

Ilk Cas9-CKO Strategy

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Project Overview

Project Name

Ilk

Project type

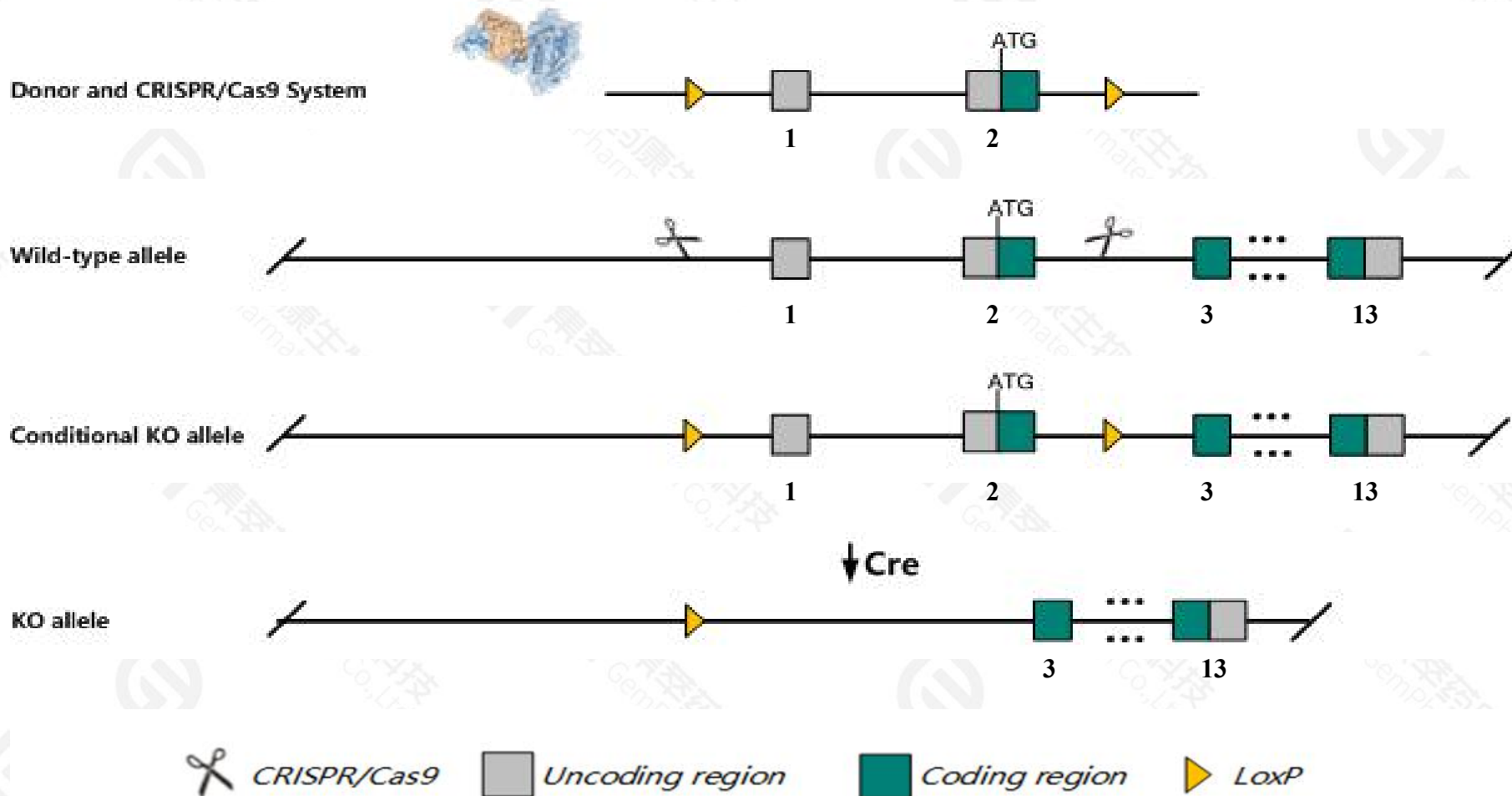
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Ilk* gene has 12 transcripts. According to the structure of *Ilk* gene, exon1-exon2 of *Ilk-201*(ENSMUST00000033182.10) transcript is recommended as the knockout region. The region contains 89bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Ilk* 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, nullizygous embryos do not polarize the epiblast and die after implantation. Mice with mutations in the ATP-binding site show aphagia, hunched posture, and neonatal death due to renal aplasia. Mice with mutations in the paxillin-binding site show vasculogenesis and growth defects, and die at ~E12.5.
- The KO region contains functional region of the *Rrp8* gene. Knockout the region may affect the function of *Rrp8* gene.
- The *Ilk* gene is located on the Chr7. 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)

Ilk integrin linked kinase [Mus musculus (house mouse)]

Gene ID: 16202, updated on 13-Mar-2020

Summary



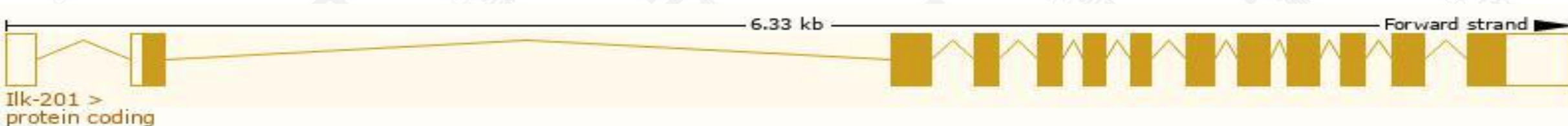
Official Symbol	Ilk provided by MGI
Official Full Name	integrin linked kinase provided by MGI
Primary source	MGI:MGI:1195267
See related	Ensembl:ENSMUSG00000030890
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AA511515, ESTM2, ESTM24
Expression	Ubiquitous expression in bladder adult (RPKM 143.4), subcutaneous fat pad adult (RPKM 90.0) and 28 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

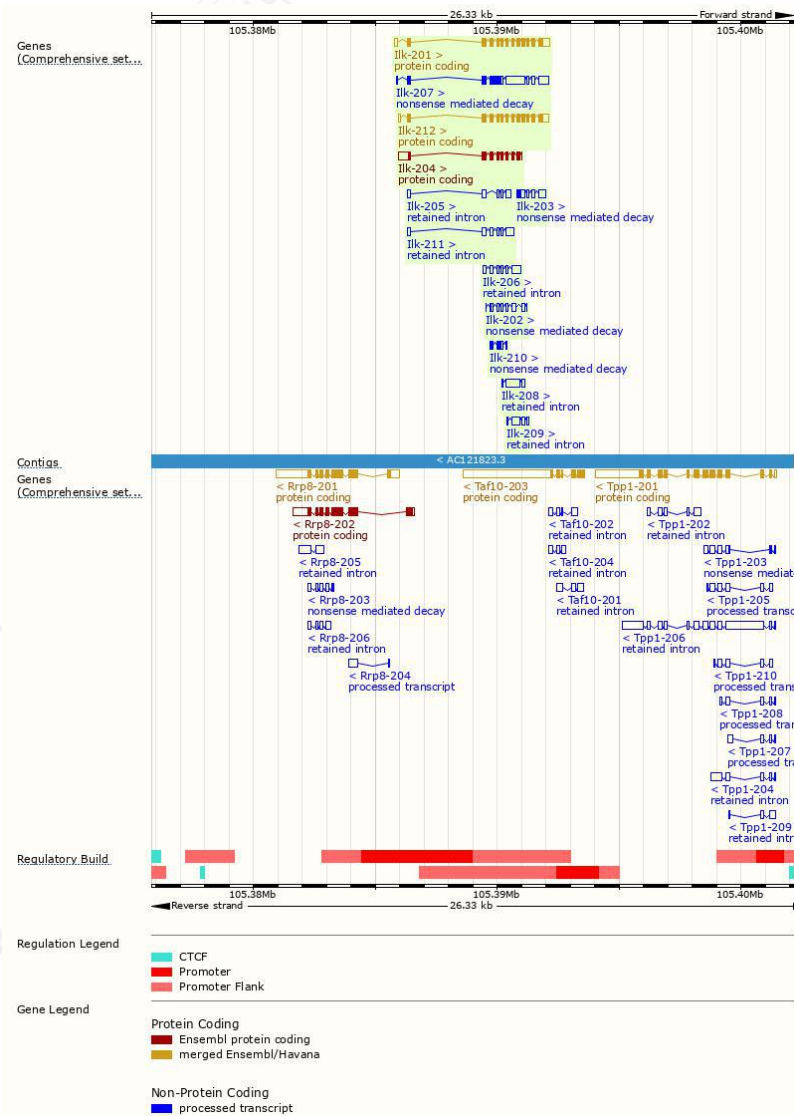
The gene has 12 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ilk-201	ENSMUST00000033182.9	1795	452aa	Protein coding	CCDS21659	O55222	TSL:1 GENCODE basic APPRIS P1
Ilk-212	ENSMUST00000163389.8	1749	452aa	Protein coding	CCDS21659	O55222	TSL:1 GENCODE basic APPRIS P1
Ilk-204	ENSMUST00000136687.8	1303	295aa	Protein coding	-	D3YZA5	CDS 3' incomplete TSL:5
Ilk-207	ENSMUST00000149695.7	2278	233aa	Nonsense mediated decay	-	A0A1B0GRF6	TSL:1
Ilk-203	ENSMUST00000130565.7	821	54aa	Nonsense mediated decay	-	A0A1B0GRW6	CDS 5' incomplete TSL:2
Ilk-202	ENSMUST00000127298.3	688	17aa	Nonsense mediated decay	-	F6Q5Z1	CDS 5' incomplete TSL:3
Ilk-210	ENSMUST00000153557.1	398	95aa	Nonsense mediated decay	-	A0A1B0GR42	CDS 5' incomplete TSL:5
Ilk-206	ENSMUST00000148971.1	909	No protein	Retained intron	-	-	TSL:2
Ilk-211	ENSMUST00000154626.7	886	No protein	Retained intron	-	-	TSL:2
Ilk-208	ENSMUST00000151108.1	756	No protein	Retained intron	-	-	TSL:5
Ilk-205	ENSMUST00000145123.7	698	No protein	Retained intron	-	-	TSL:3
Ilk-209	ENSMUST00000152508.7	608	No protein	Retained intron	-	-	TSL:3

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



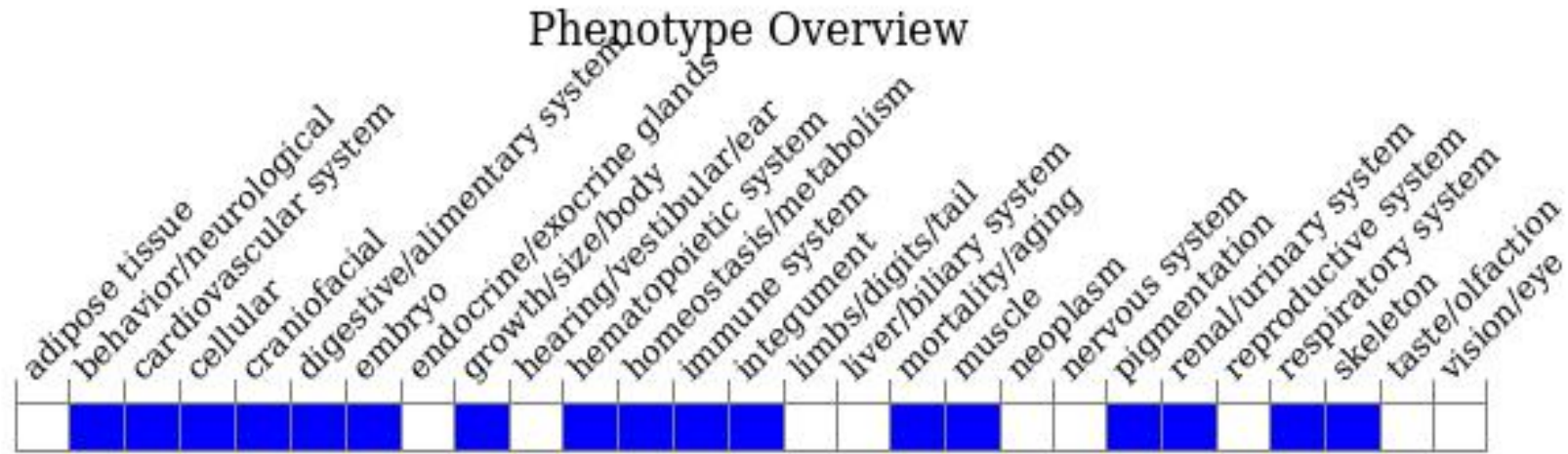
Genomic location distribution



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, nullizygous embryos do not polarize the epiblast and die after implantation. Mice with mutations in the ATP-binding site show aphagia, hunched posture, and neonatal death due to renal aplasia. Mice with mutations in the paxillin-binding site show vasculogenesis and growth defects, and die at ~E12.5.

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

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