

Keap1 Cas9-CKO Strategy

Designer:

Daohua Xu

Reviewer:

Huimin Su

Design Date:

2019-11-22

Project Overview

Project Name

Keap1

Project type

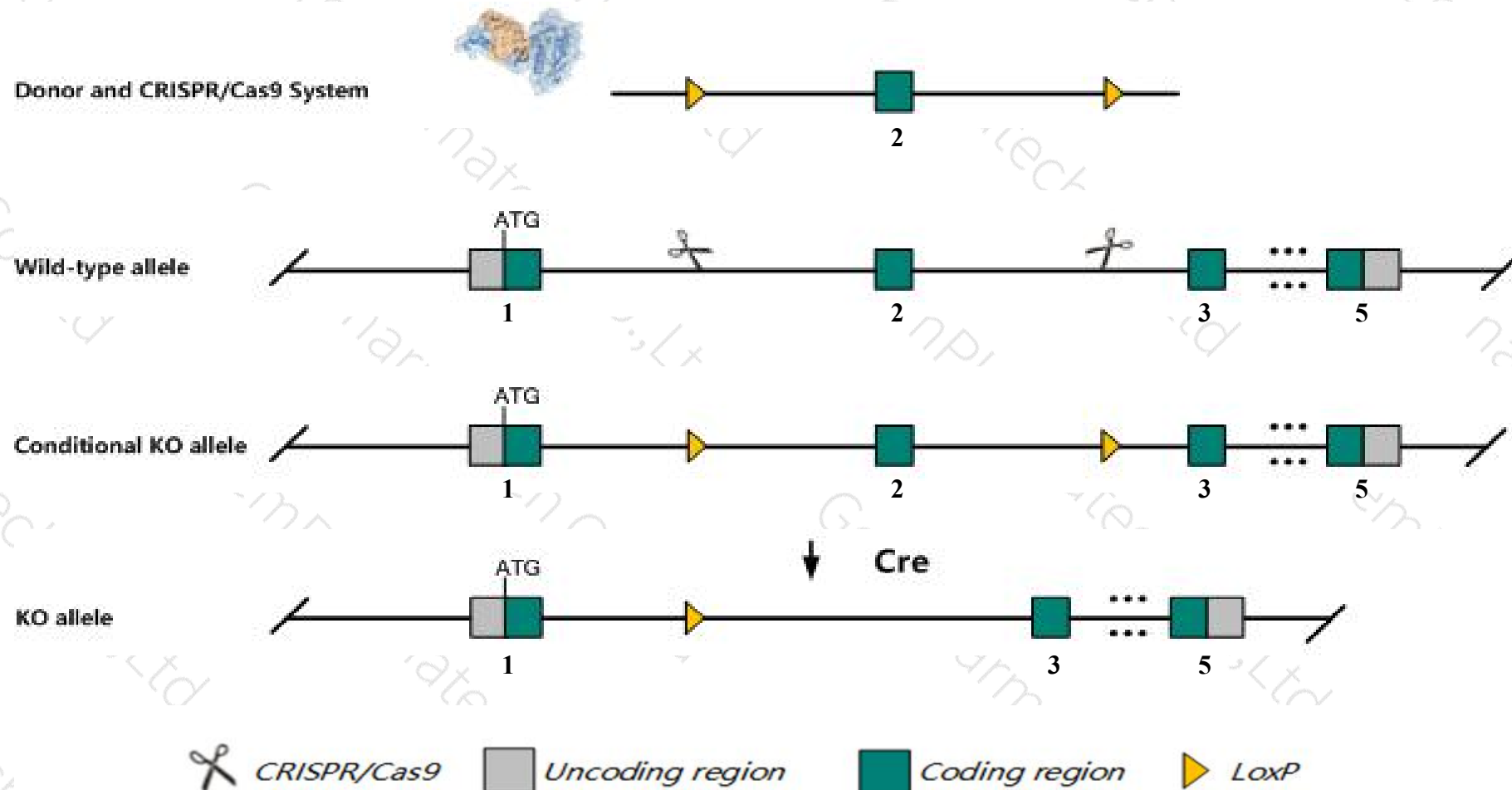
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Keap1* gene. The schematic diagram is as follows:



- The *Keap1* gene has 6 transcripts. According to the structure of *Keap1* gene, exon2 of *Keap1*-202 (ENSMUST00000164812.7) transcript is recommended as the knockout region. The region contains 686bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Keap1* 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, Homozygous null mice exhibit scaly skin, hyperkeratosis of the esophagus and stomach mucosa, and die around 3 weeks of age, putatively due to malnutrition resulting from the abnormal alimentary epithelium.
- The *Keap1* gene is located on the Chr9. 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)

Keap1 kelch-like ECH-associated protein 1 [Mus musculus (house mouse)]

Gene ID: 50868, updated on 9-Apr-2019

Summary



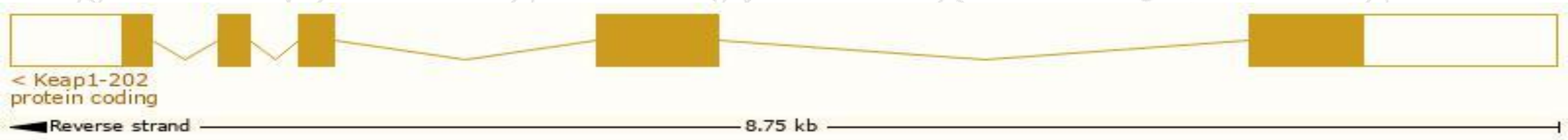
Official Symbol	Keap1 provided by MGI
Official Full Name	kelch-like ECH-associated protein 1 provided by MGI
Primary source	MGI:MGI:1858732
See related	Ensembl:ENSMUSG00000003308
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	INRF2, mKIAA0132
Expression	Ubiquitous expression in ovary adult (RPKM 41.2), adrenal adult (RPKM 36.0) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

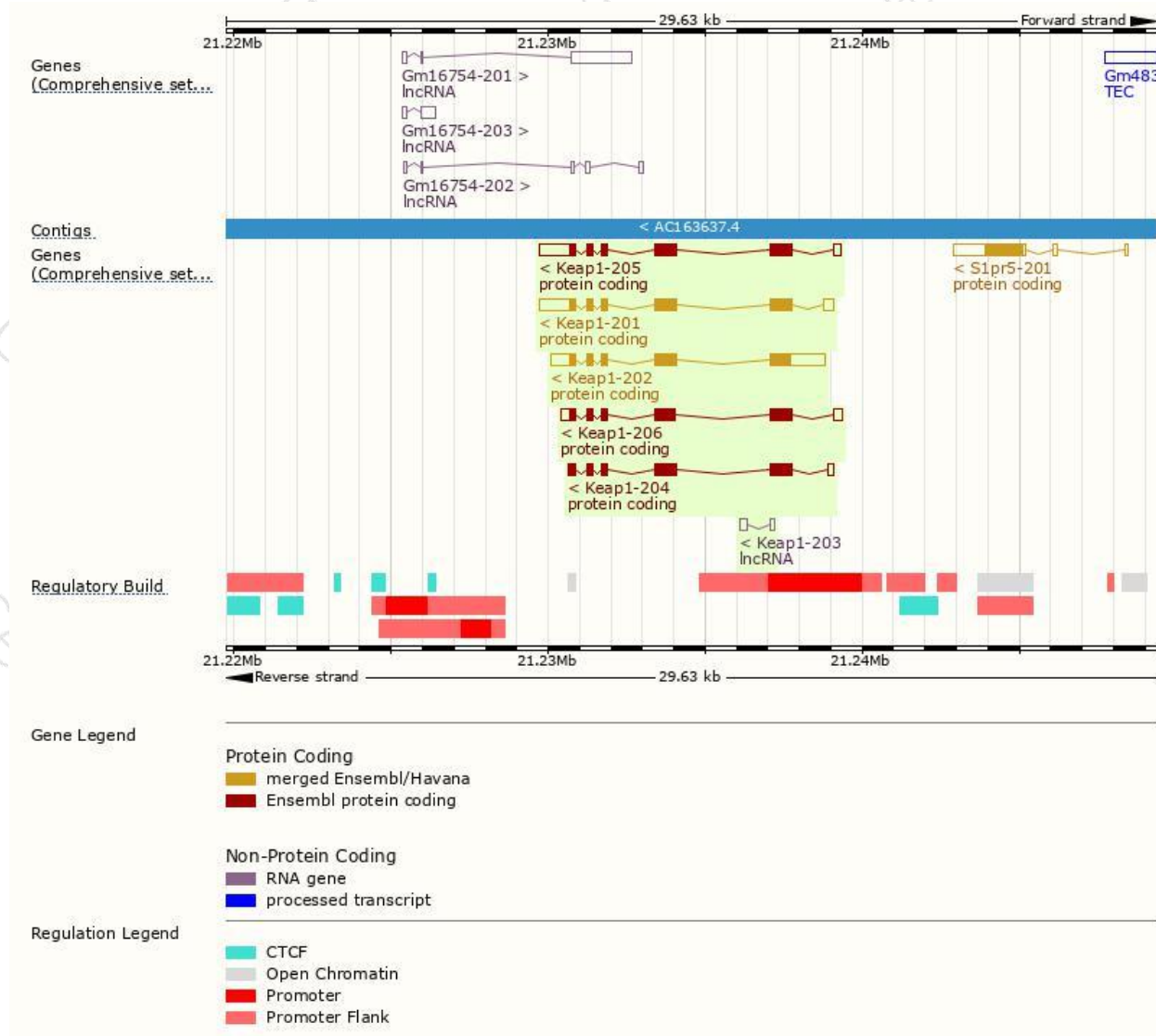
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Keap1-202	ENSMUST00000164812.7	3614	624aa	Protein coding	CCDS22897	Q9Z2X8	TSL:1 GENCODE basic APPRIS P2
Keap1-201	ENSMUST00000049567.9	3172	624aa	Protein coding	CCDS22897	Q9Z2X8	TSL:1 GENCODE basic APPRIS P2
Keap1-205	ENSMUST00000194542.5	3151	624aa	Protein coding	CCDS22897	Q9Z2X8	TSL:1 GENCODE basic APPRIS P2
Keap1-204	ENSMUST00000193982.1	2163	624aa	Protein coding	CCDS22897	Q9Z2X8	TSL:1 GENCODE basic APPRIS P2
Keap1-206	ENSMUST00000216436.1	2498	620aa	Protein coding	-	A0A1L1SS10	TSL:5 GENCODE basic APPRIS ALT2
Keap1-203	ENSMUST00000193247.1	317	No protein	lncRNA	-	-	TSL:5

The strategy is based on the design of *Keap1-202* 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 null mice exhibit scaly skin, hyperkeratosis of the esophagus and stomach mucosa, and die around 3 weeks of age, putatively due to malnutrition resulting from the abnormal alimentary epithelium.

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

Tel: 400-9660890

