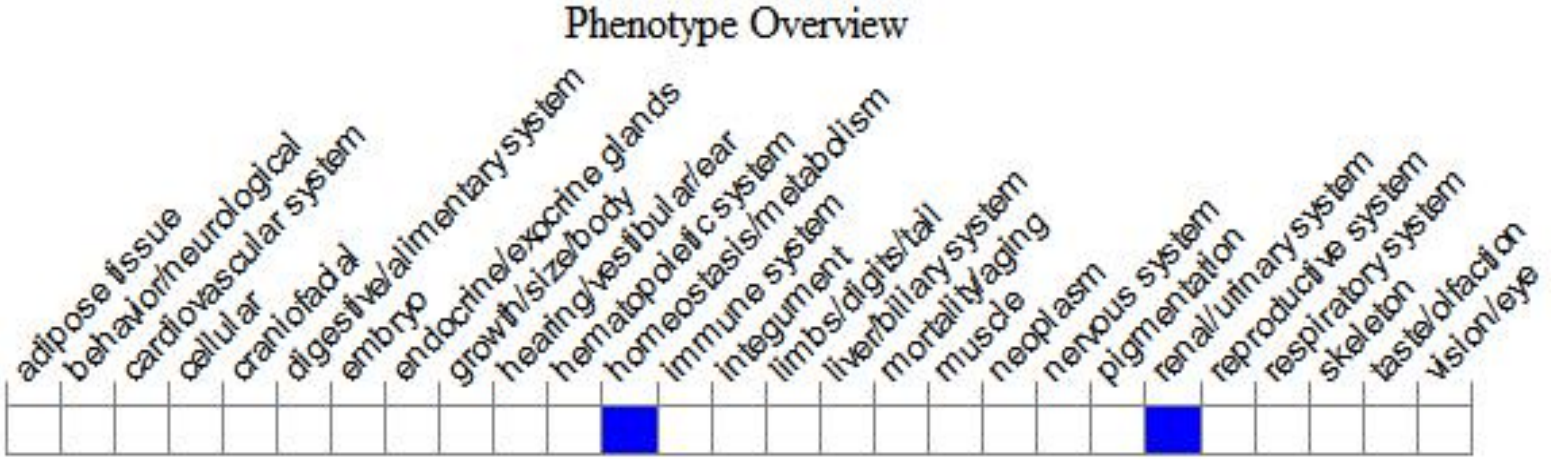
Genomic location distribution



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,cBA, C3H, DBA/2, SM and AKR have the F.1 form of this soluble liver antigen; A/J, A2G, BALB/c and C57BL/10 the F.2 form. F.2 antigen induces precipitating antibodies in F.1 but not F.2 strains and vice versa. F antigen immune response requires H2 Kk or Ak alleles.

集萃药康
GemPharmatech

If you have any questions, you are welcome to inquire.
Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd