

***Chuk* Cas9-CKO Strategy**

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Reviewer:

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Design Date:

2019-12-18

Project Overview

Project Name

Chuk

Project type

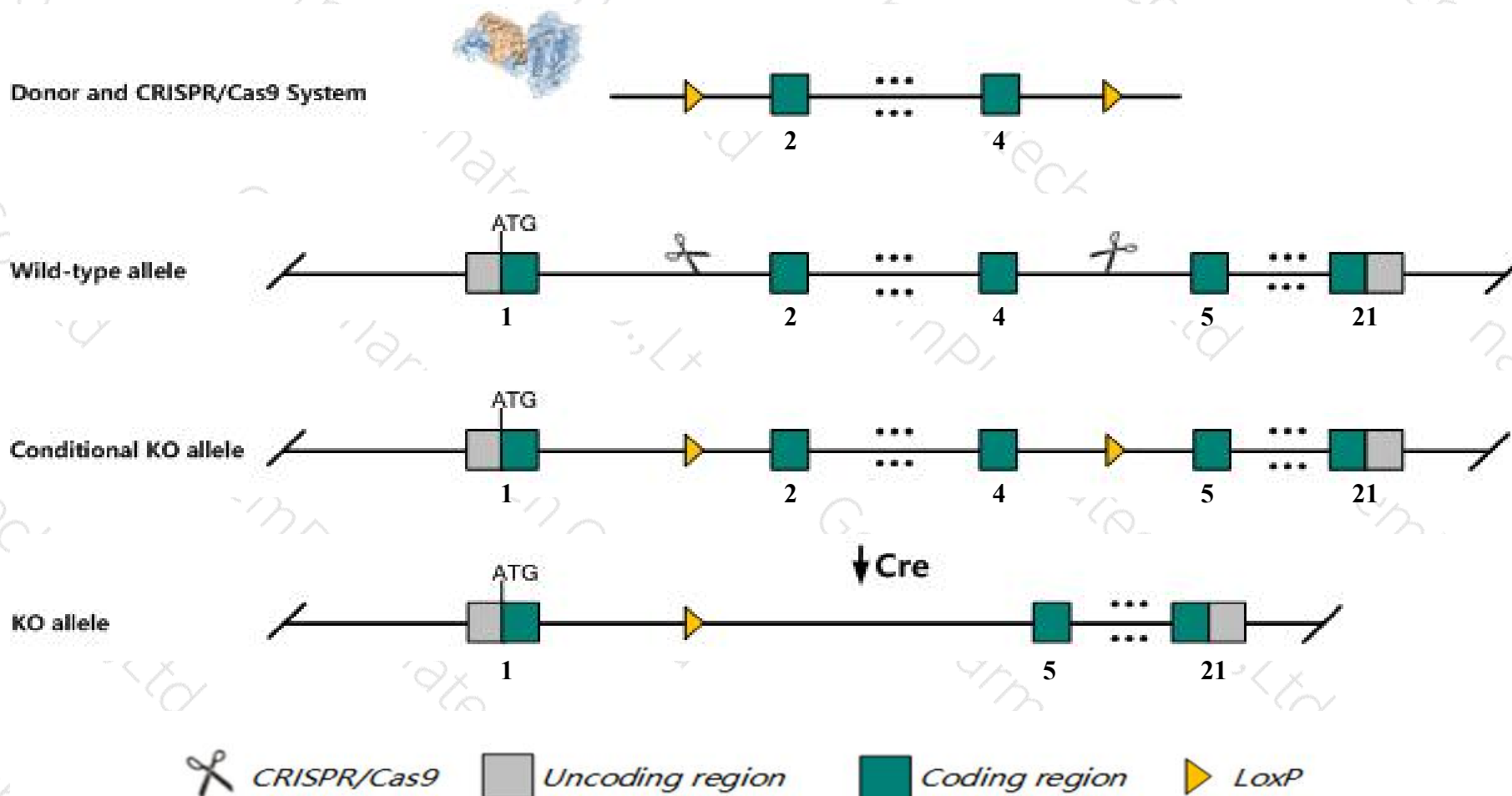
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Chuk* gene has 8 transcripts. According to the structure of *Chuk* gene, exon2-exon4 of *Chuk-201* (ENSMUST00000026217.10) transcript is recommended as the knockout region. The region contains 280bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Chuk* 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.

- According to the existing MGI data, Homozygotes for targeted null mutations die neonatally and exhibit thickened, taut, adhesive skin that prevents appendages from protruding from the trunk, absence of whiskers, skeletal abnormalities, and closed esophagus.
- The *Chuk* gene is located on the Chr19. 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)

Chuk conserved helix-loop-helix ubiquitous kinase [Mus musculus (house mouse)]

Gene ID: 12675, updated on 5-Mar-2019

Summary



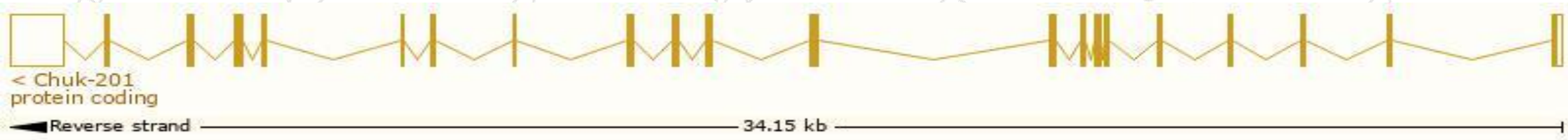
Official Symbol	Chuk provided by MGI
Official Full Name	conserved helix-loop-helix ubiquitous kinase provided by MGI
Primary source	MGI:MGI:99484
See related	Ensembl:ENSMUSG00000025199
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI256658, Chuk1, Fbx24, Fbxo24, IKBKA, IKK alpha, IKK1, Ikka, NFKBIKA
Expression	Ubiquitous expression in CNS E18 (RPKM 12.2), CNS E14 (RPKM 11.6) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

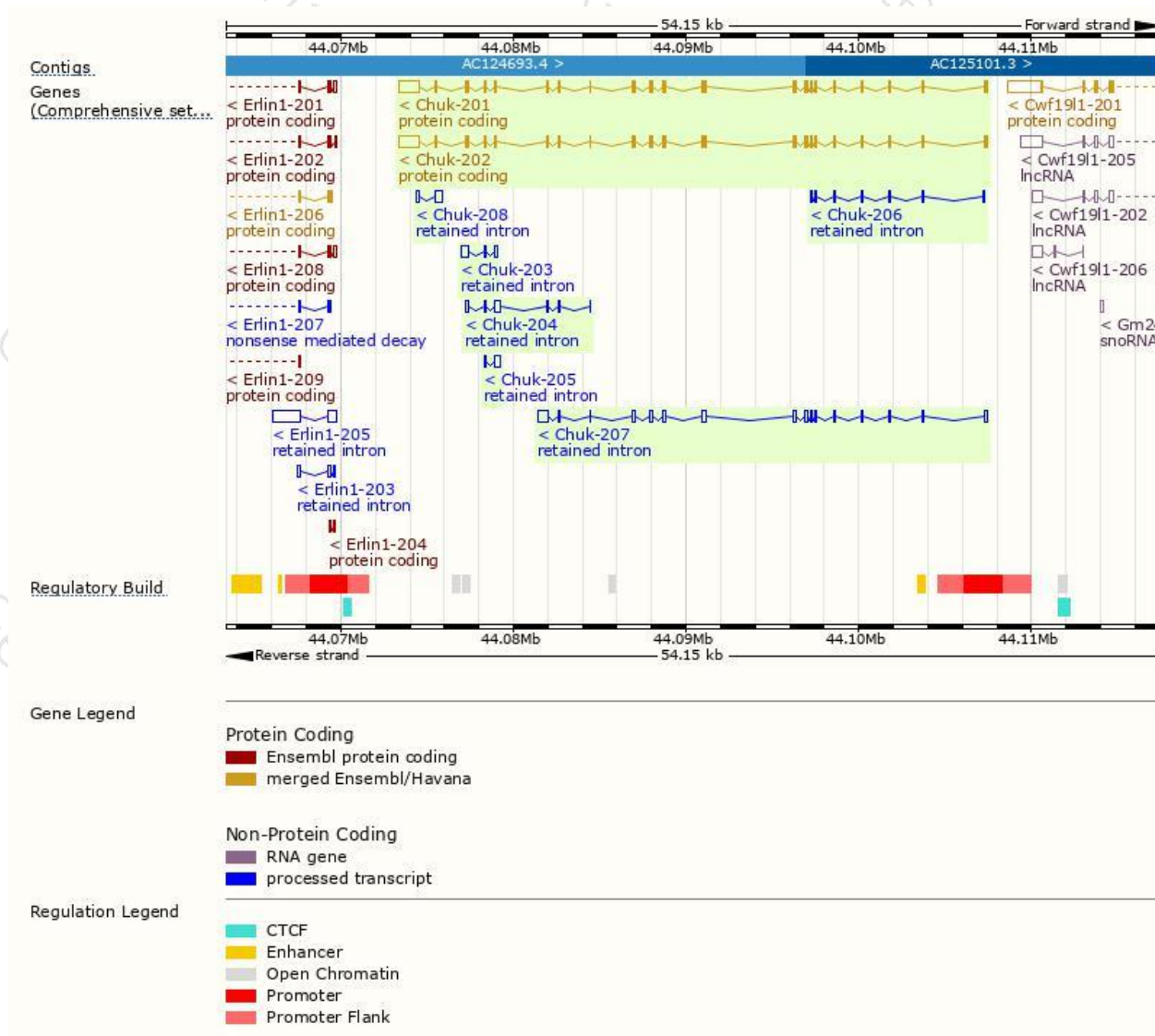
The gene has 8 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Chuk-201	ENSMUST00000026217.10	3502	745aa	Protein coding	CCDS29843	E9QNL4	TSL:1 GENCODE basic APPRIS P1
Chuk-202	ENSMUST00000119591.1	3481	719aa	Protein coding	CCDS50444	E9Q605	TSL:1 GENCODE basic
Chuk-207	ENSMUST00000147423.7	2337	No protein	Retained intron	-	-	TSL:1
Chuk-204	ENSMUST00000134495.7	855	No protein	Retained intron	-	-	TSL:3
Chuk-203	ENSMUST00000123806.7	737	No protein	Retained intron	-	-	TSL:3
Chuk-206	ENSMUST00000146861.1	656	No protein	Retained intron	-	-	TSL:3
Chuk-208	ENSMUST00000149091.1	642	No protein	Retained intron	-	-	TSL:1
Chuk-205	ENSMUST00000144286.1	545	No protein	Retained intron	-	-	TSL:5

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



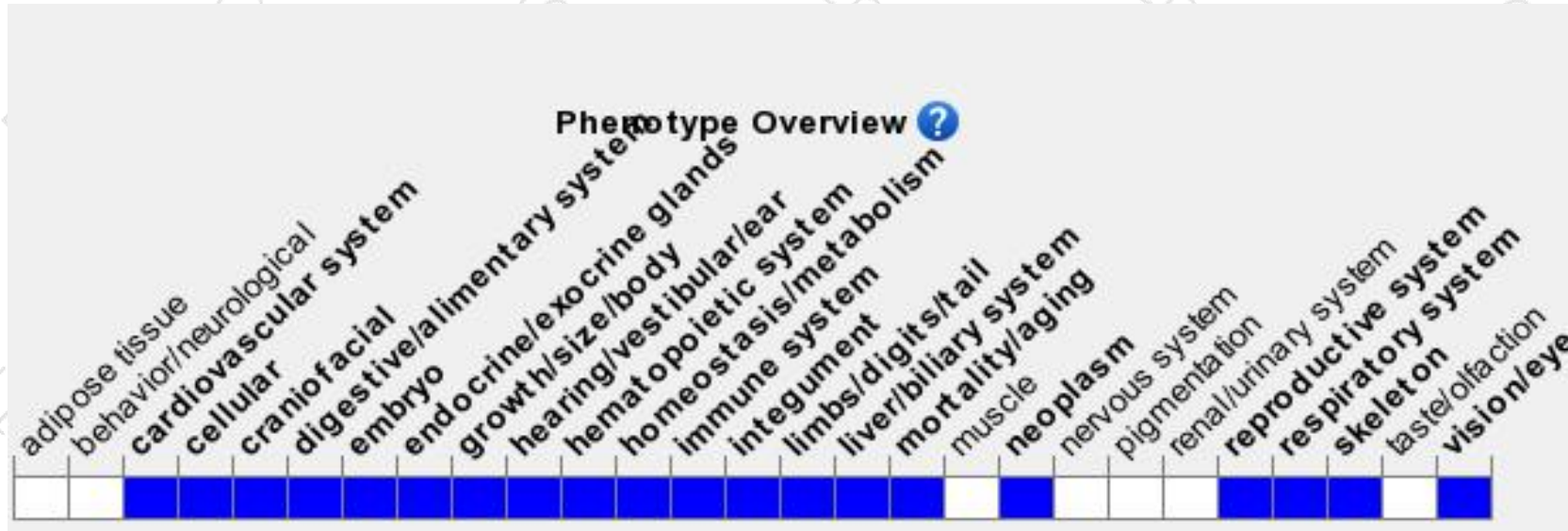
Genomic location distribution



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 for targeted null mutations die neonatally and exhibit thickened, taut, adhesive skin that prevents appendages from protruding from the trunk, absence of whiskers, skeletal abnormalities, and closed esophagus.

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

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