Foxi3 Cas9-CKO Strategy

Designer: Xiaojing Li

Reviewer: JiaYu

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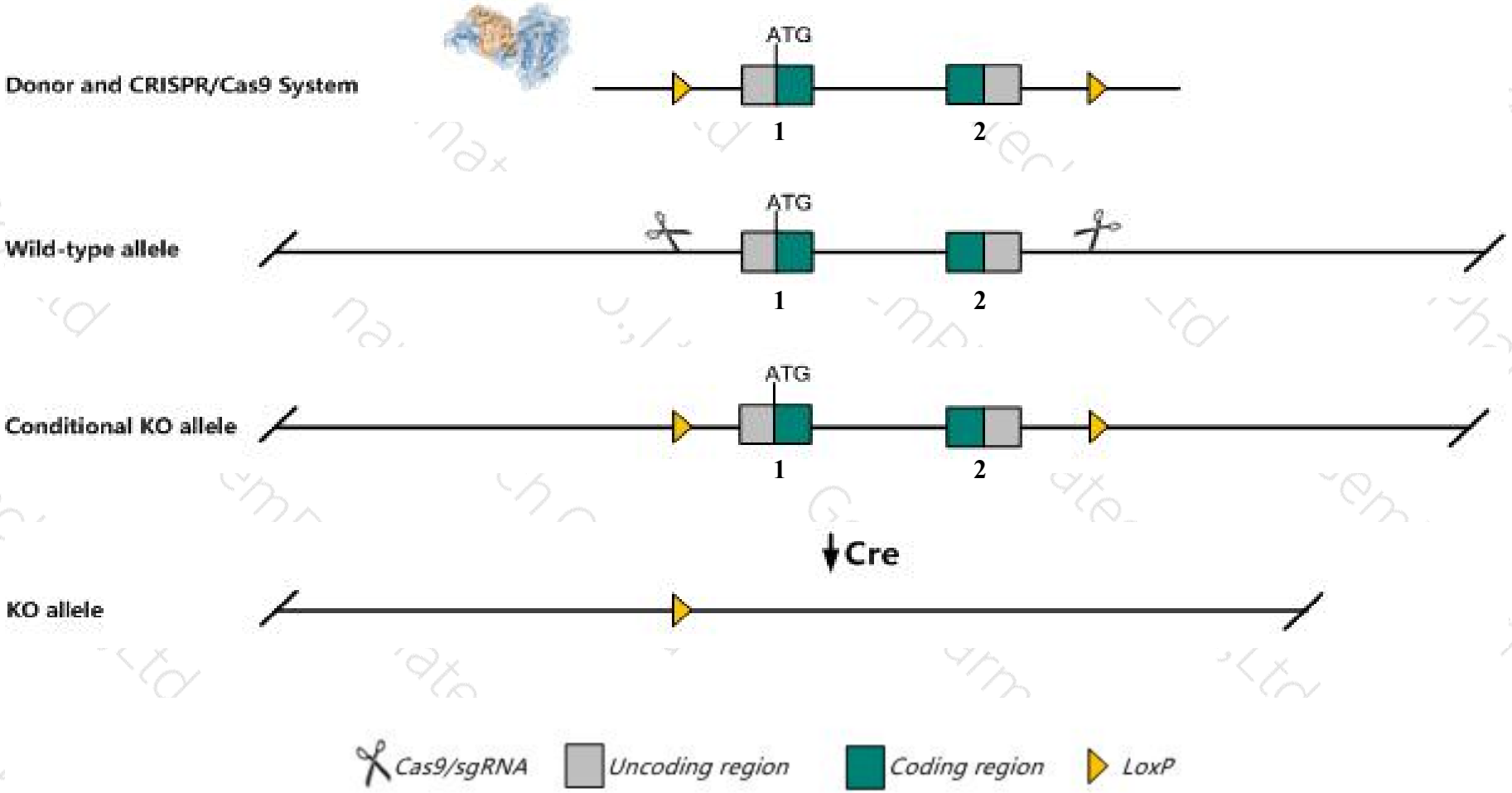
Project Overview

Project Name	***Foxi3***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Foxi3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Foxi3* gene has 2 transcripts. According to the structure of *Foxi3* gene, exon1-exon2 of *Foxi3-201*(ENSMUST00000069634.5) 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 *Foxi3* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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 null mice start dying after E9.5. Those born die neonatally, lack a mouth and whiskers, and show branchial arch-derived skeletal defects, including a reduced mandible, total absence of inner, middle and external ear structures, and increased cranial neural crest cell apoptosis.

➢ The *Foxi3* gene is located on the Chr6. 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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Foxi3 forkhead box I3 [Mus musculus (house mouse)]

Gene ID: 232077, updated on 13-Mar-2020

Summary

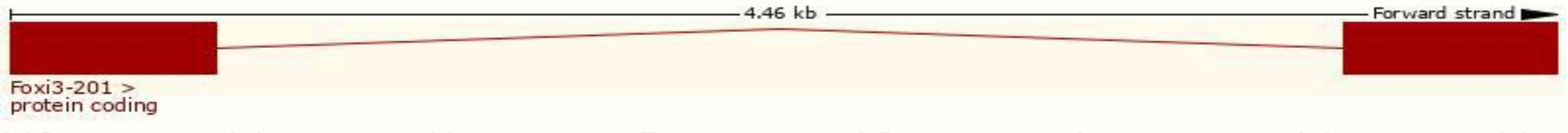
Official Symbol	Foxi3 provided by MGI
Official Full Name	forkhead box I3 provided byMGI
Primary source	MGI:MGI:3511278
See related	Ensembl:ENSMUSG00000055874
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Low expression observed in reference datasetSee more
Orthologs	human all

Transcript information（Ensembl）

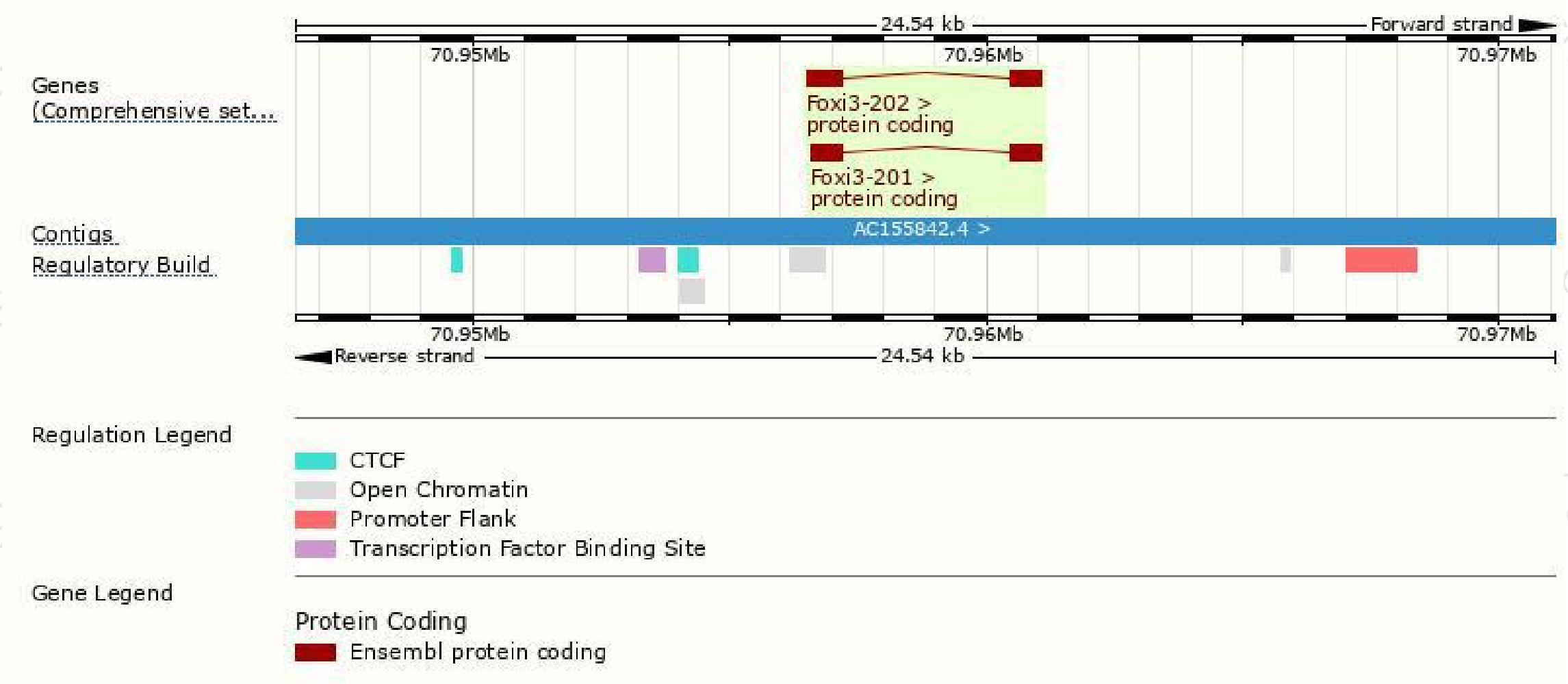
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Foxi3-201	ENSMUST00000069634.5	1206	399aa	Protein coding	CCDS51804	D3Z120	TSL:5 GENCODE basic APPRIS P2
Foxi3-202	ENSMUST00000163089.1	1278	425aa	Protein coding	-	E0CZH3	TSL:5 GENCODE basic APPRIS ALT2

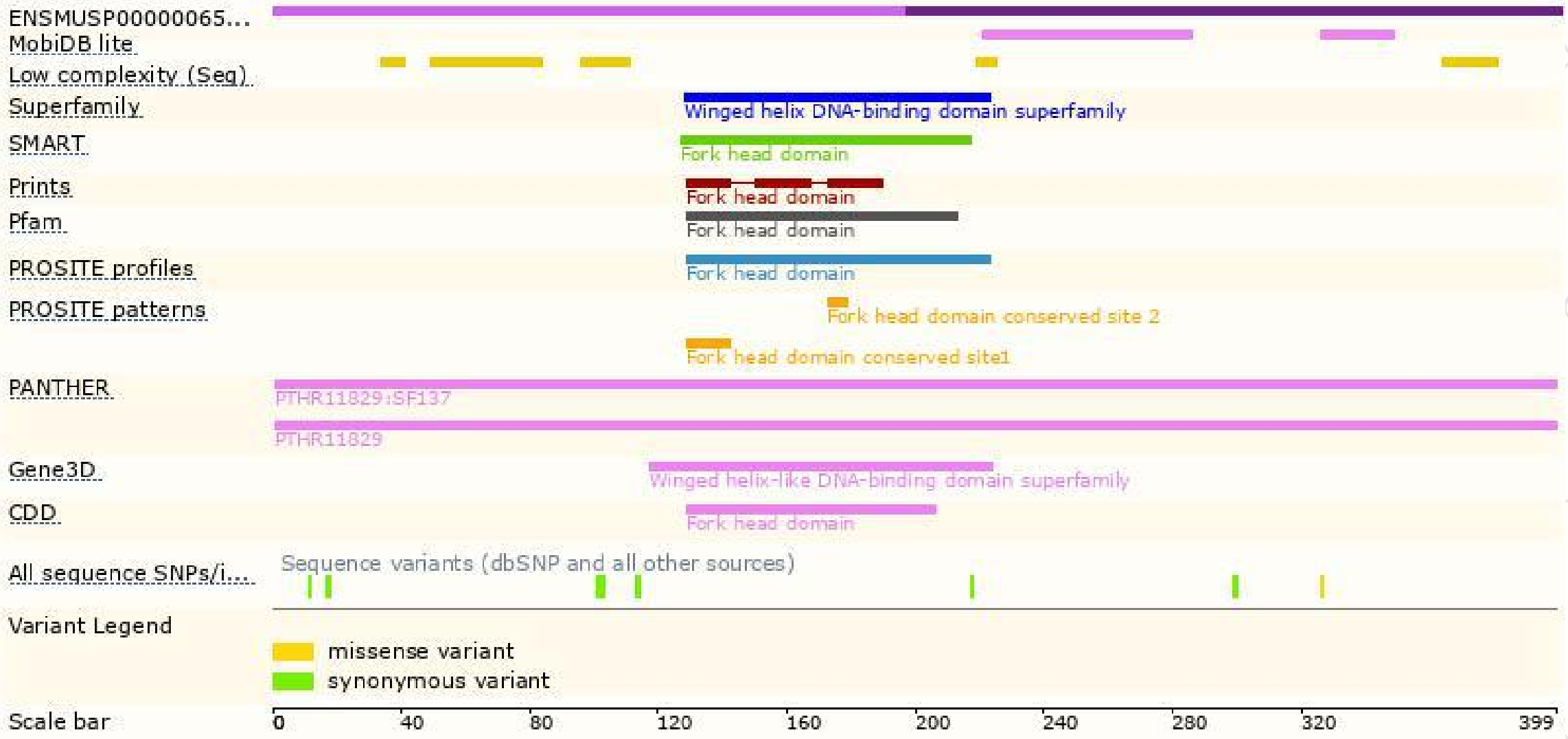
The strategy is based on the design of *Foxi3-201* transcript,the transcription is shown below:



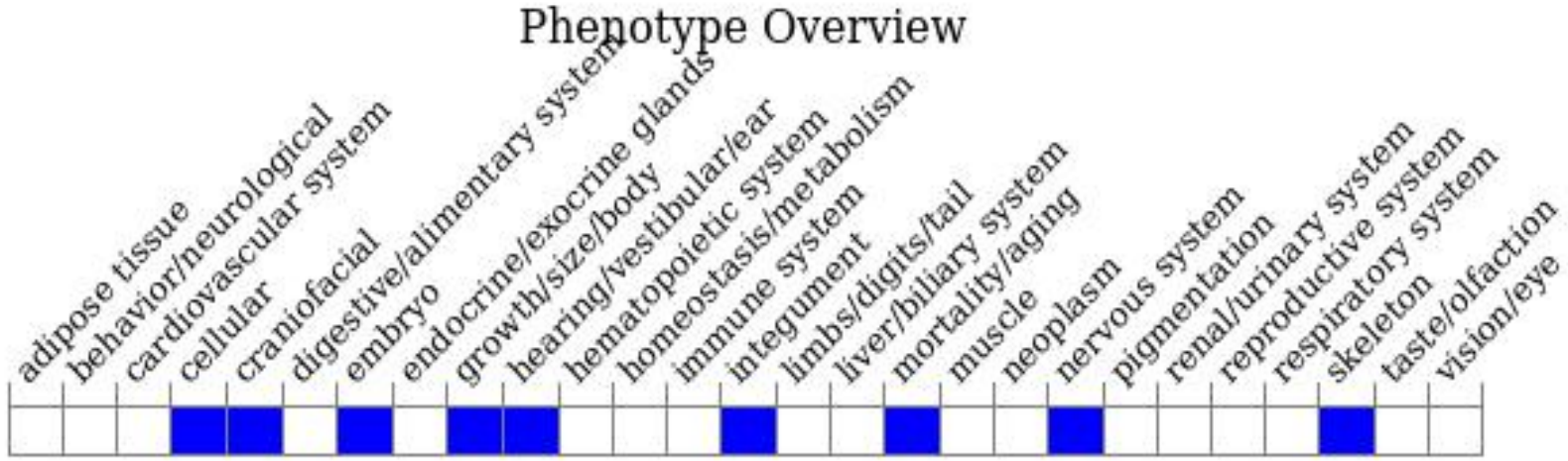
Genomic location distribution



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.

Tel: 025-5864 1534

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GemPharmatech Co.,Ltd