GemPharmatech™

Dohh Cas9-CKO Strategy

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Reviewer: Xingkai Xiao

Design Date: 2023-3-24

Overview

Target Gene Name

- *Dohh*

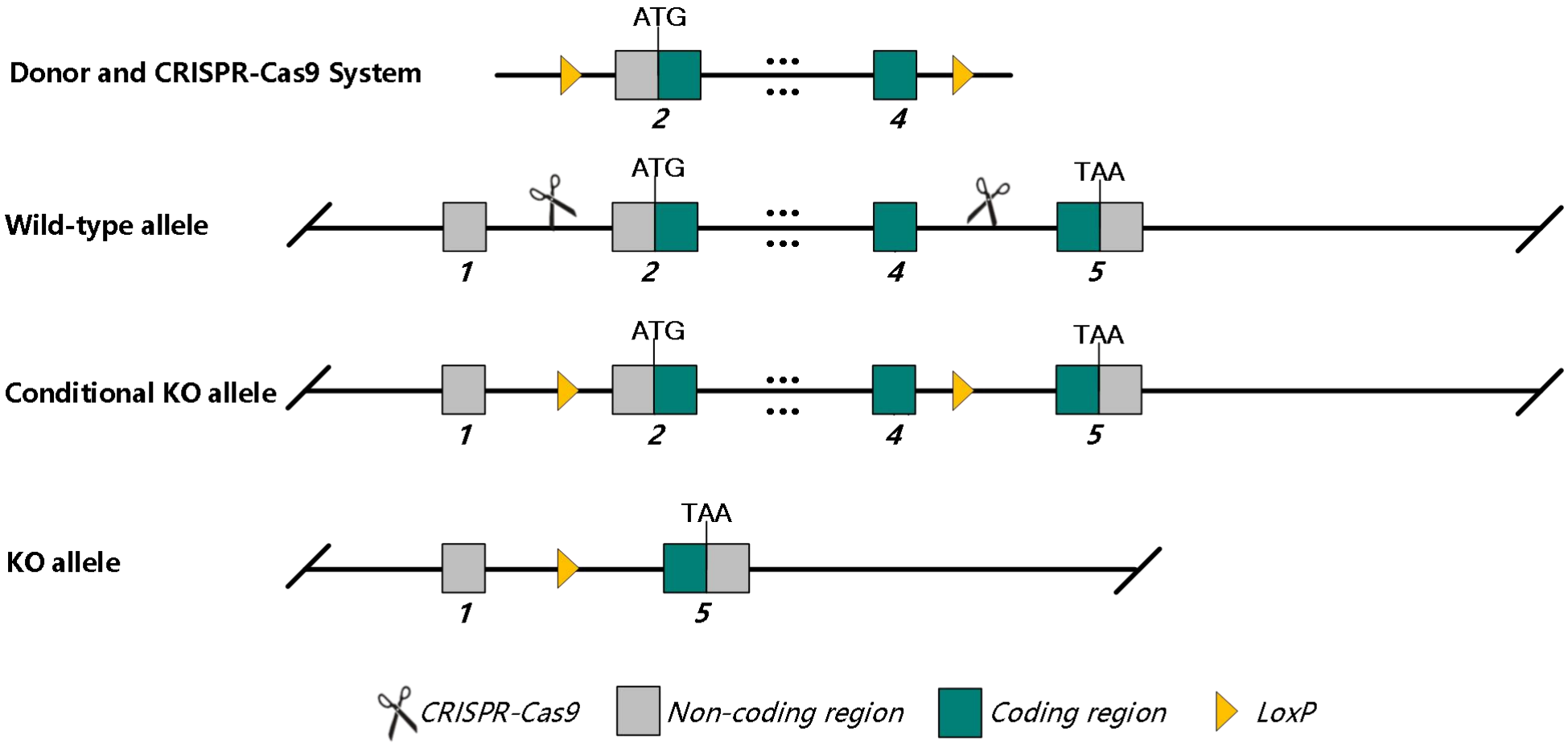
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Dohh* gene.

Technical Information

- The *Dohh* gene has 5 transcripts. According to the structure of *Dohh* gene, exon 2-4 of *Dohh*-201 (ENSMUST00000072751.13) is recommended as the knockout region. The region contains start codon. Knockout the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Dohh* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

Gene Information

Dohh deoxyhypusine hydroxylase/monooxygenase [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 102115, updated on 8-Dec-2022

Summary

Official Symbol	Dohh provided by MGI
Official Full Name	deoxyhypusine hydroxylase/monooxygenase provided by MGI
Primary source	MGI:MGI:1915964
See related	Ensembl:ENSMUSG00000078440 AllianceGenome:MGI:1915964
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	Hlrc1; 1110033C18Rik
Summary	Predicted to enable deoxyhypusine monooxygenase activity and iron ion binding activity. Acts upstream of or within peptidyl-lysine modification to peptidyl-hypusine. Is expressed in nervous system; retina; and skeletal muscle. Orthologous to human DOHH (deoxyhypusine hydroxylase). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in ovary adult (RPKM 72.2), adrenal adult (RPKM 53.2) and 28 other tissues See more
Orthologs	human all

NEW **Try the new Gene table**
Try the new Transcript table

Genomic context

Location: 10; 10 C1 — See Dohh in Genome Data Viewer

Exon count: 5

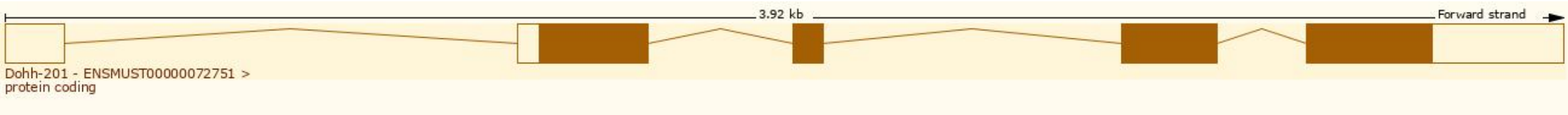
https://www.ncbi.nlm.nih.gov/gene/102115

Transcript Information

The gene has 5 transcripts, the transcripts are shown below:

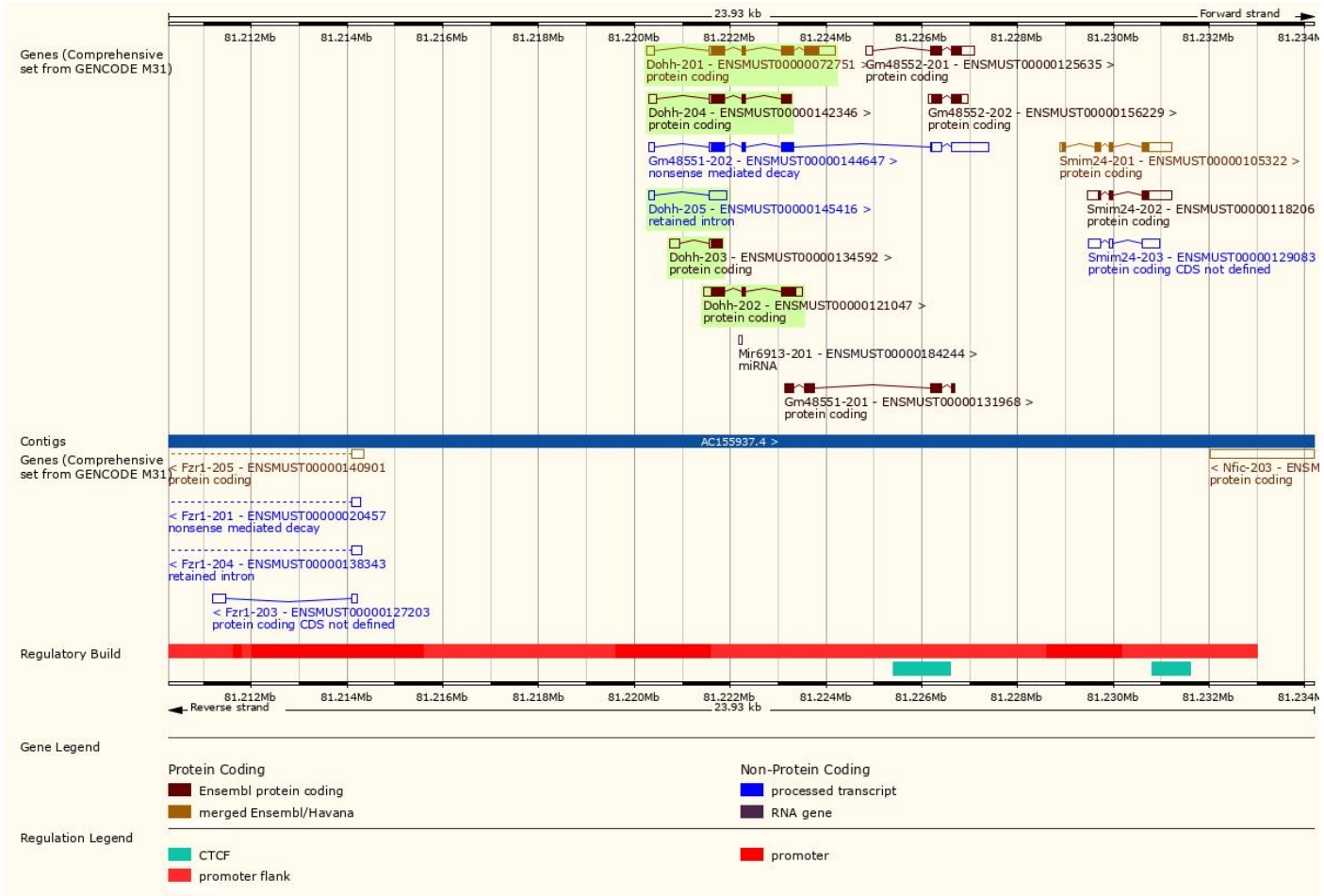
Transcript ID	Name ▲	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000072751.13	Dohh-201	1444	302aa	Protein coding	CCDS35997	Q99LN9-1	Ensembl Canonical GENCODE basic APPRIS P1 TSL:1
ENSMUST00000121047.2	Dohh-202	946	213aa	Protein coding		D3Z6Y9	GENCODE basic TSL:2
ENSMUST00000134592.2	Dohh-203	479	73aa	Protein coding		D3YZM0	TSL:3 CDS 3' incomplete
ENSMUST00000142346.8	Dohh-204	786	187aa	Protein coding		D3Z7J7	TSL:2 CDS 3' incomplete
ENSMUST00000145416.2	Dohh-205	495	No protein	Retained intron		-	TSL:2

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



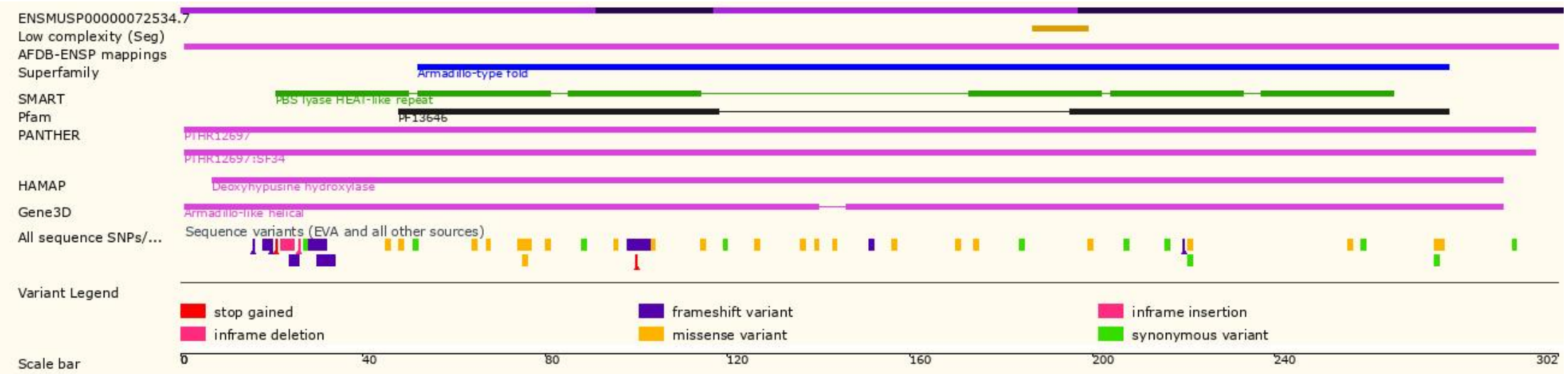
Source: http://asia.ensembl.org/

Genomic Information



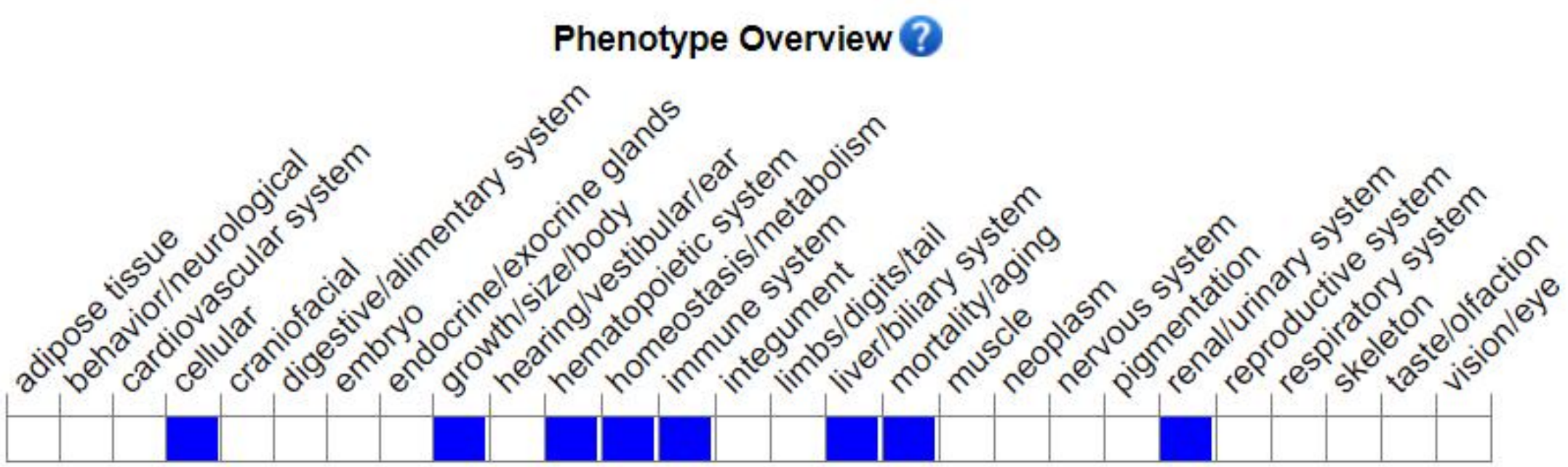
Source:http://asia.ensembl.org/

Protein Information



Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



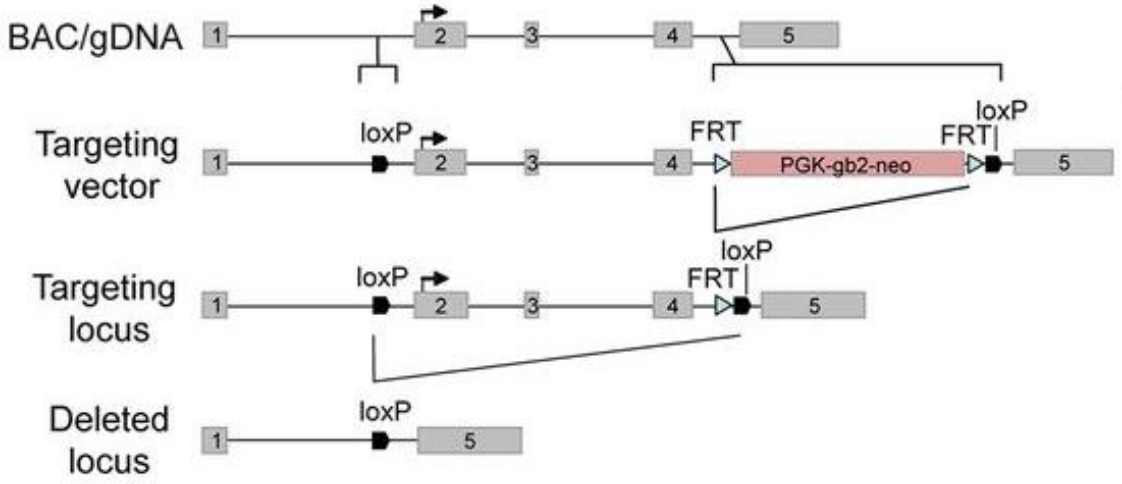
- Mice homozygous for a null mutation die prior to organogenesis.

Source: https://www.informatics.jax.org/marker/MGI:1915964

Important Information

- The loxps will insert into intron 1-2 and intron 4-5 of *Dohh*-201 transcript respectively, which may affect the regulation of this gene.
- The knockout region overlaps with *Gm48551* gene, which may affect the function of this gene.
- The knockout region contains *Mir6913* gene, this strategy will knockout this gene.
- The knockout region is about 1.3 kb away from the 5' of the *Gm48552* gene, which may affect the regulation of this gene.
- The knockout region is about 5.3 kb away from the 5' of the *Smim24* gene, which may affect the regulation of this gene.
- The knockout region is about 6.7 kb away from the 5' of the *Fzr1* gene, which may affect the regulation of this gene.
- The knockout region contains start codon, translation may recognize new start codon and form new unknown protein.
- *Dohh* is located on Chr 10. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.

Reference



To determine the molecular function of the second step of hypusine modification in mammals, we generated a mouse strain enabling conditional knockout of *Dohh* (B6.Dohh^{tm1bal}). Inactivation of *Dohh* was achieved by using the Cre/loxP approach to target exons 2-4, which include both the *Dohh* start codon and three of the four His-Glu motifs essential for DOHH function (Kim et al., 2006) (Fig. 1A). Southern blot analysis and genotyping PCR confirmed correct recombination in embryonic stem cells (ESC; Fig. 1B) and accurate Cre-mediated *Dohh* deletion, respectively (Fig. 1C). To determine the specific role of eIF5A(Dhp50) in embryonic development, *Dohh* null allele (*Dohh*$^{+/-}$) mice were generated by using CMV-Cre-deleter mice expressing Cre in early embryonic development (Schwenk et al., 1995). Heterozygous knockout mice (*Dohh*$^{+/-}$) appeared normal

[1] Sievert H, Pällmann N, Miller K K, et al. A novel mouse model for inhibition of DOHH-mediated hypusine modification reveals a crucial function in embryonic development, proliferation and oncogenic transformation[J]. Disease models & mechanisms, 2014, 7(8): 963-976.