Kalrn Cas9-CKO Strategy

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Reviewer: Xiaoying Sun

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Overview

Target Gene Name
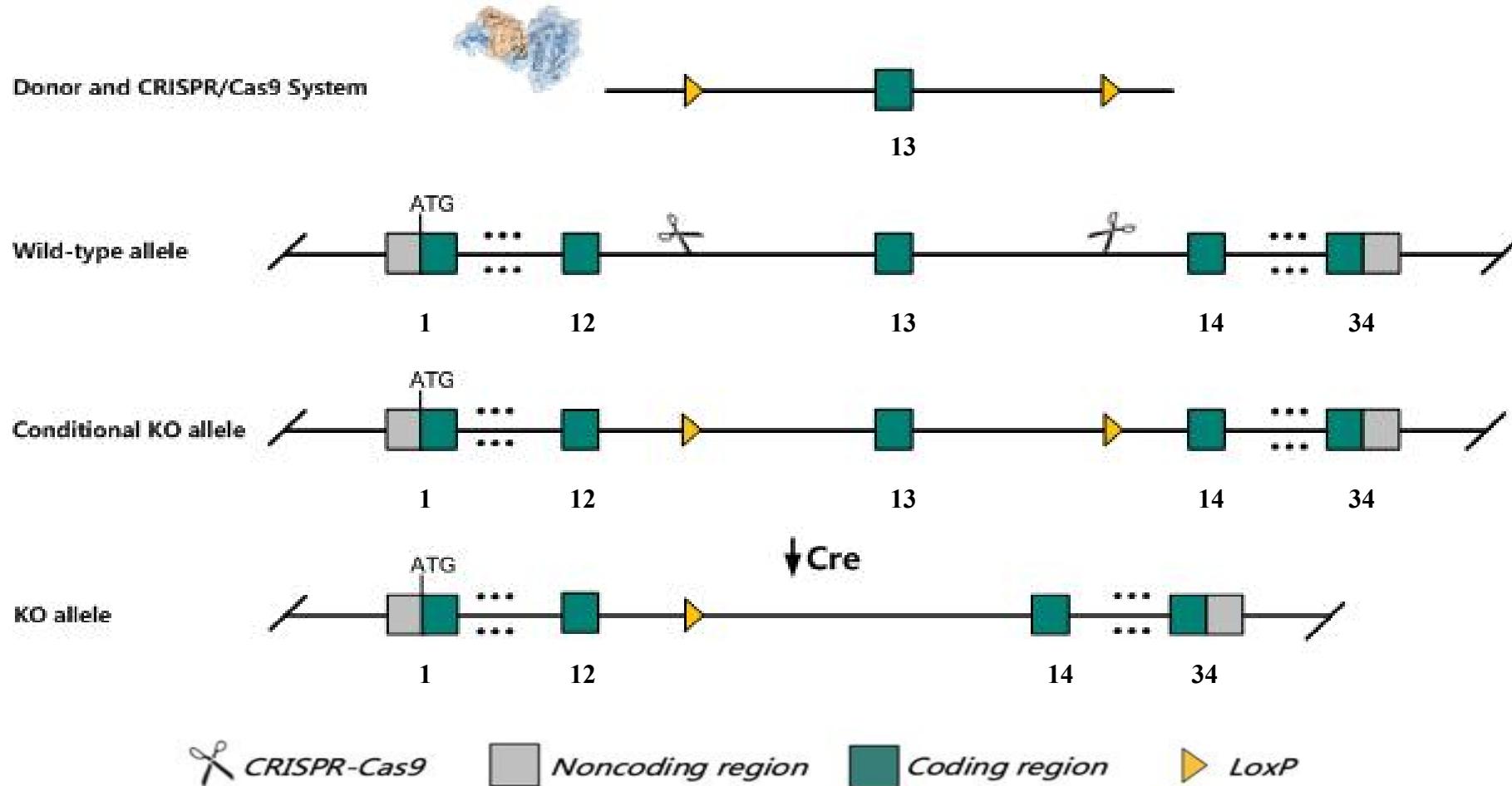
- Kalrn

Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy

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

Technical Information

- The *Kalrn* gene has 21 transcripts. According to the structure of *Kalrn* gene, exon13 of *Kalrn*-207 (ENSMUST00000114960.9) transcript is recommended as the knockout region. The region contains 175bp coding sequence. Knocking out the region will result in disruption of protein function.

- In this project we use CRISPR-Cas9 technology to modify *Kalrn* 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

Kalrn   kalirin, RhoGEF kinase [_Mus musculus_ (house mouse)]

Gene ID: 545156, updated on 5-Mar-2024

⬆ Download Datasets

▲ Summary

Official Symbol	Kalrn provided by MGI
Official Full Name	kalirin, RhoGEF kinase provided by MGI
Primary source	MGI:MGI:2685385
See related	Ensembl:ENSMUSG00000061751 AllianceGenome:MGI:2685385
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	DUET; TRAD; Gm539; Hapip; 2210407G14Rik; E530005C20Rik
Summary	Enables guanyl-nucleotide exchange factor activity. Acts upstream of or within several processes, including behavioral fear response; learning or memory; and modulation of chemical synaptic transmission. Located in postsynaptic density. Is expressed in dorsal root ganglion and spinal cord. Orthologous to human KALRN (kalirin RhoGEF kinase). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Biased expression in cortex adult (RPKM 11.3), frontal lobe adult (RPKM 7.9) and 13 other tissues See more
Orthologs	human all

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

▲ Genomic context

Location: 16 B3; 16 24.27 cM

See Kalrn in Genome Data Viewer

Exon count: 69

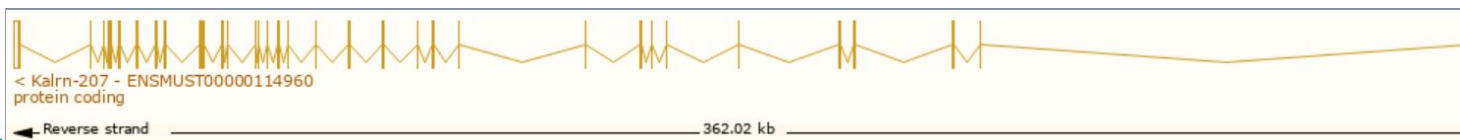| Annotation release	Status	Assembly	Chr	Location
RS_2024_02	current	GRCm39 (GCF_000001635.27)	16	NC_000082.7 (33789443..34393647, complement)
108.20200622	previous assembly	GRCm38.p6 (GCF_000001635.26)	16	NC_000082.6 (33969073..34573277, complement)

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

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Transcript Information

The gene has 21 transcripts,all transcripts are shown below:

Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000076810.12	Kalrn-201	8940	2964aa	Protein coding		A2CG49	Ensembl Canonical GENCODE basic APPRIS P1 TSL:5
ENSMUST00000114973.9	Kalrn-212	10438	1295aa	Protein coding		D3Z4R2	GENCODE basic TSL:5
ENSMUST00000142817.8	Kalrn-217	7636	2371aa	Protein coding		F6QYT9	TSL:5 CDS 5' incomplete
ENSMUST00000089655.12	Kalrn-202	6506	1663aa	Protein coding		A2CG49-8	GENCODE basic TSL:5
ENSMUST00000114953.8	Kalrn-205	4722	1022aa	Protein coding		A2CG49-5	GENCODE basic TSL:1
ENSMUST00000114954.8	Kalrn-206	4677	1022aa	Protein coding		A2CG49-5	GENCODE basic TSL:1
ENSMUST00000151491.8	Kalrn-218	4209	1403aa	Protein coding		B1B1A7	TSL:5 CDS 5' and 3' incomplete
ENSMUST00000114966.8	Kalrn-211	3857	705aa	Protein coding		D3Z532	GENCODE basic TSL:5
ENSMUST00000114949.8	Kalrn-204	3618	1013aa	Protein coding		A2CG49-10	GENCODE basic TSL:5
ENSMUST00000114963.8	Kalrn-209	3207	739aa	Protein coding		D3Z535	GENCODE basic TSL:5
ENSMUST00000114947.2	Kalrn-203	2922	823aa	Protein coding		D3Z560	GENCODE basic TSL:5
ENSMUST00000114964.8	Kalrn-210	2693	674aa	Protein coding		D3Z534	GENCODE basic TSL:5
ENSMUST00000232157.2	Kalrn-221	2690	673aa	Protein coding		A2CG49-4	GENCODE basic
ENSMUST00000114961.8	Kalrn-208	6128	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000231657.2	Kalrn-220	686	No protein	Protein coding CDS not defined		-	-
ENSMUST00000124430.2	Kalrn-213	534	No protein	Protein coding CDS not defined		-	TSL:2
ENSMUST00000132569.2	Kalrn-215	523	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000156668.2	Kalrn-219	373	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000137216.2	Kalrn-216	2668	No protein	Retained intron		-	TSL:1
ENSMUST00000132002.2	Kalrn-214	2118	No protein	Retained intron		-	TSL:1
ENSMUST00000114960.9	Kalrn-207	6452	1654aa	Protein coding	CCDS49836	A2CG49-9	GENCODE basic TSL:1

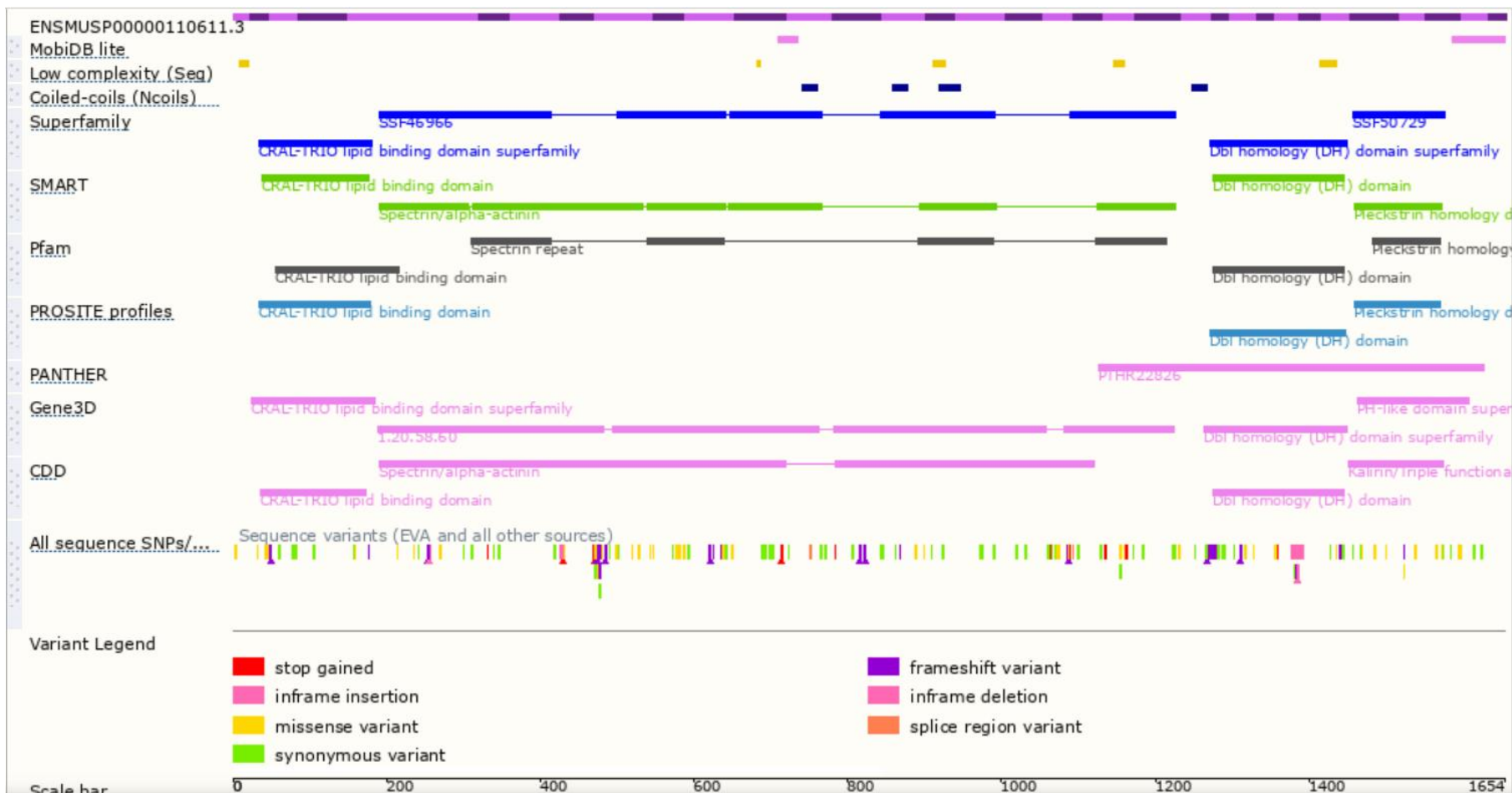The strategy is based on the design of *Kalrn*-207 transcript, the transcription is shown below:
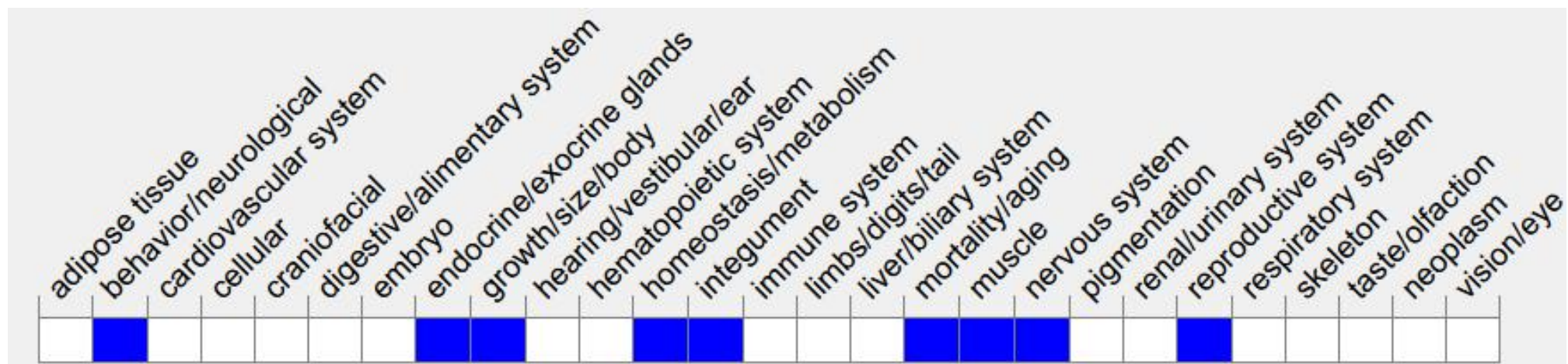

Source: https://www.ensembl.org

Genomic Information

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

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Protein Information
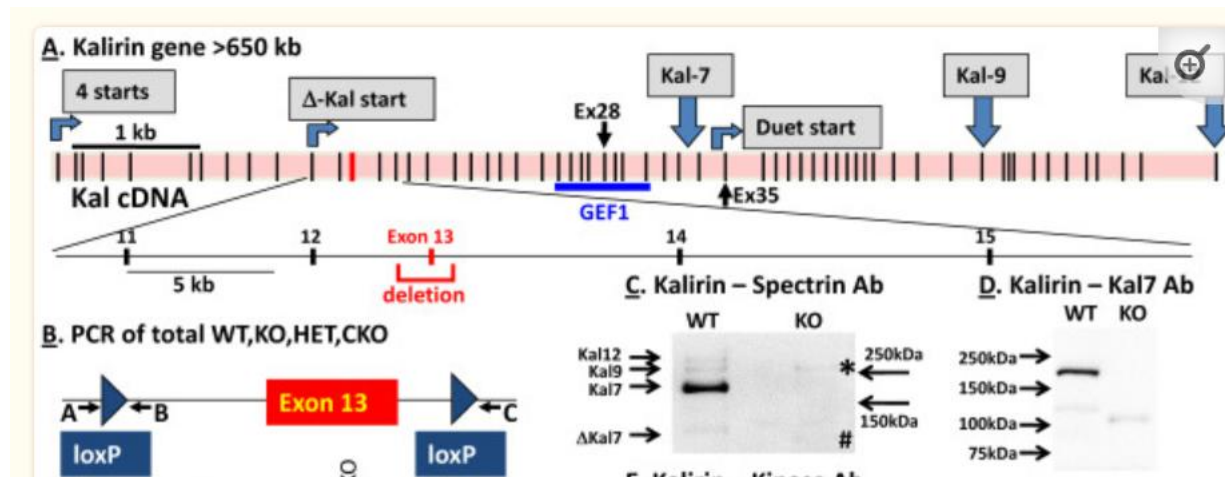

Source: : https://www.ensembl.org
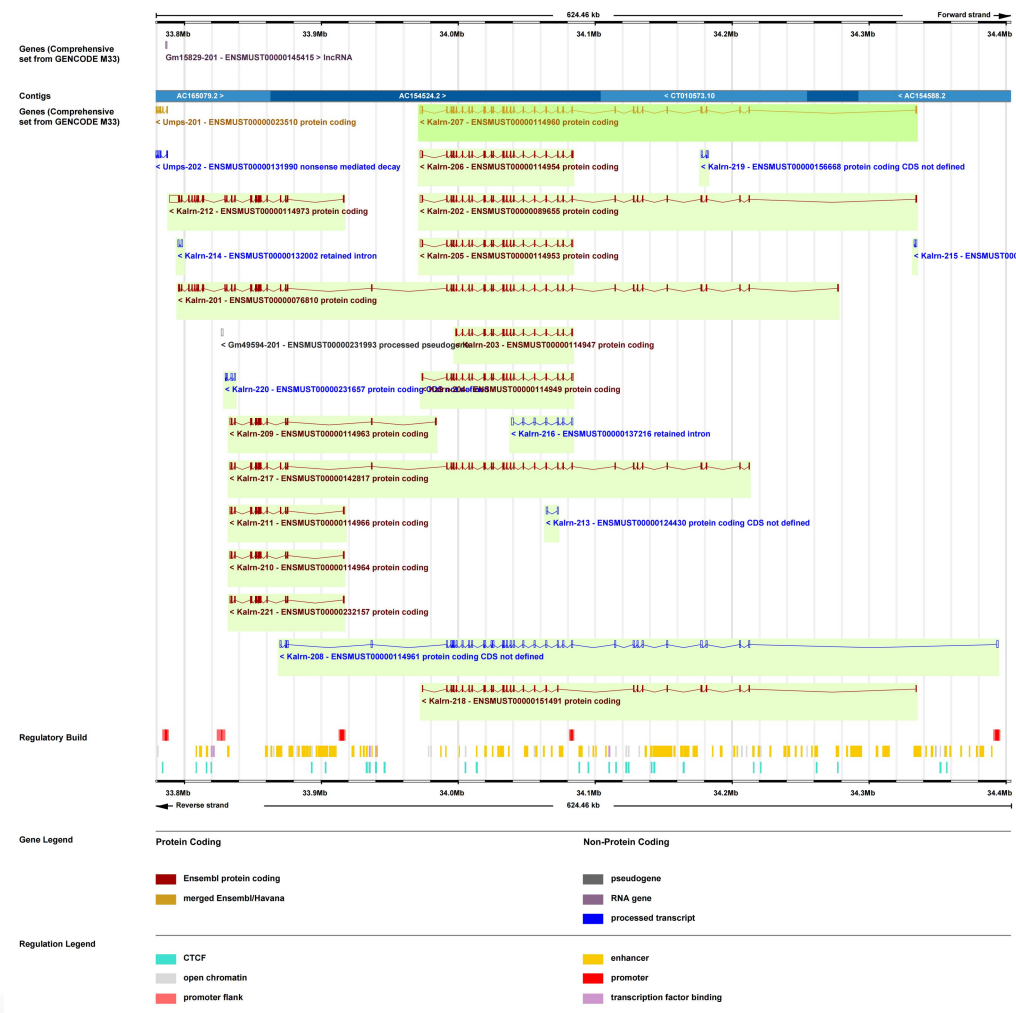
Mouse Phenotype Information (MGI)

- Mice homozygous for a knock-out allele specific for isoform 7 exhibit decreased anxiety-related behavior, contextual conditioning, and synapse formation. Mice homozygous for another knock-out allele exhibit impaired AMPA-mediated synaptic currents and abnormal behavior.

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

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Model Information

A. Kalirin gene >650 kb

B. PCR of total WT,KO,HET,CKO

C. Kalirin – Spectrin Ab

D. Kalirin – Kal7 Ab

Table 1

Primers specific for Exon 13 and Duet

Kalirin domain	Oligo name	Sequence	Tm(°C)	Length (nt)
Exon 13	Ex13-for	CTCAGCGATGTCCAACAACAAGACACC	61	121
	Ex13-rev	GAAGAGCTGTTTCACGAGCGGAAGATC	61	
Duet	Duet-for	CTGAAGTTTCCTACCGCCGCGC	60	122
	Duet-rev	AGCCCAAAGAGGGACCTCGGG	60	

Creation of global and nervous system specific *Kalrn* knockout mice

The basic strategy for ablating exon 13 was the same as for the Kalirin7-specific exon [13]. The Δ isoforms of Kalirin start at exon 11; exon 13 was chosen because exon 12 would have to be spliced to exon 28, in the middle of the GEF domain (Figure 2), to remain in the correct reading frame. Lox-p sites were introduced 1.6 kb upstream (nucleotide 34254054 on chromosome 16, mm9, July 2007) and 0.6 kb downstream of exon 13 (a 175 nt exon) (nucleotide 34251804). The strategy for removing the neomycin resistance cassette using flipper mice, breeding the conditional knockout mice into C57Bl/6 (Jackson Laboratories) and eliminating exon 13 using Hprt-Cre females was as described [13]. Mice with Lox-p sites flanking exon 13 are referred to as Kalirin Spectrin Repeat Conditional Knockout (KalSR^CKO) mice; after Cre-mediated excision of exon 13, mice are referred to as Kalirin Spectrin Repeat Knockout (KalSR^KO) mice. Both strains have been bred more than 10 generations into the C57Bl/6

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Important Information

- This stratergy may not affect *Kalrn* -209, *Kalrn* -210, *Kalrn* - 211, *Kalrn* - 221and *Kalrn* -212 coding transcript.

- A part of amino acid sequence (721 aa) will still remain at the N-terminal of *Kalrn*-207.

- *Kalrn* is located on Chr16. 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.