GemPharmatech™

Ung Cas9-KO Strategy

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Reviewer: Jia Yu

Design Date: 2023-4-3

Overview

Target Gene Name

- *Ung*

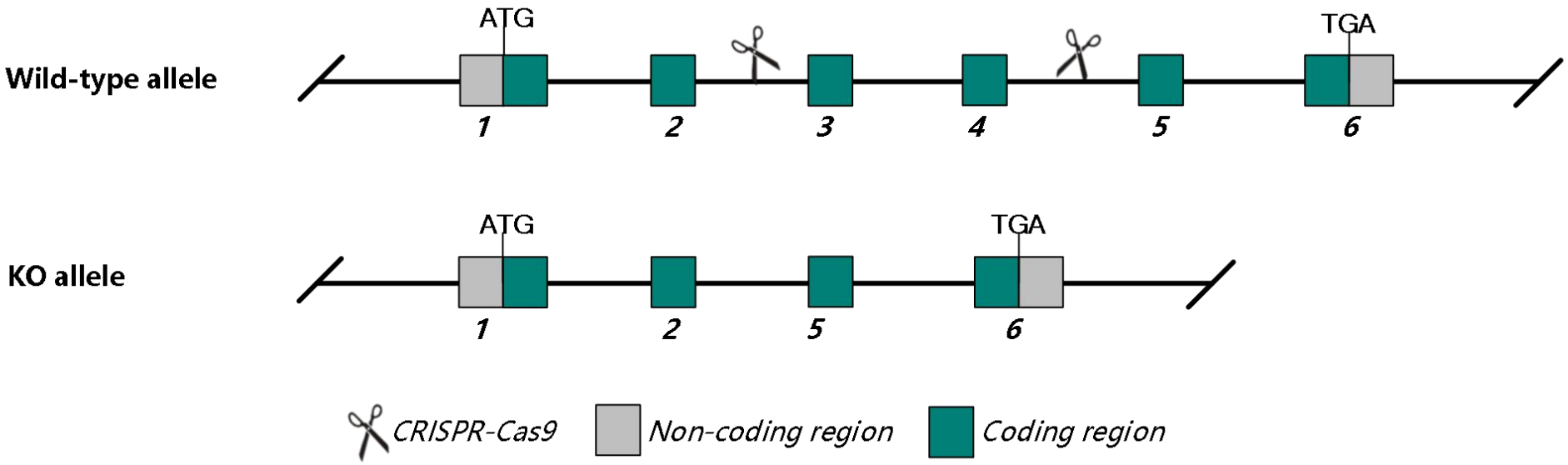
Project Type

- Cas9-KO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Ung* gene.

Technical Information

- The *Ung* gene has 6 transcripts. According to the structure of *Ung* gene, exon 3-4 of *Ung*-202 (ENSMUST00000102584.11) is recommended as the knockout region. The region contains 187 bp of coding sequence. Knockout the region will result in disruption of gene function.
- In this project we use CRISPR-Cas9 technology to modify *Ung* gene. The brief process is as follows: gRNAs were transcribed in vitro. Cas9 and gRNAs 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.

Gene Information

Ung uracil DNA glycosylase [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 22256, updated on 9-Mar-2023

Summary

Official Symbol	Ung provided by MGI
Official Full Name	uracil DNA glycosylase provided by MGI
Primary source	MGI:MGI:109352
See related	Ensembl:ENSMUSG00000029591 AllianceGenome:MGI:109352
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	UNG1; UNG2
Summary	Enables uracil DNA N-glycosylase activity. Acts upstream of or within isotype switching and somatic hypermutation of immunoglobulin genes. Located in mitochondrion and nucleus. Is expressed in several structures, including alimentary system; brain; genitourinary system; lung; and olfactory epithelium. Human ortholog(s) of this gene implicated in dysgammaglobulinemia and immunodeficiency with hyper IgM type 5. Orthologous to human UNG (uracil DNA glycosylase). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Ubiquitous expression in limb E14.5 (RPKM 8.0), CNS E11.5 (RPKM 7.9) and 28 other tissues See more
Orthologs	human all

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

Genomic context

Location: 5; 5 F

See Ung in Genome Data Viewer

Exon count: 8

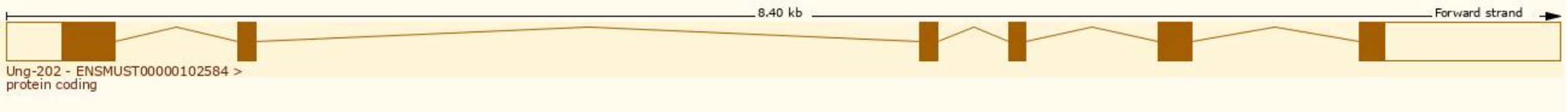
https://www.ncbi.nlm.nih.gov/gene/22256

Transcript Information

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

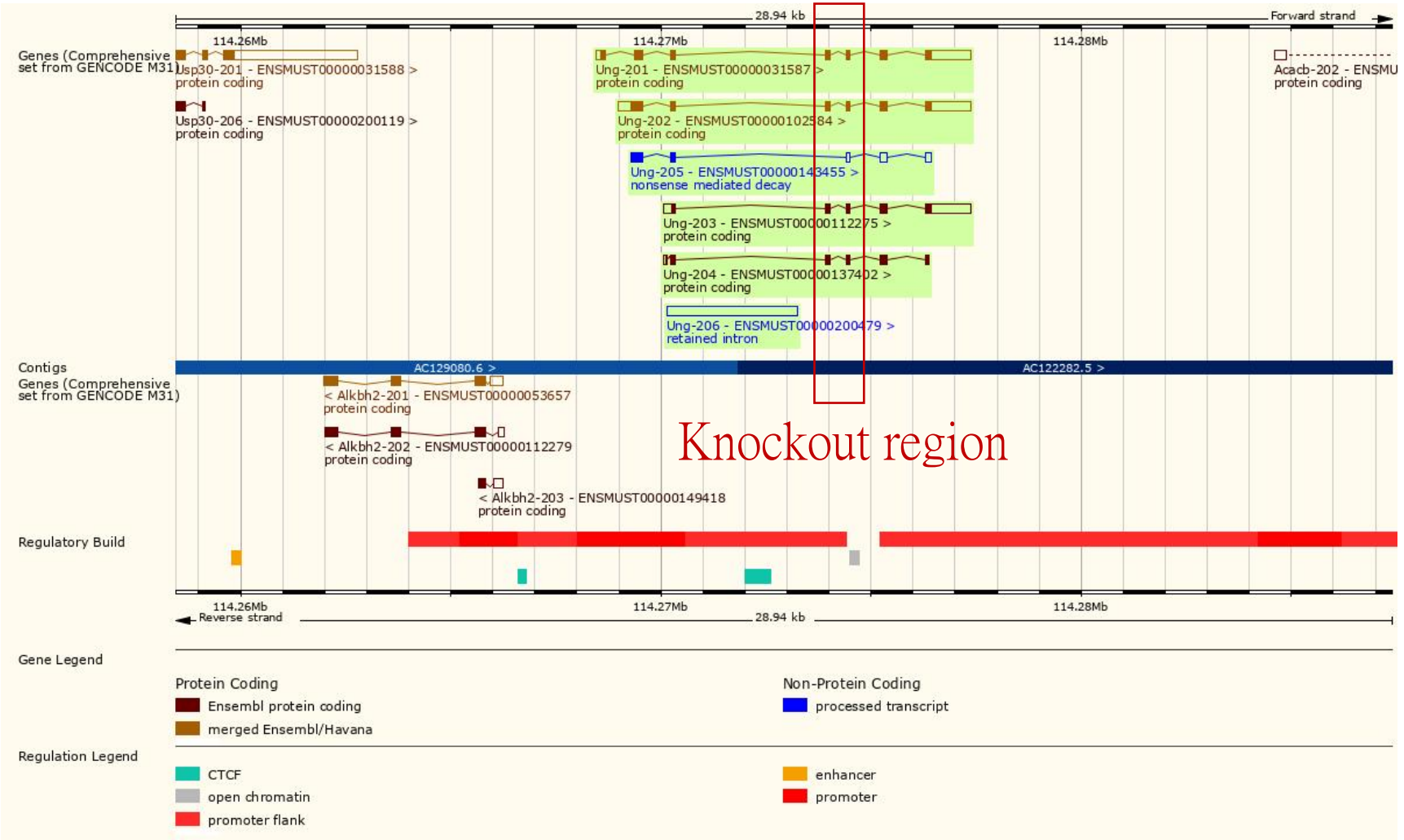
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000031587.13	Ung-201	1985	306aa	Protein coding	CCDS39221	P97931-1	Ensembl Canonical, GENCODE basic, APPRIS P1, TSL:1
ENSMUST00000102584.11	Ung-202	2136	295aa	Protein coding	CCDS19560	P97931-2	GENCODE basic, TSL:1
ENSMUST00000112275.8	Ung-203	1733	199aa	Protein coding		D3Z1G1	GENCODE basic, TSL:1
ENSMUST00000137402.2	Ung-204	609	185aa	Protein coding		D3YW18	TSL:5, CDS 3' incomplete
ENSMUST00000143455.6	Ung-205	792	132aa	Nonsense mediated decay		A0A0G2JDS8	TSL:5
ENSMUST00000200479.2	Ung-206	3099	No protein	Retained intron		-	TSL:NA

The strategy is based on the design of *Ung*-202 transcript, the transcription is shown below:



Source: http://asia.ensembl.org/

Genomic Information



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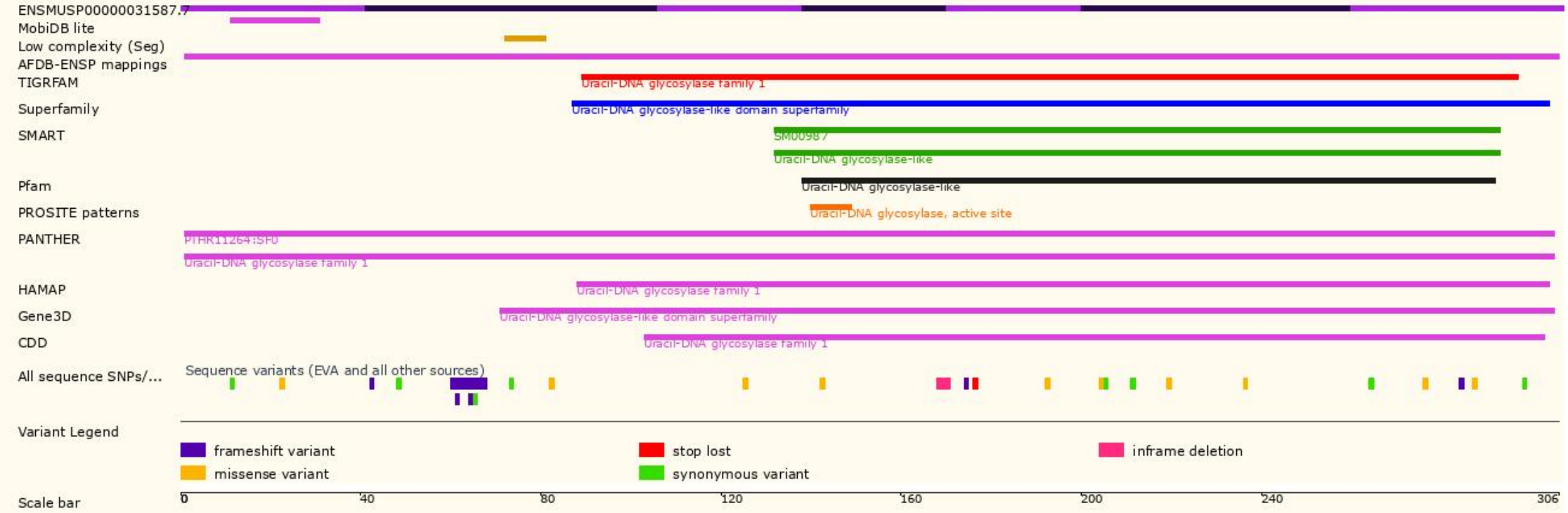
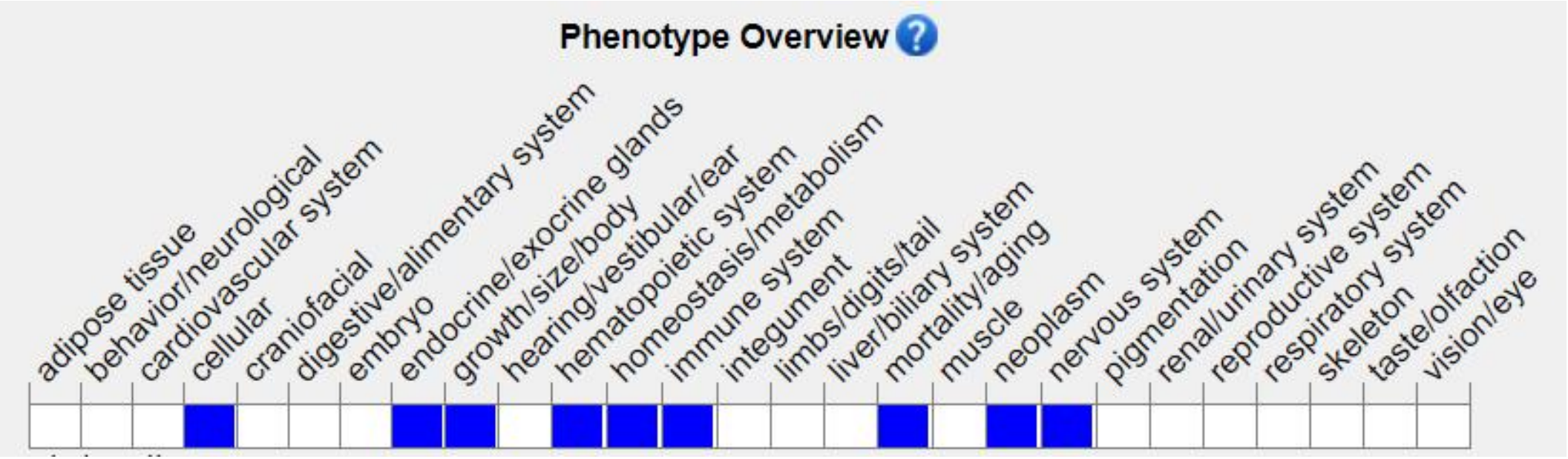
Source:http://asia.ensembl.org/

Protein Information



Source:http://asia.ensembl.org/

Mouse Phenotype Information (MGI)



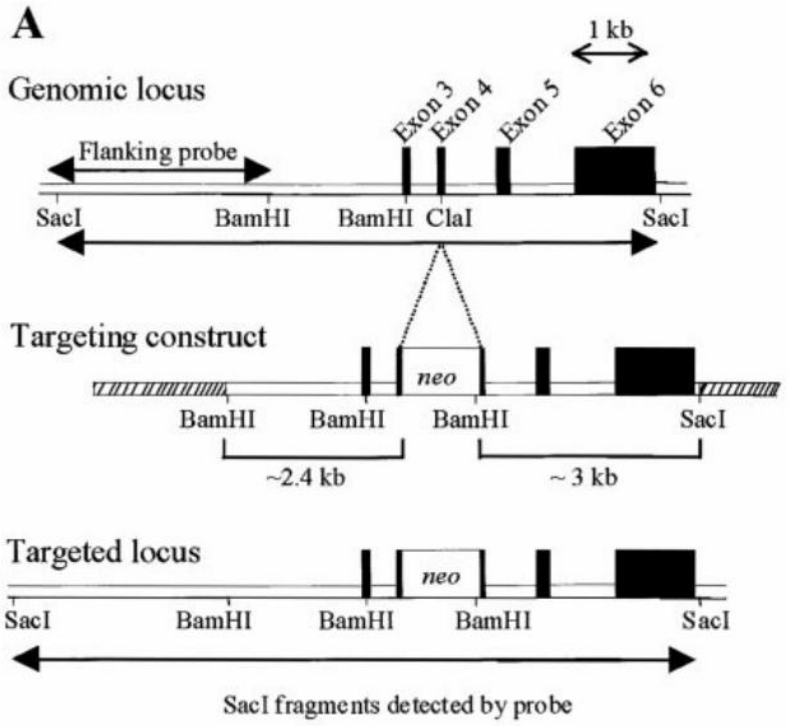
- Homozygous null mutants incorporate an elevated level of uracil into DNA of dividing cells. In hypermutation at immunoglobulin genes, mutations at C/G pairs are shifted toward transitions, and class-switch recombination is reduced. Homozygous null mutants display increased ischemic brain injury.

Source: https://www.informatics.jax.org/marker/MGI:109352

Important Information

- This strategy may have no effect on the *Ung*-206 transcript.
- The knockout region is about 7.2 kb away from the 5' of the *Alkbh2* gene, which may affect the regulation of this gene.
- *Ung* is located on Chr 5. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.

Reference



Generation of *ung* Null Mice

Homozygous null mice deficient in both nuclear (UNG2) and mitochondrial (UNG1) isoforms of uracil-DNA glycosylase were generated by targeted insertion of a *Neo* cassette into exon 4 of the murine *Ung* gene in embryonic stem (ES) cells (Figure 1A); this exon encodes residues of the uracil-binding pocket within the catalytic domain of the enzyme (Slupphaug et al., 1996). Genotyping of live-born mice from intermatings of $ung^{+/-}$ heterozygotes (Figure 1B) showed that *ung* null mice are viable and were recovered in Mendelian ratios. The $ung^{-/-}$ mice were fertile, developed normally into adulthood with no overt phenotype, and remained tumor free (comparison of >100 *ung* null versus wild-type mice of 12–18 months). Detailed histopathological examination of a male and female sacrificed at 1 year showed no abnormalities (G. Stamp and D. E. B., unpublished data).

[1] Nilsen H, Rosewell I, Robins P, et al. Uracil-DNA glycosylase (UNG)-deficient mice reveal a primary role of the enzyme during DNA replication[J]. Molecular cell, 2000, 5(6): 1059-1065.