Six1 Cas9-CKO Strategy

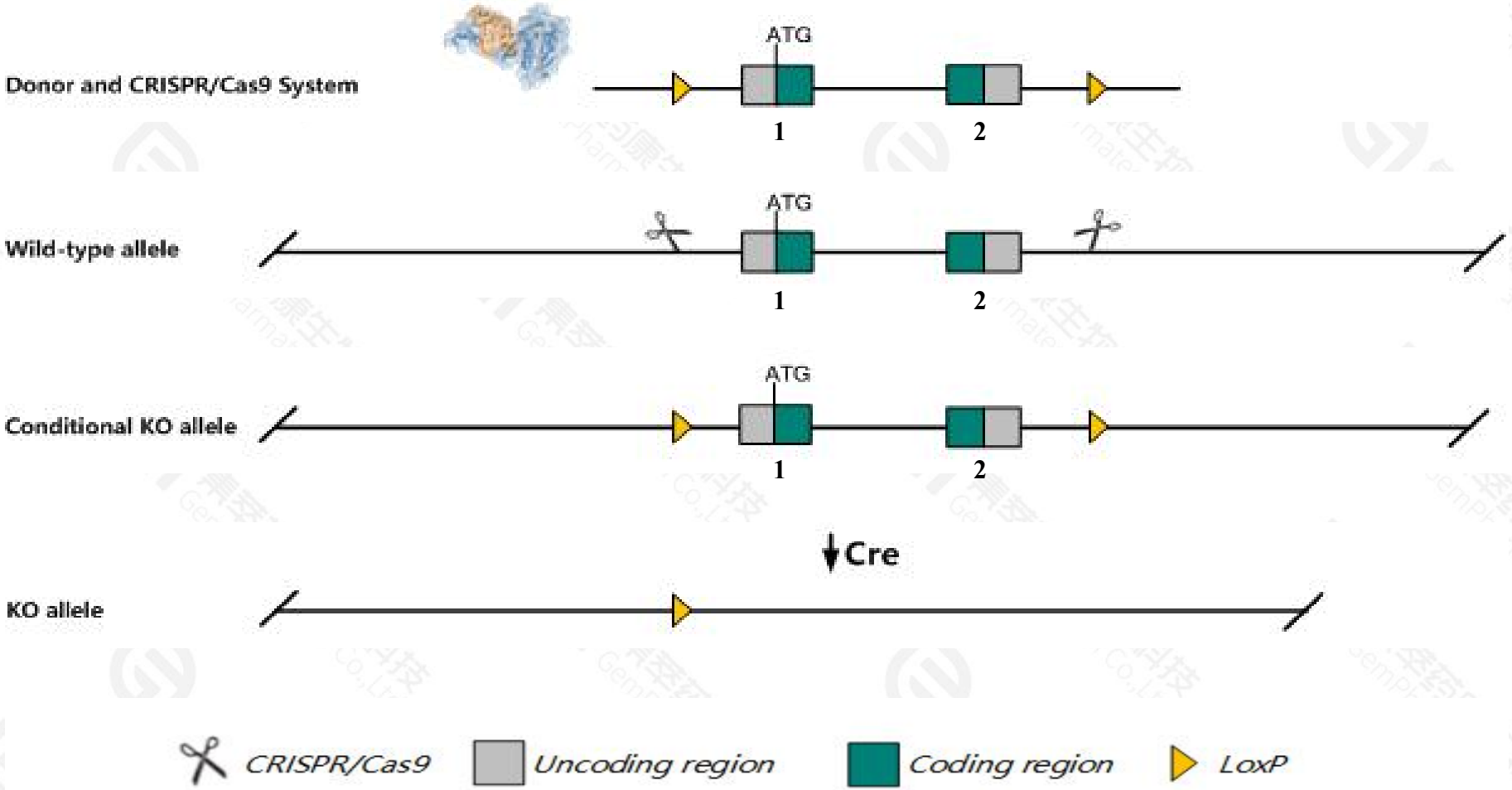
Designer:Qiong Zhou

Project Overview

Project Name	***Six1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Six1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Six1* gene has 4 transcripts. According to the structure of *Six1* gene, exon1-exon2 of *Six1-201*(ENSMUST00000050029.8) 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 *Six1* 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,homozygous inactivation of this gene causes perinatal lethality associated with severe muscle hypoplasia, rib defects, absence of kidneys and thymus, craniofacial anomalies, as well as defects in neurogenesis and ear, nasal, and gland development. Heterozygotes may show variable hearing loss.

➢ The *Six1* gene is located on the Chr12. 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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Six1 sine oculis-related homeobox 1 [Mus musculus (house mouse)]

Gene ID: 20471, updated on 13-Mar-2020

Summary

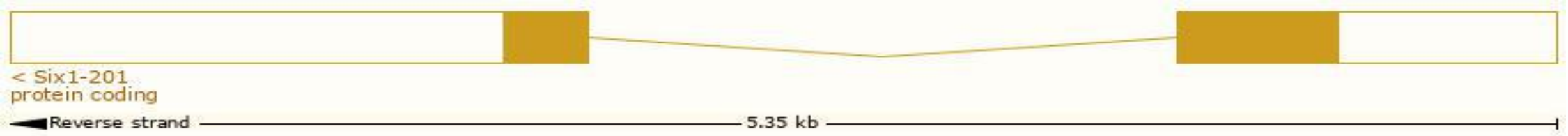
Official Symbol	Six1 provided by MGI
Official Full Name	sine oculis-related homeobox 1 provided byMGI
Primary source	MGI:MGI:102780
See related	Ensembl:ENSMUSG00000051367
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BB138287
Expression	Biased expression in limb E14.5 (RPKM 14.6), CNS E11.5 (RPKM 6.0) and 7 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

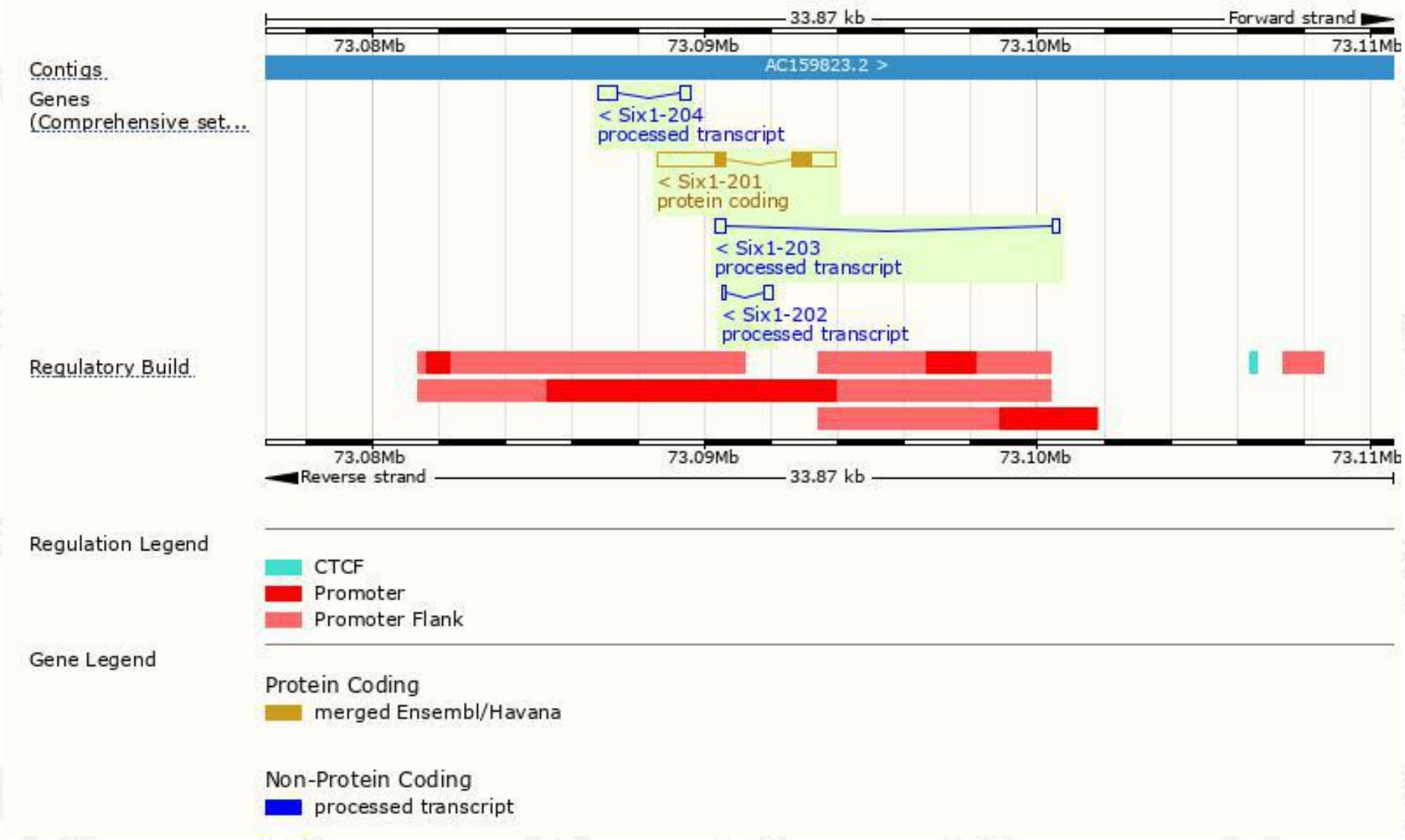
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Six1-201	ENSMUST00000050029.7	3316	284aa	Protein coding	CCDS25973	Q3V2C3 Q62231	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Six1-204	ENSMUST00000176310.1	885	No protein	Processed transcript	-	-	TSL:3
Six1-203	ENSMUST00000176091.1	528	No protein	Processed transcript	-	-	TSL:2
Six1-202	ENSMUST00000175677.1	334	No protein	Processed transcript	-	-	TSL:3

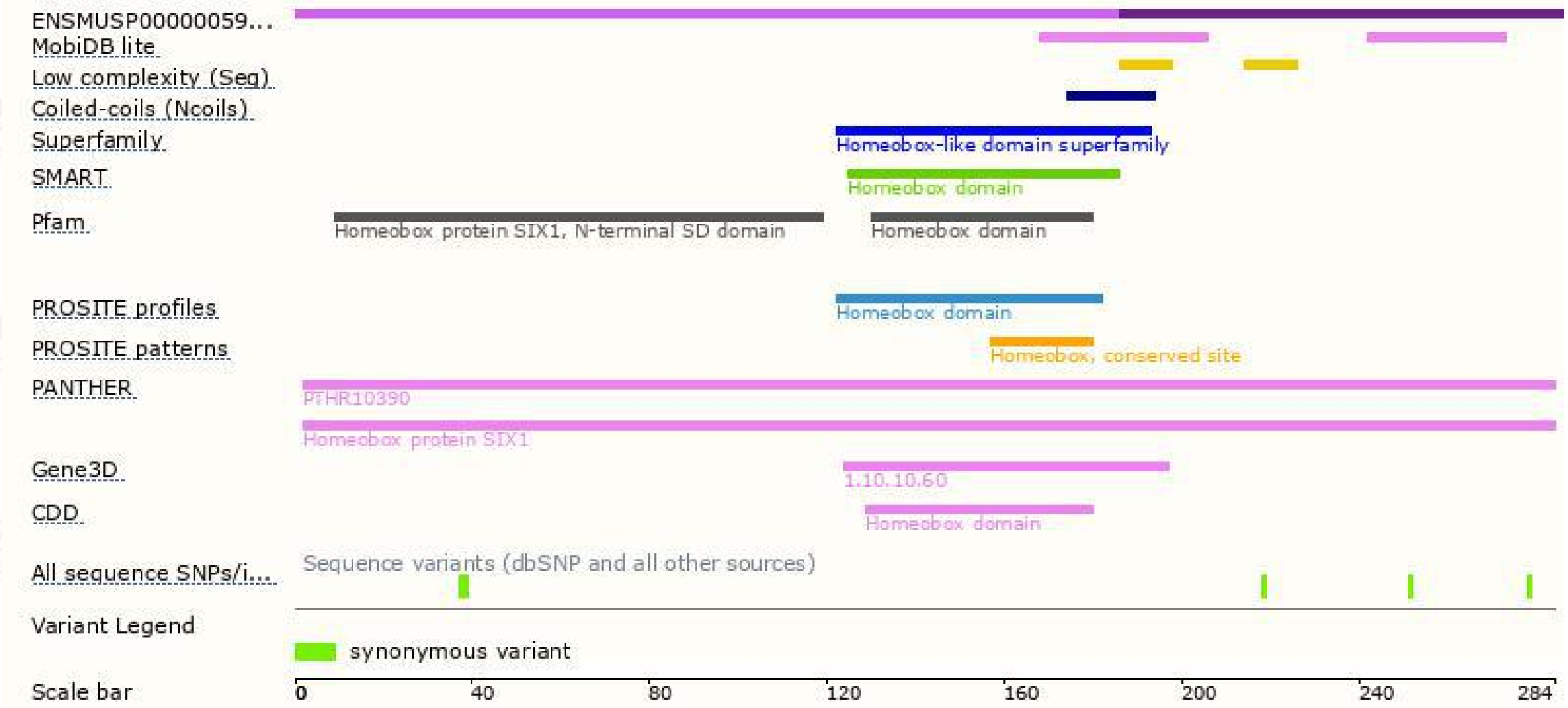
The strategy is based on the design of *Six1-201* transcript,the transcription is shown below:



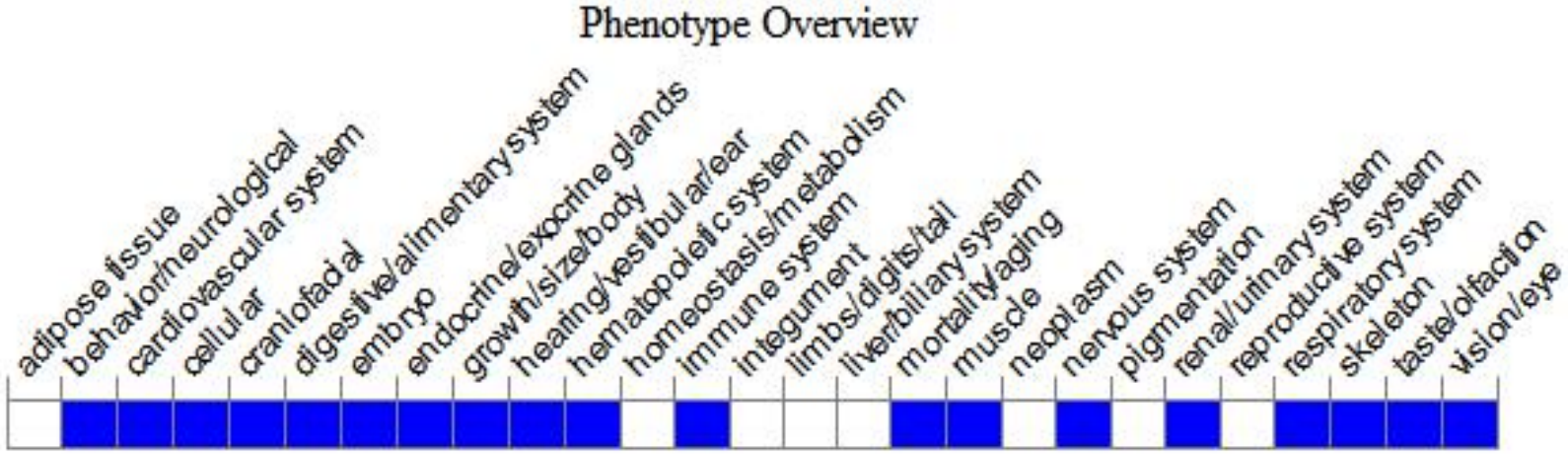
Genomic location distribution



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,homozygous inactivation of this gene causes perinatal lethality associated with severe muscle hypoplasia, rib defects, absence of kidneys and thymus, craniofacial anomalies, as well as defects in neurogenesis and ear, nasal, and gland development. Heterozygotes may show variable hearing loss.

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