

***Knl1* Cas9-CKO Strategy**

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Project Overview

Project Name

Kn1

Project type

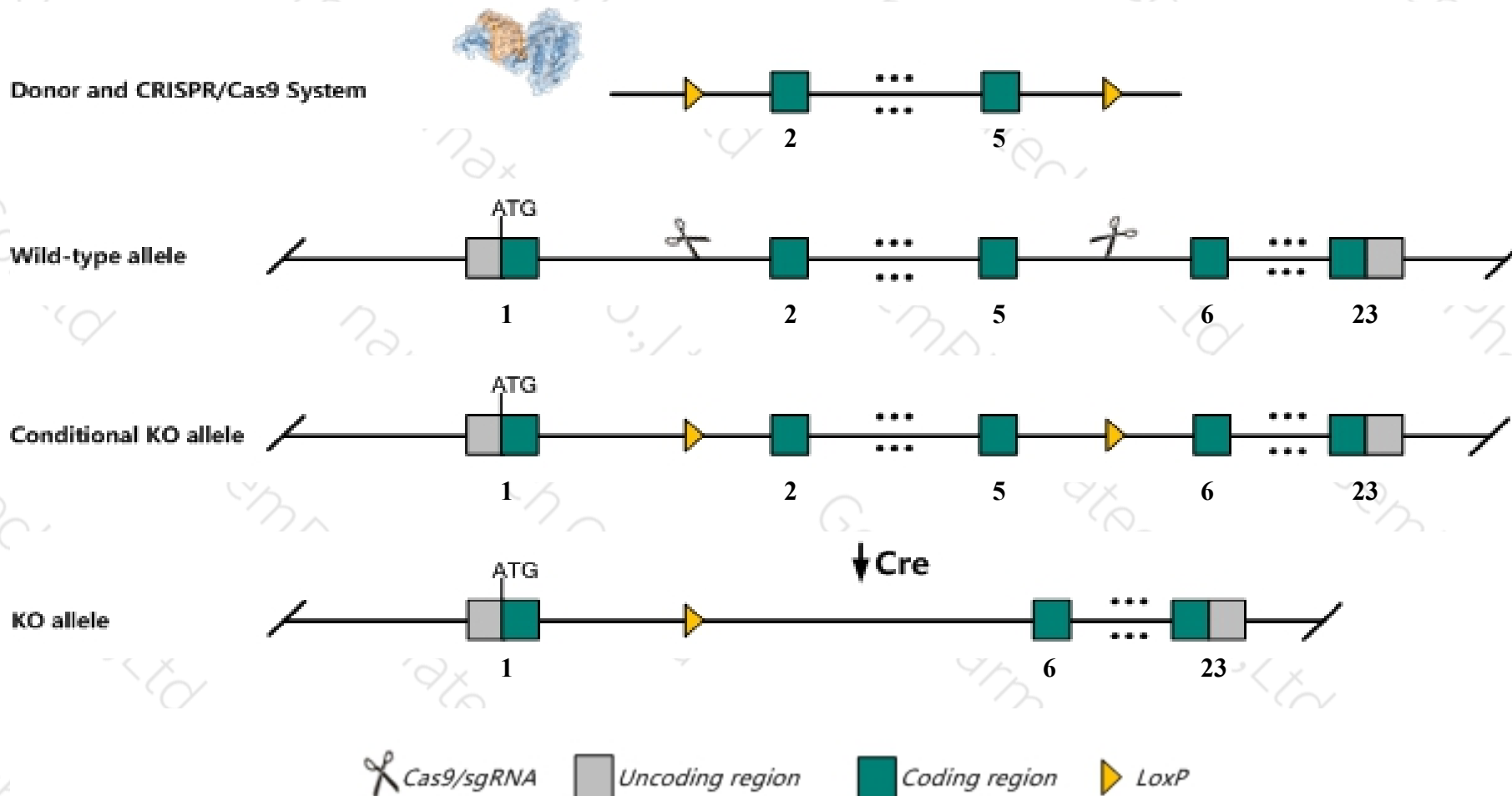
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Kn1l* gene. The schematic diagram is as follows:



- The *Knll* gene has 5 transcripts. According to the structure of *Knll* gene, exon2-exon5 of *Knll*-202(ENSMUST00000028802.2) transcript is recommended as the knockout region. The region contains 215bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Knll* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed. Cas9, sgRNA 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.
- The flox mice was 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.

- According to the existing MGI data, mice homozygous for a transgenic gene disruption exhibit embryonic lethality at E6. Mice homozygous for a conditional allele activated in NPCs exhibit postnatal and premature death and microcephaly associated with NPC apoptosis and premature differentiation.
- The *Kn11* gene is located on the Chr2. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes 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 existing technological level.

Gene information (NCBI)

Kn11 kinetochore scaffold 1 [Mus musculus (house mouse)]

Gene ID: 76464, updated on 13-Mar-2020

Summary



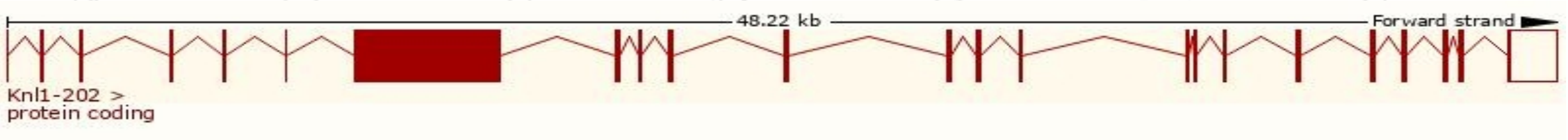
Official Symbol	Kn11 provided by MGI
Official Full Name	kinetochore scaffold 1 provided by MGI
Primary source	MGI:MGI:1923714
See related	Ensembl:ENSMUSG00000027326
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2310043D08Rik, 5730505K17Rik, Casc5
Expression	Biased expression in liver E14 (RPKM 4.5), CNS E11.5 (RPKM 3.4) and 10 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

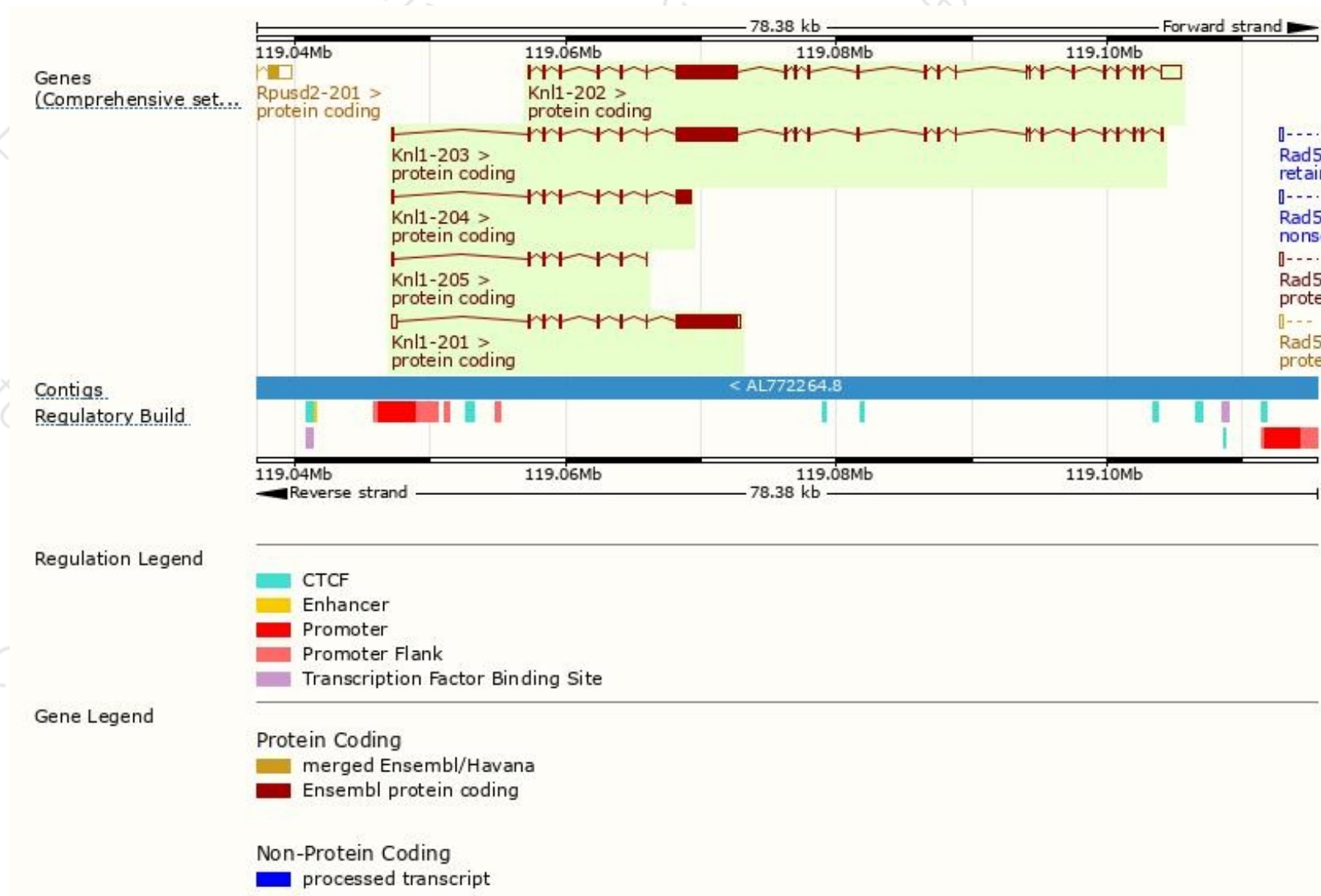
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kn11-202	ENSMUST00000028802.2	7800	2119aa	Protein coding	CCDS38203	A3KGI3	TSL:5 GENCODE basic APPRIS P1
Kn11-203	ENSMUST00000099542.8	6525	2119aa	Protein coding	CCDS38203	A3KGI3	TSL:5 GENCODE basic APPRIS P1
Kn11-201	ENSMUST00000028799.11	5527	1612aa	Protein coding	-	Q66JQ7	TSL:1 GENCODE basic
Kn11-204	ENSMUST00000152380.7	1573	484aa	Protein coding	-	A3KGI5	CDS 3' incomplete TSL:1
Kn11-205	ENSMUST00000153300.7	370	91aa	Protein coding	-	A3KGI6	CDS 3' incomplete TSL:5

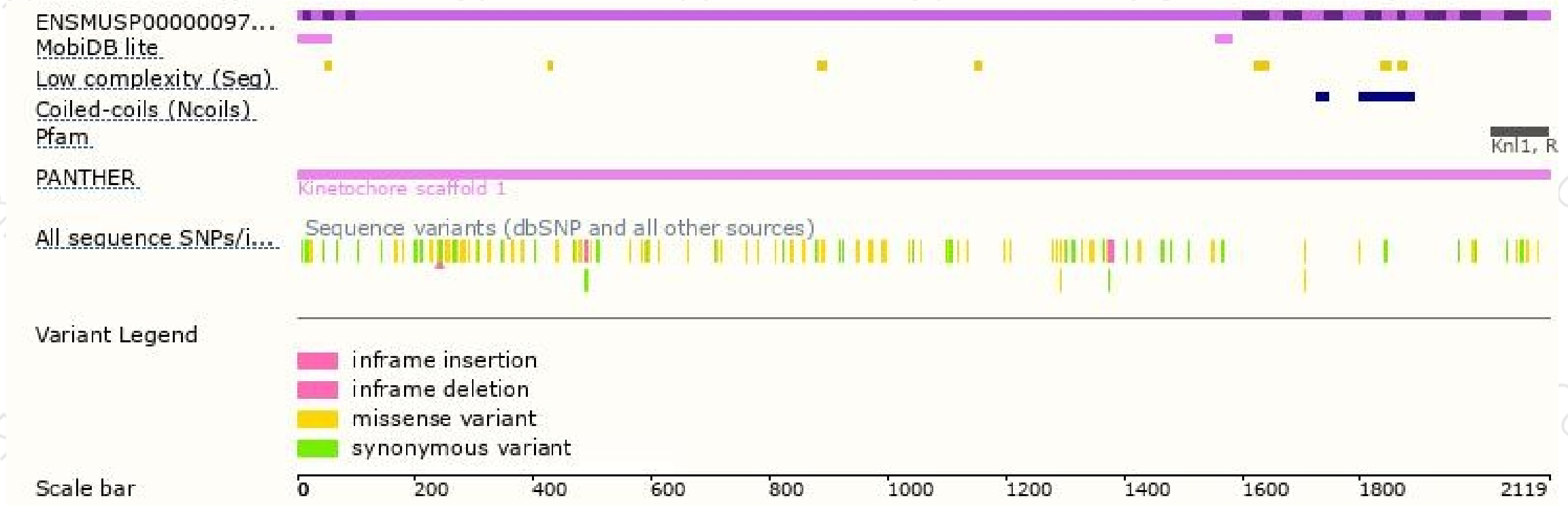
The strategy is based on the design of *Kn11-202* transcript,the transcription is shown below:



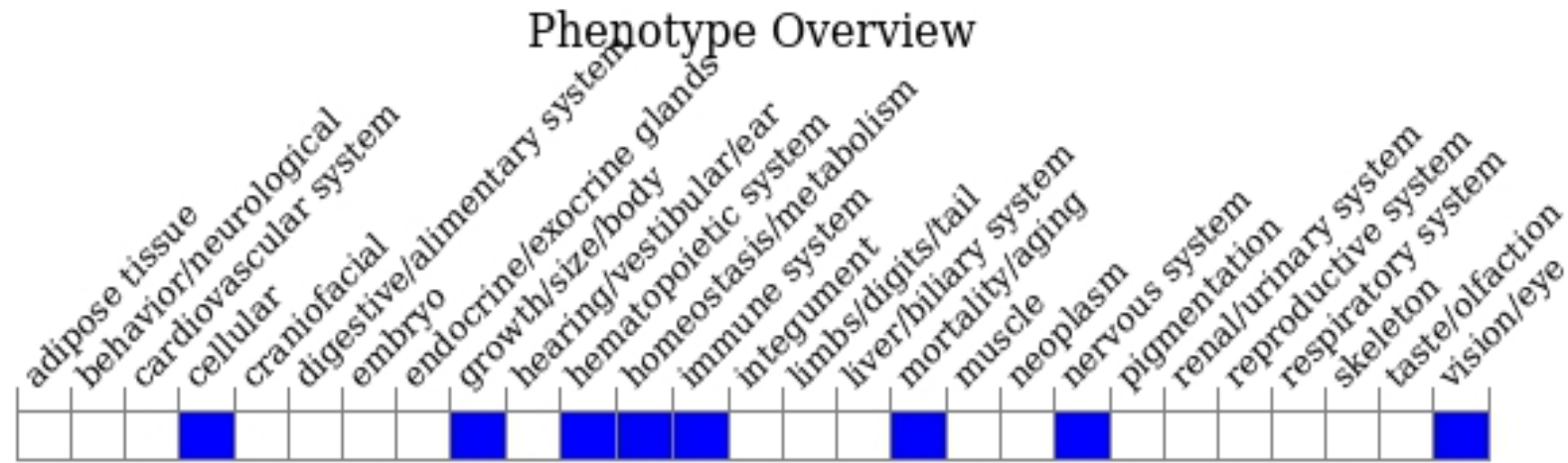
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).

According to the existing MGI data, mice homozygous for a transgenic gene disruption exhibit embryonic lethality at E6. Mice homozygous for a conditional allele activated in NPCs exhibit postnatal and premature death and microcephaly associated with NPC apoptosis and premature differentiation.

If you have any questions, you are welcome to inquire.

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