

Hoxb5 Cas9-KO Strategy

Designer:

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Project Overview

Project Name

Hoxb5

Project type

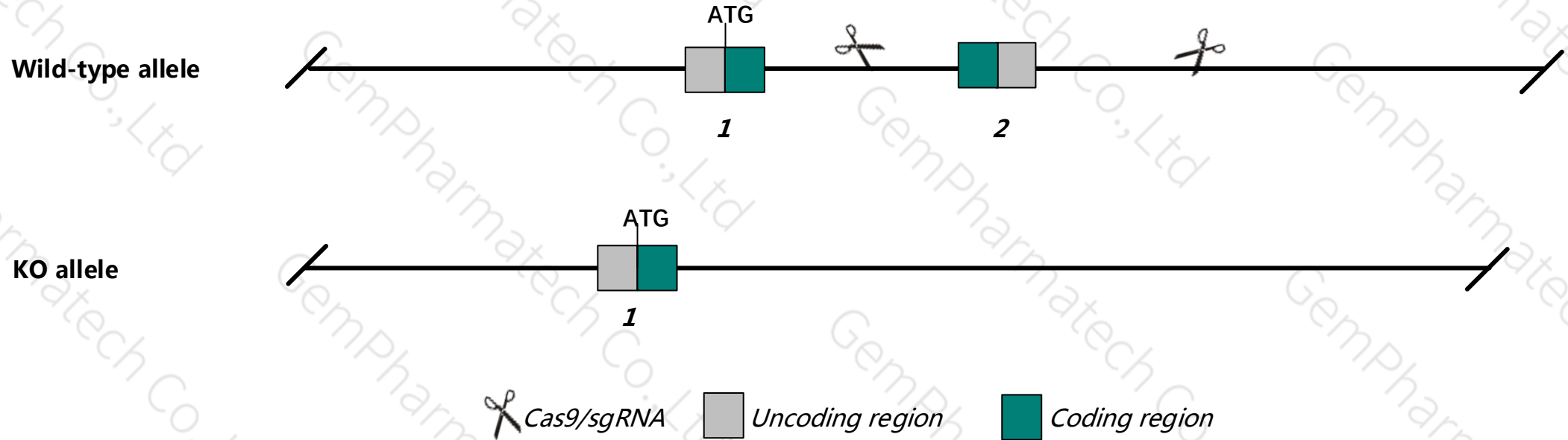
Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Hoxb5* gene. The schematic diagram is as follows:



Technical routes

- The *Hoxb5* gene has 2 transcripts. According to the structure of *Hoxb5* gene, exon2 of *Hoxb5*-201 (ENSMUST00000049272.4) transcript is recommended as the knockout region. The region contains key coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Hoxb5* gene. The brief process is as follows: sgRNA was transcribed in vitro. Cas9, sgRNA 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.

- According to the existing MGI data , Mice homozygous for a knock-out allele are viable and fertile but exhibit a rostral shift of the shoulder girdle resulting in altered position of the forelimbs, and show variable anteriorizing homeotic transformations of cervicothoracic vertebrae C6 through T1.
- The KO region contains functional region of the *Hoxb5os* gene. Knockout the region may affect the function of *Hoxb5os* gene.
- The *Hoxb5* gene is located on the Chr11. 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 knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



Hoxb5 homeobox B5 [*Mus musculus* (house mouse)]

Gene ID: 15413, updated on 10-Oct-2019

Summary

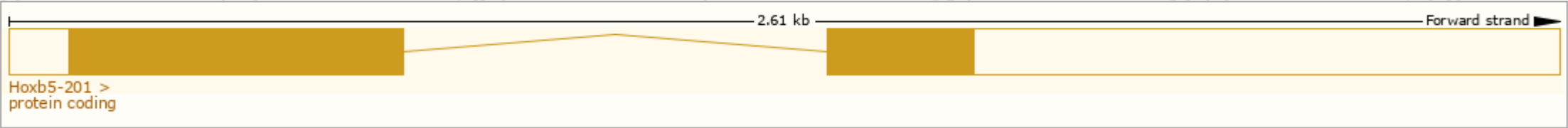
Official Symbol	Hoxb5 provided by MGI
Official Full Name	homeobox B5 provided by MGI
Primary source	MGI:MGI:96186
See related	Ensembl:ENSMUSG00000038700
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Hox-2.1; AI385717
Expression	Broad expression in adrenal adult (RPKM 20.9), CNS E14 (RPKM 8.8) and 15 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

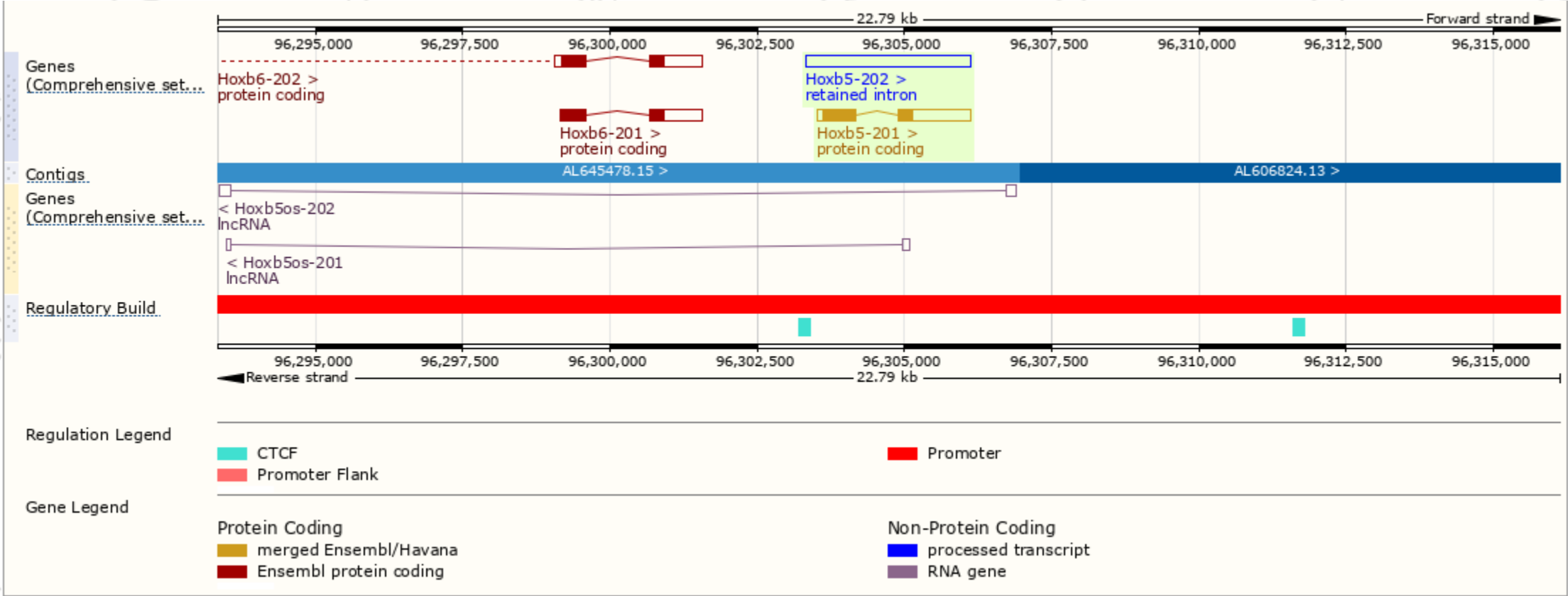
The gene has 2 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hoxb5-201	ENSMUST00000049272.4	1895	269aa	Protein coding	CCDS25296	P09079	TSL:1 Gencode basic APPRIS P1
Hoxb5-202	ENSMUST00000190470.1	2786	No protein	Retained intron	-	-	TSL:NA

The strategy is based on the design of *Hoxb5*-201 transcript, The transcription is shown below



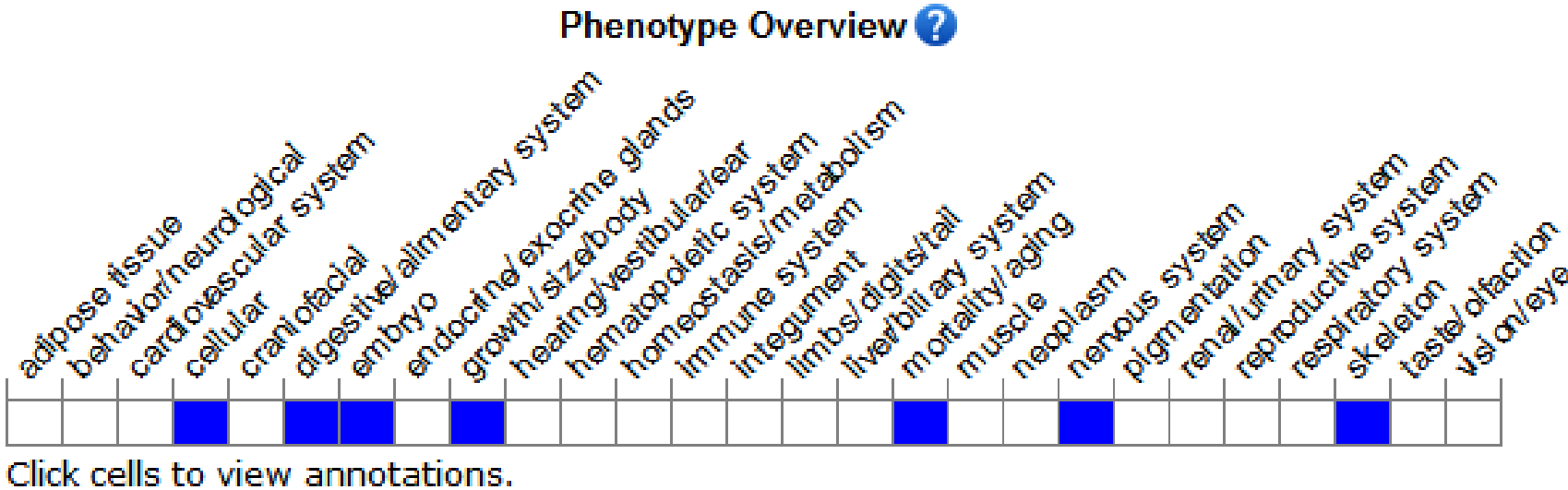
Genomic location distribution



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 knock-out allele are viable and fertile but exhibit a rostral shift of the shoulder girdle resulting in altered position of the forelimbs, and show variable anteriorizing homeotic transformations of cervicothoracic vertebrae C6 through T1.

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