

***Hoxd13* Cas9-CKO Strategy**

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Project Overview

Project Name

Hoxd13

Project type

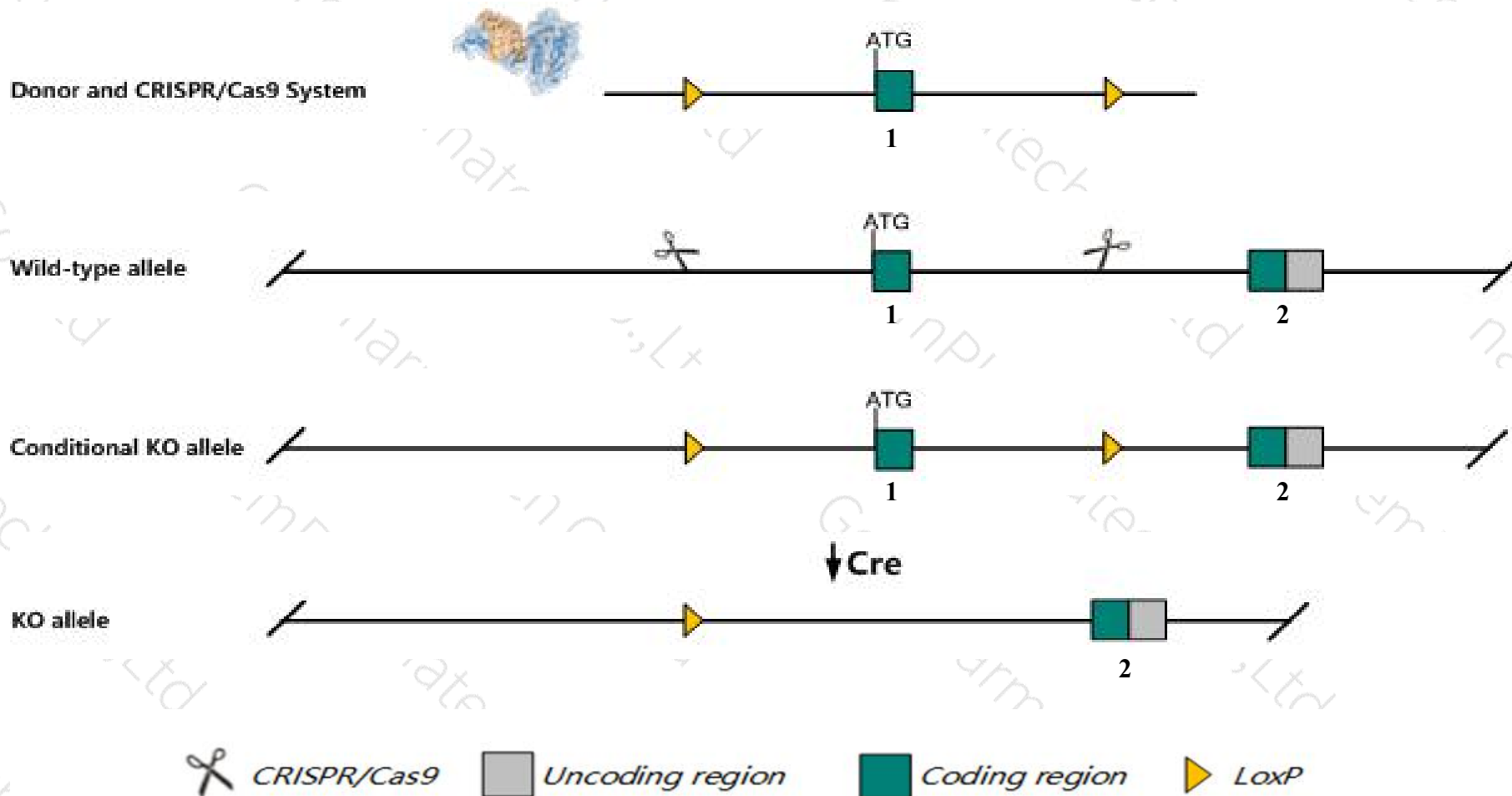
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Hoxd13* gene. The schematic diagram is as follows:



- The *Hoxd13* gene has 1 transcript. According to the structure of *Hoxd13* gene, exon1 of *Hoxd13-201* (ENSMUST00000001872.4) 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 *Hoxd13* 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.

- According to the existing MGI data, Homozygotes for targeted and spontaneous mutations exhibit abnormalities of the axial skeleton, especially limbs, and of the male accessory organs, and agenesis of the preputial glands. Mutant males are sterile.
- The *Hoxd13* 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)

Hoxd13 homeobox D13 [*Mus musculus* (house mouse)]

Gene ID: 15433, updated on 5-Nov-2019

Summary

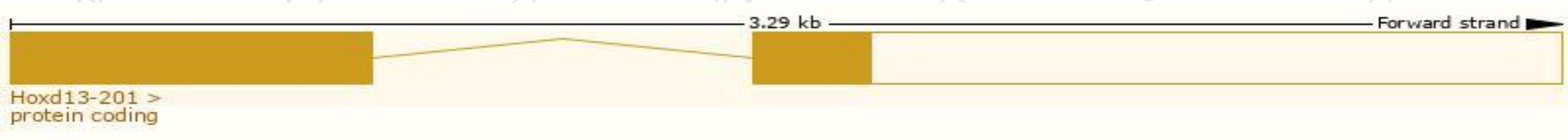
Official Symbol	Hoxd13 provided by MGI
Official Full Name	homeobox D13 provided by MGI
Primary source	MGI:MGI:96205
See related	Ensembl:ENSMUSG00000001819
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	spdh; Hox-4.8
Expression	Biased expression in limb E14.5 (RPKM 44.7), colon adult (RPKM 15.1) and 1 other tissue See more
Orthologs	human all

Transcript information (Ensembl)

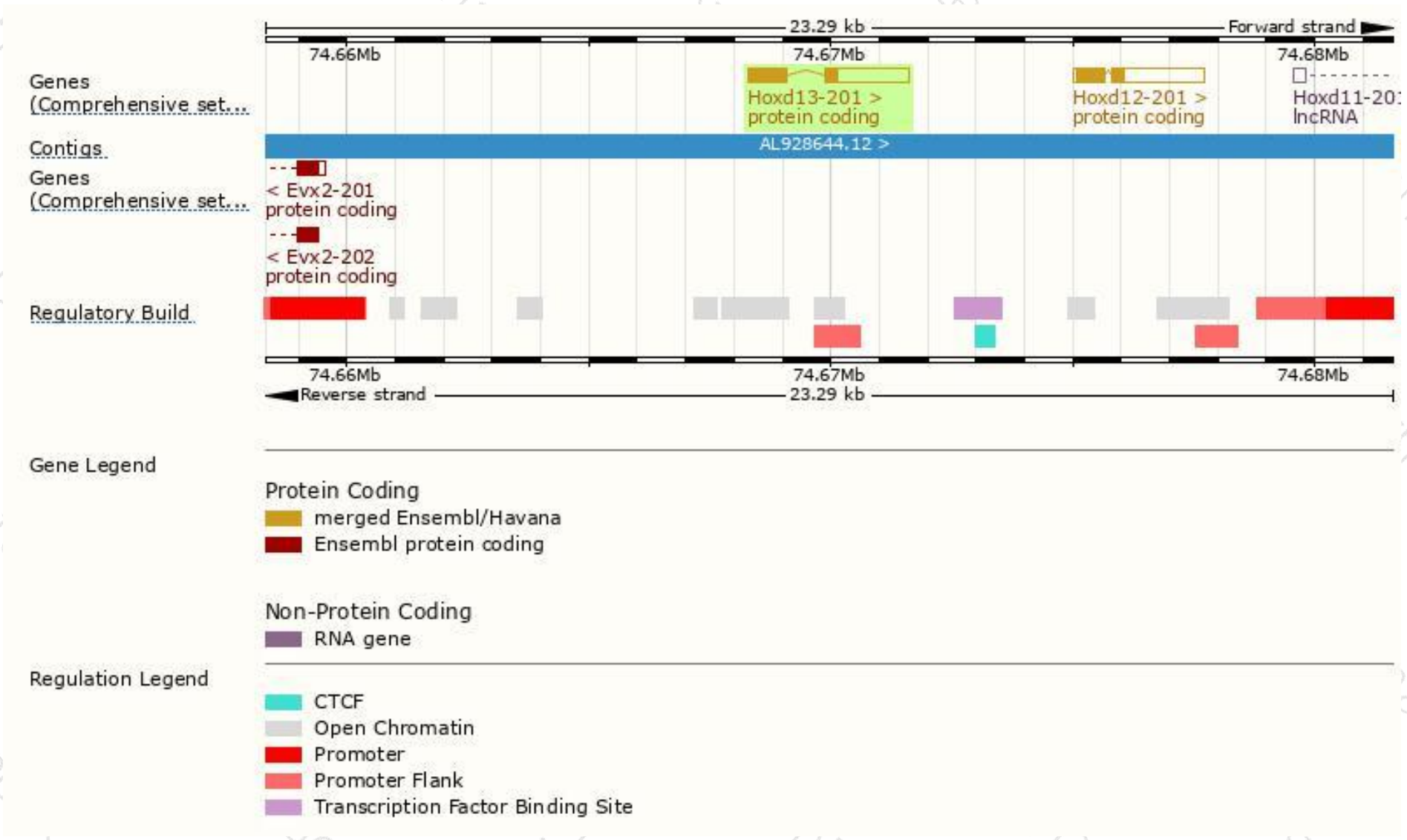
The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hoxd13-201	ENSMUST00000001872.4	2483	339aa	Protein coding	CCDS16139	P70217	TSL:1 GENCODE basic APPRIS P1

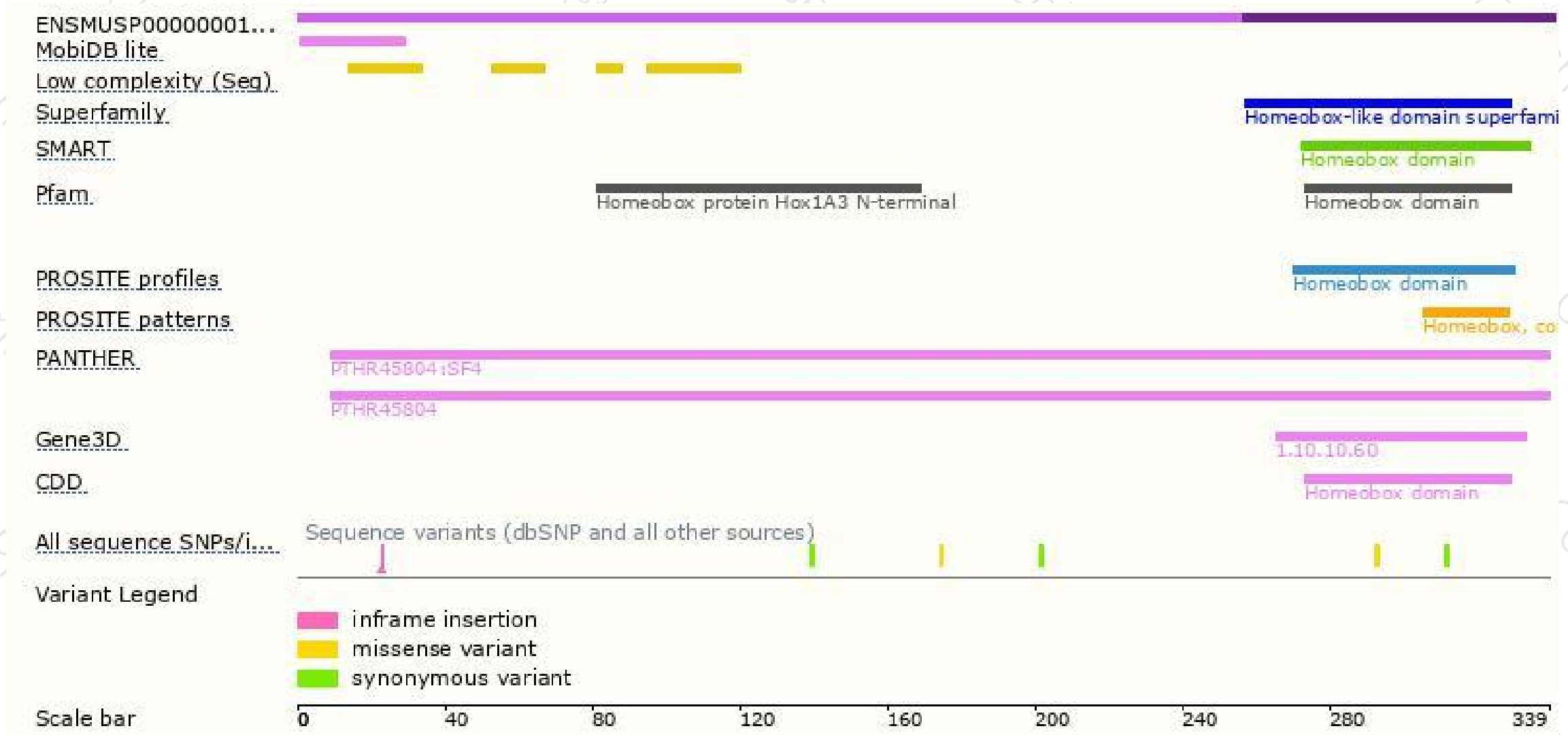
The strategy is based on the design of *Hoxd13-201* transcript, The transcription is shown below



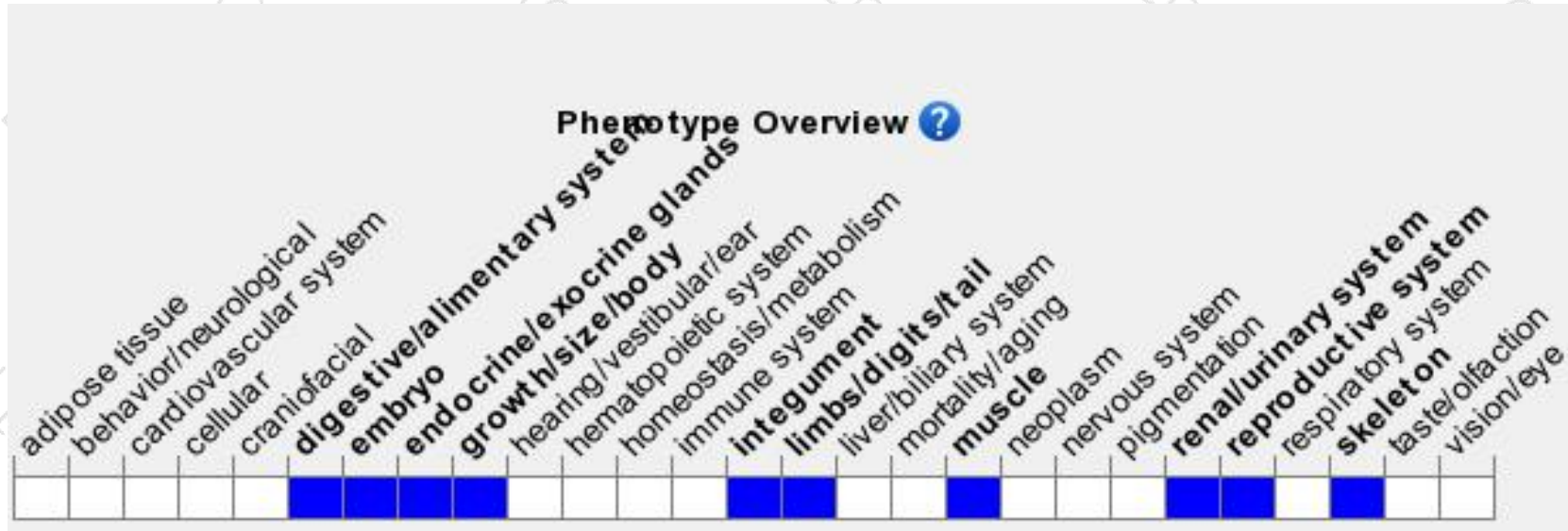
Genomic location distribution



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, Homozygotes for targeted and spontaneous mutations exhibit abnormalities of the axial skeleton, especially limbs, and of the male accessory organs, and agenesis of the preputial glands. Mutant males are sterile.

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

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