Trp53 Cas9-CKO Strategy

Designer: Jing Jin

Reviewer: Yang Zeng

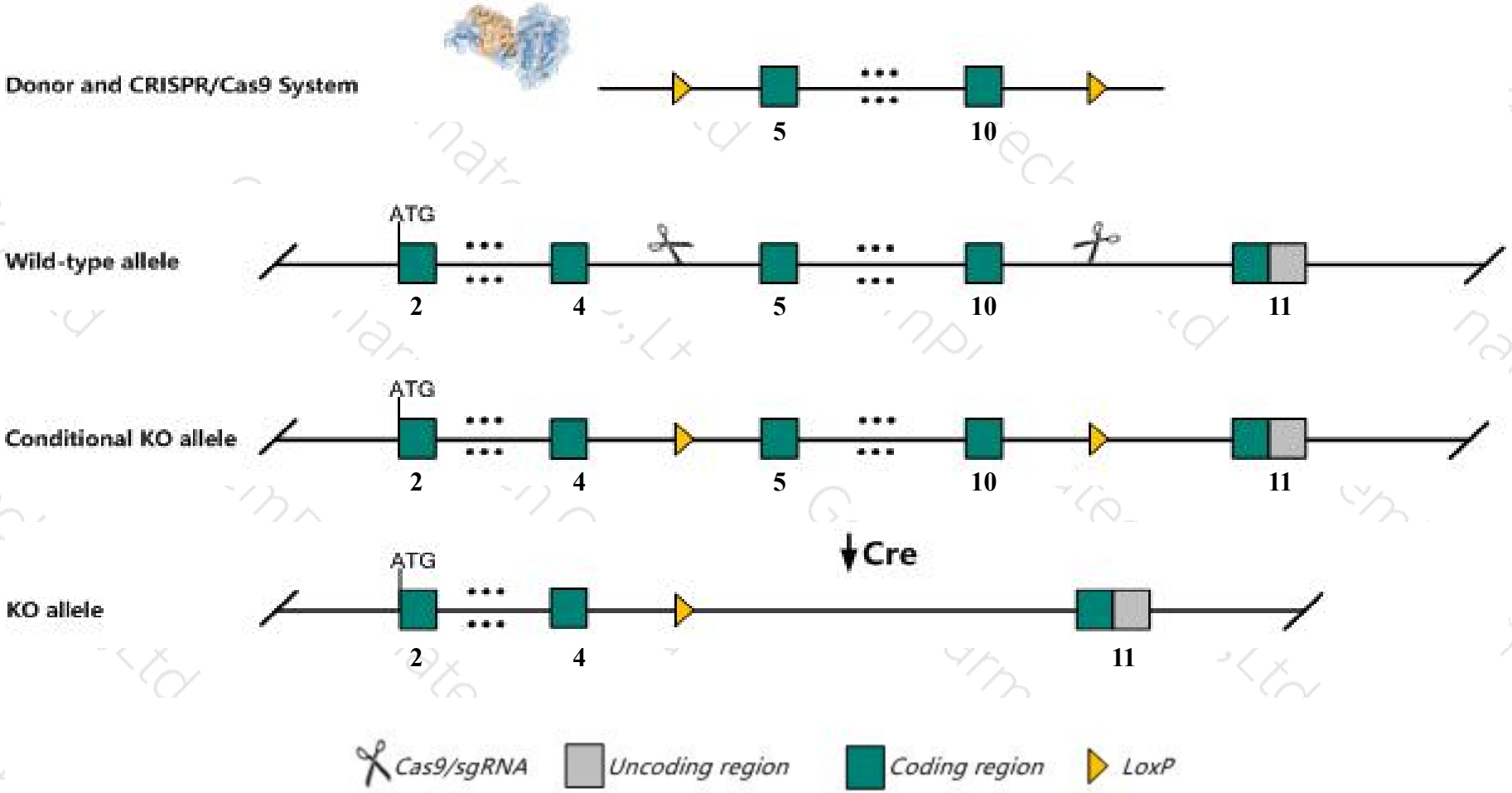
Design Date: 2018-6-25

Project Overview

Project Name	***Trp53***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Trp53* gene. The schematic diagram is as follows:



Technical routes

集萃药康 GemPharmatech

- The *Trp53* gene has 6 transcripts. According to the structure of *Trp53* gene, exon5-exon10 of *Trp53-206* (ENSMUST00000171247.7) transcript is recommended as the knockout region.The region contains 725bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Trp53* 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,Mutations in this locus affect cell-cycle regulation and apoptosis. Null homozygotes show high, early-onset tumor incidence; some have persistent hyaloid vasculature and cataracts. Truncated or temperature-sensitive alleles cause early aging phenotypes.

➢ The KO region is close to 5'UTR region of the *Wrap53* gene.Knockout the region may affect the regulation of *Wrap53* gene.

➢ The *Trp53* gene is located on the Chr11. 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

Trp53 transformation related protein 53 [Mus musculus (house mouse)]

Gene ID: 22059, updated on 9-Apr-2019

Summary

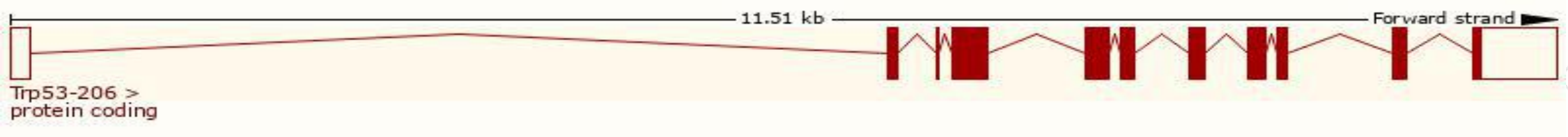
Official Symbol	Trp53 provided by MGI
Official Full Name	transformation related protein 53 provided byMGI
Primary source	MGI:MGI:98834
See related	Ensembl:ENSMUSG00000059552
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Tp53, bbl, bfy, bhy, p44, p53
Summary	This gene encodes tumor protein p53, which responds to diverse cellular stresses to regulate target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. p53 protein is expressed at low level in normal cells and at a high level in a variety of transformed cell lines, where it's believed to contribute to transformation and malignancy. p53 is a DNA-binding protein containing transcription activation, DNA-binding, and oligomerization domains. It is postulated to bind to a p53-binding site and activate expression of downstream genes that inhibit growth and/or invasion, and thus function as a tumor suppressor. Mice deficient for this gene are developmentally normal but are susceptible to spontaneous tumors. Evidence to date shows that this gene contains one promoter, in contrast to alternative promoters of the human gene, and transcribes a few of splice variants which encode different isoforms, although the biological validity or the full-length nature of some variants has not been determined. [provided by RefSeq, Jul 2008]
Expression	Ubiquitous expression in thymus adult (RPKM 69.1), ovary adult (RPKM 56.5) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

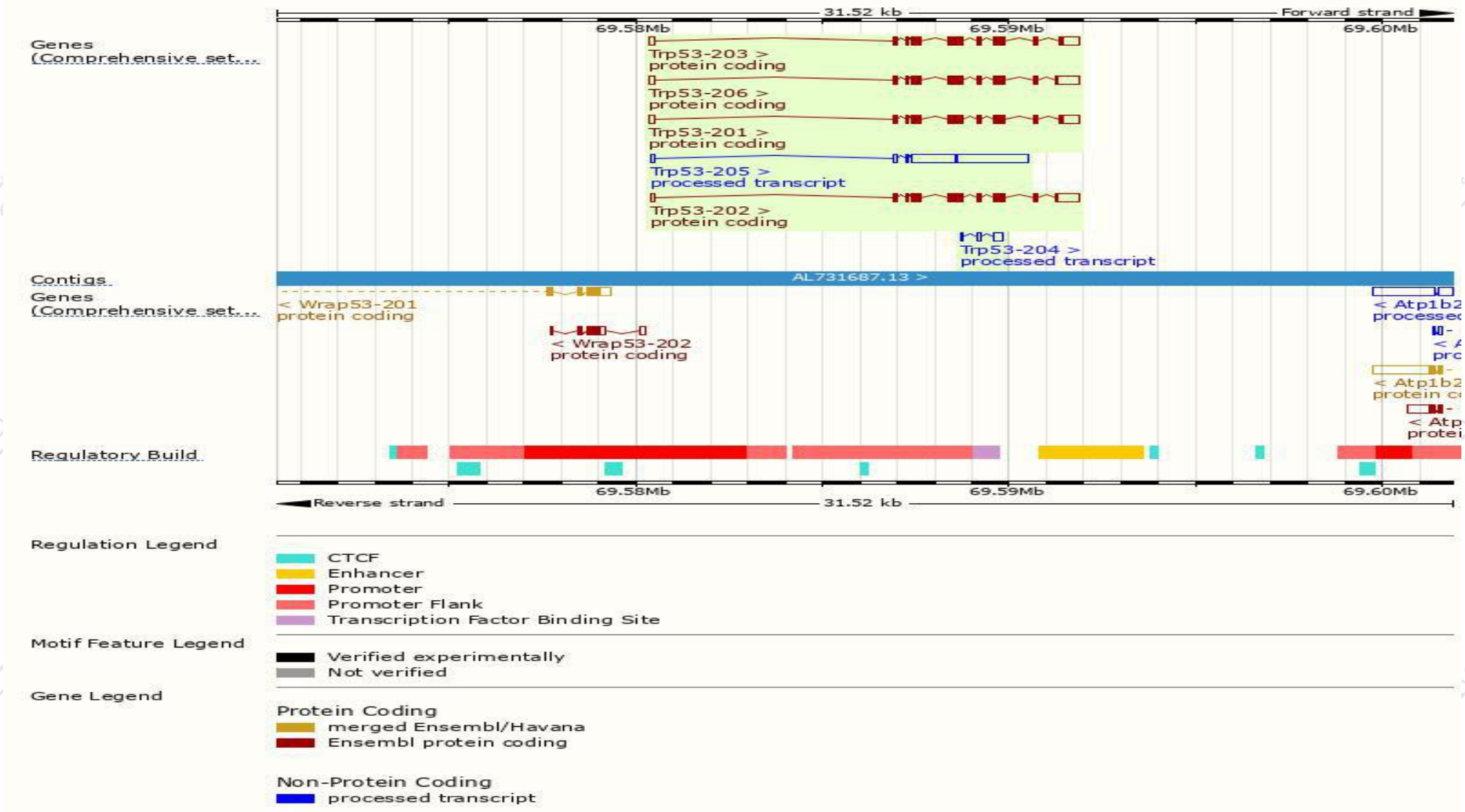
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Trp53-206	ENSMUST00000171247.7	1867	381aa	Protein coding	CCDS48826	Q80ZA1	TSL:1 GENCODE basic APPRIS ALT2
Trp53-203	ENSMUST00000108658.9	1771	390aa	Protein coding	CCDS36193	P02340 Q549C9	TSL:1 GENCODE basic APPRIS P3
Trp53-202	ENSMUST00000108657.3	1822	378aa	Protein coding	-	I7HIK9	TSL:1 GENCODE basic APPRIS ALT2
Trp53-201	ENSMUST00000005371.11	1772	387aa	Protein coding	-	A0A158SIS7	TSL:1 GENCODE basic APPRIS ALT2
Trp53-205	ENSMUST00000147512.1	3275	No protein	Processed transcript	-	-	TSL:1
Trp53-204	ENSMUST00000130540.1	403	No protein	Processed transcript	-	-	TSL:3

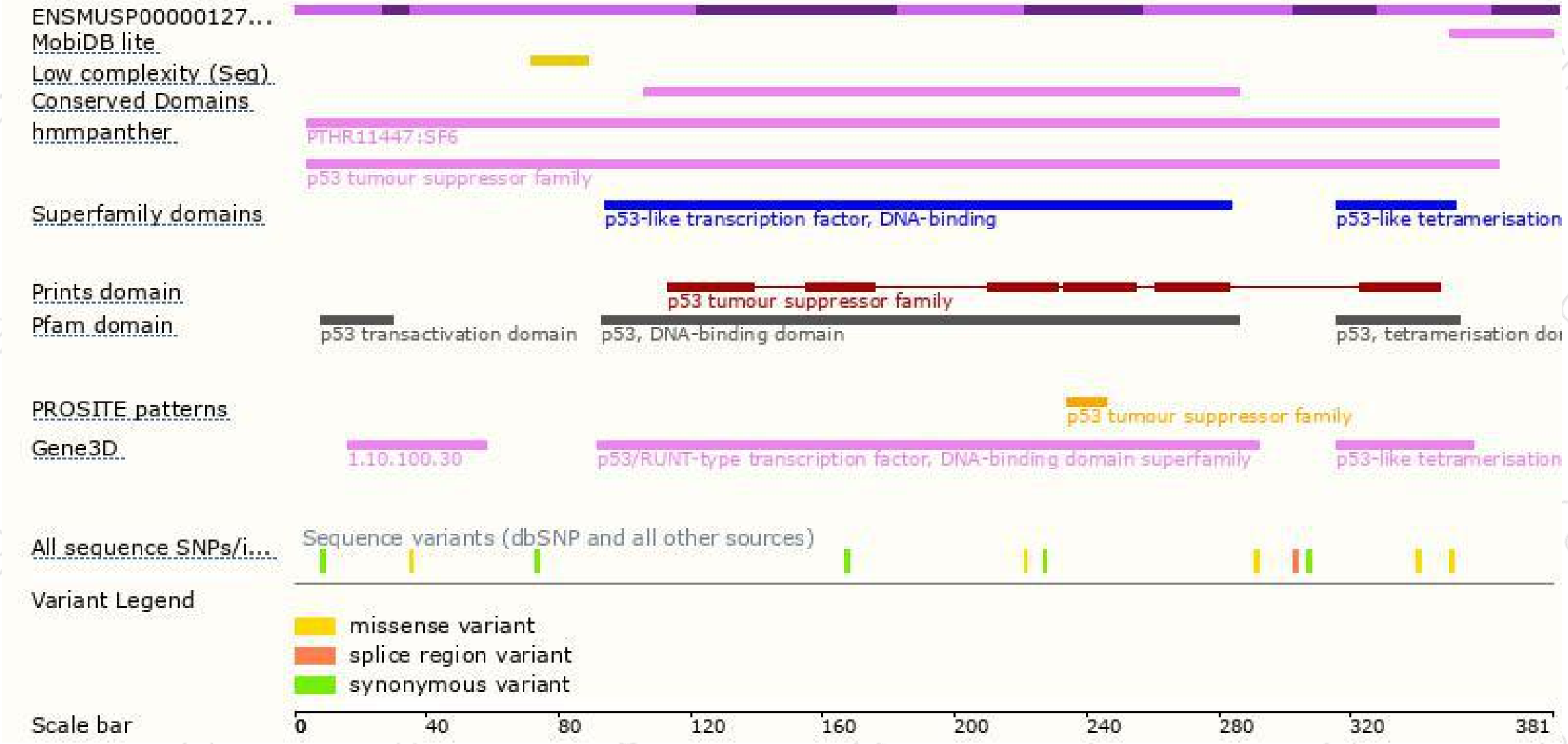
The strategy is based on the design of *Trp53-206* transcript,The transcription is shown below



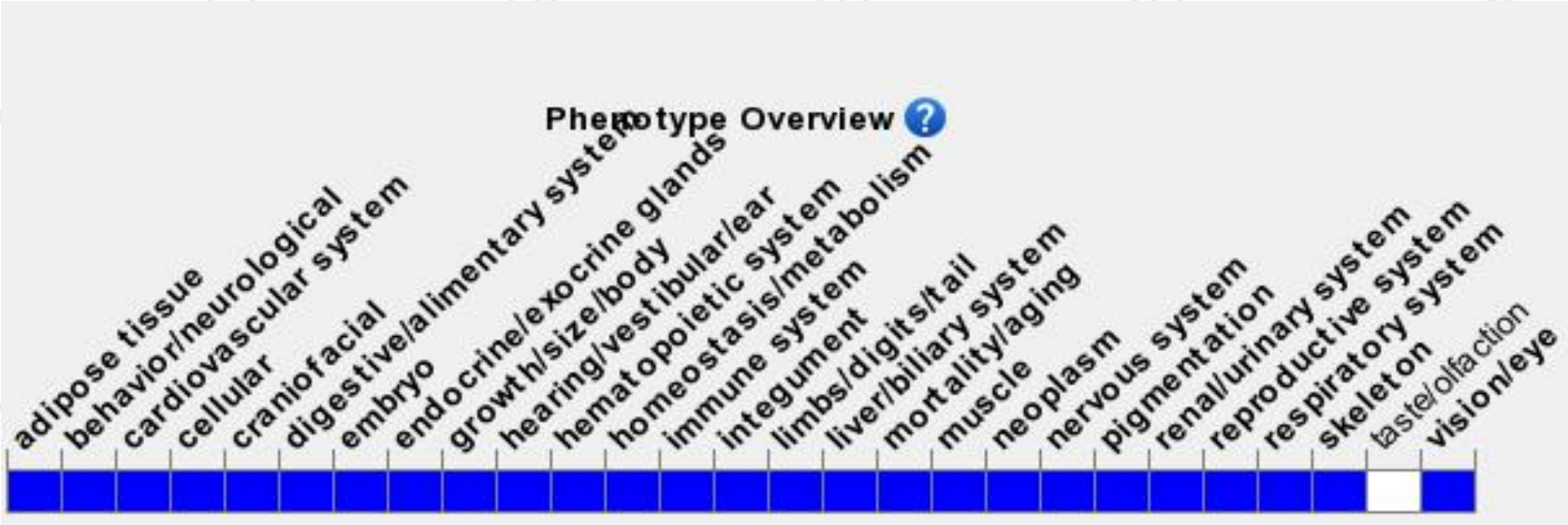
Genomic location distribution



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,Mutations in this locus affect cell-cycle regulation and apoptosis. Null homozygotes show high, early-onset tumor incidence; some have persistent hyaloid vasculature and cataracts. Truncated or temperature-sensitive alleles cause early aging phenotypes.

集萃药康
GemPharmatech

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

Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd