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Psmc3 Cas9-CKO Strategy

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Design Date: 2023-10-16

Overview

Target Gene Name

- Psmc3

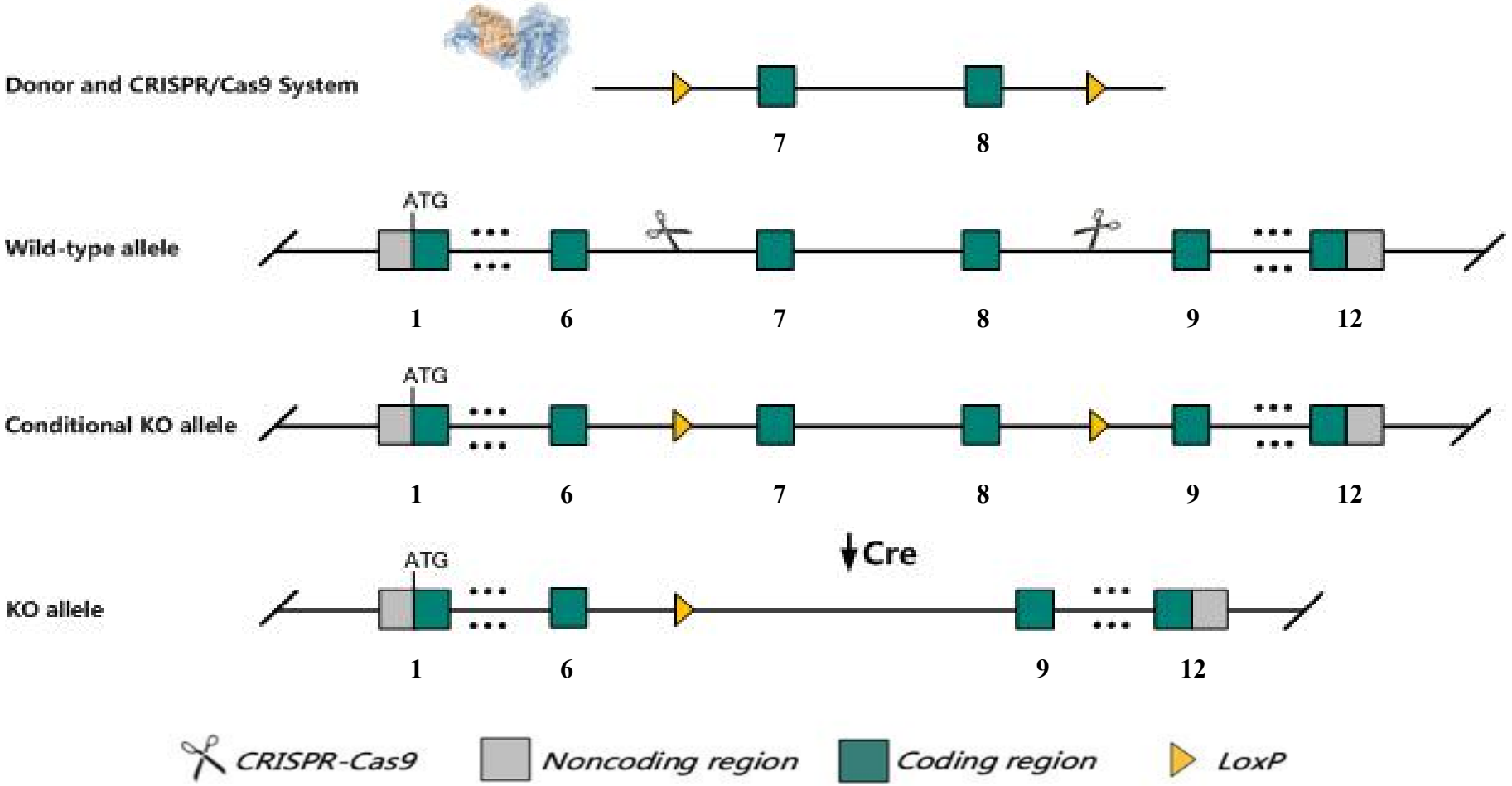
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Psmc3* gene.

Technical Information

- The *Psmc3* gene has 10 transcripts. According to the structure of *Psmc3* gene, exon7-exon8 of *Psmc3*-202 (ENSMUST00000067663.14) transcript is recommended as the knockout region. The region contains 293bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Psmc3* 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 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.
- 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.

Gene Information

Psmc3 proteasome (prosome, macropain) 26S subunit, ATPase 3 [Mus musculus (house mouse)]

Gene ID: 19182, updated on 13-Mar-2020

Summary

Official Symbol	Psmc3 provided by MGI
Official Full Name	proteasome (prosome, macropain) 26S subunit, ATPase 3 provided by MGI
Primary source	MGI:MGI:1098754
See related	Ensembl:ENSMUSG00000002102
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	TBP-1
Expression	Ubiquitous expression in testis adult (RPKM 179.9), CNS E11.5 (RPKM 103.6) and 28 other tissues See more
Orthologs	human all

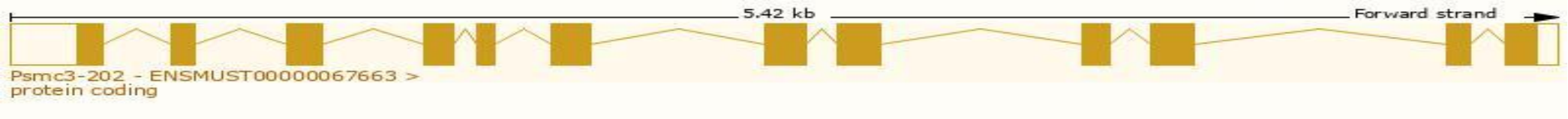
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 10 transcripts,all transcripts are shown below:

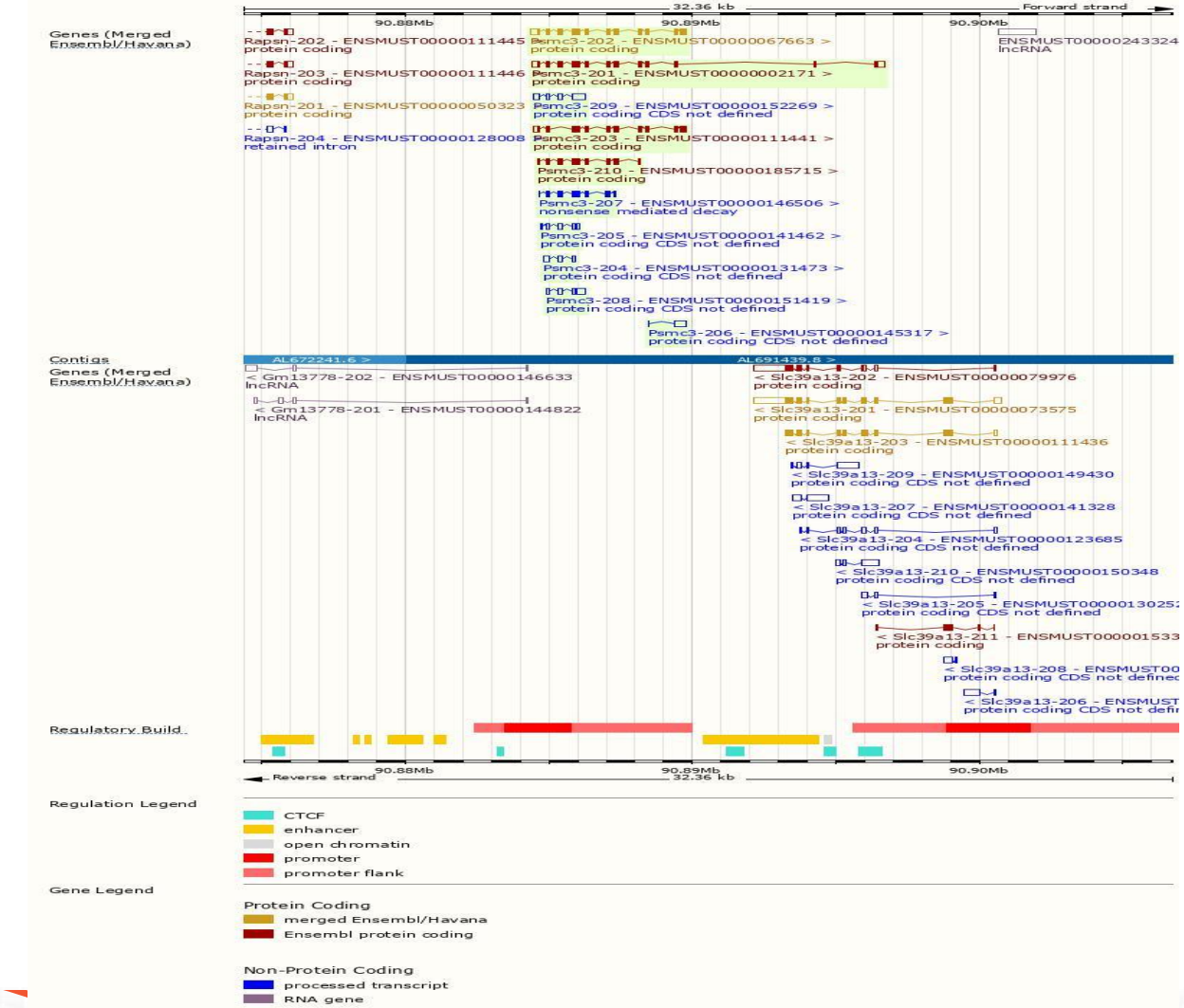
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Psmc3-202	ENSMUST00000067663.13	1636	442aa	Protein coding	CCDS16423	O88685	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Psmc3-201	ENSMUST00000002171.13	1819	451aa	Protein coding	-	B7ZCF1	TSL:1 GENCODE basic
Psmc3-203	ENSMUST00000111441.9	1419	400aa	Protein coding	-	A2AGN7	TSL:5 GENCODE basic
Psmc3-210	ENSMUST00000185715.6	944	305aa	Protein coding	-	A0A087WPH7	CDS 3' incomplete TSL:3
Psmc3-207	ENSMUST00000146506.1	792	203aa	Nonsense mediated decay	-	F6Q2E3	CDS 5' incomplete TSL:3
Psmc3-209	ENSMUST00000152269.7	925	No protein	Processed transcript	-	-	TSL:2
Psmc3-208	ENSMUST00000151419.1	598	No protein	Processed transcript	-	-	TSL:3
Psmc3-206	ENSMUST00000145317.1	436	No protein	Processed transcript	-	-	TSL:3
Psmc3-205	ENSMUST00000141462.7	429	No protein	Processed transcript	-	-	TSL:5
Psmc3-204	ENSMUST00000131473.7	403	No protein	Processed transcript	-	-	TSL:2

The strategy is based on the design of *Psmc3*-202 transcript, the transcription is shown below:



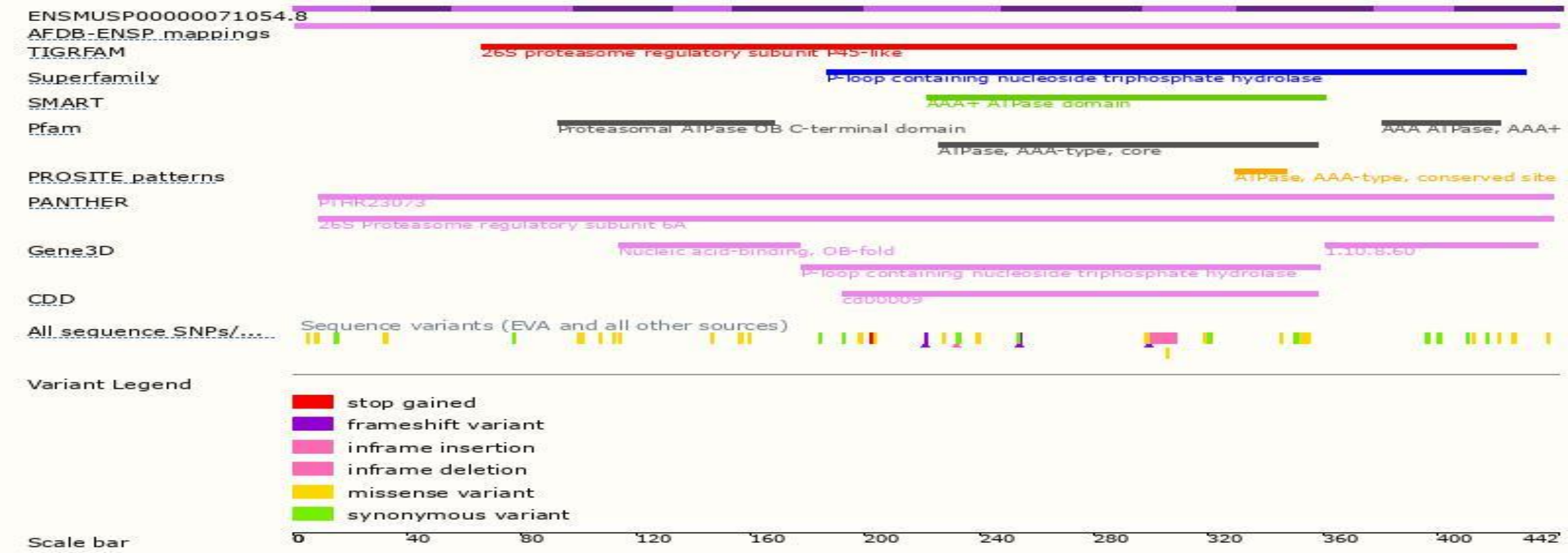
Source: https://www.ensembl.org

Genomic Information



Source: : https://www.ensembl.org

Protein Information



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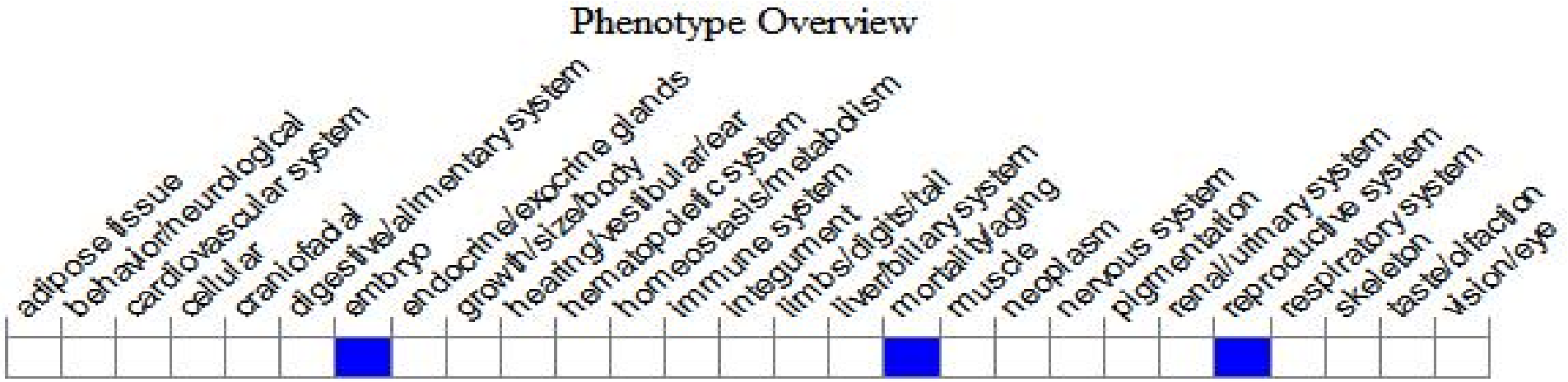
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



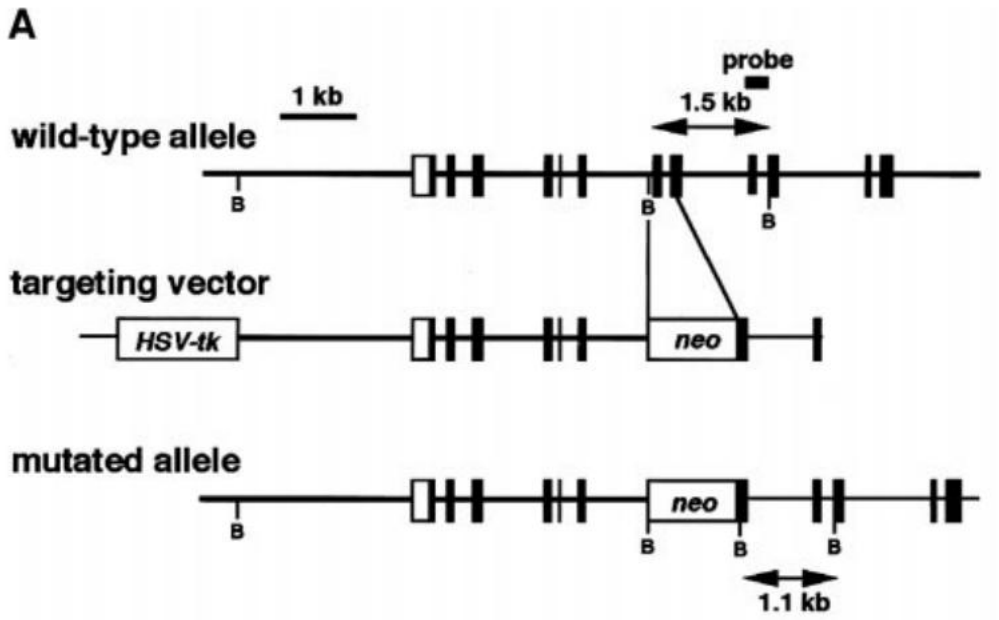
- Mice homozygous for disruptions in this gene die as embryos.

Source: https://www.informatics.jax.org

Important Information

- According to the MGI information,mice homozygous for disruptions in this gene die as embryos.
- The floxed region is near to the N-terminal of *Gm13778* gene,the strategy may affect the function of the N-terminal of *Gm13778* gene.
- *Psmc3* is located on Chr2. 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 Psmc3- and Psmc4-deficient mice. A targeting vector of Psmc3 was designed to replace an approximately 300-bp genomic fragment, containing exon 7 and a part of exon 8, with pMC1-neo (Stratagene). The targeting vector was flanked by the 5.5-kb fragment at the 5′ end and the 1.2-kb fragment at the 3′ end and contains a HSV-tk cassette at the 5′ end of the vector. A targeting vector of Psmc4 was constructed by replacing an approximately 1.0-kb genomic fragment, containing exons 8, 9, and 10 and part of exon 11, with a pMC1-neo cassette. The targeting vector was flanked by the 5.7-kb fragment at the 5′ end and the 1.3-kb fragment at the 3′ end. An HSV-tk cassette was inserted into the 5′ end of the vector. The targeting vector was linearized with *Sal*I and electroporated into E14.1 embryonic stem cells. The clones resistant to both G418 and gancyclovir were screened by polymerase chain reaction (PCR) for homologous recombination and confirmed by Southern blot analysis with the probes shown in Fig. 4. Generation of chimeric mice and mutant mice was essentially as described previously (Takeda *et al.*, 1996).

Sakao Y, et al., Mouse proteasomal ATPases Psmc3 and Psmc4: genomic organization and gene targeting. Genomics. 2000 Jul 1;67(1):1-7