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Ltk Cas9-CKO Strategy

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Reviewer:Xiangli Bian

Design Date: 2024.1.15

Overview

Target Gene Name

- Ltk

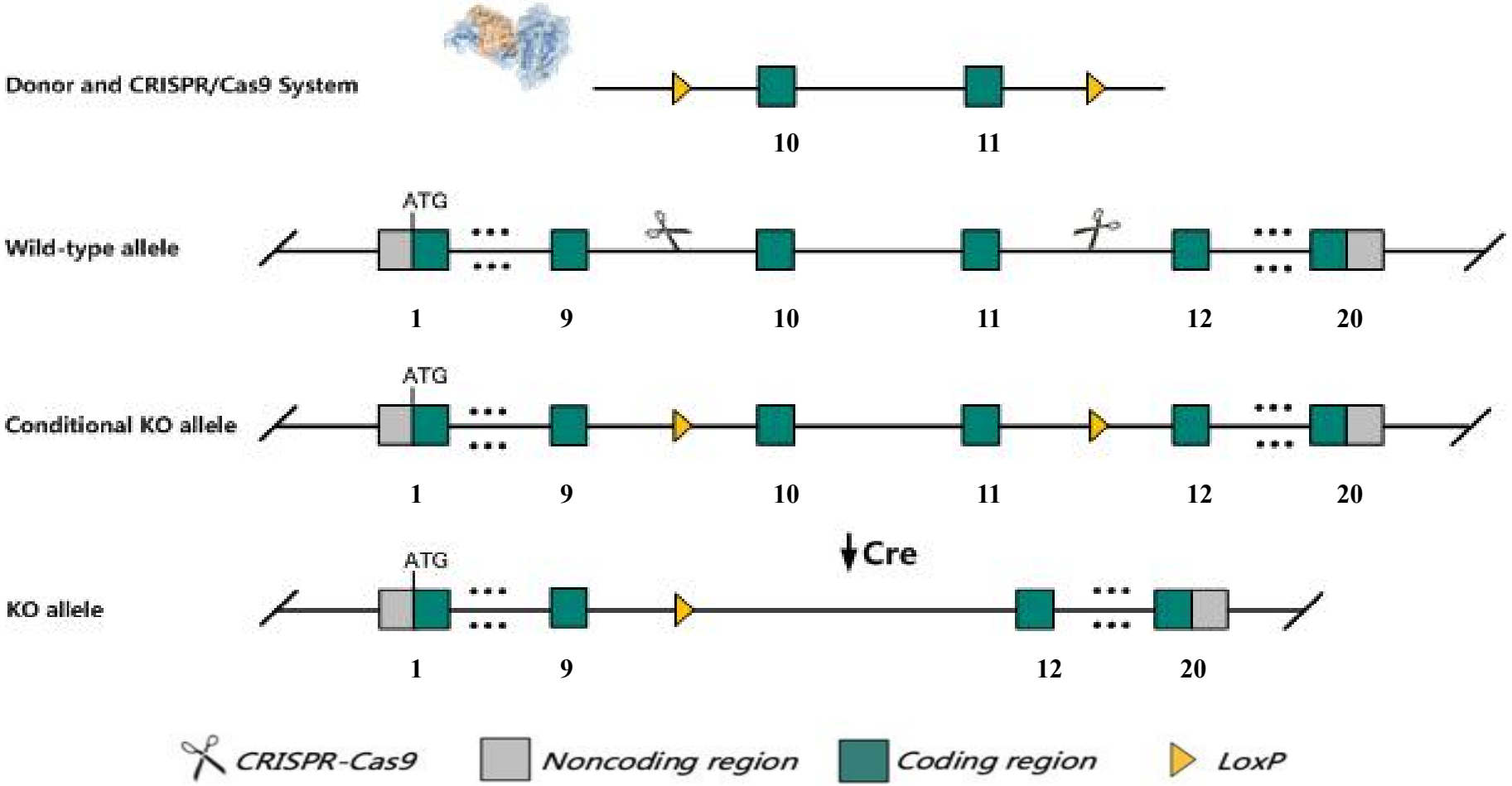
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy

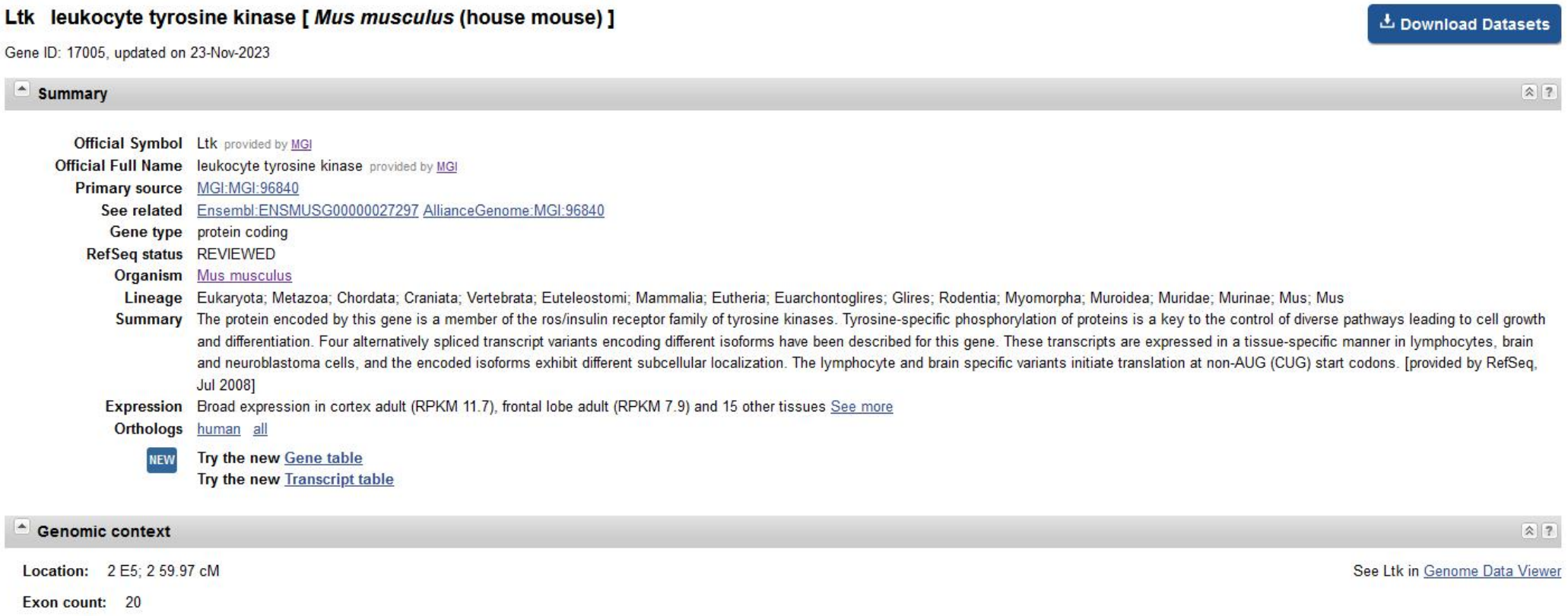


Schematic representation of CRISPR-Cas9 engineering used to edit the *Ltk* gene.

Technical Information

- The *Ltk* gene has 6 transcripts. According to the structure of *Ltk* gene, exon10-exon11 of *Ltk*-201 (ENSMUST00000028759.13) transcript is recommended as the knockout region. The region contains 289bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Ltk* 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



Ltk leukocyte tyrosine kinase [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 17005, updated on 23-Nov-2023

Summary

Official Symbol	Ltk provided by MGI
Official Full Name	leukocyte tyrosine kinase provided by MGI
Primary source	MGI:MGI:96840
See related	Ensembl:ENSMUSG00000027297 AllianceGenome:MGI:96840
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
Summary	The protein encoded by this gene is a member of the ros/insulin receptor family of tyrosine kinases. Tyrosine-specific phosphorylation of proteins is a key to the control of diverse pathways leading to cell growth and differentiation. Four alternatively spliced transcript variants encoding different isoforms have been described for this gene. These transcripts are expressed in a tissue-specific manner in lymphocytes, brain and neuroblastoma cells, and the encoded isoforms exhibit different subcellular localization. The lymphocyte and brain specific variants initiate translation at non-AUG (CUG) start codons. [provided by RefSeq, Jul 2008]
Expression	Broad expression in cortex adult (RPKM 11.7), frontal lobe adult (RPKM 7.9) and 15 other tissues See more
Orthologs	human all

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

Genomic context

Location: 2 E5; 2 59.97 cM — See Ltk in Genome Data Viewer

Exon count: 20

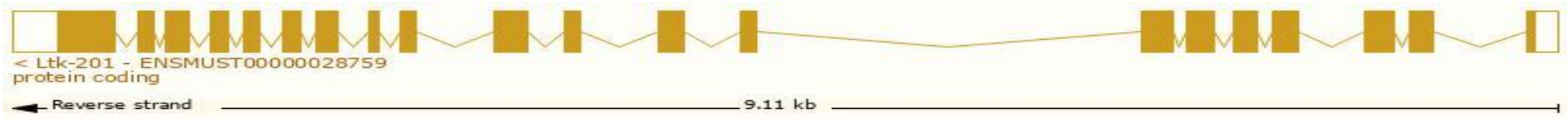
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

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

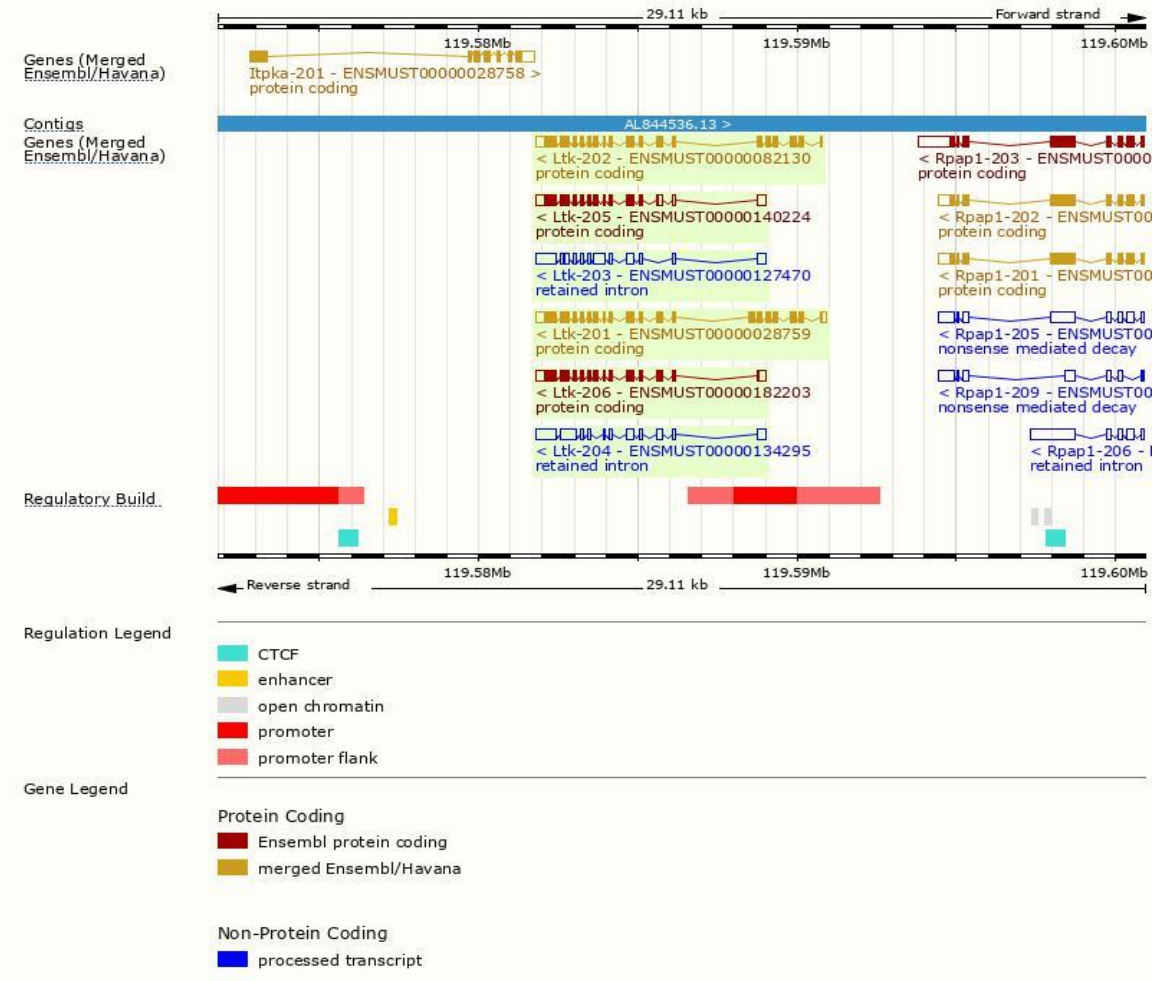
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000028759.13	Ltk-201	3080	888aa	Protein coding	CCDS16608	P08923	Ensembl Canonical GENCODE basic APPRIS P2 TSL:1
ENSMUST00000082130.13	Ltk-202	2767	827aa	Protein coding	CCDS16609	P08923-4	GENCODE basic APPRIS ALT2 TSL:1
ENSMUST00000127470.8	Ltk-203	2234	No protein	Retained intron		-	TSL:1
ENSMUST00000134295.2	Ltk-204	2299	No protein	Retained intron		-	TSL:2
ENSMUST00000140224.8	Ltk-205	2240	476aa	Protein coding		F6V2R5	GENCODE basic APPRIS ALT2 TSL:1
ENSMUST00000182203.3	Ltk-206	2232	576aa	Protein coding	CCDS50674	A0A804AW03	GENCODE basic APPRIS ALT2 TSL:1

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



Source: https://www.ensembl.org

Genomic Information



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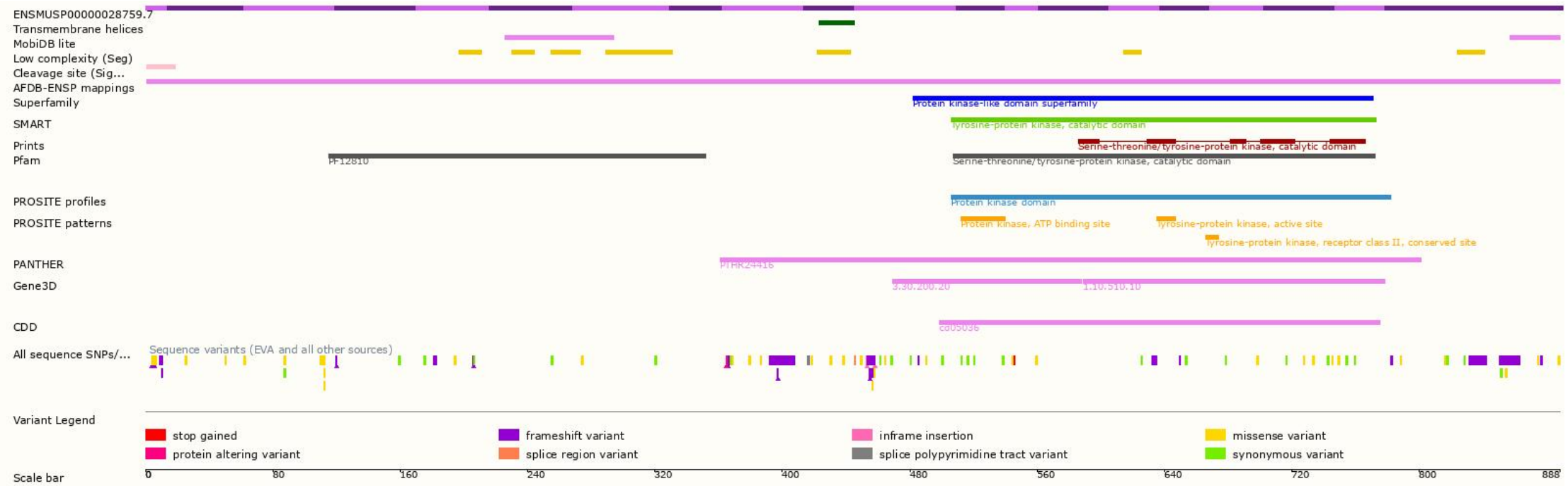
Source: https://www.ensembl.org

Protein Information



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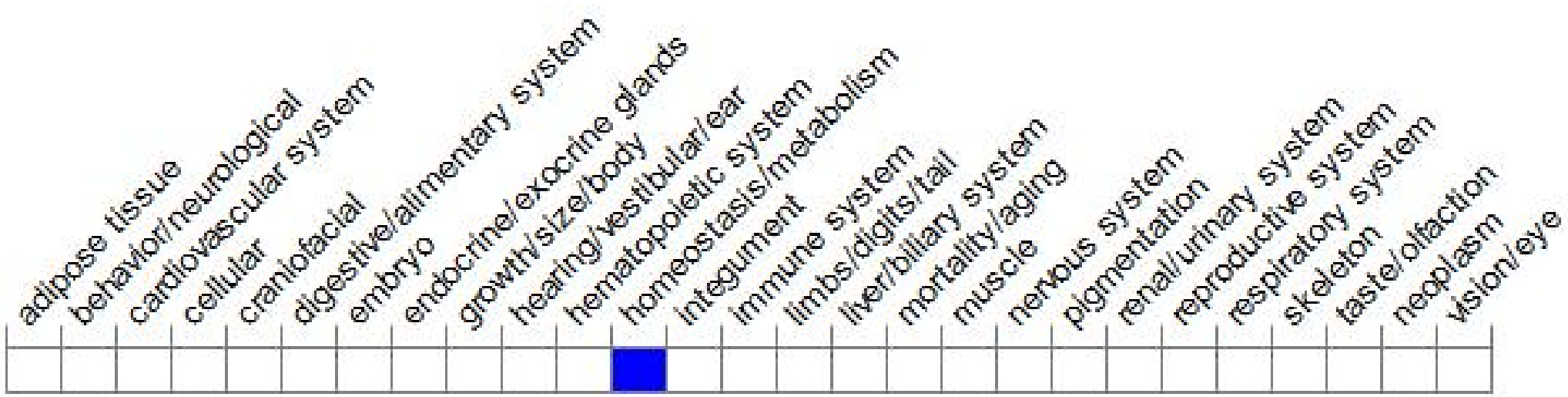
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



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

Reference

Targeted mutations in Alk and Ltk were induced by homologous recombination at each locus in mouse ES cells. The targeting vectors encode neomycin resistance and their integration results in deletion of essential coding sequences. In the Alk locus exons 20 and 21 are deleted, a total of 93 amino acids. The deleted exons encode the entire intracellular juxtamembrane domain and the initial portion of the tyrosine kinase catalytic domain. In the Ltk locus exons 10 and 11 are deleted, a total of 66 amino acids. The domains deleted include the entire transmembrane domain required for anchoring the mature receptor in the plasma membrane. Targeted mutations were generated in 129 mouse strain ES cells, which were injected into C57Bl6 blastocysts to create 129/C57Bl6 chimeric mice. Subsequent to conventional breeding strategies to generate homozygous Alk and Ltk single-mutant mice, the animals were bred onto a pure C57Bl6 background for 10 generations (Stephan W. Morris, MD, personal communication). Homozygous Alk and Ltk single-null animals were crossed to create double-mutant mice. Genotyping was performed by PCR using genomic DNA and primers specific for the neomycin resistance transgene and the deleted regions as well as a novel junctional fragment created at the Alk locus.

Weiss JB, Xue C, Benice T, Xue L, Morris SW, Raber J. Anaplastic lymphoma kinase and leukocyte tyrosine kinase: functions and genetic interactions in learning, memory and adult neurogenesis. Pharmacol Biochem Behav. 2012 Jan;100(3):566-74.

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

Important Information

- According to MGI, knocking out this gene has not yet detected the lethal phenotype.
- The length of intron9-10(457bp) and intron9-10(466bp) are relatively short, and the insertion of loxp may affect normal splicing.
- *Ltk* 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.