Twist1 Cas9-CKO Strategy

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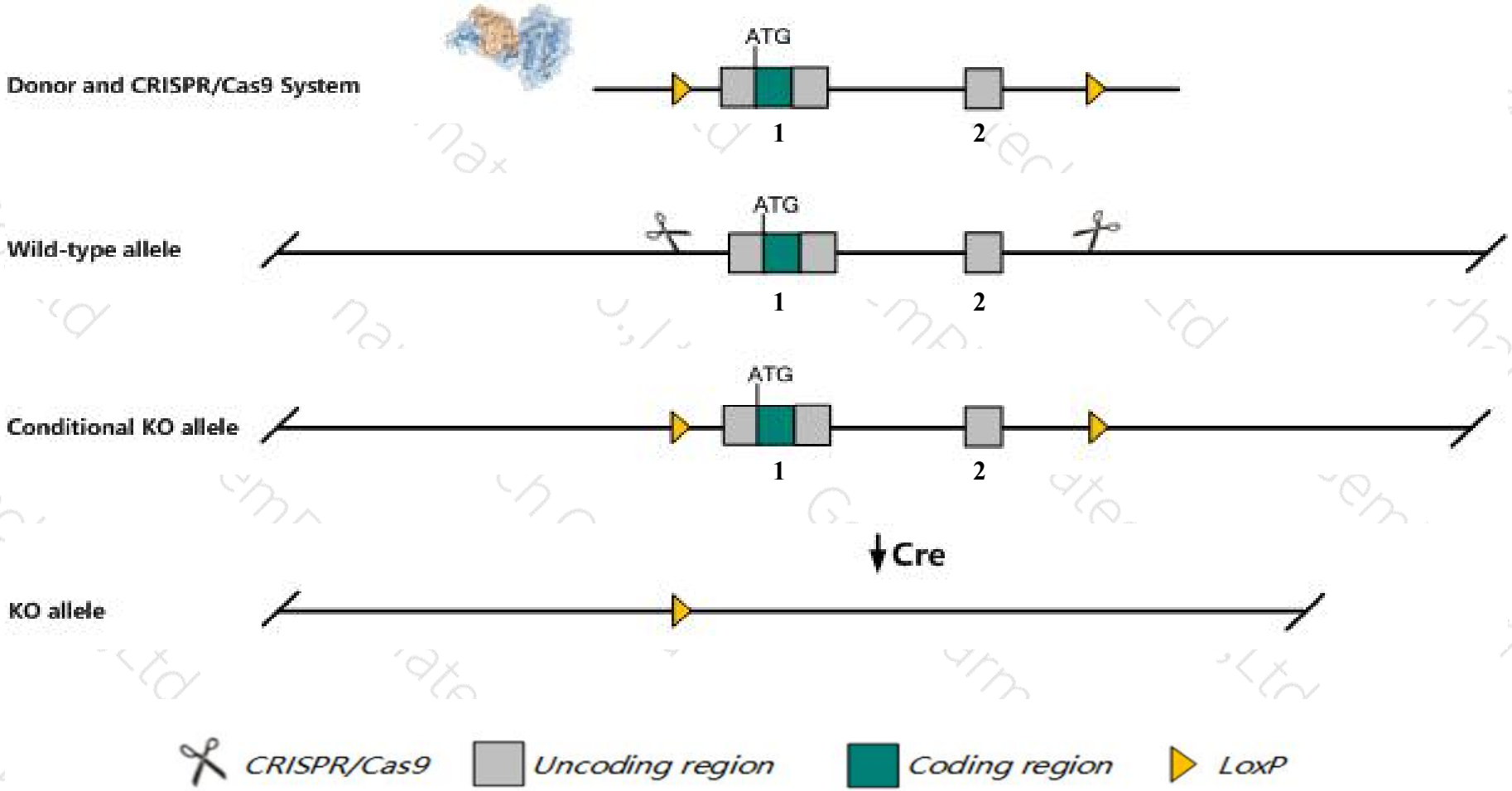
Project Overview

Project Name	***Twist1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Twist1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Twist1* gene has 1 transcript. According to the structure of *Twist1* gene, exon1-exon2 of *Twist1-201*(ENSMUST00000049089.6) 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 *Twist1* 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 embyros have neural tube defects and die around E11. Heterozygous mutants are viable and exhibit features of human Saethre-Chotzen syndrome, including hindlimb polydactyly, craniofacial defects, long bone abnormalities, an abnormal gait and a small size.

➢ The *Twist1* 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）

集萃药康 GemPharmatech

Twist1 twist basic helix-loop-helix transcription factor 1 [Mus musculus (house mouse)]

Gene ID: 22160, updated on 13-Mar-2020

Summary

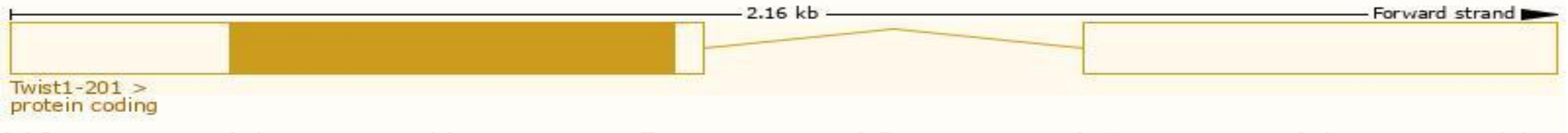
Official Symbol	Twist1 provided by MGI
Official Full Name	twist basic helix-loop-helix transcription factor 1 provided by MGI
Primary source	MGI:MGI:98872
See related	Ensembl:ENSMUSG00000035799
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	M-Twist, Pde, Ska10, Ska, Twist, bHLHa38, pdt
Summary	Basic helix-loop-helix (bHLH) transcription factors have been implicated in cell lineage determination and differentiation. This gene encodes a bHLH transcription factor that is evolutionarily conserved from invertebrates to humans, and was originally identified in Drosophila as an essential gene involved in early mesoderm development and dorsal-ventral patterning in the embryo. This protein plays a role in cancer by regulating the epithelial-mesenchymal transition (EMT), a process that is critical for metastasis initiation, and promoting tumor progression. Mutations in the human gene are associated with Saethre-Chotzen syndrome (SCS). Mice with heterozygous mutations in this gene exhibit cranofacial and structural defects similar to those seen in human SCS patients. [provided by RefSeq, Sep 2015]
Expression	Biased expression in limb E14.5 (RPKM 91.2), CNS E11.5 (RPKM 22.7) and 6 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

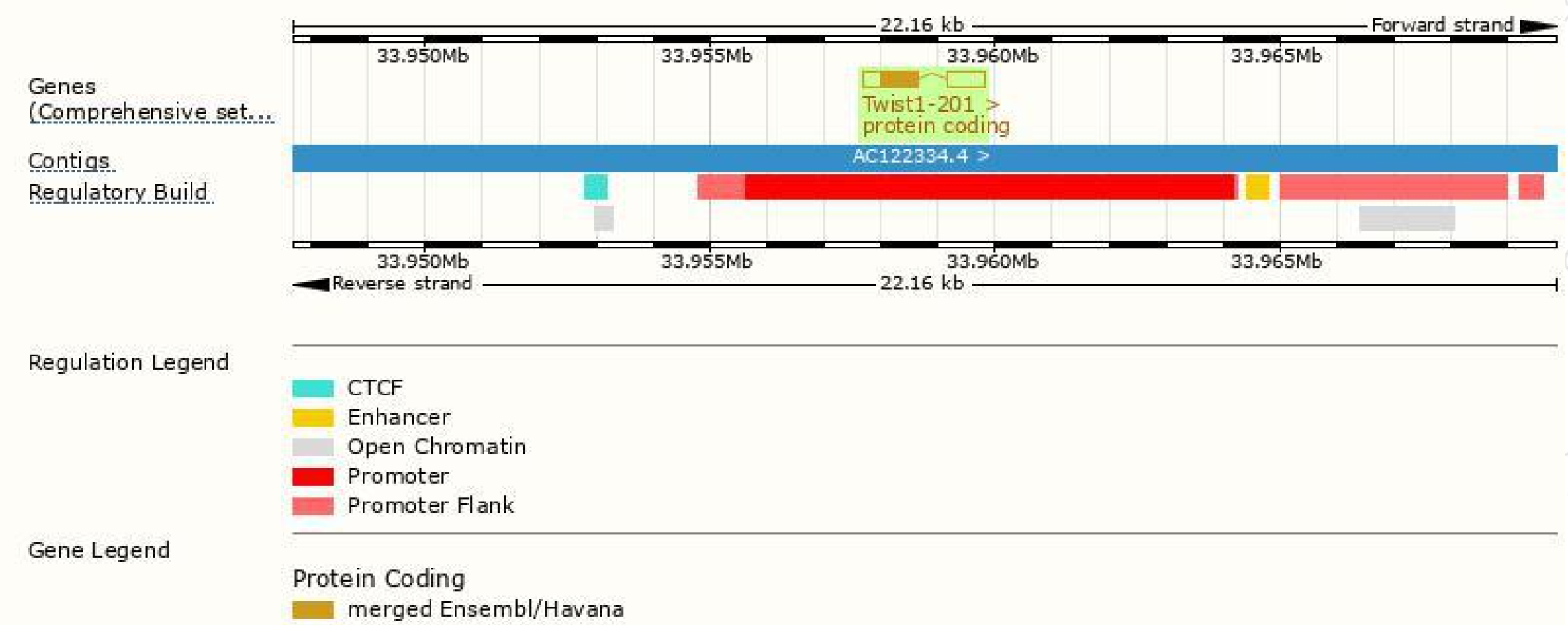
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Twist1-201	ENSMUST00000049089.6	1628	206aa	Protein coding	CCDS25879	P26687	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

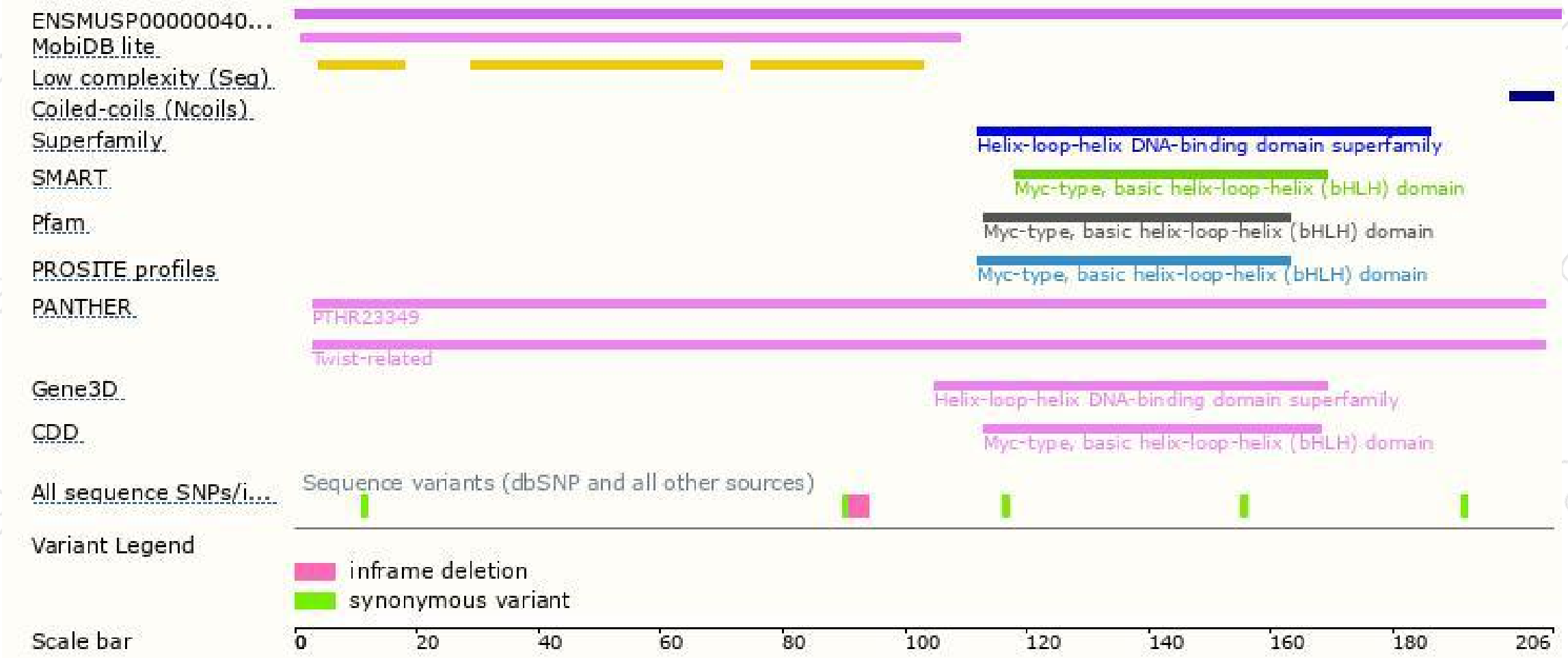
The strategy is based on the design of *Twist1-201* transcript,the transcription is shown below:



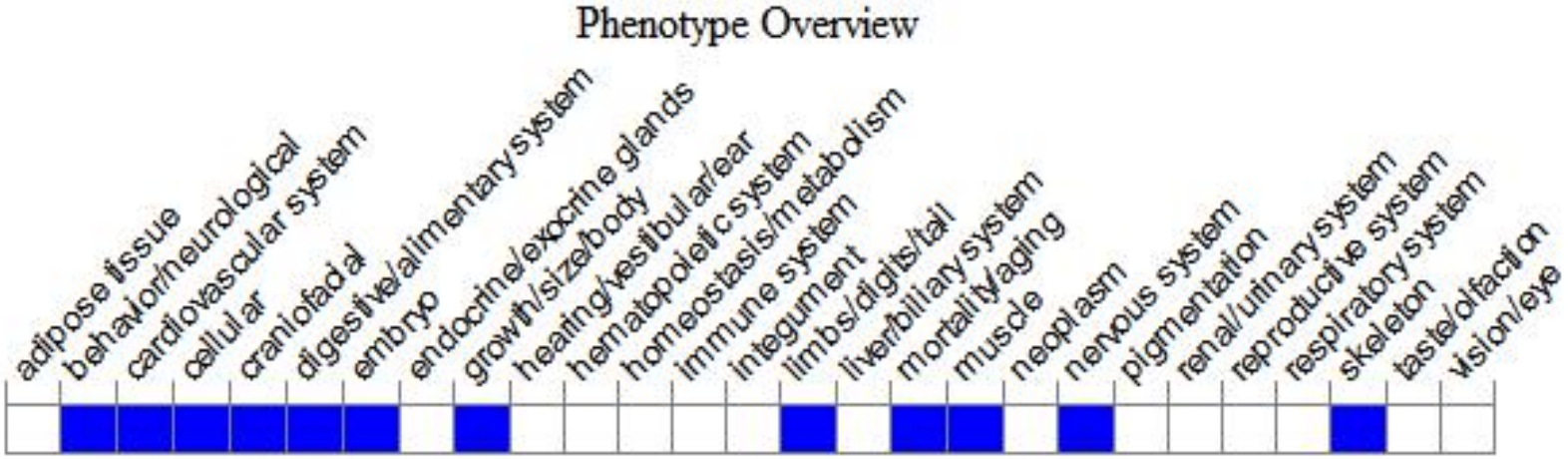
Genomic location distribution



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/).

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

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