Foxa2 Cas9-CKO Strategy

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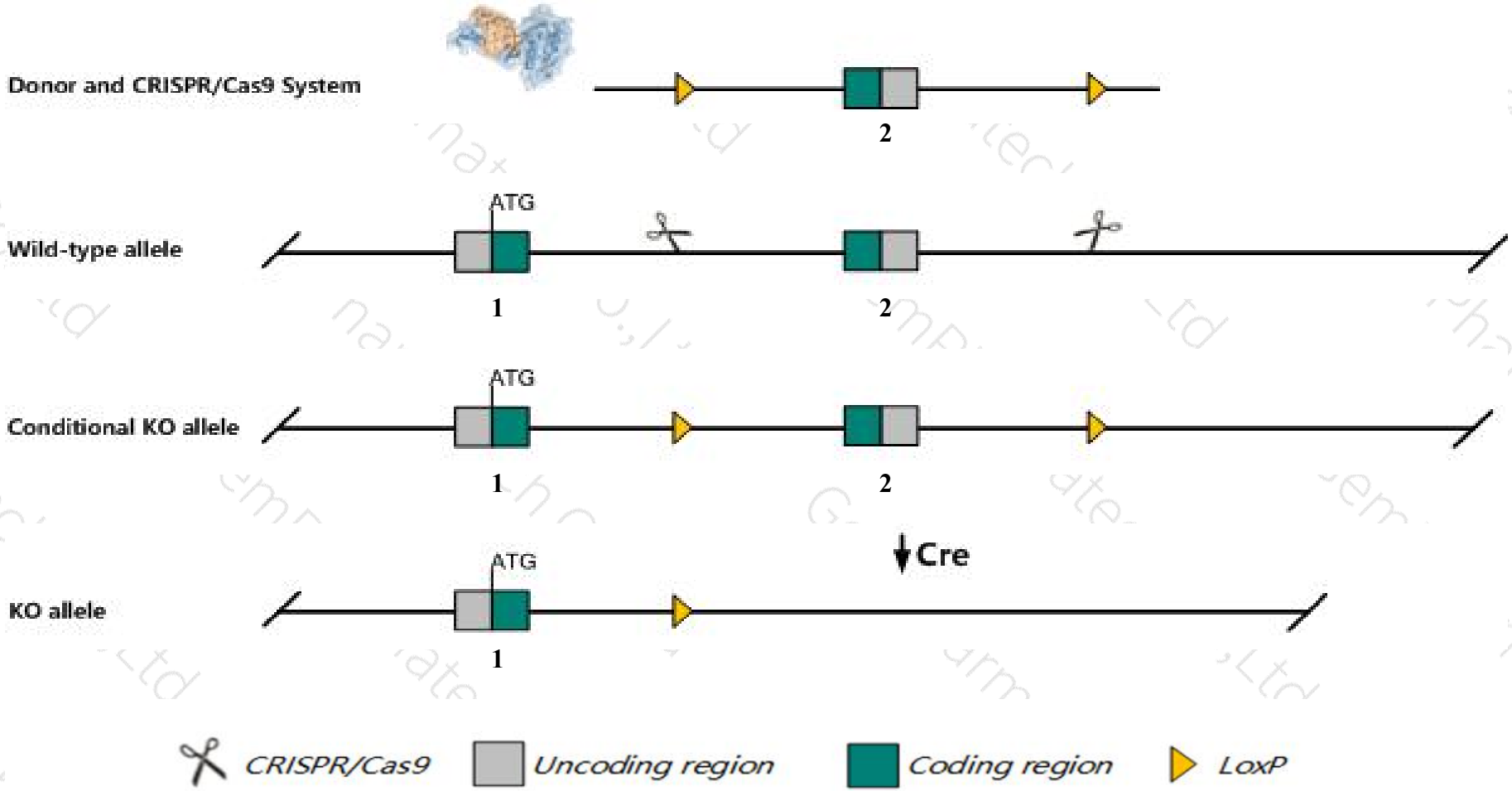
Project Overview

Project Name	***Foxa2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Foxa2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Foxa2* gene has 4 transcripts. According to the structure of *Foxa2* gene, exon2 of *Foxa2-202*(ENSMUST00000109964.7) transcript is recommended as the knockout region.The region contains most 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 *Foxa2* 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,embryos homozygous for targeted null mutations fail to form a distinct node, lack a notochord, and die by embryonic day 10 or 11. Mutants also exhibit defects of somite and neural tube organization, and lack a floor plate and motor neurons.

➢ The KO region contains functional region of the 9030622O22Rik gene.Knockout the region may affect the function of 9030622O22Rik gene.

➢ The *Foxa2* 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）

集萃药康 GemPharmatech

Foxa2 forkhead box A2 [Mus musculus (house mouse)]

Gene ID: 15376, updated on 13-Mar-2020

Summary

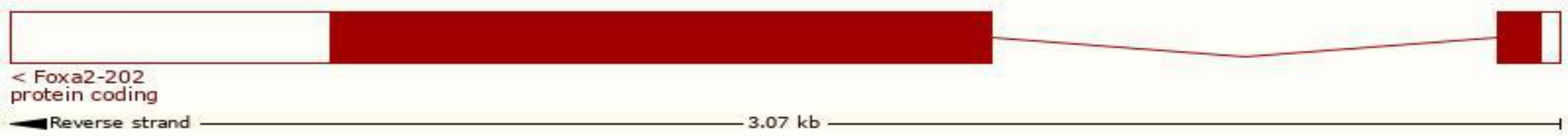
Official Symbol	Foxa2 provided by MGI
Official Full Name	forkhead box A2 provided by MGI
Primary source	MGI:MGI:1347476
See related	Ensembl:ENSMUSG00000037025
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	HNF3-beta, HNF3beta, Hnf-3b, Hnf3b, Tcf-3b, Tcf3b
Expression	Biased expression in colon adult (RPKM 33.4), stomach adult (RPKM 27.8) and 11 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

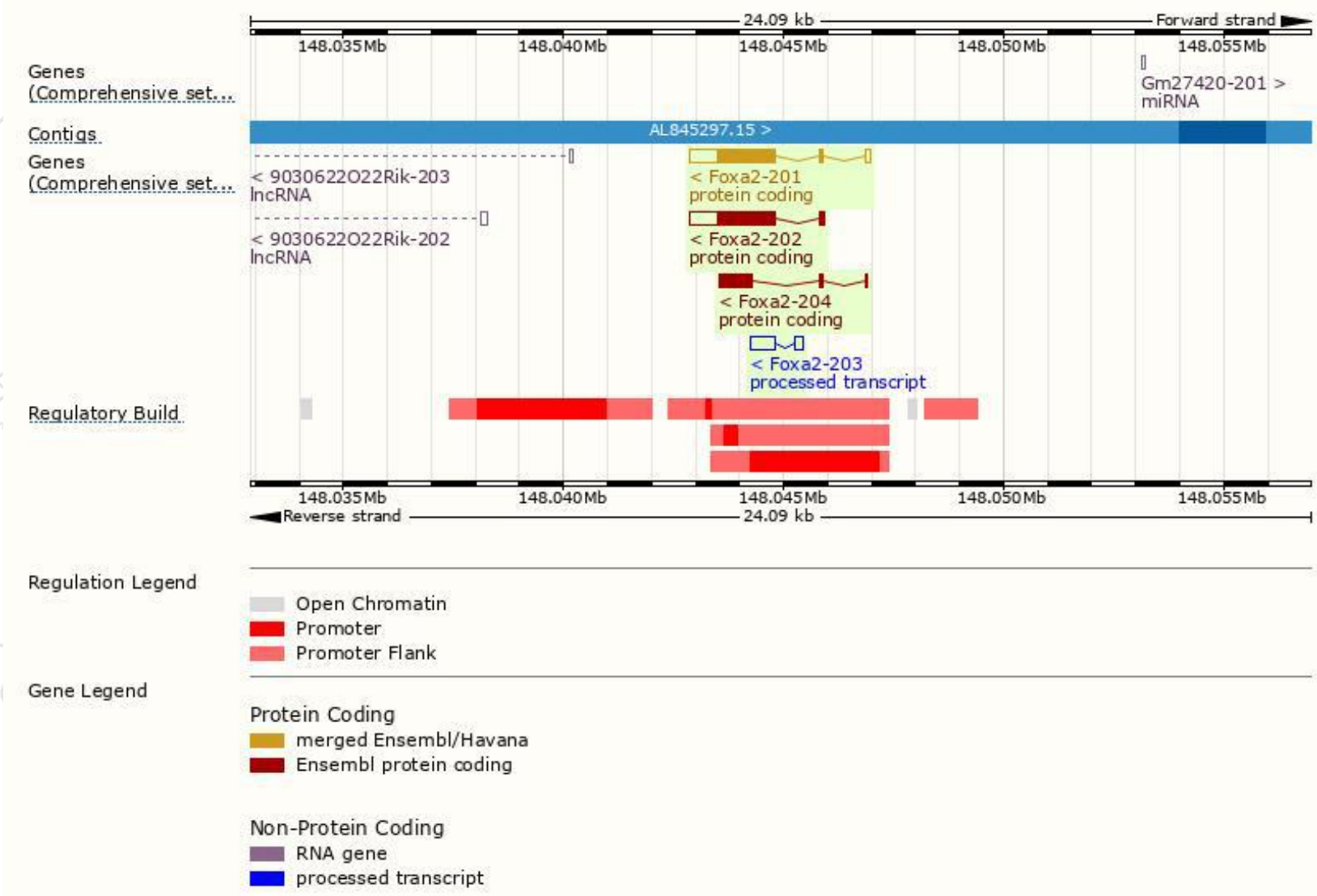
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Foxa2-201	ENSMUST00000047315.9	2128	459aa	Protein coding	CCDS16836	P35583	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 P3
Foxa2-202	ENSMUST00000109964.7	2070	465aa	Protein coding	CCDS71157	G5E8P5	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 ALT1
Foxa2-204	ENSMUST00000172928.1	865	275aa	Protein coding	-	G3UYH0	CDS 3' incomplete TSL:5
Foxa2-203	ENSMUST00000146242.1	775	No protein	Processed transcript	-	-	TSL:2

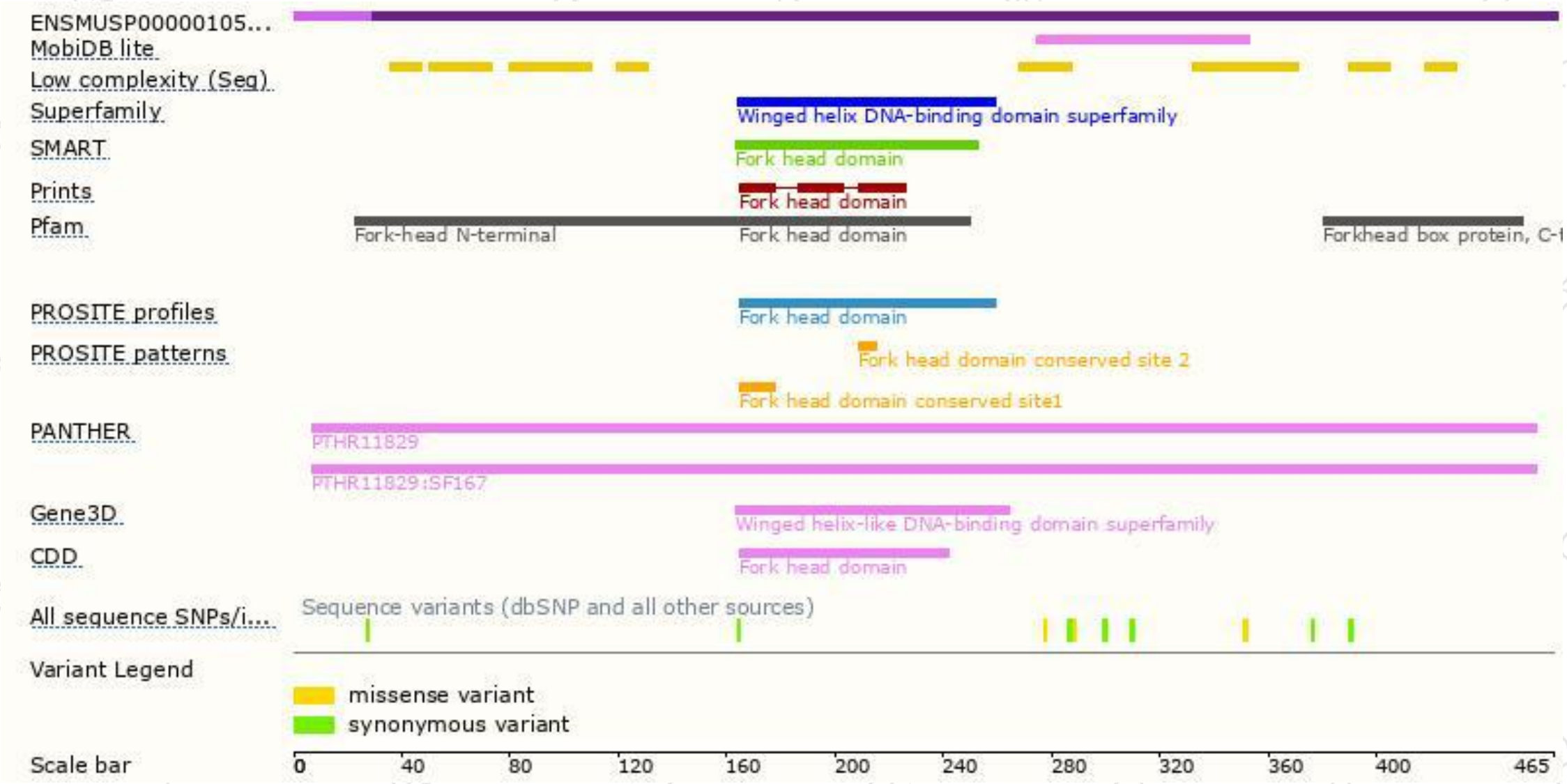
The strategy is based on the design of *Foxa2-202* transcript,the transcription is shown below:



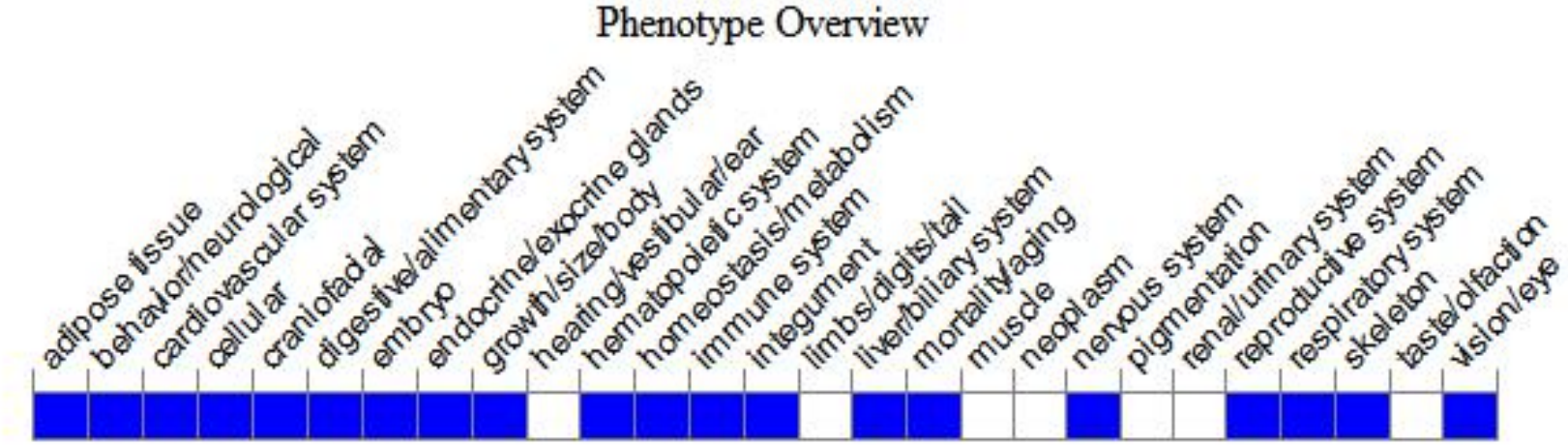
Genomic location distribution



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,embryos homozygous for targeted null mutations fail to form a distinct node, lack a notochord, and die by embryonic day 10 or 11. Mutants also exhibit defects of somite and neural tube organization, and lack a floor plate and motor neurons.

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

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