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Akt1 Cas9-CKO Strategy

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Reviewer: Daohua Xu

Design Date: 2024-2-27

Overview

Target Gene Name

- Akt1

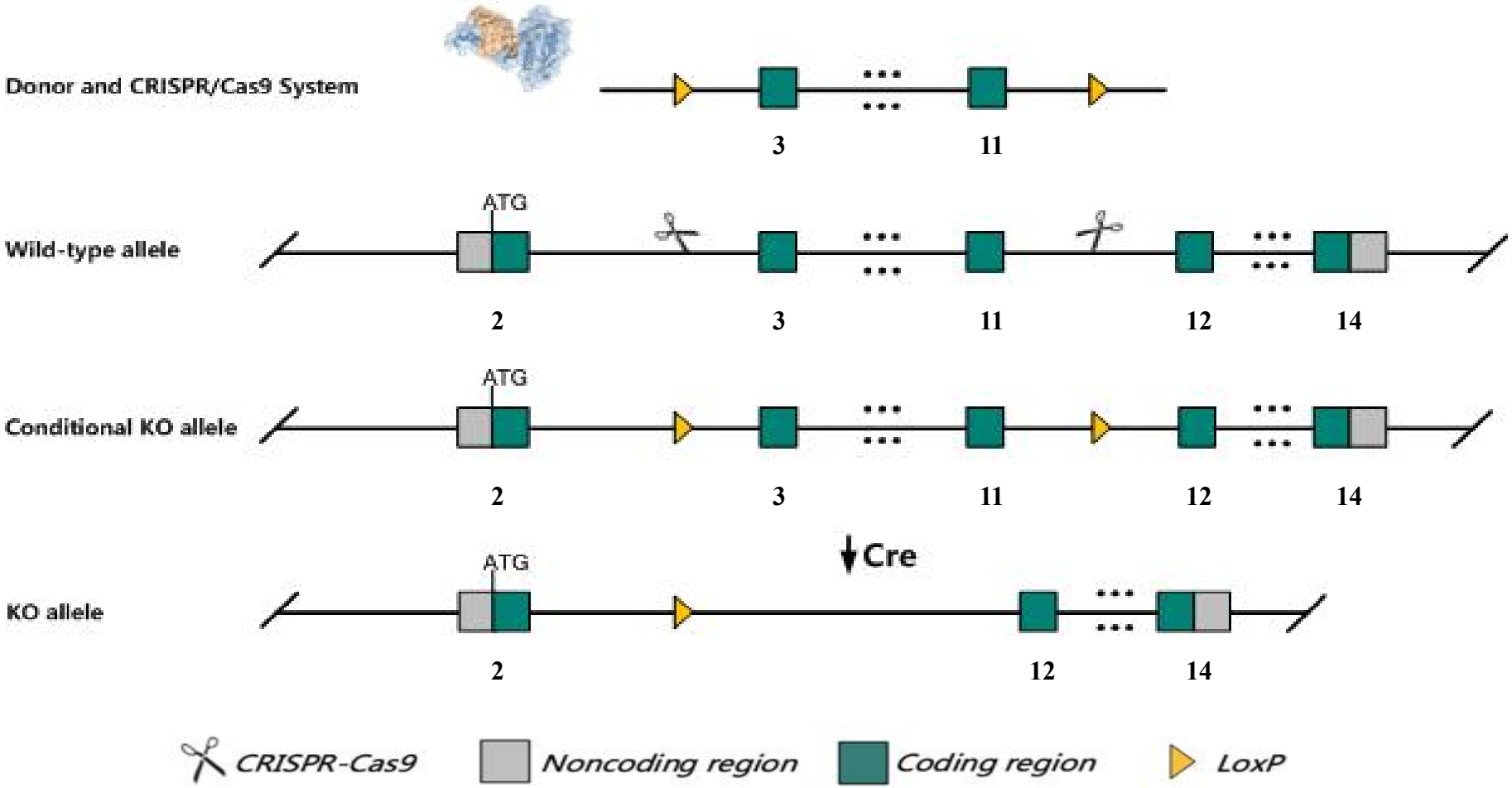
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Akt1* gene.

Technical Information

- The *Akt1* gene has 9 transcripts. According to the structure of *Akt1* gene, exon3-exon11 of *Akt1*-201 (ENSMUST00000001780.10) transcript is recommended as the knockout region. The region contains 1126bp coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Akt1* 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

Akt1 thymoma viral proto-oncogene 1 [*Mus musculus* (house mouse)]

Download Datasets

Gene ID: 11651, updated on 13-Feb-2024

Summary

Official Symbol	Akt1 provided by MGI
Official Full Name	thymoma viral proto-oncogene 1 provided by MGI
Primary source	MGI:MGI:87986
See related	Ensembl:ENSMUSG00000001729 AllianceGenome:MGI:87986
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	Akt; PKB; Rac; LTR-akt; PKB/Akt; PKBalpha
Summary	This gene encodes the founding member of the Akt serine-threonine protein kinase gene family that also includes Akt2 and Akt3. This kinase is a major downstream effector of the phosphatidylinositol 3-kinase (PI3K) pathway that mediates the effects of various growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin and insulin-like growth factor I (IGF-I). It is activated through recruitment to cellular membranes by PI3K lipid products and by phosphorylation by 3-phosphoinositide dependent kinase-1. It then further phosphorylates different downstream proteins in response to various extracellular signals and thus plays a pivotal role in mediating a variety of cellular processes, such as glucose metabolism, glycogen biosynthesis, protein synthesis and turn over, inflammatory response, cell survival (anti-apoptosis) and development. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Sep 2009]
Expression	Ubiquitous expression in adrenal adult (RPKM 140.9), limb E14.5 (RPKM 84.5) and 28 other tissues See more
Orthologs	human all

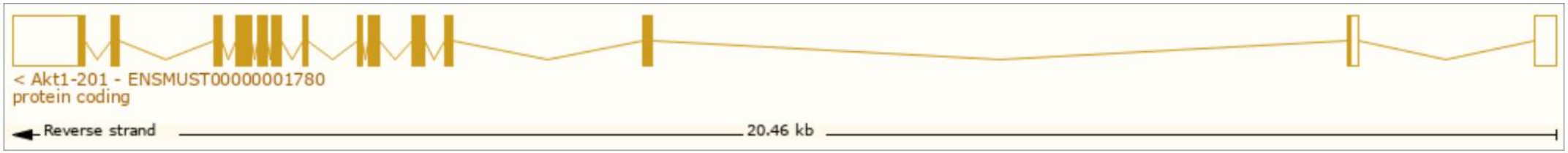
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 9 transcripts,all transcripts are shown below:

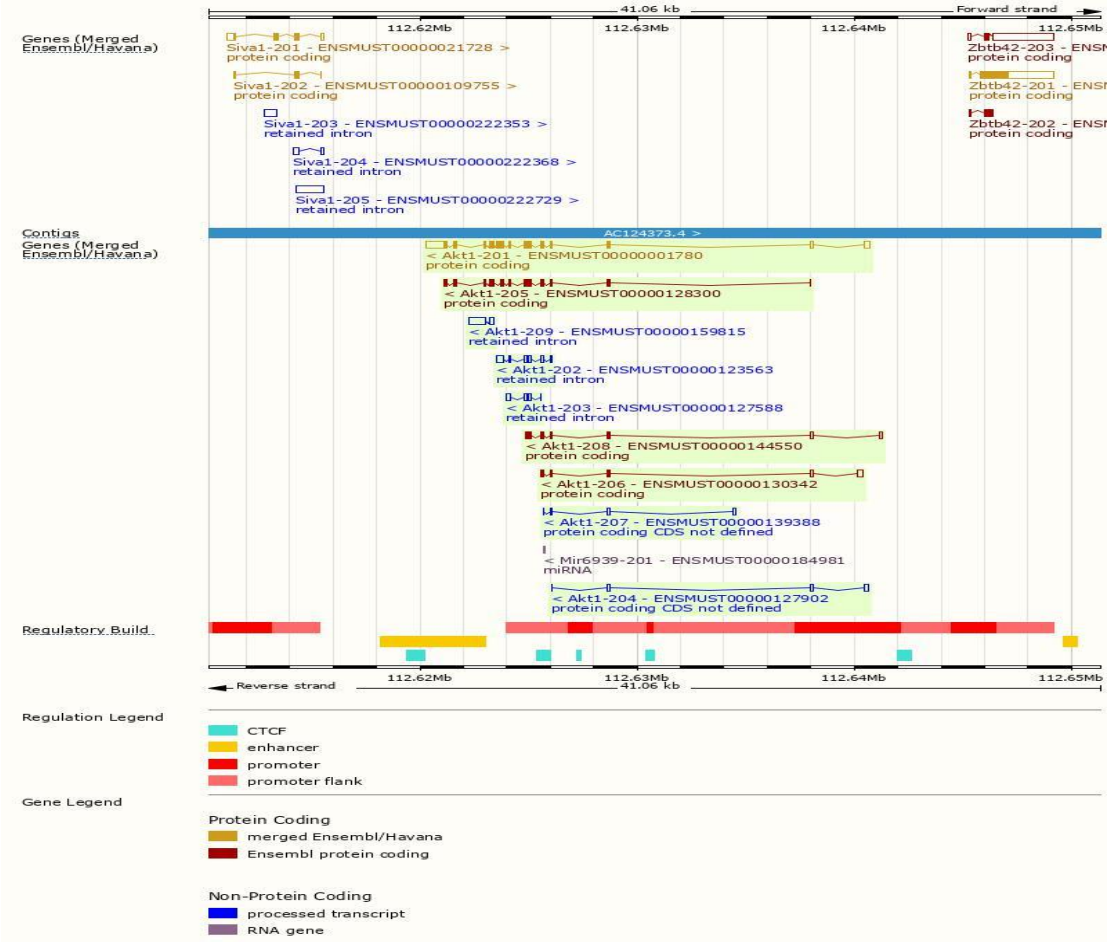
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000001780.10	Akt1-201	2690	480aa	Protein coding	CCDS26194	P31750	Ensembl Canonical, GENCODE basic, APPRIS P1, TSL:1
ENSMUST00000128300.9	Akt1-205	1342	437aa	Protein coding		D3Z783	GENCODE basic, TSL:5
ENSMUST00000144550.9	Akt1-208	862	202aa	Protein coding		D3YXX3	TSL:5, CDS 3' incomplete
ENSMUST00000130342.2	Akt1-206	736	134aa	Protein coding		D3YYP9	TSL:3, CDS 3' incomplete
ENSMUST00000127902.2	Akt1-204	526	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000139388.3	Akt1-207	397	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000159815.2	Akt1-209	944	No protein	Retained intron		-	TSL:2
ENSMUST00000123563.9	Akt1-202	803	No protein	Retained intron		-	TSL:2
ENSMUST00000127588.3	Akt1-203	386	No protein	Retained intron		-	TSL:2

The strategy is based on the design of *Akt1*-201 transcript, the transcription is shown below:



Source: https://www.ensembl.org

Genomic Information



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

Protein Information

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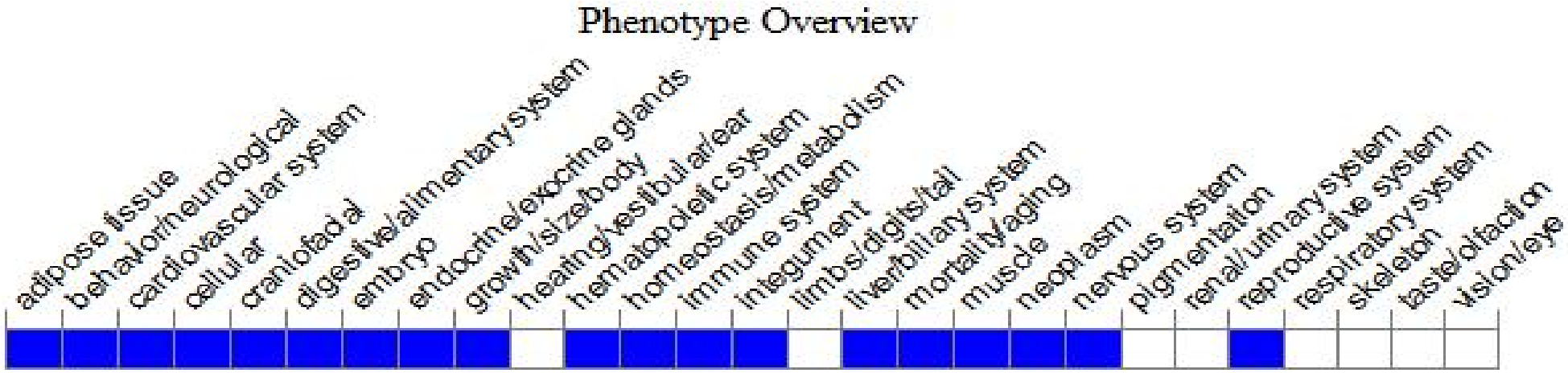
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



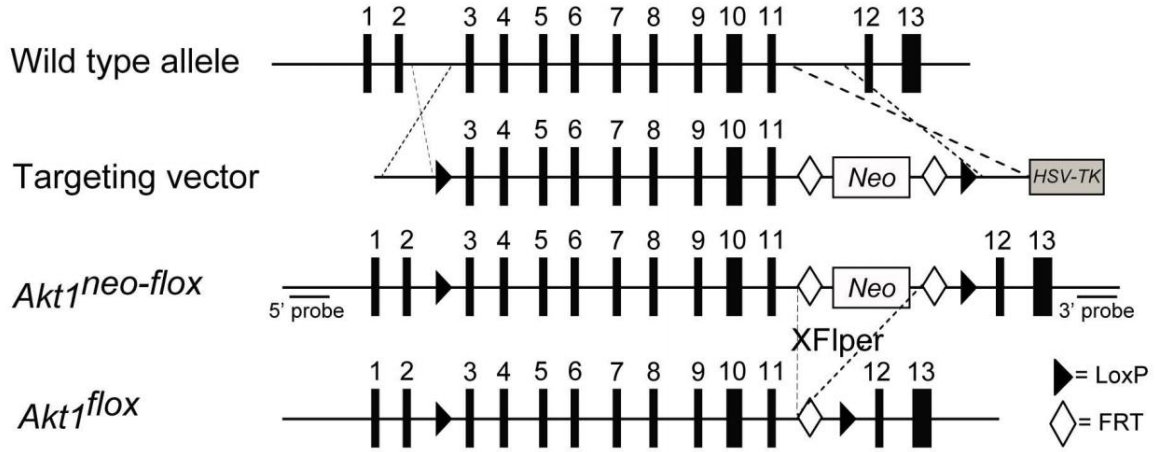
- Mutant homozygotes are smaller than sibs due to retarded prenatal and postnatal growth and exhibit increased apoptosis and decreased lifespan with genotoxic stress. Mice are fertile, but males have attenuated spermatogenesis and abnormal testes.

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

Important Information

- According to MGI information, mutant homozygotes are smaller than sibs due to retarded prenatal and postnatal growth and exhibit increased apoptosis and decreased lifespan with genotoxic stress. Mice are fertile, but males have attenuated spermatogenesis and abnormal testes.
- This strategy intron11-12 is only 187bp, and the insertion of loxp may affect the normal splicing of the target gene.
- *Akt1* is located on Chr12. 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



Since *Akt1*$^{-/-}$ mice are perinatal lethal [1, 2], we generated floxed *Akt1* mice. The gene targeting strategy was shown in Fig. S1A. Two loxP sites were inserted into introns 2 and 11 separately. After obtaining *Akt1*$^{f/+}$, we intercrossed them to get *Akt1*$^{f/f}$. To

Wang L, Cheng S, Yin Z, Xu C, Lu S, Hou J, Yu T, Zhu X, Zou X, Peng Y, Xu Y, Yang Z, Chen G. Conditional inactivation of Akt three isoforms causes tau hyperphosphorylation in the brain. Mol Neurodegener. 2015 Jul 31;10:33. doi: 10.1186/s13024-015-0030-y. PMID: 26227811; PMCID: PMC4521471.