Hoxd3 Cas9-CKO Strategy

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Project Overview

Project Name	***Hoxd3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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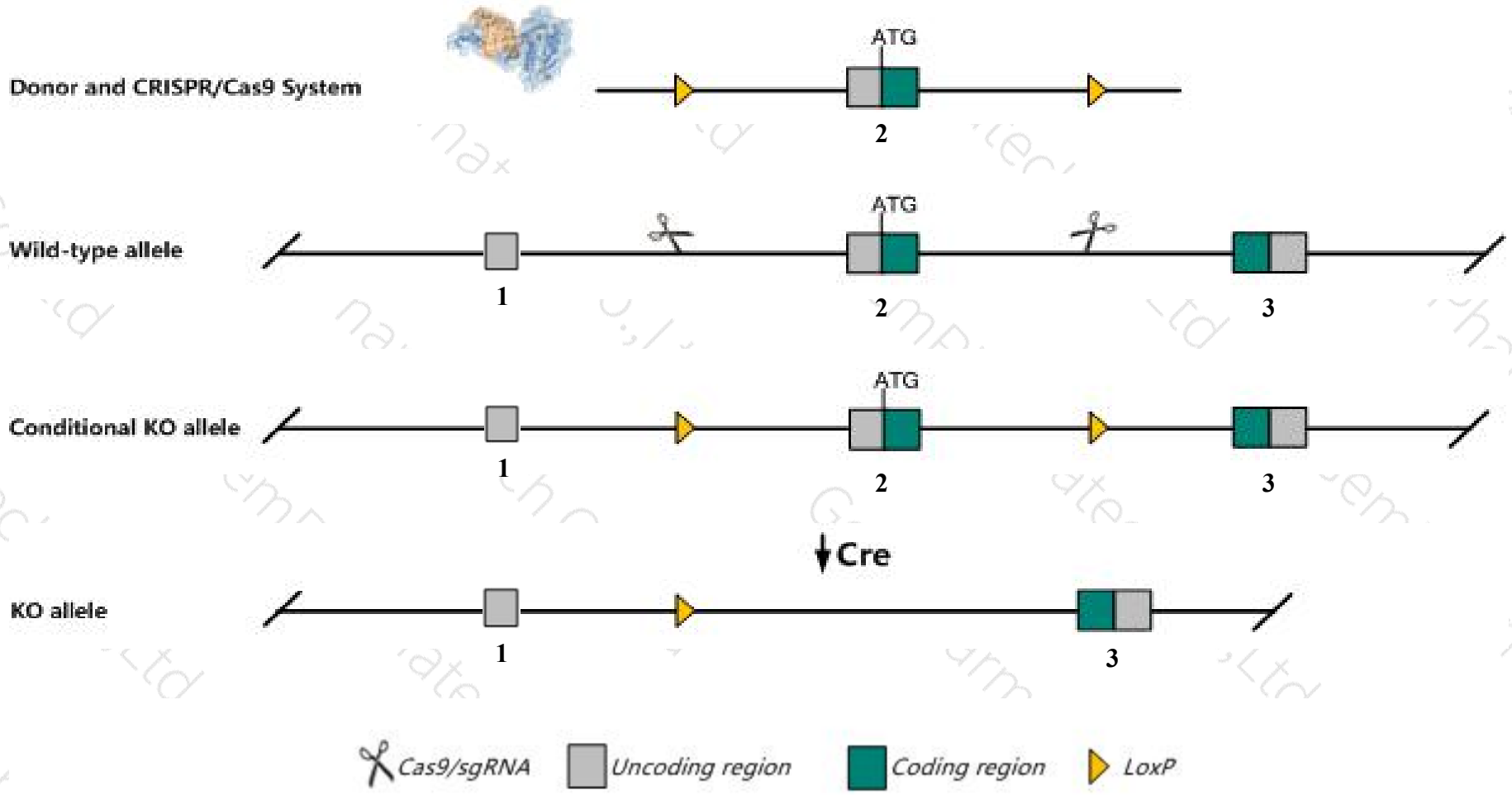
This model will use CRISPR/Cas9 technology to edit the *Hoxd3* gene. The schematic diagram is as follows:



Technical routes

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- The *Hoxd3* gene has 6 transcripts. According to the structure of *Hoxd3* gene, exon2 of *Hoxd3-203* (ENSMUST00000111983.8) transcript is recommended as the knockout region.The region contains start codon ATG. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Hoxd3* 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,mice homozygous for a knock-out allele show partial postnatal lethality, asymmetric rib-sternum attachment, and anterior transformations of the cervical vertebrae i (atlas) and ii (axis). mice homozygous for a different knock-out allele lack the anteriorarch of the atlas and the dens of the axis.
- The knockout region contains exon3 of *Gm28230*, and the effect on *Gm28230* is unknown.
- *Hoxd3-204* transcript may not be affect.
- The *Hoxd3* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Hoxd3 homeobox D3 [*Mus musculus* (house mouse)]

Gene ID: 15434, updated on 13-Mar-2020

Summary

Official Symbol	Hoxd3 provided by MGI
Official Full Name	homeobox D3 provided by MGI
Primary source	MGI:MGI:96207
See related	Ensembl:ENSMUSG00000079277
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	Hox-4.1; Hox-5.5
Expression	Biased expression in ovary adult (RPKM 19.8), genital fat pad adult (RPKM 17.5) and 11 other tissues See more
Orthologs	human all

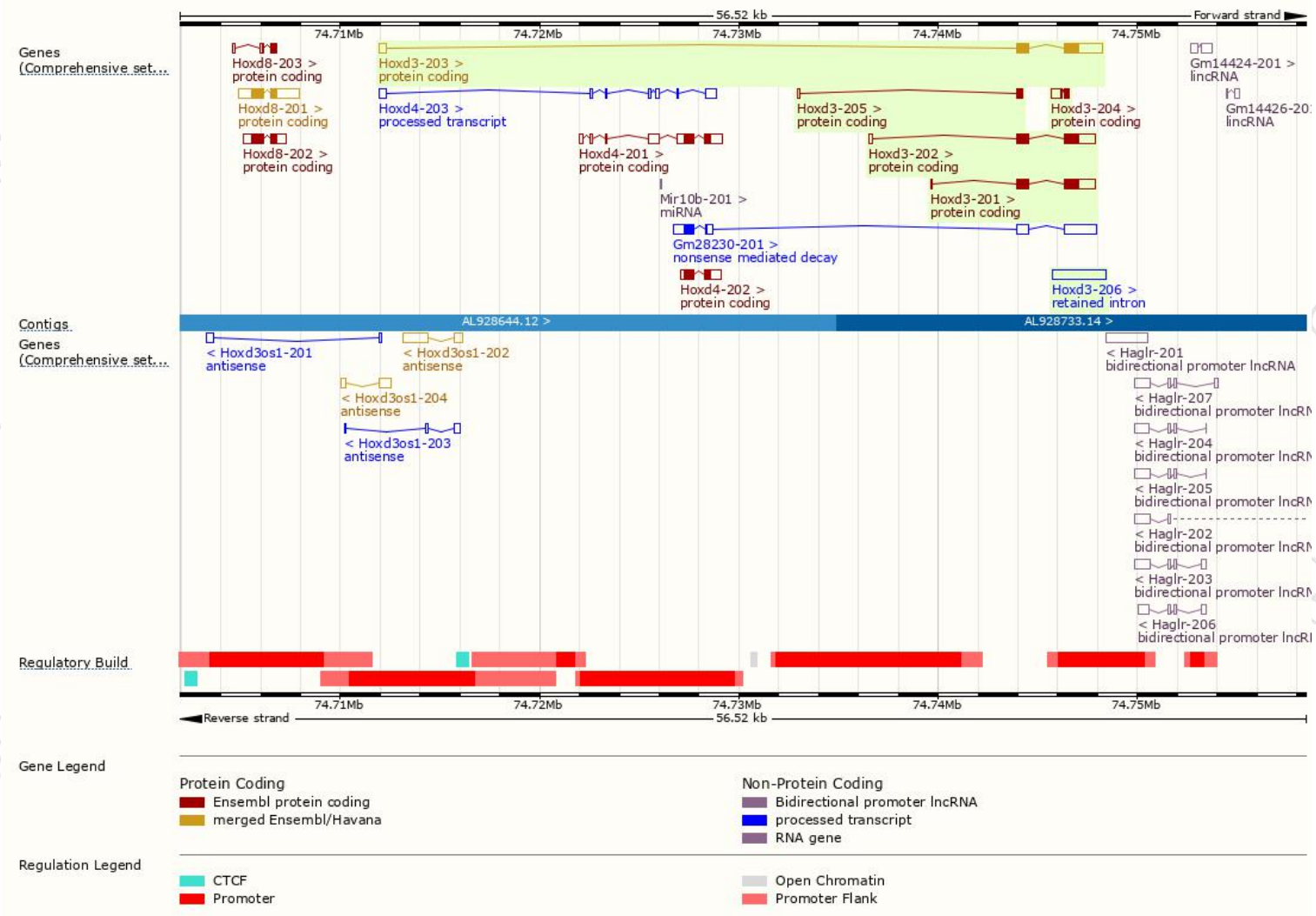
Transcript information（Ensembl）

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

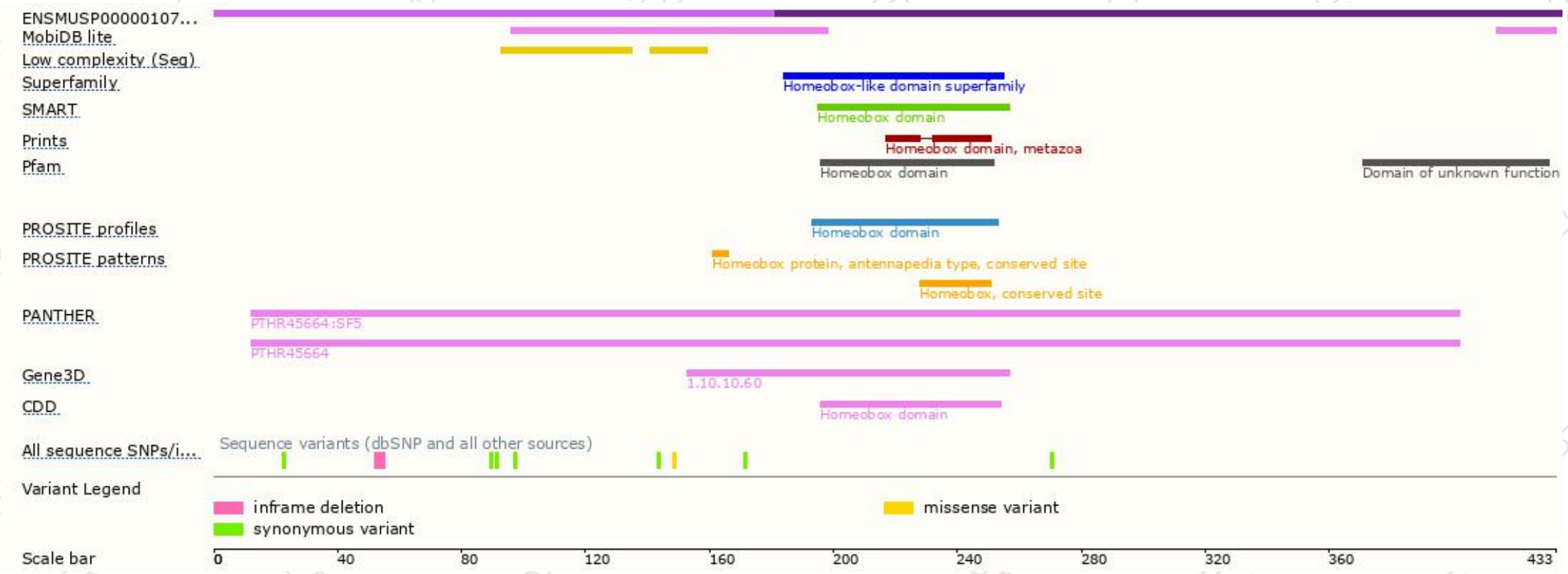
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hoxd3-203	ENSMUST00000111983.8	2968	433aa	Protein coding	CCDS38149	P09027 Q3UZR4	TSL:1 GENCODE basic APPRIS P1
Hoxd3-202	ENSMUST00000111982.7	2413	433aa	Protein coding	CCDS38149	P09027 Q3UZR4	TSL:1 GENCODE basic APPRIS P1
Hoxd3-201	ENSMUST00000047830.7	2292	433aa	Protein coding	CCDS38149	P09027 Q3UZR4	TSL:5 GENCODE basic APPRIS P1
Hoxd3-204	ENSMUST00000140666.1	822	113aa	Protein coding	-	G3UZS5	CDS 3' incomplete TSL:3
Hoxd3-205	ENSMUST00000144544.2	415	67aa	Protein coding	-	G3UZU1	CDS 3' incomplete TSL:3
Hoxd3-206	ENSMUST00000190553.1	2721	No protein	Retained intron	-	-	TSL:NA

The strategy is based on the design of *Hoxd3-203* transcript, the transcription is shown below

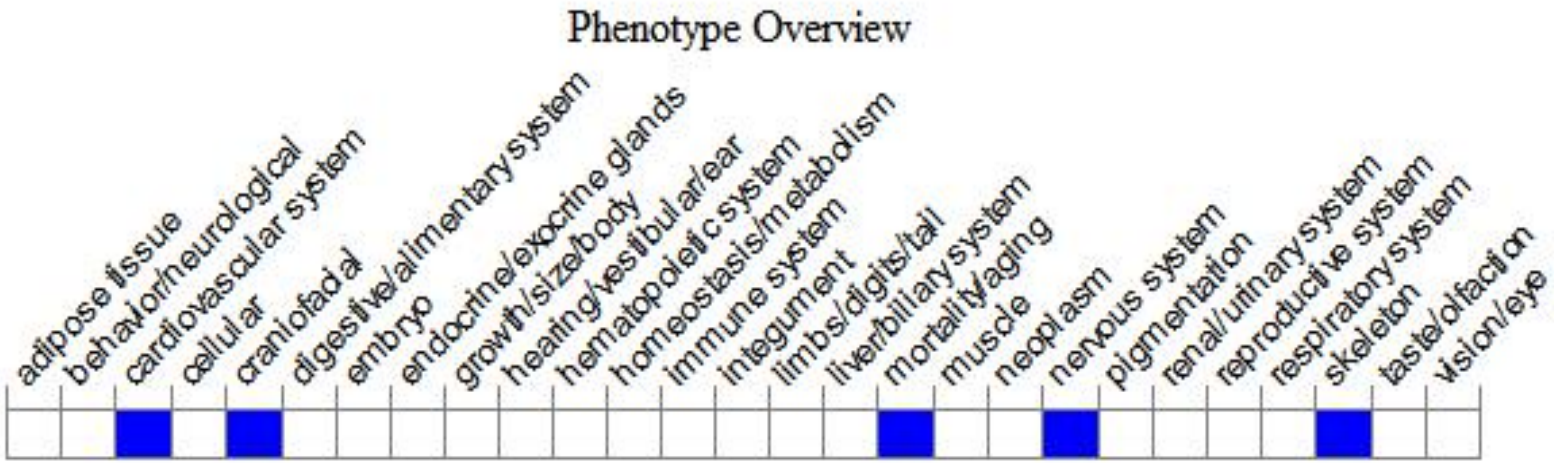
Genomic location distribution



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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