Pla2g4f Cas9-KO Strategy

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Reviewer: Daohua Xu

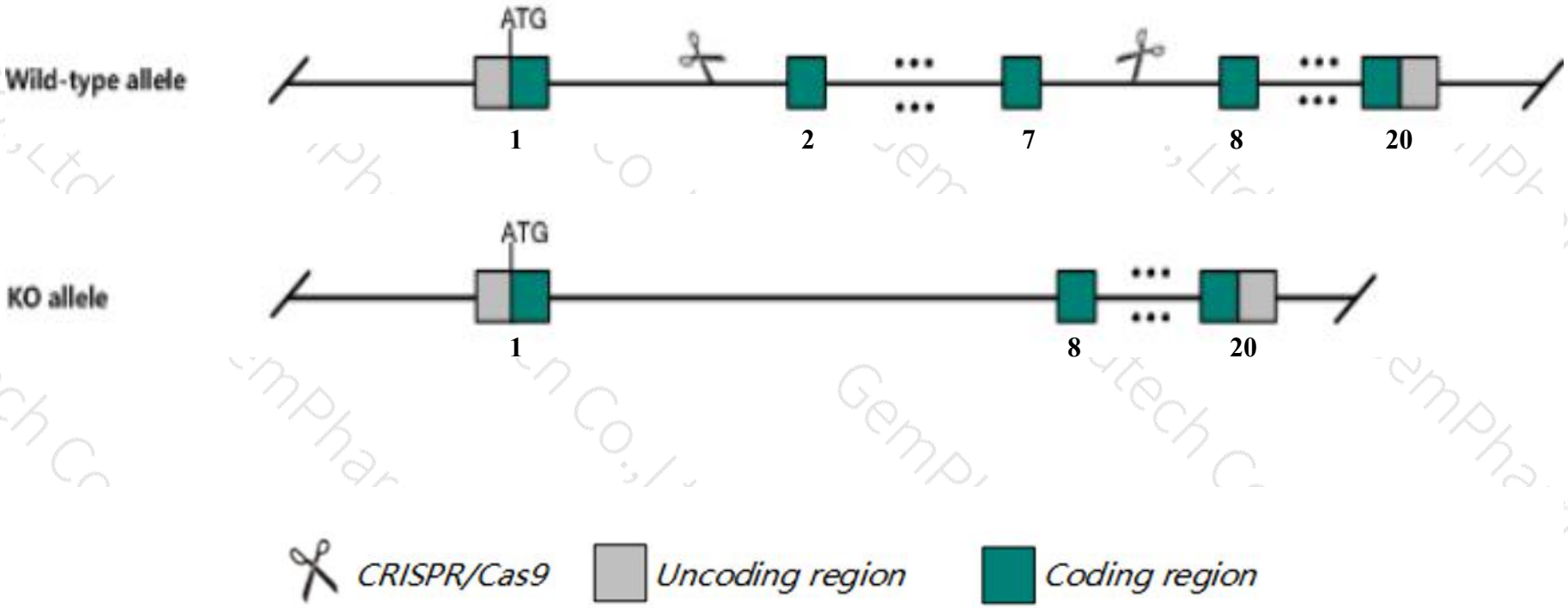
Design Date: 2020-9-29

Project Overview

Project Name	***Pla2g4f***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pla2g4f* gene. The schematic diagram is as follows:



Technical routes

➢ The *Pla2g4f* gene has 2 transcripts. According to the structure of *Pla2g4f* gene, exon2-exon7 of *Pla2g4f-201*(ENSMUST00000054651.7) transcript is recommended as the knockout region.The region contains 487bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Pla2g4f* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice

➢ According to the existing MGI data,lung fibroblasts from homozygous mutant mice show a normal response to the calcium ionophore A23187.

➢ The *Pla2g4f* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Pla2g4f phospholipase A2, group IVF [Mus musculus (house mouse)]

Gene ID: 271844, updated on 20-Mar-2020

Summary

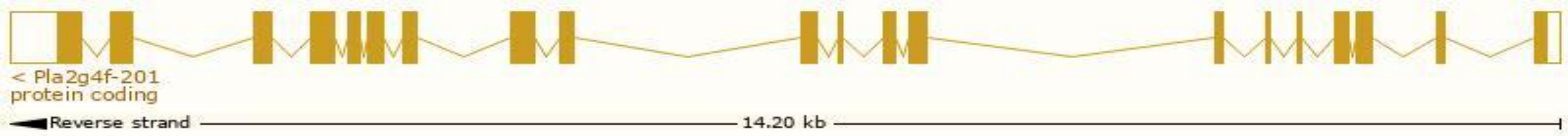
Official Symbol	Pla2g4f provided by MGI
Official Full Name	phospholipase A2, group IVF provided byMGI
Primary source	MGI:MGI:2685493
See related	Ensembl:ENSMUSG00000046971
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	4732472I07Rik, Gm647, Pla2zeta
Expression	Biased expression in stomach adult (RPKM 8.2), placenta adult (RPKM 7.1) and 6 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

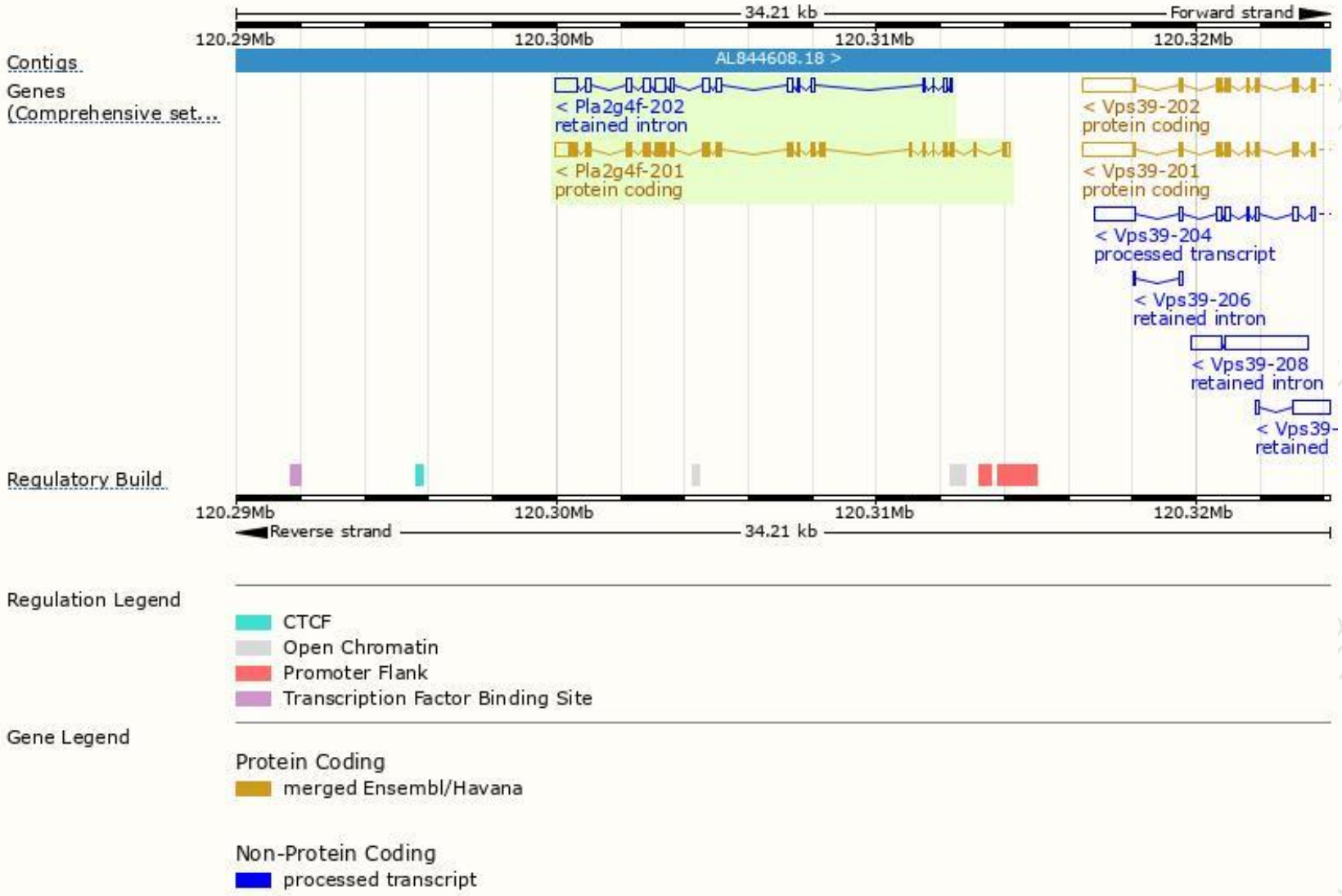
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pla2g4f-201	ENSMUST00000054651.7	3116	855aa	Protein coding	CCDS16618	Q50L41	TSL:1 GENCODE basic APPRIS P1
Pla2g4f-202	ENSMUST00000142183.1	2610	No protein	Retained intron	-	-	TSL:2

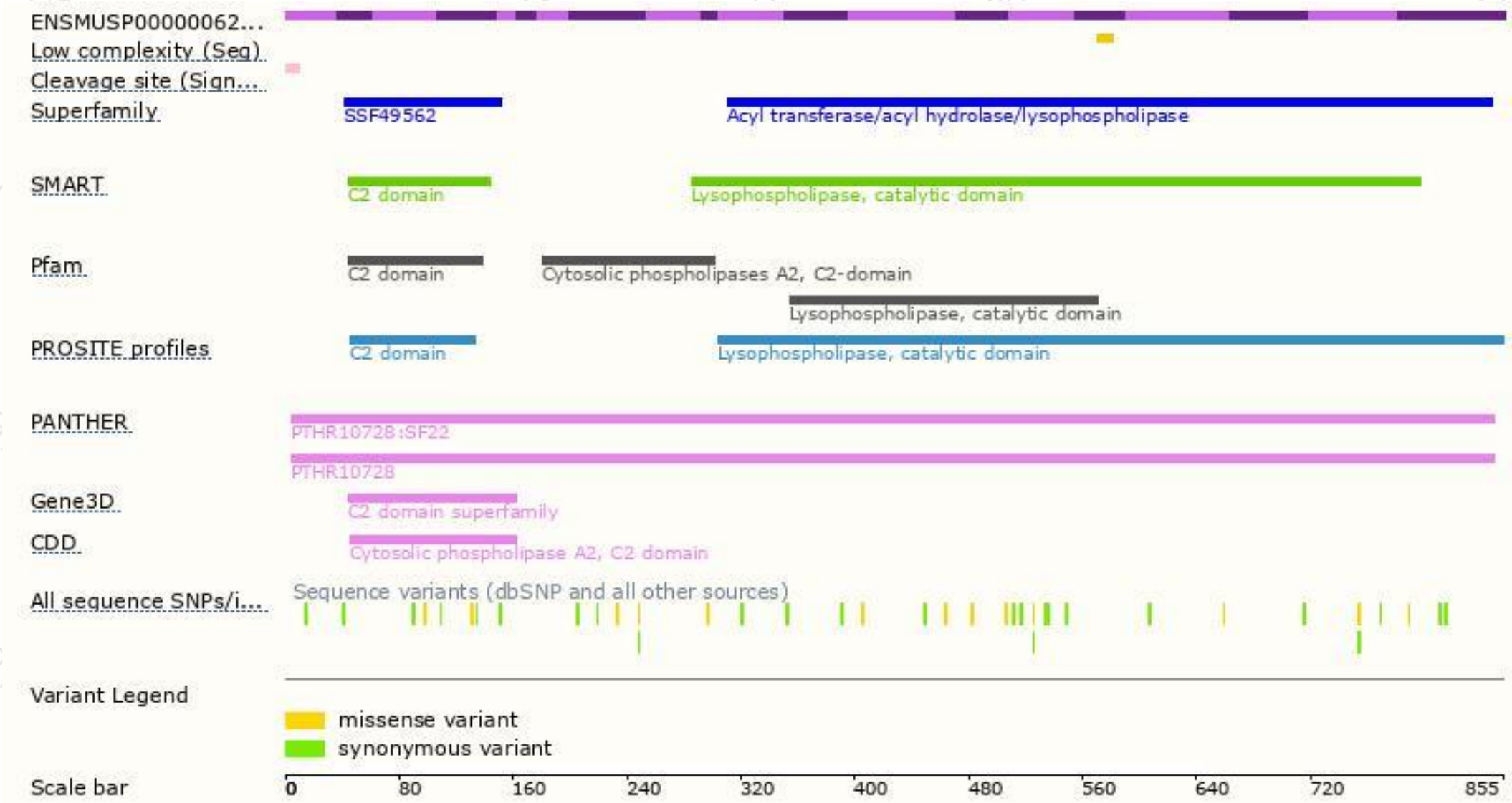
The strategy is based on the design of *Pla2g4f-201* transcript,the transcription is shown below:



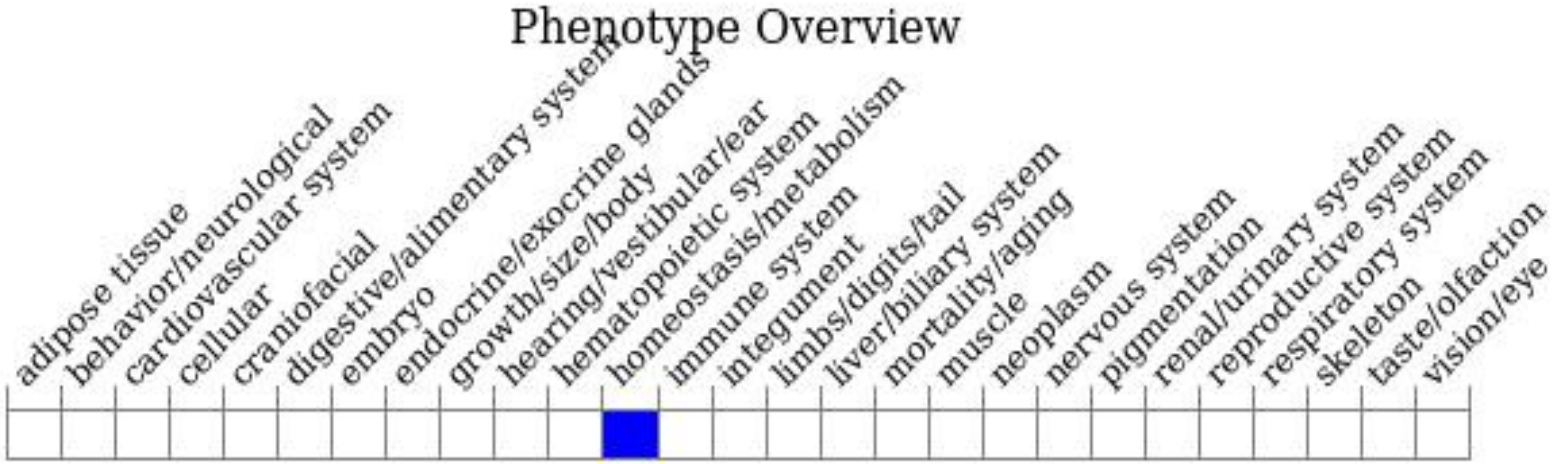
Genomic location distribution



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,lung fibroblasts from homozygous mutant mice show a normal response to the calcium ionophore A23187.

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

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