

# Krt2 Cas9-CKO Strategy

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Reviewer: Ruirui Zhang

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## **Project Overview**



**Project Name** 

Krt2

**Project type** 

Cas9-CKO

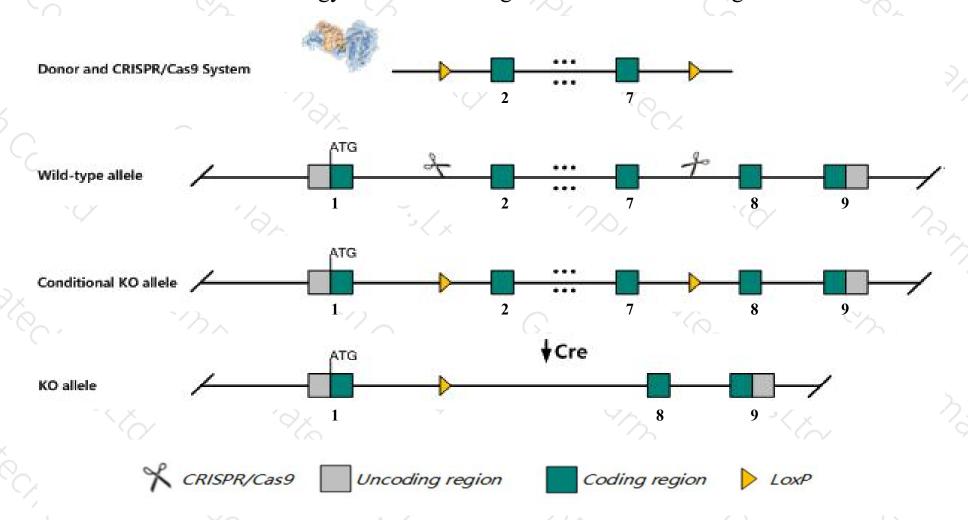
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Krt2 gene. The schematic diagram is as follows:



### Technical routes



- ➤ The *Krt2* gene has 1 transcript. According to the structure of *Krt2* gene, exon2-exon7 of *Krt2-201*(ENSMUST00000023712.7) transcript is recommended as the knockout region. The region contains 884bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Krt2* 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.

### **Notice**



- According to the existing MGI data, ENU-induced mutant mice exhibit scaly skin and increased pigmentation in the tail, ears and feet. Mice homozygous for a knock-out allele show scaly skin, acanthosis, hyperkeratosis, increased water loss, ear skin inflammation, and aberrant aggregation of keratin 10 in suprabasal epidermal keratinocytes.
- The *Krt2* gene is located on the Chr15. 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)



#### Krt2 keratin 2 [ Mus musculus (house mouse) ]

Gene ID: 16681, updated on 3-Sep-2019

#### Summary

☆ ?

Official Symbol Krt2 provided by MGI
Official Full Name keratin 2 provided by MGI

Primary source MGI:MGI:96699

See related Ensembl: ENSMUSG00000064201

Gene type protein coding
RefSeq status VALIDATED
Organism <u>Mus musculus</u>

Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae;

Murinae: Mus; Mus

Also known as Krt2e; Krt2-2; Krt2-17; BB005427

Expression Low expression observed in reference dataset See more

Orthologs human all

#### Genomic context

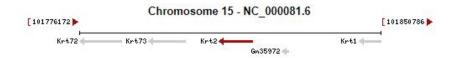


Location: 15 F2; 15 57.03 cM

See Krt2 in Genome Data Viewer

Exon count: 11

Annotation release	Status	Assembly	Chr	Location
<u>108</u>	current	GRCm38.p6 (GCF_000001635.26)	15	NC_000081.6 (101810689101818956, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	15	NC_000081.5 (101641120101648600, complement)



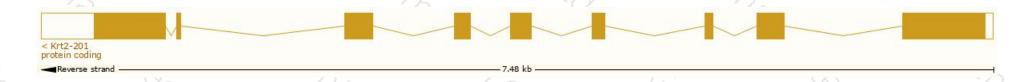
## Transcript information (Ensembl)



The gene has 1 transcript, and the transcript is shown below:

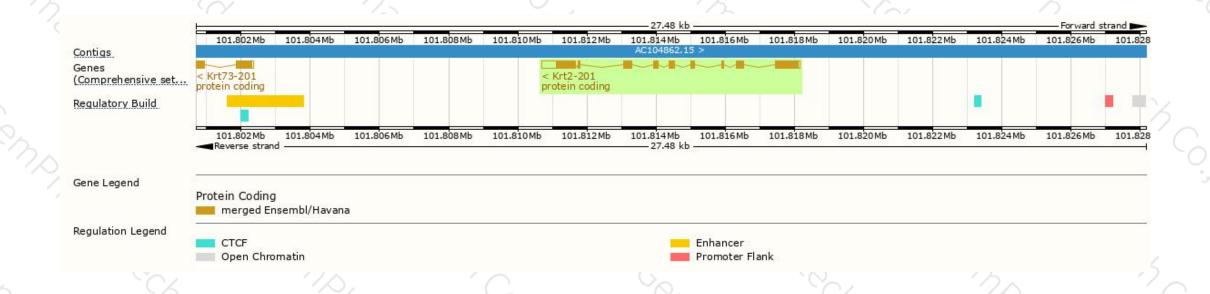
Name 🍦	Transcript ID	bp 🌲	Protein 👙	Biotype	CCDS 🍦	UniProt +	Flags		
Krt2-201	ENSMUST00000023712.7	2613	<u>707aa</u>	Protein coding	CCDS37220 ₽	Q3TTY5₽	TSL:1	GENCODE basic	APPRIS P1

The strategy is based on the design of *Krt2-201* transcript, The transcription is shown below



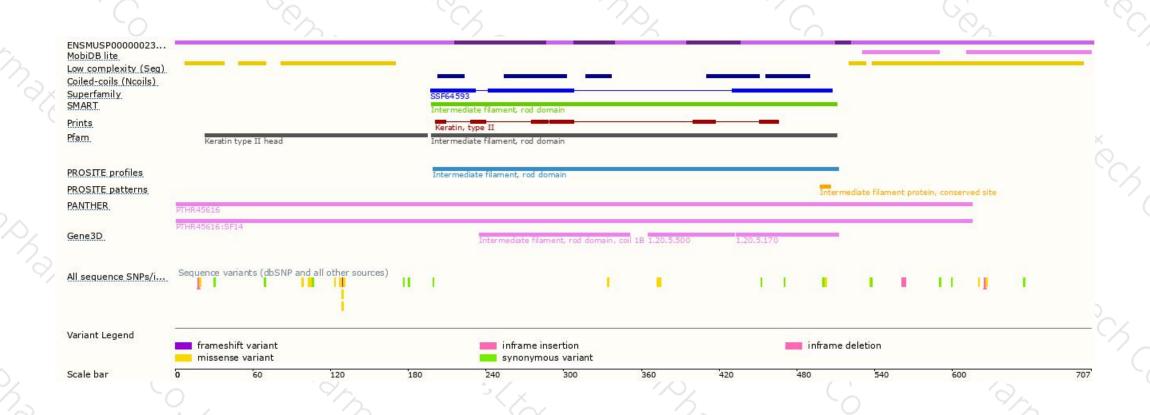
### Genomic location distribution





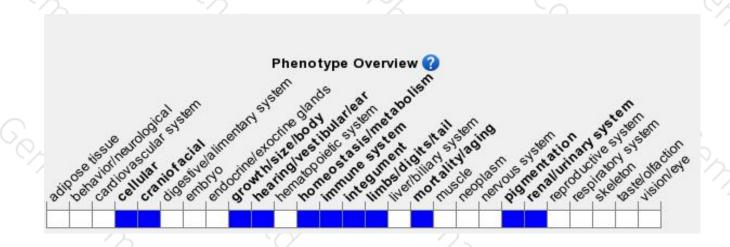
### Protein domain





## 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, ENU-induced mutant mice exhibit scaly skin and increased pigmentation in the tail, ears and feet. Mice homozygous for a knock-out allele show scaly skin, acanthosis, hyperkeratosis, increased water loss, ear skin inflammation, and aberrant aggregation of keratin 10 in suprabasal epidermal keratinocytes.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





