Crkl Cas9-CKO Strategy

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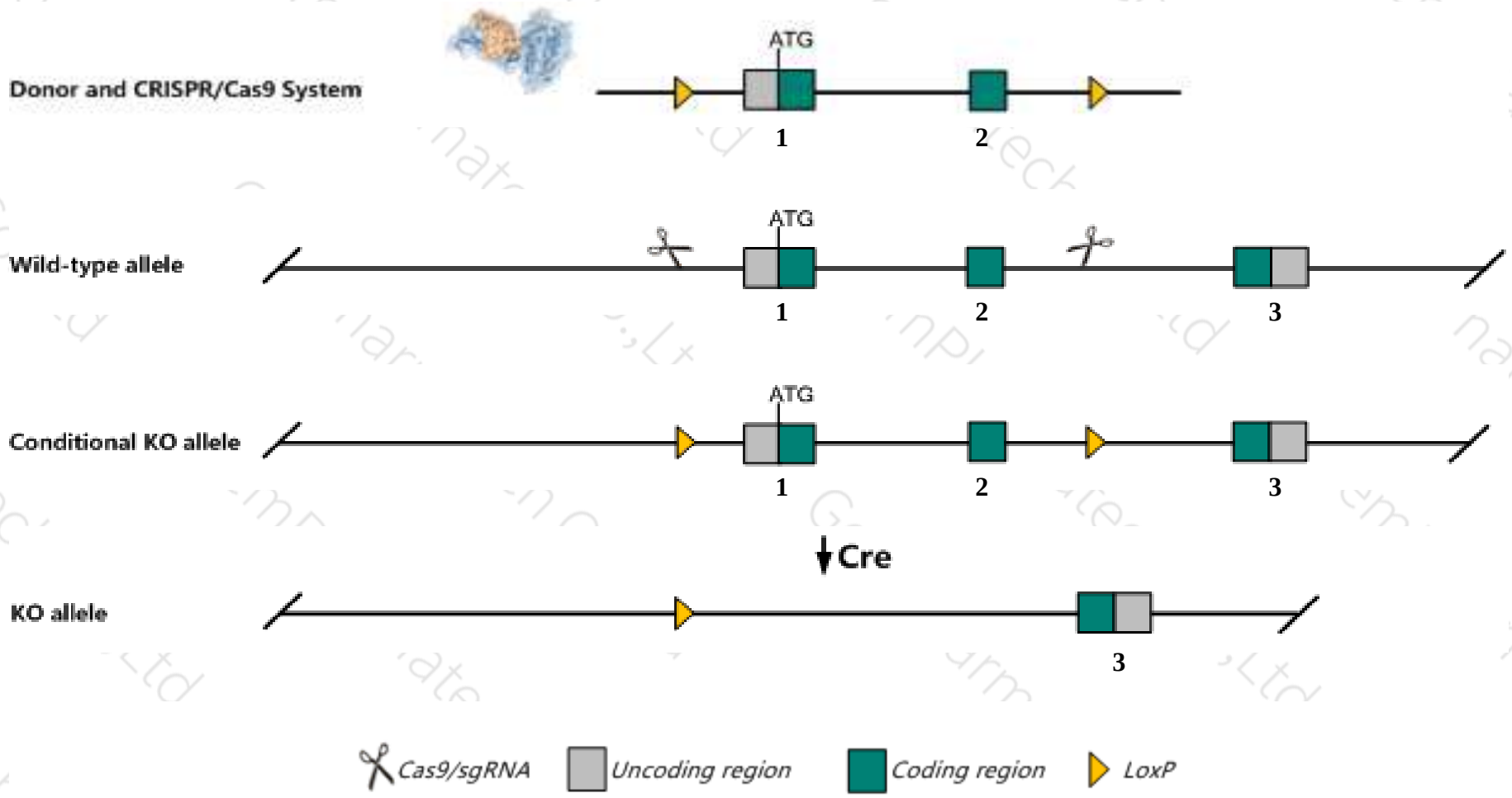
Project Overview

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

Conditional Knockout strategy

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



Technical routes

- The *Crkl* gene has 3 transcripts. According to the structure of *Crkl* gene, exon1-exon2 of *Crkl-201* (ENSMUST00000006293.4) 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 *Crkl* 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,Mice homozygous for a null allele exhibit fetal lethality with abnormal heart, craniofacial, and brain morphology.

- The *Crkl* gene is located on the Chr16. 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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Crkl v-crk avian sarcoma virus CT10 oncogene homolog-like [*Mus musculus* (house mouse)]

Gene ID: 12929, updated on 24-Oct-2019

Summary

Official Symbol	Crkl provided by MGI
Official Full Name	v-crk avian sarcoma virus CT10 oncogene homolog-like provided by MGI
Primary source	MGI:MGI:104686
See related	Ensembl:ENSMUSG00000006134
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	Crkol; snoop; AA589403; AI325100; 1110025F07Rik
Summary	This gene is part of a family of adapter proteins that mediate formation of signal transduction complexes in response to extracellular stimuli, such as growth and differentiation factors. Protein-protein interactions occur through the SH2 domain, which binds phosphorylated tyrosine residues, and the SH3 domain, which binds proline-rich peptide motifs. These interactions promote recruitment and activation of effector proteins to regulate cell migration, adhesion, and proliferation. In certain mouse genetic backgrounds this protein is essential for embryonic development. It is important for neural crest cell differentiation and survival and is proposed to play an important role in transducing the oncogenic signal of Bcr/Abl. Deletion of this gene in mouse mimics the phenotype of DiGeorge/velocardiofacial syndrome in human. Alternative splicing results in multiple transcript variants that encode different protein isoforms. [provided by RefSeq, Mar 2013]
Expression	Ubiquitous expression in thymus adult (RPKM 14.0), whole brain E14.5 (RPKM 13.0) and 28 other tissues See more
Orthologs	human all

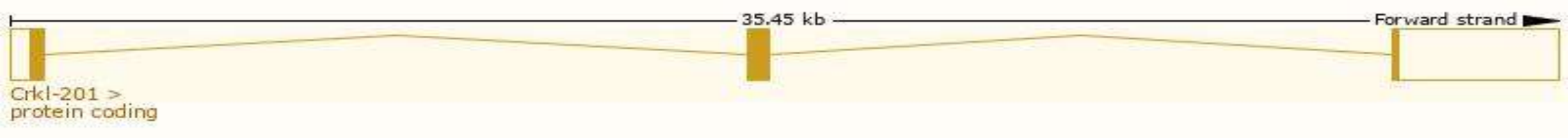
Chromosome 16 - NC_000082.6

Transcript information（Ensembl）

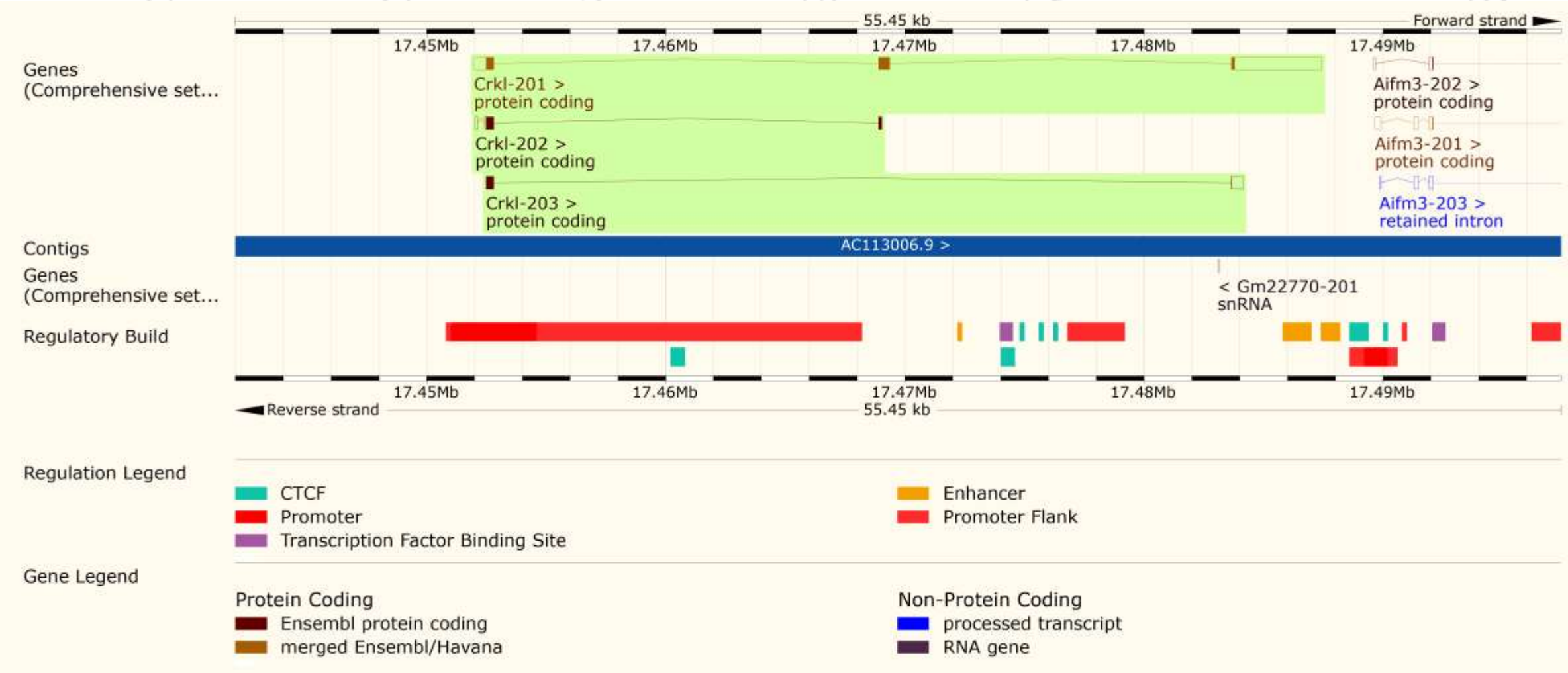
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Translation ID	Biotype	CCDS	UniProt	Flags
Crkl-201	ENSMUST00000006293.4	5042	303aa	ENSMUSP00000006293.3	Protein coding	CCDS28002	A2RS58 P47941	TSL:1 GENCODE basic APPRIS P1
Crkl-203	ENSMUST00000231629.1	780	108aa	ENSMUSP00000156063.1	Protein coding	-	A0A338P6Q0	GENCODE basic
Crkl-202	ENSMUST00000231228.1	577	148aa	ENSMUSP00000155860.1	Protein coding	-	A0A338P675	CDS 3' incomplete

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

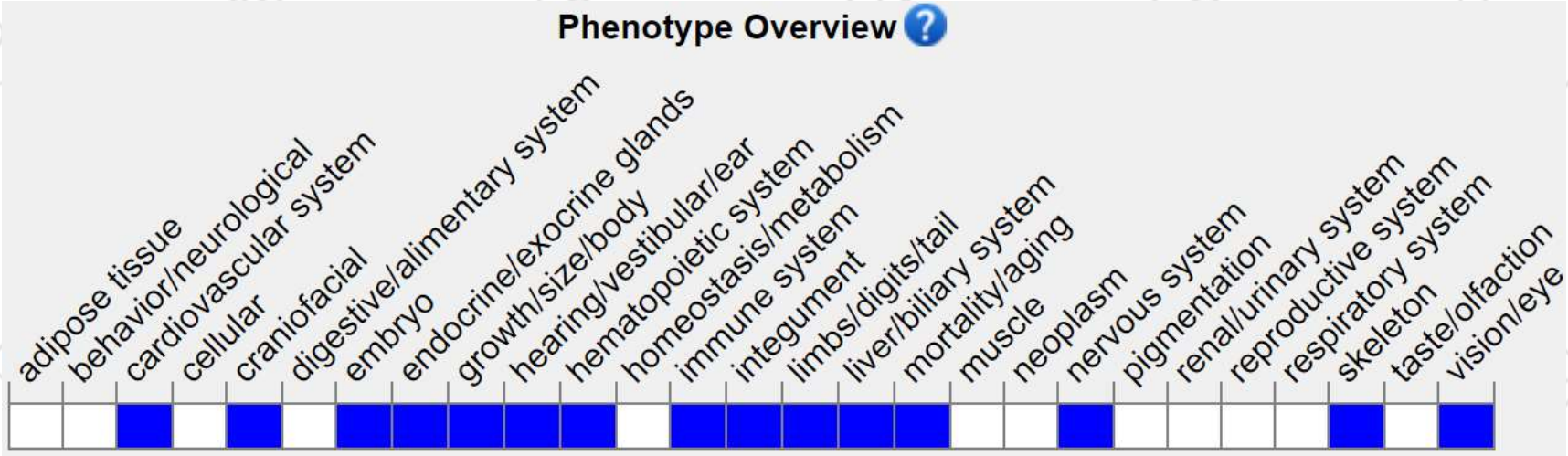


Genomic location distribution



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,Mice homozygous for a null allele exhibit fetal lethality with abnormal heart, craniofacial, and brain morphology.

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

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