Gdf5 Cas9-CKO Strategy

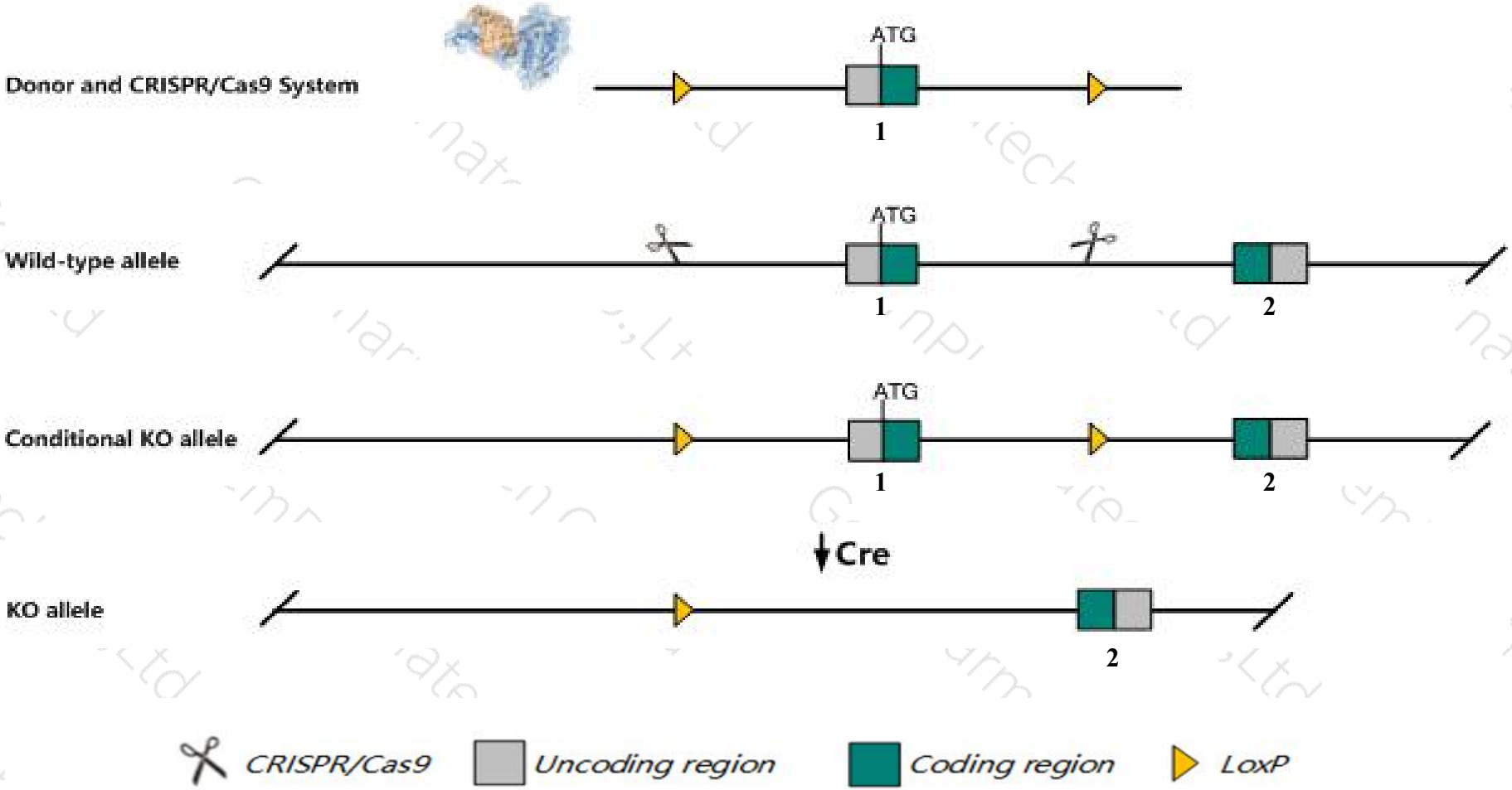
Designer: Daohua Xu
Reviewer: Huimin Su
Design Date: 2019-9-28

Project Overview

Project Name	***Gdf5***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Gdf5* gene. The schematic diagram is as follows:



Technical routes

集萃药康 GemPharmatech

- The *Gdf5* gene has 1 transcript. According to the structure of *Gdf5* gene, exon1 of *Gdf5-201* (ENSMUST00000040162.2) 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 *Gdf5* 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 mutations in this gene can cause joint patterning defects leading to complete or partial fusions between specific skeletal elements and alterations in the patterns of repeating structures in the digits, wrists and ankles.

➢ The *Gdf5* 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

Gdf5 growth differentiation factor 5 [Mus musculus (house mouse)]

Gene ID: 14563, updated on 5-Feb-2019

Summary

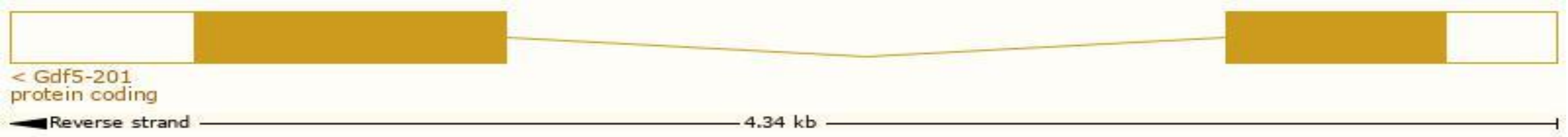
Official Symbol	Gdf5 provided by MGI
Official Full Name	growth differentiation factor 5 provided by MGI
Primary source	MGI:MGI:95688
See related	Ensembl:ENSMUSG00000038259
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	BMP-14, Cdmp-1, bp, brp
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 the development of numerous tissue and cell types, including cartilage, joints, brown fat, teeth, and the growth of neuronal axons and dendrites. Mice with a mutation in this gene exhibit enhanced tooth enamel formation. [provided by RefSeq, Aug 2016]
Expression	Biased expression in limb E14.5 (RPKM 16.4) and subcutaneous fat pad adult (RPKM 1.2)See more
Orthologs	human all

Transcript information（Ensembl）

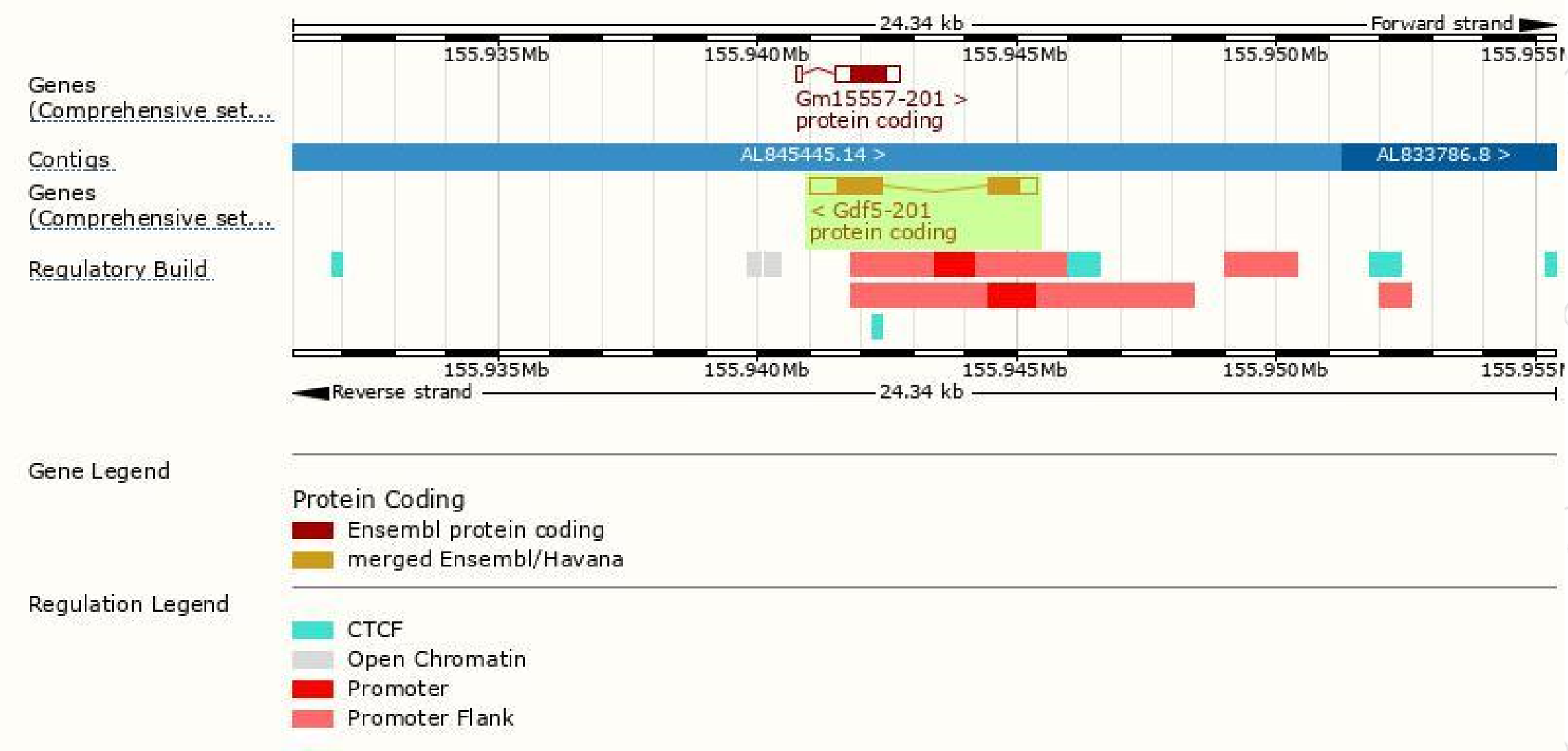
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gdf5-201	ENSMUST00000040162.2	2320	495aa	Protein coding	CCDS16958	P43027	TSL:1 GENCODE basic APPRIS P1

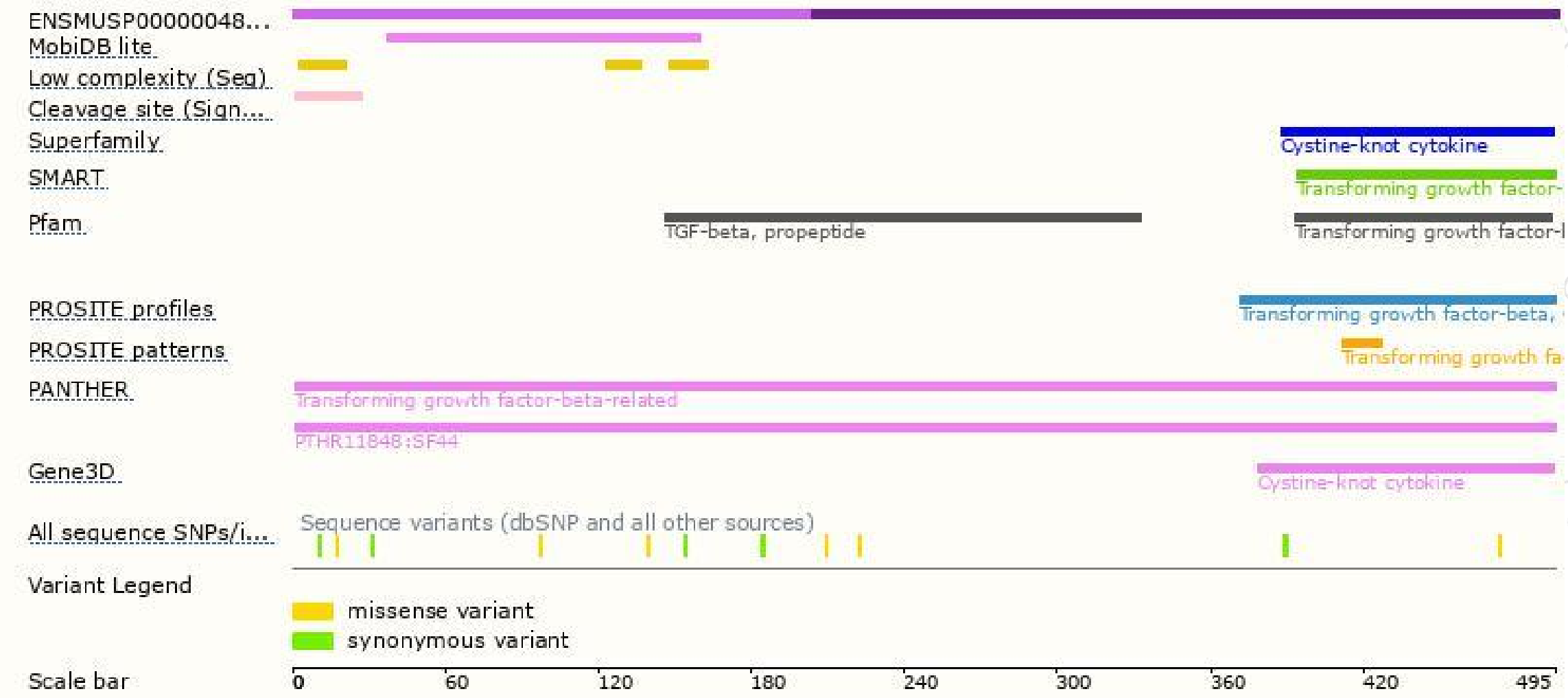
The strategy is based on the design of *Gdf5-201* transcript,The transcription is shown below



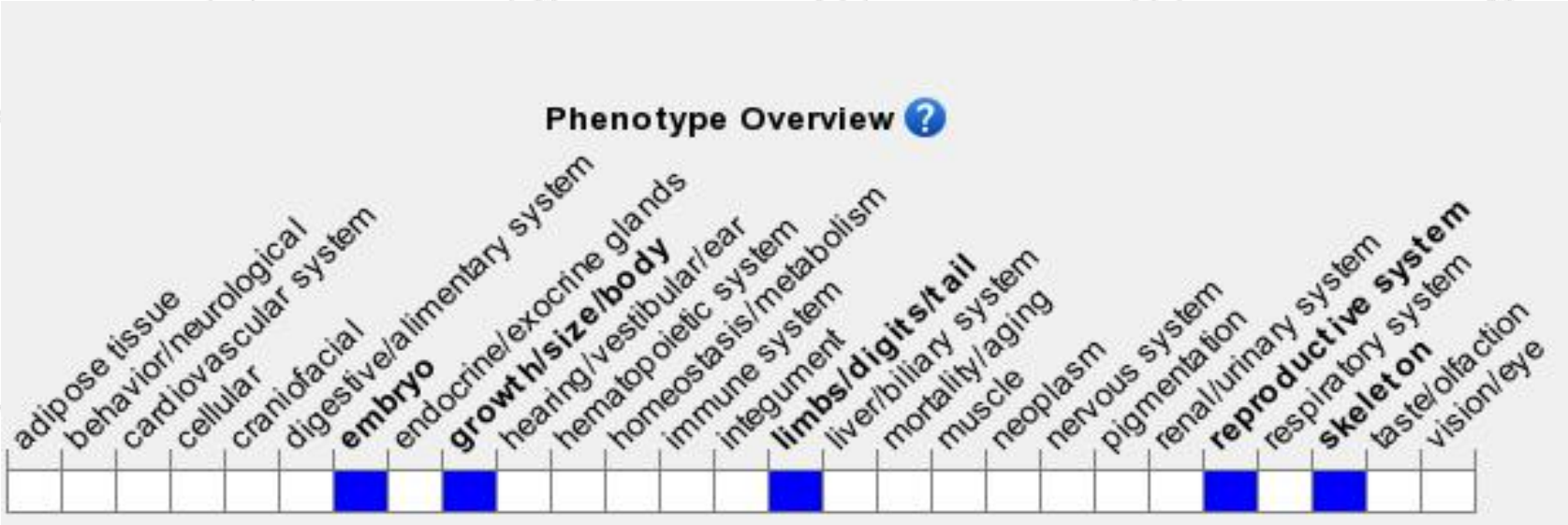
Genomic location distribution



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 mutations in this gene can cause joint patterning defects leading to complete or partial fusions between specific skeletal elements and alterations in the patterns of repeating structures in the digits, wrists and ankles.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd