Bmp4 Cas9-CKO Strategy

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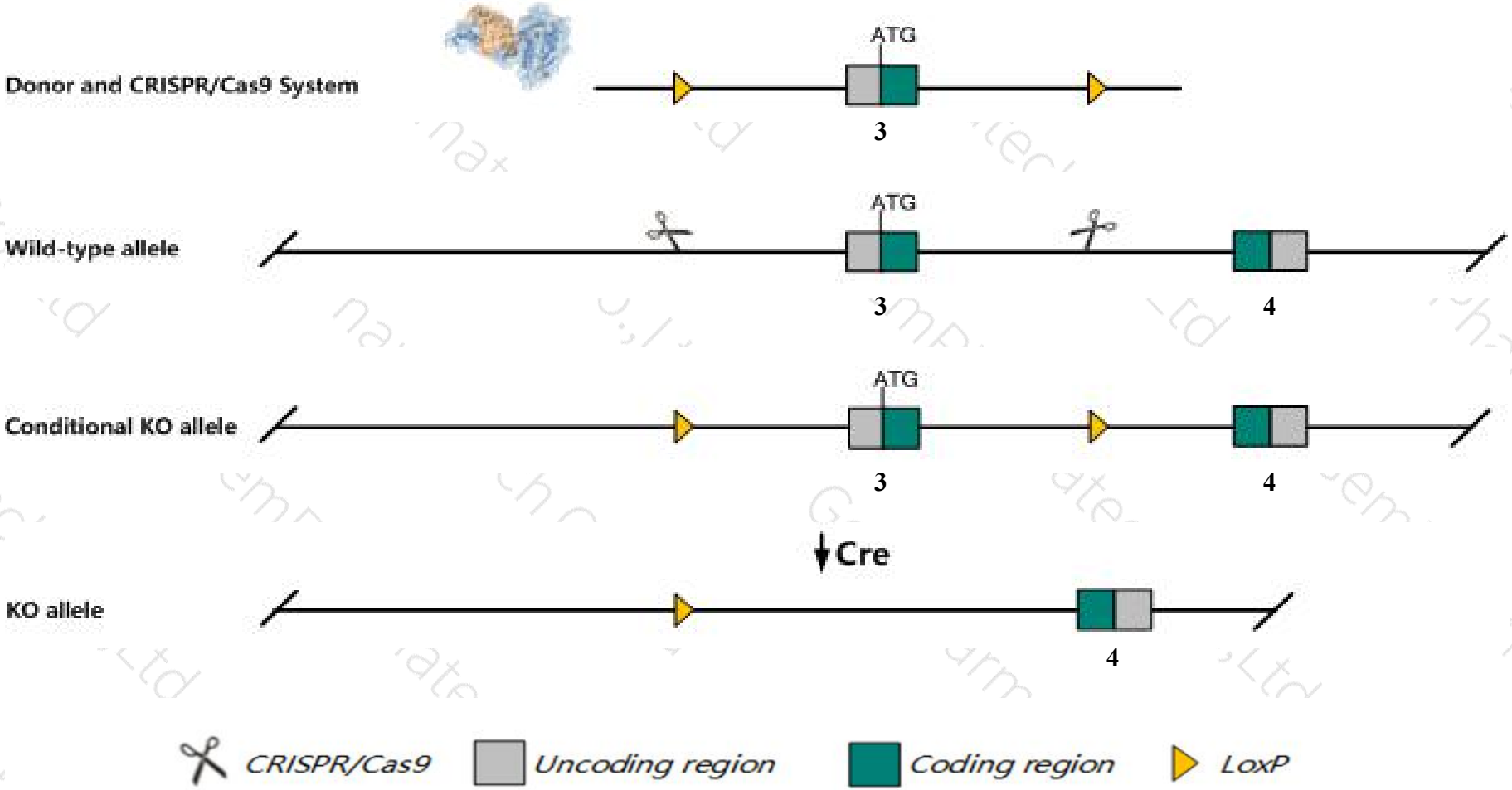
Project Overview

Project Name	***Bmp4***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Bmp4* gene. The schematic diagram is as follows:



Technical routes

➢ The *Bmp4* gene has 4 transcripts. According to the structure of *Bmp4* gene, exon3 of *Bmp4-201*(ENSMUST00000074077.11) 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 *Bmp4* 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,targeted mutants have wide ranging effects, including embryonic lethality, aberrant mesoderm differentation, developmental retardation and disorganized posterior structures; heterozygous null mutants display anomalies of the kidney and urinary tract; other targeted mutants display failure of lens induction and lack primordial germ cells.

➢ The floxed region is near to the N-terminal of *Gm15222* gene,this strategy may influence the regulatory function of the N-terminal of *Gm15222* gene.

➢ The *Bmp4* gene is located on the Chr14. 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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Bmp4 bone morphogenetic protein 4 [Mus musculus (house mouse)]

Gene ID: 12159, updated on 22-Mar-2020

Summary

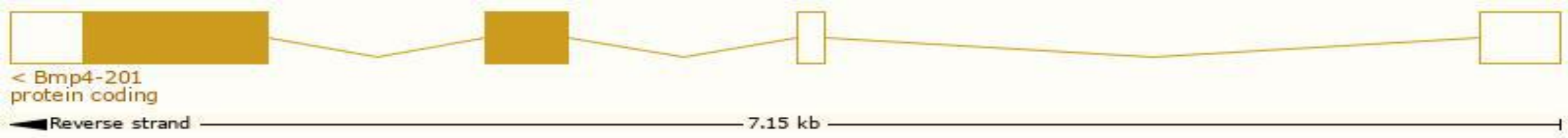
Official Symbol	Bmp4 provided by MGI
Official Full Name	bone morphogenetic protein 4 provided byMGI
Primary source	MGI:MGI:88180
See related	Ensembl:ENSMUSG00000021835
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Bmp-4, Bmp2b, Bmp2b-1, Bmp2b1
Summary	This gene encodes a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Ligands of this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. The encoded preproprotein is proteolytically processed to generate each subunit of the disulfide-linked homodimer. This protein regulates heart development and adipogenesis. Homozygous knockout mice die in utero, while a conditional knockout mouse exhibits defects in heart development. Transgenic mice overexpressing this gene in a neuron-specific manner exhibit a phenotype resembling the rare hereditary connective tissue disease, fibrodysplasia ossificans progressiva. [provided by RefSeq, Jul 2016]
Expression	Broad expression in bladder adult (RPKM 26.3), lung adult (RPKM 20.8) and 18 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

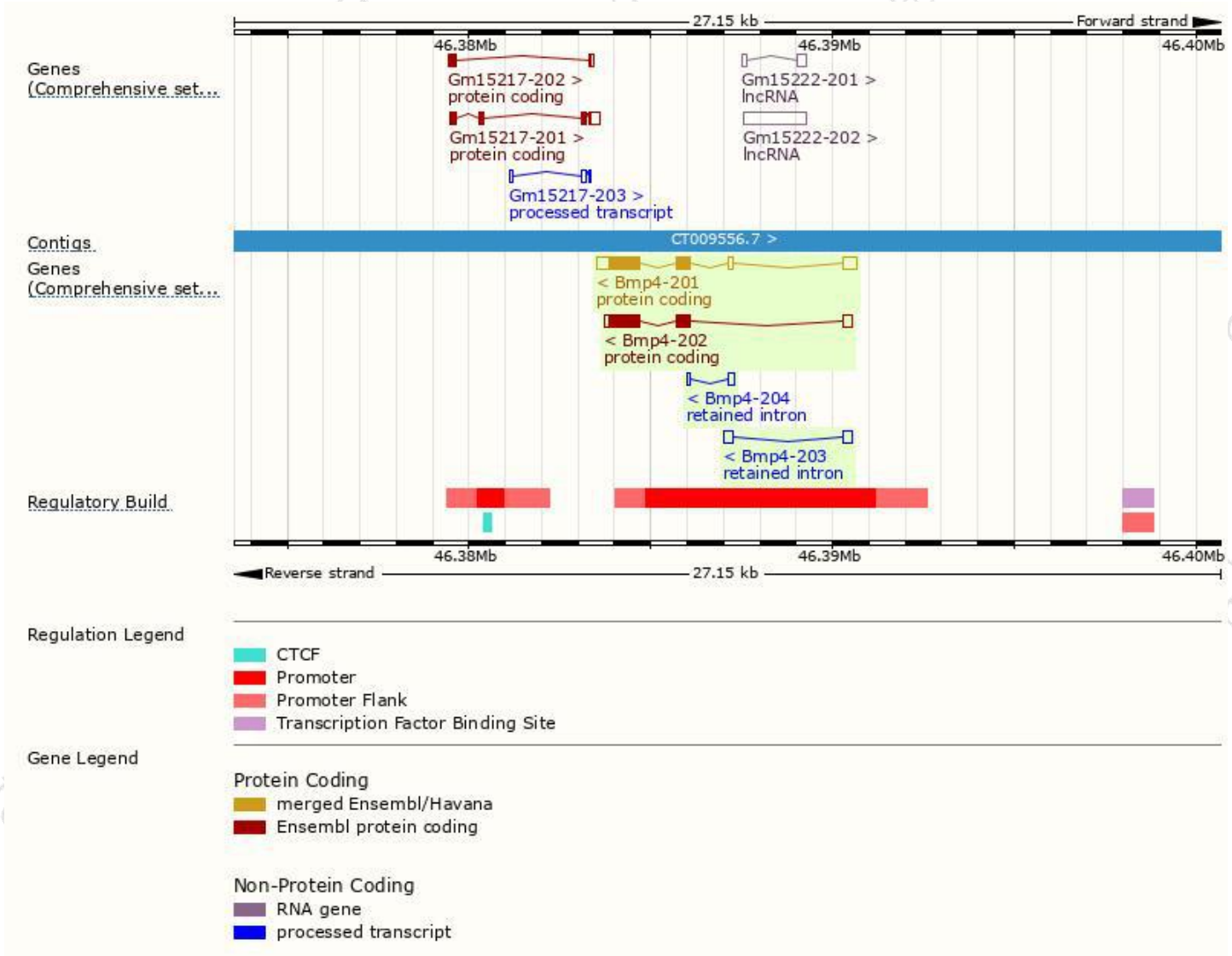
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Bmp4-201	ENSMUST00000074077.11	2064	408aa	Protein coding	CCDS36897	P21275 Q3ULR1	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
Bmp4-202	ENSMUST00000100676.2	1633	408aa	Protein coding	CCDS36897	P21275 Q3ULR1	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
Bmp4-203	ENSMUST00000135408.1	517	No protein	Retained intron	-	-	TSL:2
Bmp4-204	ENSMUST00000226759.1	258	No protein	Retained intron	-	-	

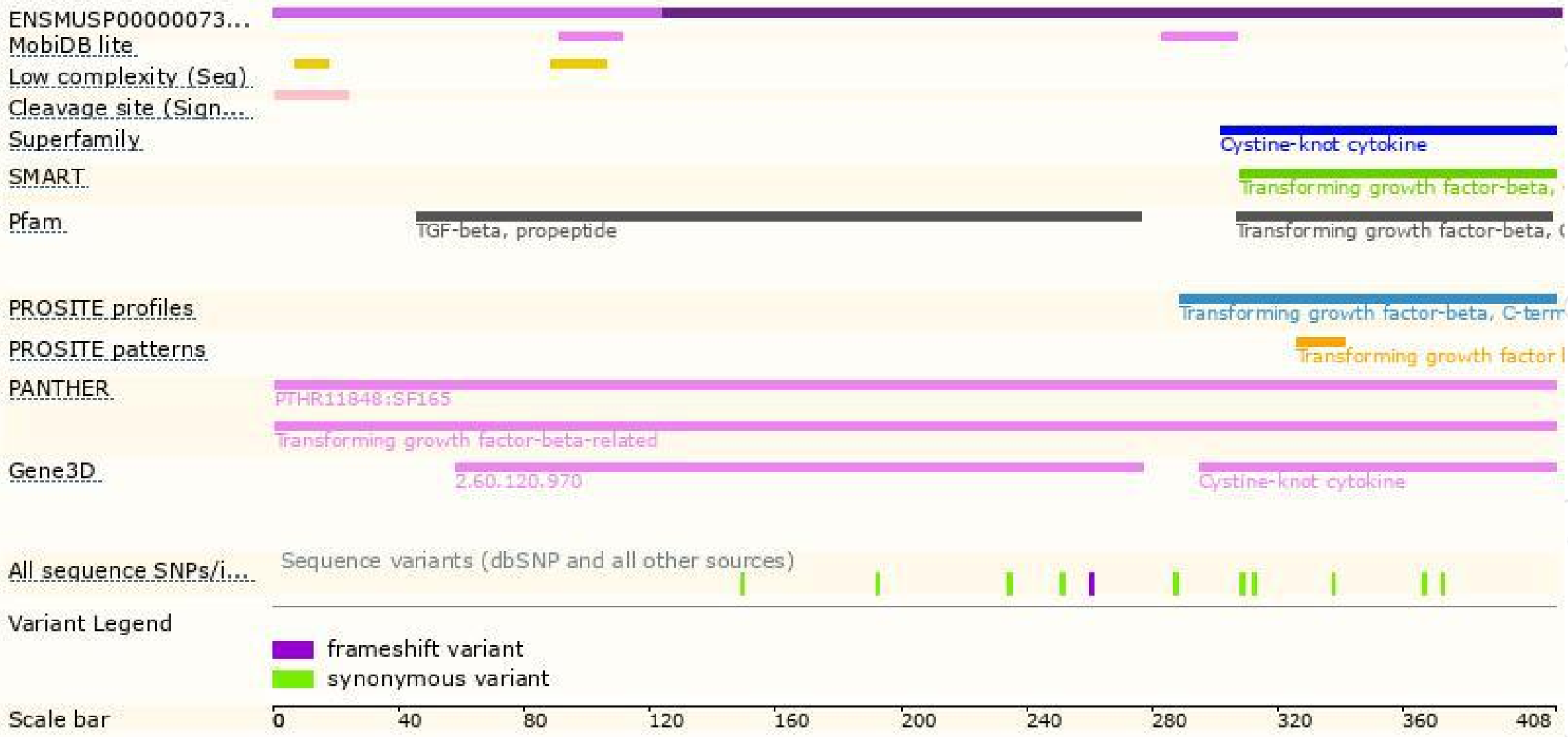
The strategy is based on the design of *Bmp4-201* transcript,the transcription is shown below:



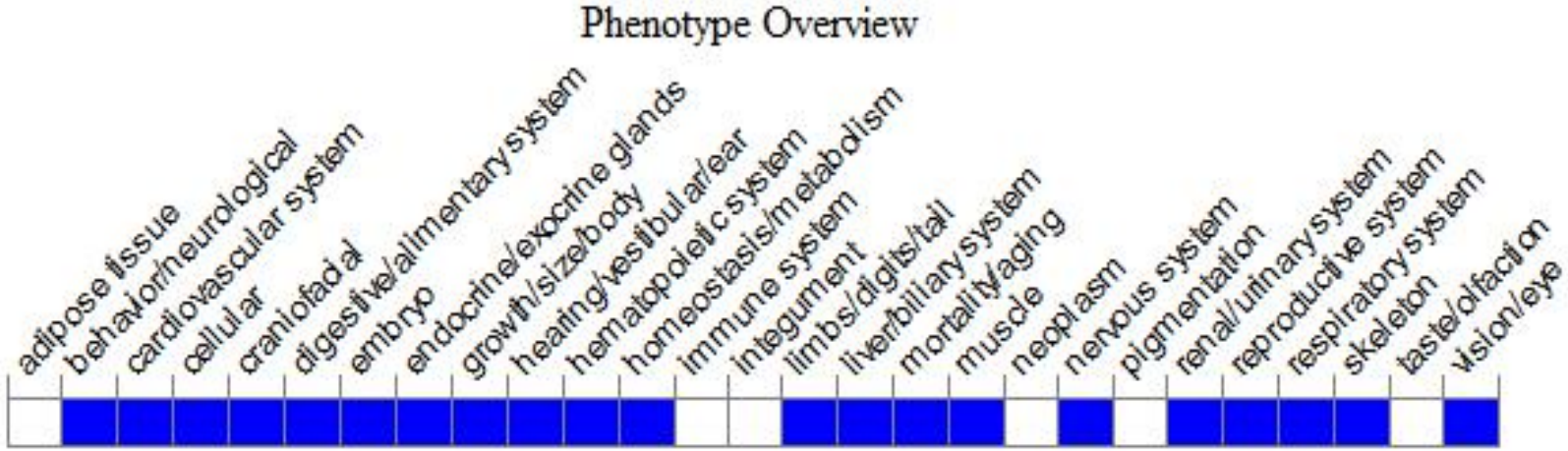
Genomic location distribution



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,targeted mutants have wide ranging effects, including embryonic lethality, aberrant mesoderm differentation, developmental retardation and disorganized posterior structures; heterozygous null mutants display anomalies of the kidney and urinary tract; other targeted mutants display failure of lens induction and lack primordial germ cells.

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

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