Krt19-DreERT2-polyA cas9-ki Strategy

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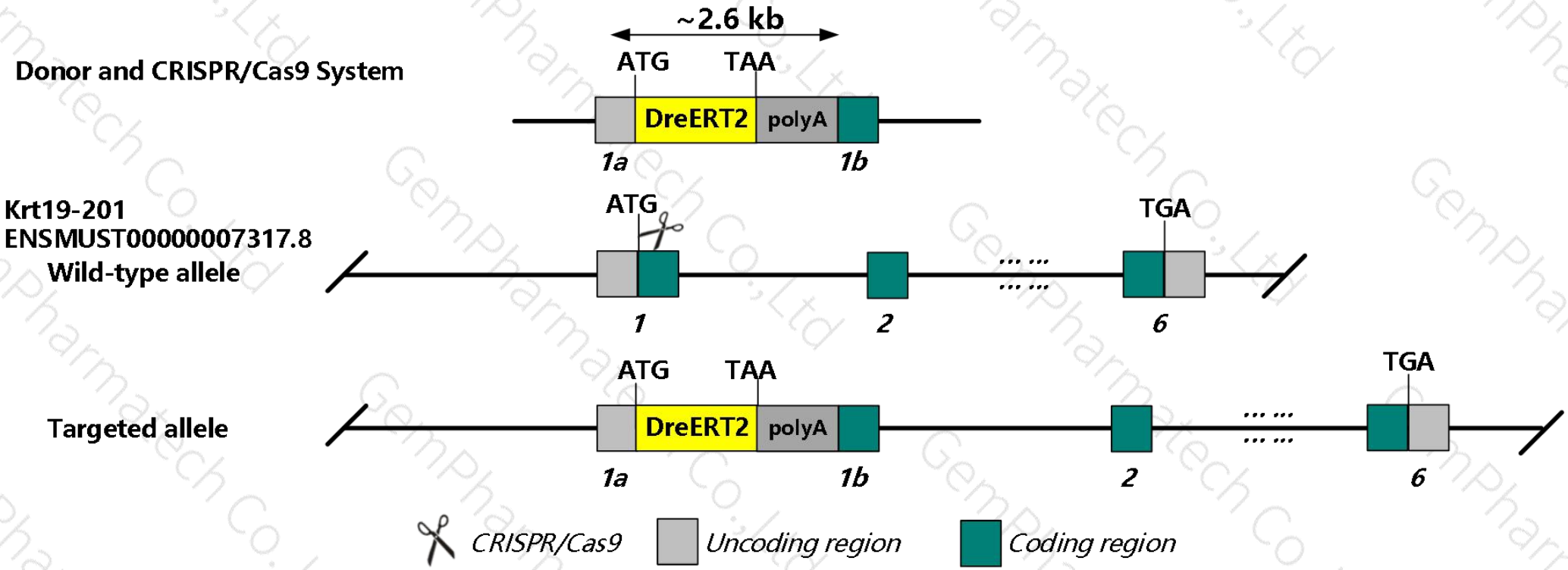
Design Date:	**2021-08-27**

Project Overview

Project Name	**Krt19-DreERT2-polyA**
Project type	**cas9-ki**
Strain background	**C57BL/6JGpt**

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Krt19* gene. The schematic diagram is as follows:



Technical routes

- The *Krt19* gene has 3 transcripts. According to the reference and structure of *Krt19* gene, transcript *Krt19*-201(ENSMUST00000007317.8) is selected for presentation of the recommended strategy.

- *Krt19*-201 transcript has 6 exons, with the ATG start codon in exon1 and TGA stop codon in exon6.

- We make *Krt19-DreERT2-polyA* knockin mice via CRISPR/Cas9 system. CRISPR/Cas9 system and donor will be co-injected into zygotes. Cas9 endonuclease cleavage near start codon(ATG) of exon1 of *Krt19* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in *DreERT2-polyA* after start coding(ATG) of *Krt19* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

Notice

- According to the existing MGI data, mice homozygous for a knock-in allele are viable. Mice homozygous for a reporter allele show partial and strain-dependent preweaning lethality but no anatomical or behavioral defects. Mice that are either homozygous or heterozygous for a targeted insertion into intron 6 exhibit sperm tail defects.
- There may be 1 to 2 amino acid mutation in exon1 of *Krt19* gene in this strategy.
- The effect of transcript 202,203 is unknown.
- The *Krt19* gene is located on the Chr11. If the knockin mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene after the gene coding region may affect the expression of endogenous and foreign genes. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

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Krt19 keratin 19 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 16669, updated on 3-Aug-2021

Summary

Official Symbol	Krt19 provided by MGI
Official Full Name	keratin 19 provided by MGI
Primary source	MGI:MGI:96693
See related	Ensembl:ENSMUSG00000020911
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	End; K19; CK-19; EndoC; Krt1-1; Krt-1.1; Krt1-19; AI663979; Krt-1.19
Summary	The protein encoded by this gene is a member of the keratin family. The keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells and are subdivided into cytokeratins and hair keratins. The type I cytokeratins consist of acidic proteins which are arranged in pairs of heterotypic keratin chains. Unlike its related family members, this smallest known acidic cytokeratin is not paired with a basic cytokeratin in epithelial cells. It is specifically expressed in the periderm, the transiently superficial layer that envelopes the developing epidermis. Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Sep 2015]
Expression	Biased expression in colon adult (RPKM 2054.0), stomach adult (RPKM 1224.1) and 8 other tissues See more
Orthologs	human all

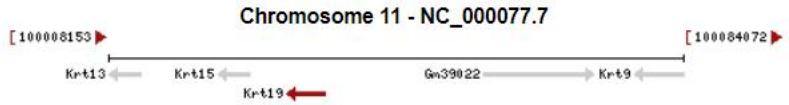
NEW **Try the new Gene table**
Try the new Transcript table

Genomic context

Location: 11 D; 11 63.42 cM

See Krt19 in Genome Data Viewer

Exon count: 7

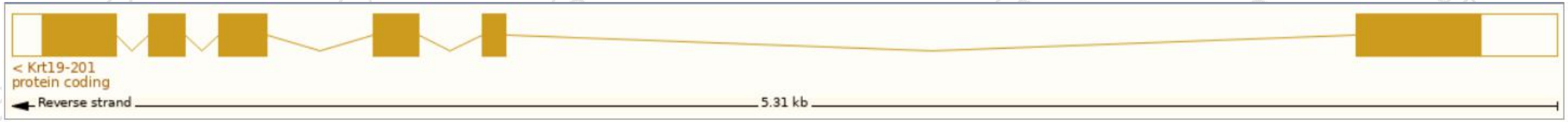


Transcript information （Ensembl）

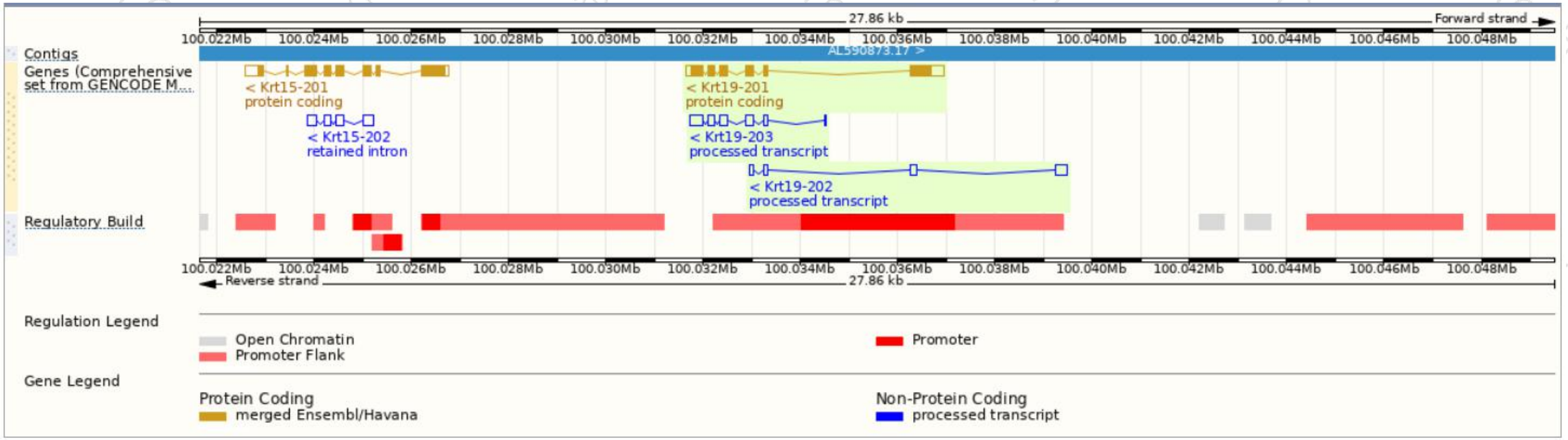
The gene has 3 transcripts, and all transcripts are shown below：

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Krt19-201	ENSMUST00000007317.8	1576	403aa	Protein coding	CCDS25411	B1AQ78 P19001	GENCODE basic APPRIS P1 TSL:1
Krt19-202	ENSMUST00000125888.2	546	No protein	Processed transcript	-	-	TSL:5
Krt19-203	ENSMUST00000126460.8	823	No protein	Processed transcript	-	-	TSL:3

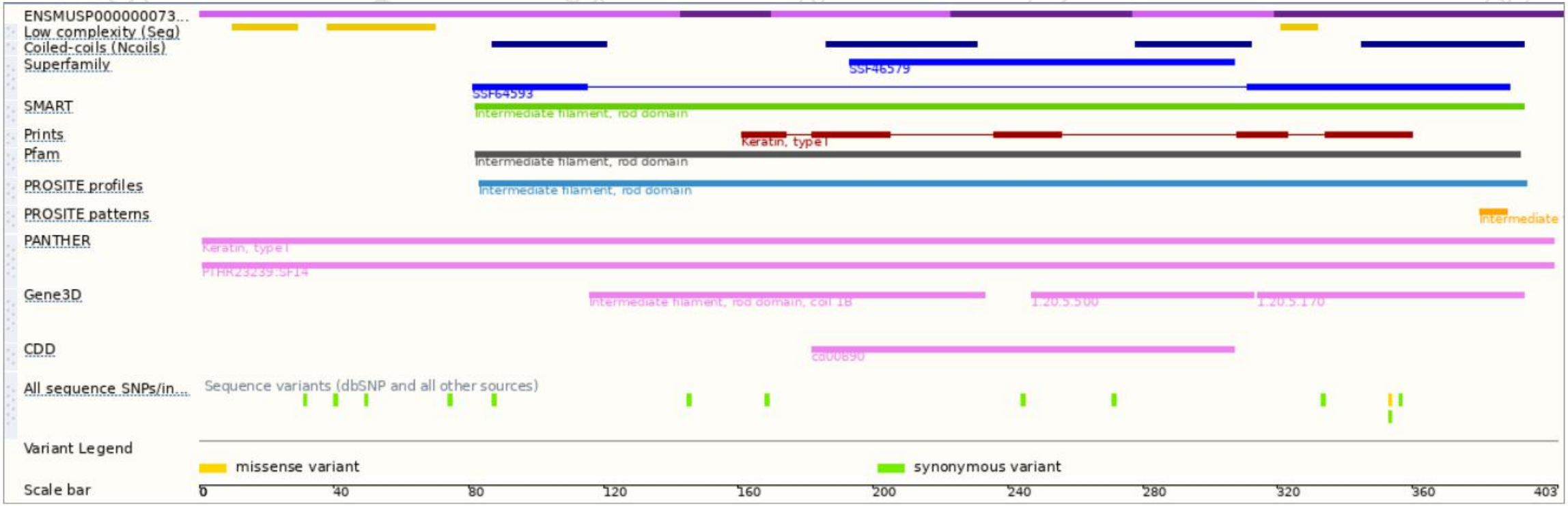
The strategy is based on the design of *Krt19-201* transcript,the transcription is shown below



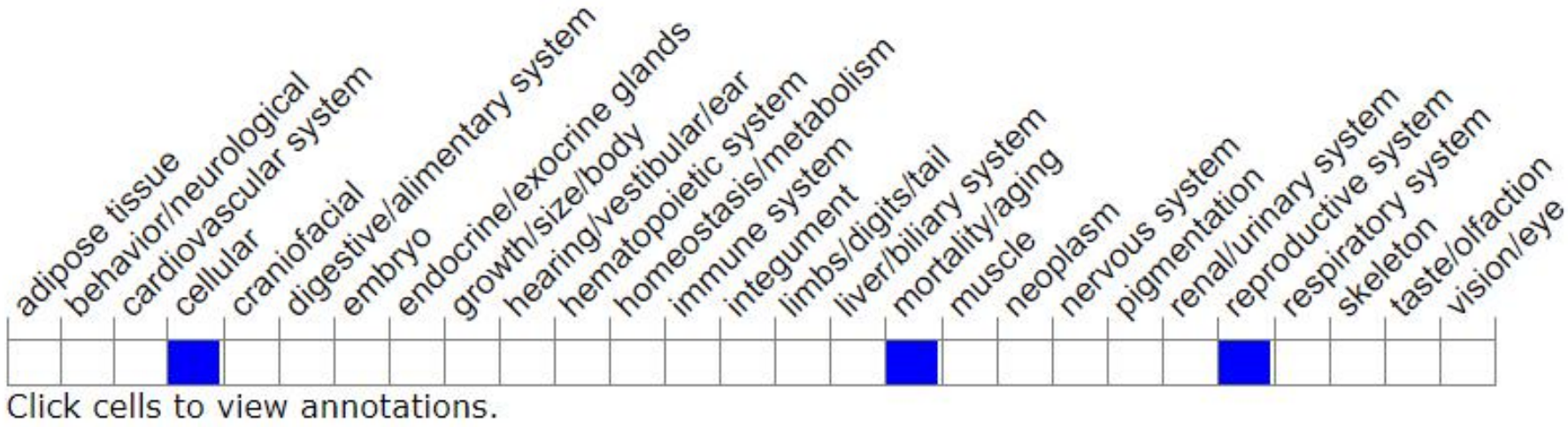
Genomic location distribution



Protein domain



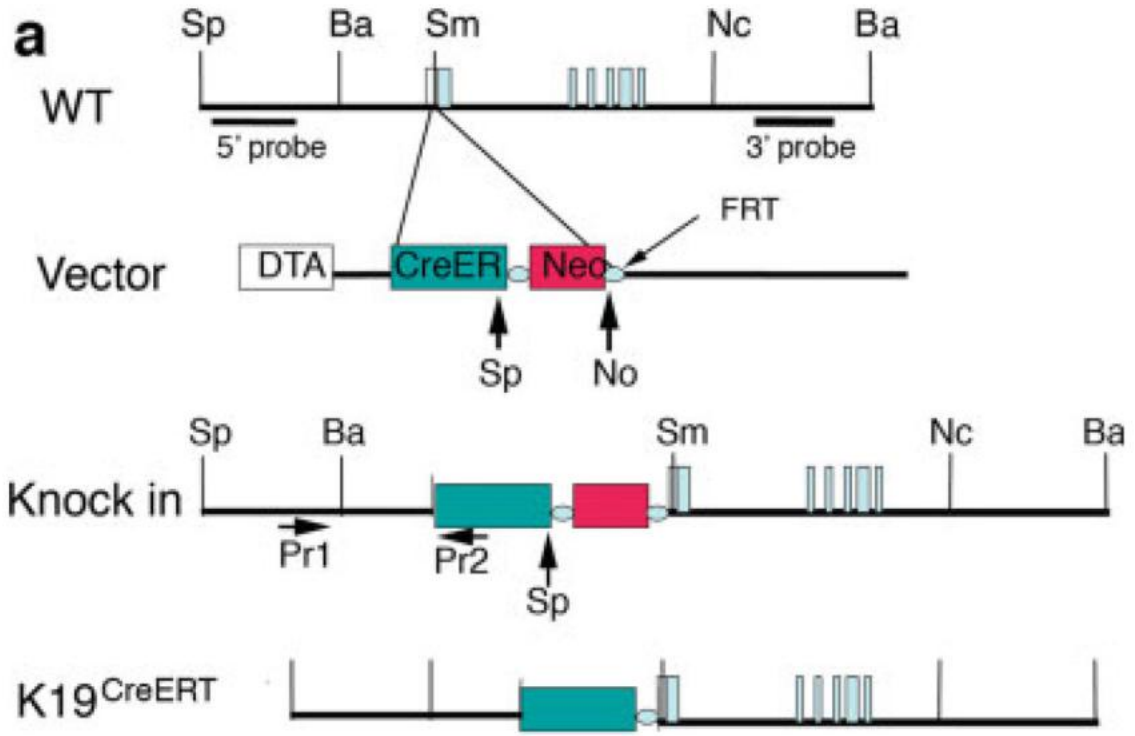
Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/marker/MGI:96693) .

Mice homozygous for a knock-in allele are viable. Mice homozygous for a reporter allele show partial and strain-dependent preweaning lethality but no anatomical or behavioral defects. Mice that are either homozygous or heterozygous for a targeted insertion into intron 6 exhibit sperm tail defects.

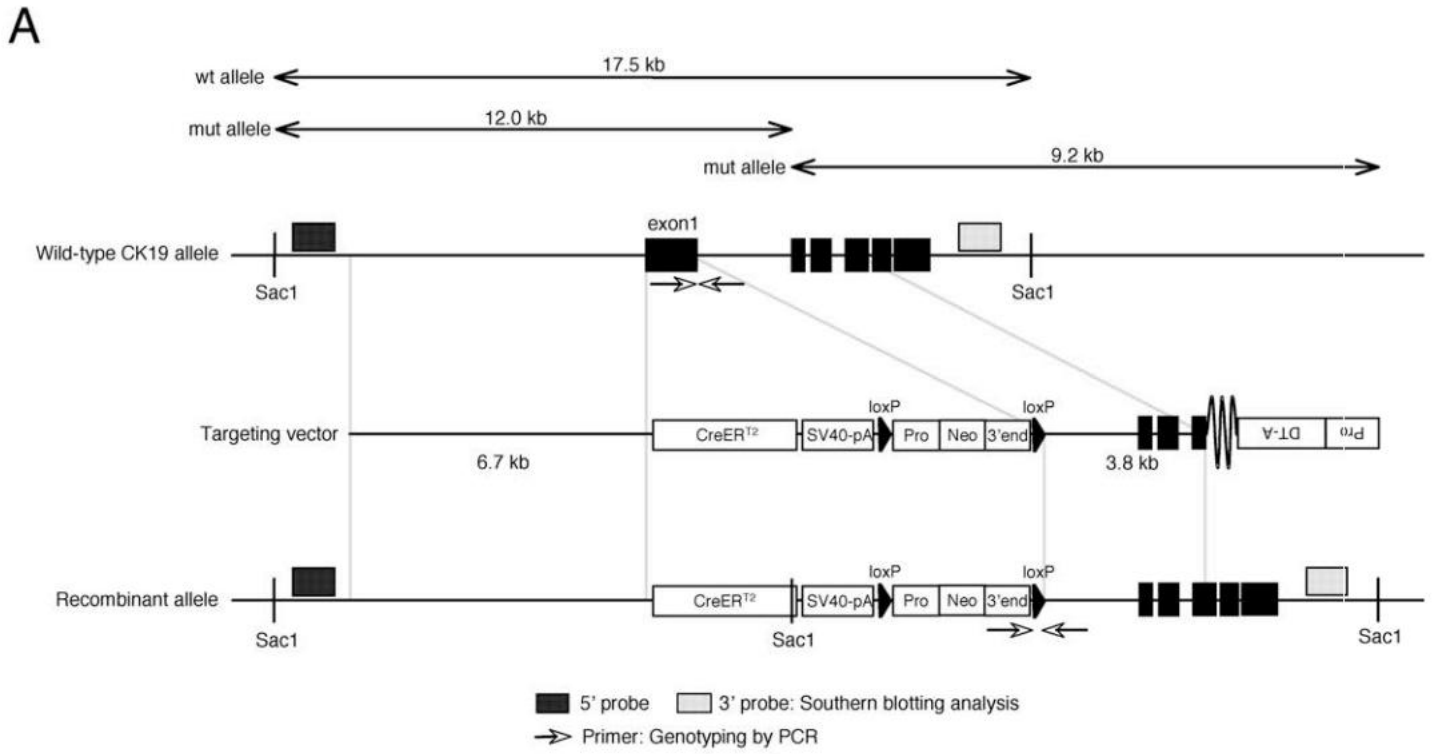
Reference



We derived a *K19*^{CreERT} allele by replacing *K19* ATG with a CreER^{T}-cDNA followed by a SV40 polyadenylation signal (see Fig. 1). This design minimally altered *K19* transcription regulatory elements while producing a CreER^{T} message with a short 3′-UTR. Inclusion of a

Means AL; Xu Y; Zhao A; Ray KC; Gu G. 2008. A CK19(CreERT) knockin mouse line allows for conditional DNA recombination in epithelial cells in multiple endodermal organs. Genesis 46(6):318-23

Reference



Sekiya S, et al., Intrahepatic cholangiocarcinoma can arise from Notch-mediated conversion of hepatocytes. J Clin Invest. 2012 Nov 1;122(11):3914-8

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

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