Rps6ka3 Cas9-KO *Strategy*

Designer: **Bingxuan Li**

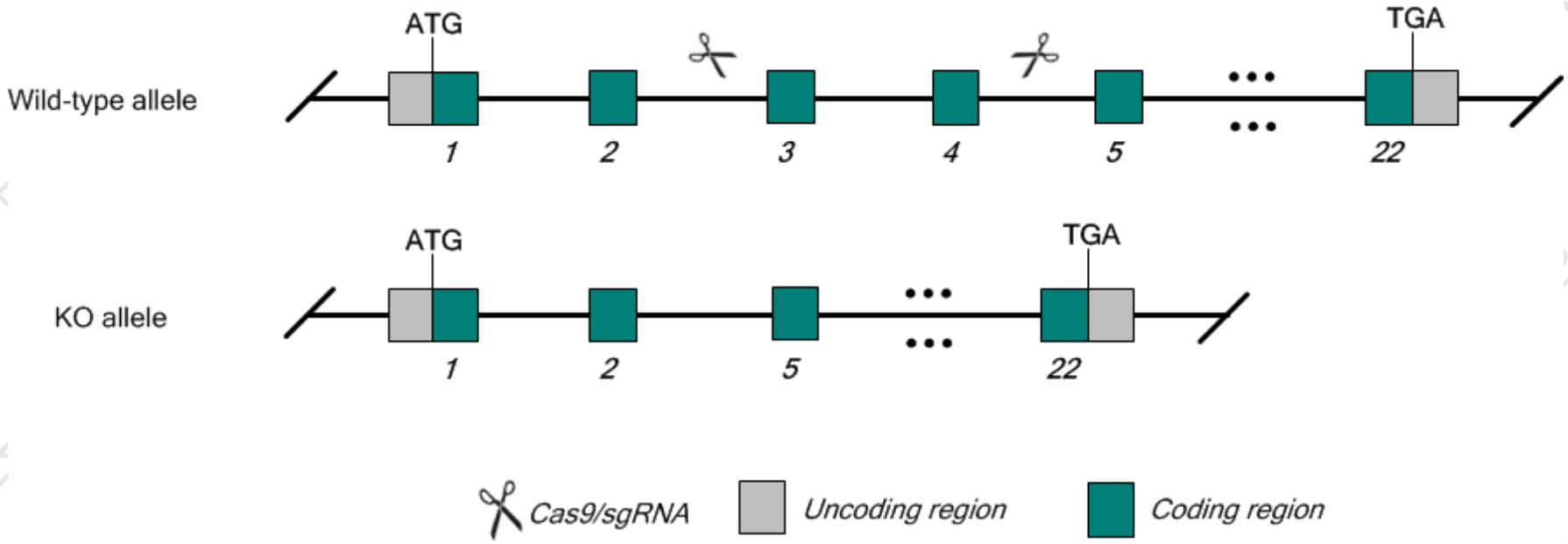
Design Date: **2019-9-5**

Project Overview

Project Name	***Rps6ka3***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rps6ka3* gene. The schematic diagram is as follows:



Technical routes

➢ The *Rps6ka3* gene has 9 transcripts. According to the structure of *Rps6ka3* gene, exon3-exon4 of *Rps6ka3-201* (ENSMUST00000033671.12) transcript is recommended as the knockout region.The region contains 199bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Rps6ka3* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

Notice

➢ According to the existing MGI data, Homozygotes/hemizygotes are a model for Coffin-Lowry Syndrome. One allele shows impaired osteoblast function with reduced long and craniofacial bones. Another allele shows infertility, lipodystrophy, impaired glycogen, glucose, and insulin metabolism, and impaired memory and coordination.

➢ The *Rps6ka3* gene is located on the ChrX. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

Rps6ka3 ribosomal protein S6 kinase polypeptide 3 [*Mus musculus* (house mouse)]

Gene ID: 110651, updated on 14-Aug-2019

Summary

Official Symbol	Rps6ka3 provided by MGI
Official Full Name	ribosomal protein S6 kinase polypeptide 3 provided by MGI
Primary source	MGI:MGI:104557
See related	Ensembl:ENSMUSG00000031309
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	Rsk2; MPK-9; p90RSK3; pp90RSK2; MAPKAPK-1b; S6K-alpha3
Expression	Ubiquitous expression in subcutaneous fat pad adult (RPKM 6.8), genital fat pad adult (RPKM 5.9) and 26 other tissues See more
Orthologs	human all

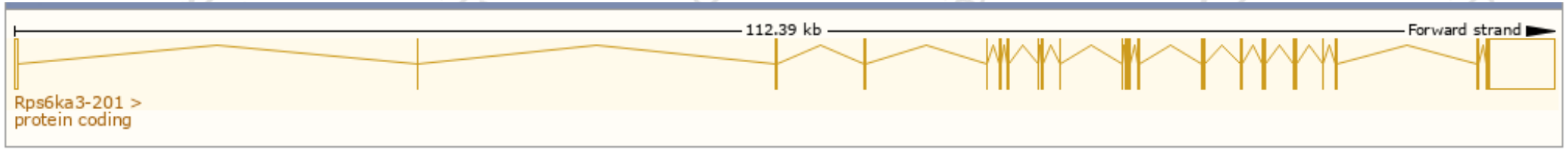
Transcript information（Ensembl）

The gene has 9 transcripts,all transcripts are shown below:

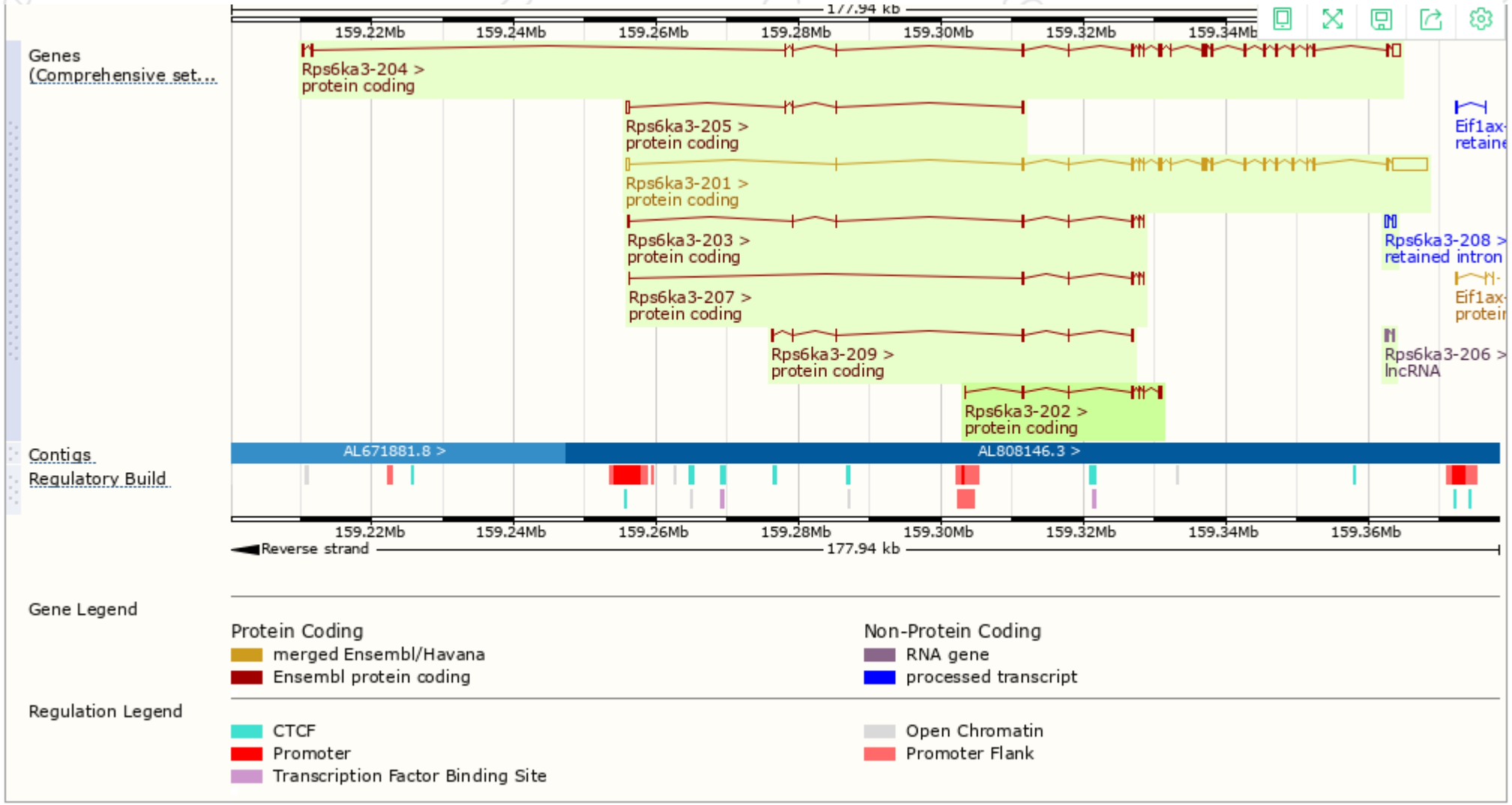
Show/hide columns (1 hidden) Filter

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rps6ka3-201	ENSMUST00000033671.12	7244	740aa	Protein coding	CCDS30502	P18654	TSL:1 GENCODE basic APPRIS P3
Rps6ka3-202	ENSMUST00000112491.1	716	211aa	Protein coding	-	B1AXP0	CDS 3' incomplete TSL:3
Rps6ka3-203	ENSMUST00000112492.7	722	135aa	Protein coding	-	B1AXN8	CDS 3' incomplete TSL:5
Rps6ka3-204	ENSMUST00000112493.7	3671	712aa	Protein coding	CCDS85822	B1AXN9	TSL:5 GENCODE basic APPRIS ALT1
Rps6ka3-205	ENSMUST00000126686.7	657	34aa	Protein coding	-	B1AXN7	CDS 3' incomplete TSL:2
Rps6ka3-206	ENSMUST00000142668.1	382	No protein	lncRNA	-	-	TSL:3
Rps6ka3-207	ENSMUST00000148570.7	517	172aa	Protein coding	-	B1AXN5	CDS 5' and 3' incomplete TSL:2
Rps6ka3-208	ENSMUST00000149939.1	755	No protein	Retained intron	-	-	TSL:2
Rps6ka3-209	ENSMUST00000156648.7	386	129aa	Protein coding	-	F6UG16	CDS 5' and 3' incomplete TSL:3

The strategy is based on the design of *Rps6ka3-201* transcript,The transcription is shown below:

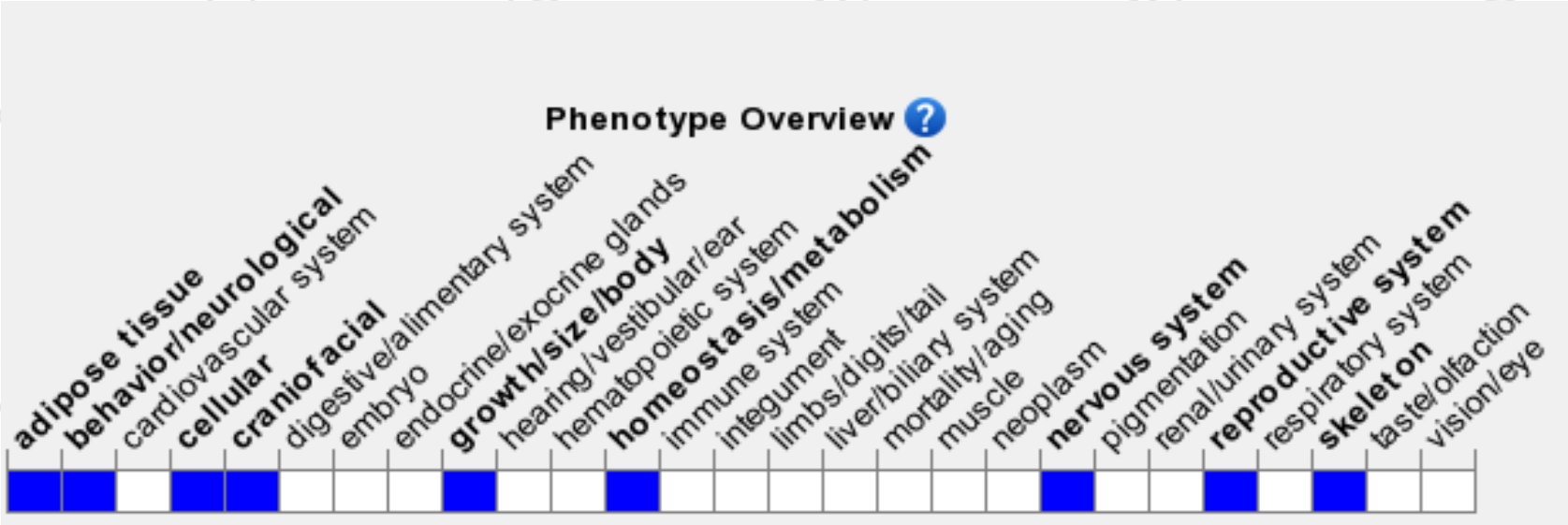


Genomic location distribution



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,Homozygotes/hemizygotes are a model for Coffin-Lowry Syndrome. One allele shows impaired osteoblast function with reduced long and craniofacial bones. Another allele shows infertility, lipodystrophy, impaired glycogen, glucose, and insulin metabolism, and impaired memory and coordination.

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