

# ***Hoxd1 Cas9-CKO Strategy***

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**Reviewer :**

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# Project Overview

**Project Name**

***Hoxd1***

**Project type**

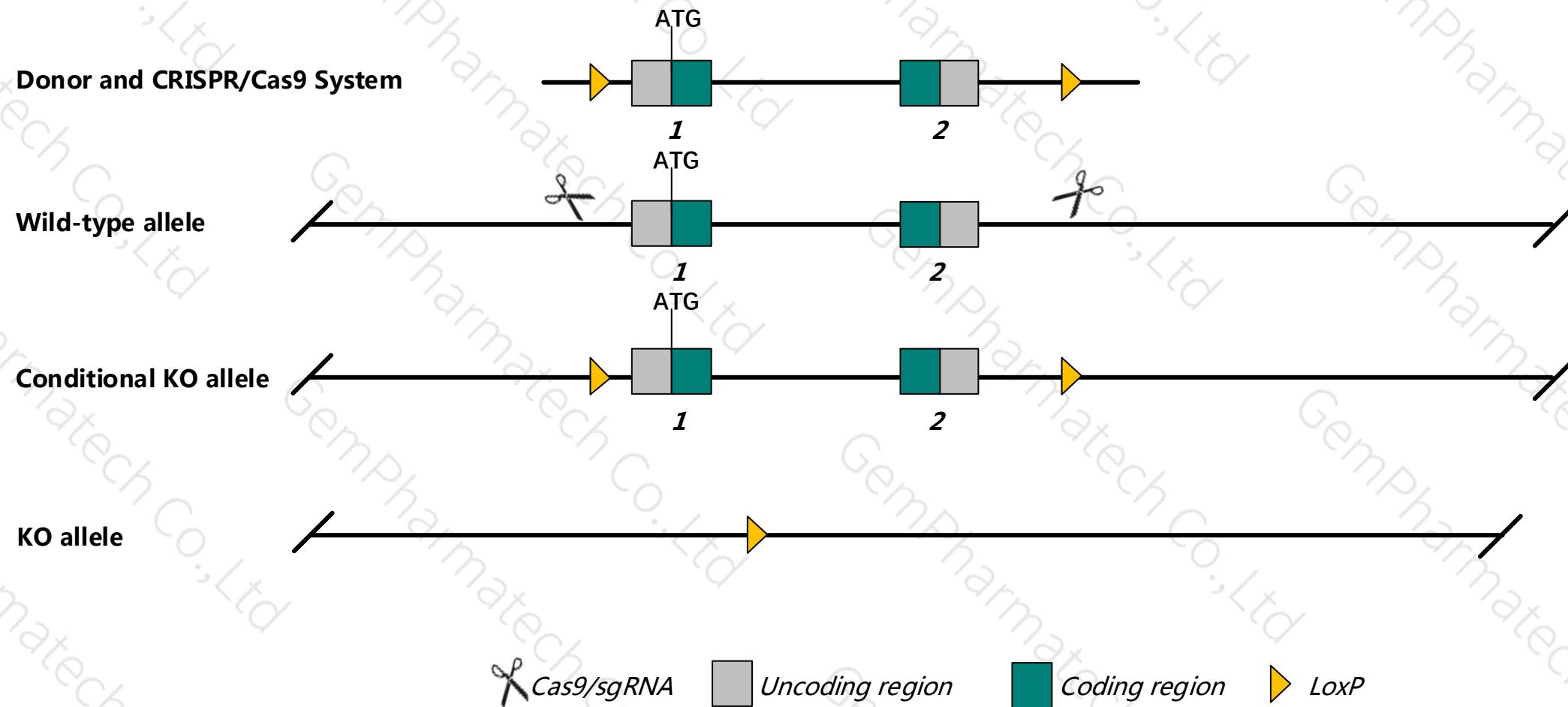
**Cas9-CKO**

**Strain background**

**C57BL/6JGpt**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Hoxd1* gene. The schematic diagram is as follows:



# Technical routes



- The *Hoxd1* gene has 1 transcript. According to the structure of *Hoxd1* gene, exon1-exon2 of *Hoxd1*-201 (ENSMUST00000047793.5) 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 *Hoxd1* 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/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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

# Notice

- According to the existing MGI data , Mice homozygous for a reporter allele display abnormal cervical vertebrae. Mice homozygous for a knock-out allele exhibit abnormal nociceptor innervation of the skin, aberrant nociceptor axonal projections in the spinal cord, and deficits in pain sensitivity.
- The KO region contains functional region of the *Haglr* gene.Knockout the region may affect the function of *Haglr* gene.
- The *Hoxd1* gene is located on the Chr2. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

# Gene information ( NCBI )

## Hoxd1 homeobox D1 [ *Mus musculus* (house mouse) ]

Gene ID: 15429, updated on 12-Aug-2019

### Summary



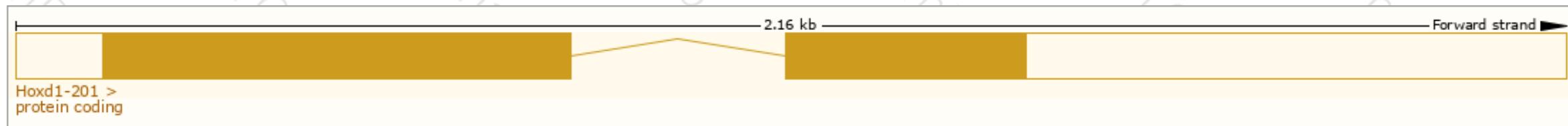
Official Symbol	Hoxd1 provided by <a href="#">MGI</a>
Official Full Name	homeobox D1 provided by <a href="#">MGI</a>
Primary source	<a href="#">MGI:MGI:96201</a>
See related	<a href="#">Ensembl:ENSMUSG00000042448</a>
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<a href="#">Mus musculus</a>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Hox-4.9
Expression	Low expression observed in reference dataset <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

# Transcript information ( Ensembl )

The gene has 1 transcript, and the transcripts is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hoxd1-201	<a href="#">ENSMUST00000047793.5</a>	1862	<a href="#">328aa</a>	Protein coding	<a href="#">CCDS16147</a>	<a href="#">Q01822</a>	TSL:1 GENCODE basic APPRIS P1

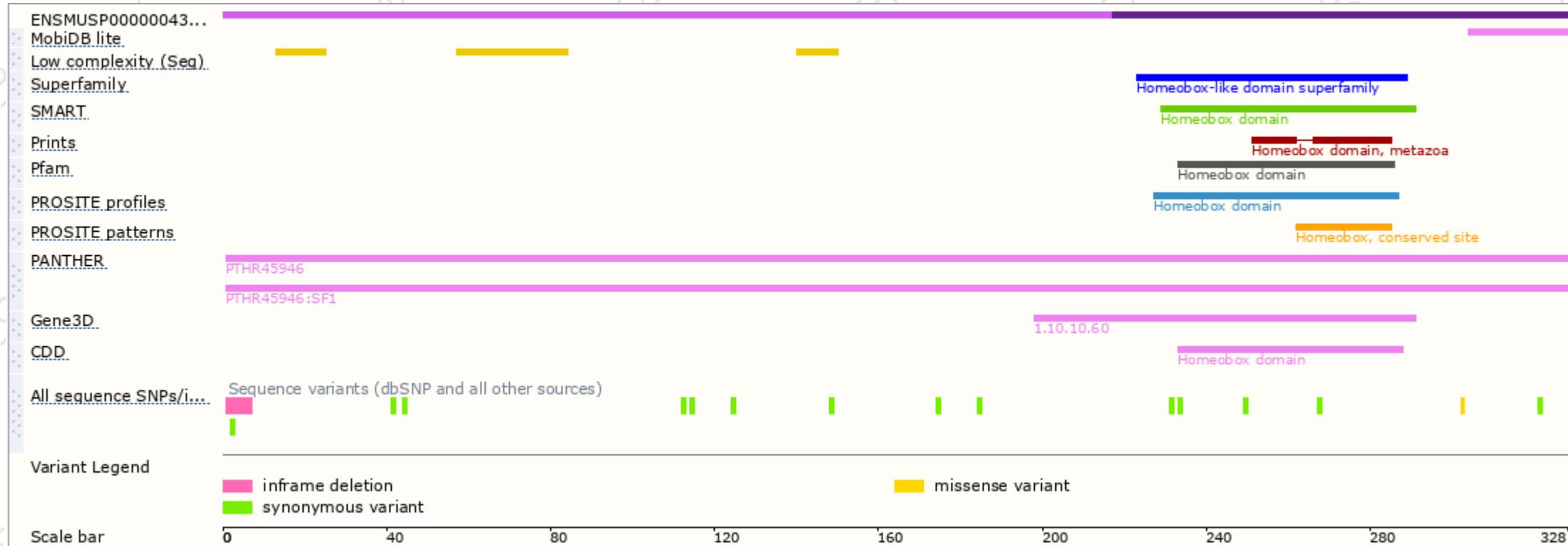
The strategy is based on the design of *Hoxd1-201* transcript, The transcription is shown below



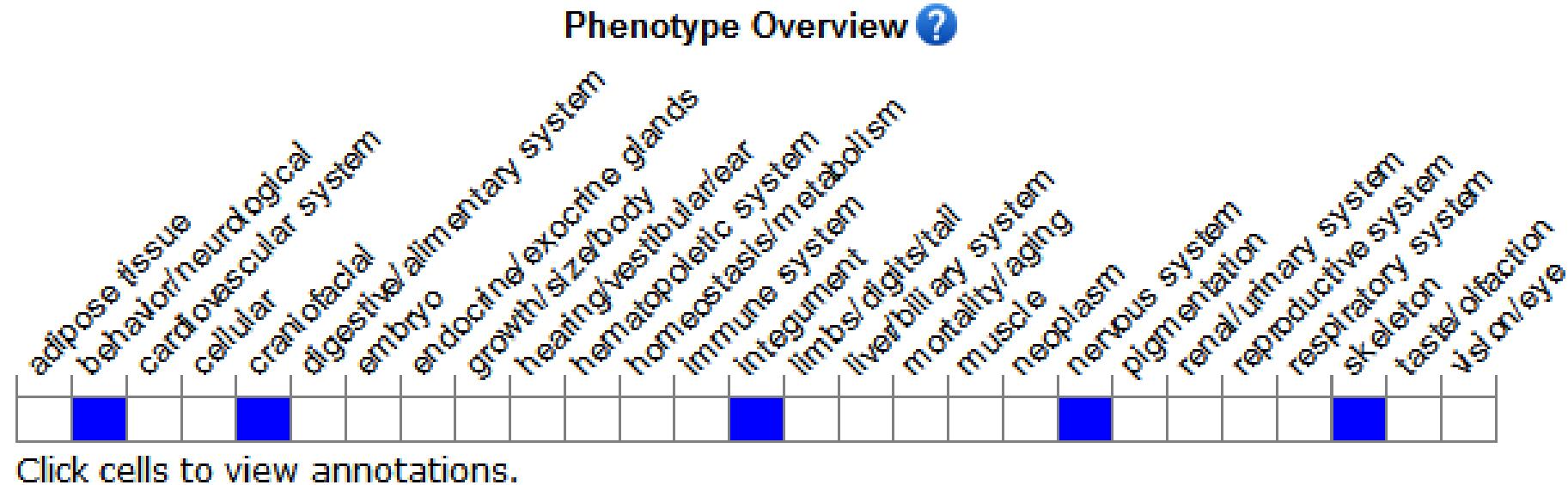
# Genomic location distribution



# 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, Mice homozygous for a reporter allele display abnormal cervical vertebrae.

Mice homozygous for a knock-out allele exhibit abnormal nociceptor innervation of the skin, aberrant nociceptor axonal projections in the spinal cord, and deficits in pain sensitivity.

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

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