

A white laboratory mouse is being held gently in a green nitrile glove. The mouse is looking directly at the camera and has its paws near its mouth. The background is a soft-focus blue with a faint DNA double helix structure on the left side.

Models to
Accelerate Innovation



***Sftpc-IRES-CreERT2* Cas9-KI**

Mouse Model Strategy

-CRISPR-Cas9 Technology

Designer

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Reviewer

Qin Xia

Date

2023-11-02

Project Overview



Project Name

Sftpc-IRES-CreERT2

Project Type

Cas9-KI

Background

C57BL/6JGpt

Timeline

6-8 Months

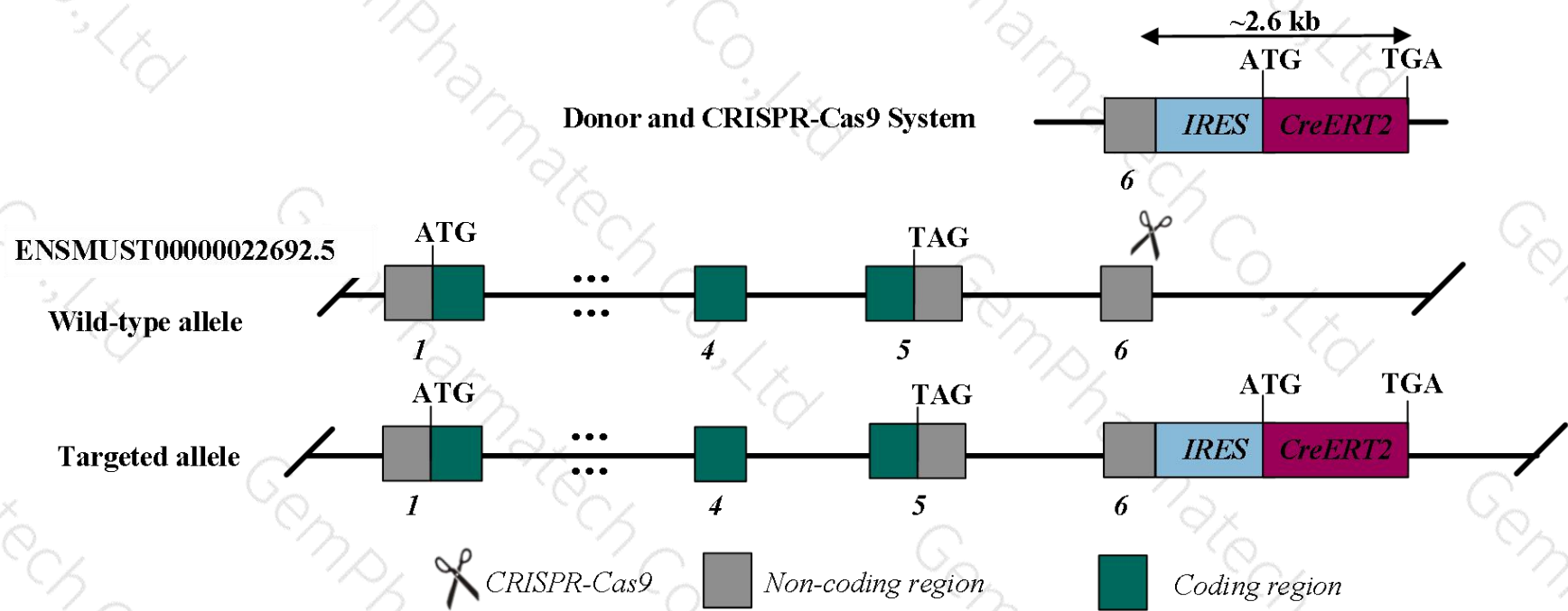
Deliverable

3~5 F1 Heterozygous Mice



Strategy

The schematic diagram is as follows:





Technical Description

- The *Sftpc* gene has 2 transcripts. According to the structure of *Sftpc* gene, *Sftpc-201*(ENSMUST00000022692.5) is selected for presentation of the recommended strategy.
- *Sftpc-201* gene has 6 exons, with the ATG start codon in exon1 and TAG stop codon in exon5.
- We make *Sftpc-IRES-CreERT2* knockin mice via CRISPR-Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage in exon6 of *Sftpc* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in IRES-CreERT2 after exon6 of *Sftpc* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.



Note

- According to the existing MGI data, mice homozygous for disruptions in this gene display respiratory abnormalities similar to emphysema.
- The IRES-linked *Sftpc* gene and the CreERT2 gene are expressed by the same promoter driver. The transcription levels are consistent but the translation is independent. Often the latter gene translates at a lower level than the former.
- Insertion of CreERT2 may affect the regulation of the 3' end of the *Sftpc* gene.
- There will be 2 to 4 base mutations in exon6 of *Sftpc* gene in this strategy.
- The distance between the site of insertion and *Lgi3/Bmp1* gene is about 9.9kb/0.8kb, insertion of IRES-CreERT2 may influence the 5' regulation of these two genes.
- The *Sftpc* gene is located on the Chr14. If the knockin 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 gene transcription and translation processes, all risks cannot be predicted under existing information.



Target Gene

Gene name	<i>Sftpc</i>
Gene ID (NCBI)	20389
Gene link (NCBI)	https://www.ncbi.nlm.nih.gov/gene/20389
Gene link (Ensembl)	http://asia.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG000000022097;r=14:70758389-70761521
Chromosome location	Chr 14

Gene Information (NCBI)



Sftpc surfactant associated protein C [*Mus musculus* (house mouse)]

Gene ID: 20389, updated on 31-Oct-2023

[Download Datasets](#)

Summary

Official Symbol

Sftpc provided by MGI

Official Full Name

surfactant associated protein C provided by MGI

Primary source

[MGI:MGI:109517](#)

See related

[Ensembl:ENSMUSG00000022097](#); [AllianceGenome:MGI:109517](#)

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

SP5; SPC; SP-C; Sftp2; Bricd6; Sftp-2; pro-SpC

Summary

Predicted to enable identical protein binding activity. Located in cytoplasm and extracellular region. Is expressed in several structures, including lung and main bronchus epithelium. Used to study desquamative interstitial pneumonia. Human ortholog(s) of this gene implicated in asthma; lung disease (multiple); and respiratory syncytial virus infectious disease. Orthologous to human SFTPC (surfactant protein C). [provided by Alliance of Genome Resources, Apr 2022]

Expression

Restricted expression toward lung adult (RPKM 8709.5) [See more](#)

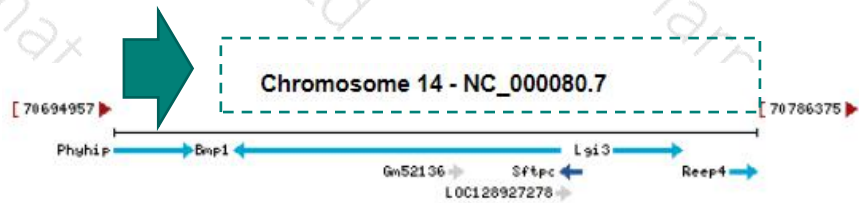
Orthologs

[human](#) [all](#)

NEW

Try the new [Gene table](#)

Try the new [Transcript table](#)



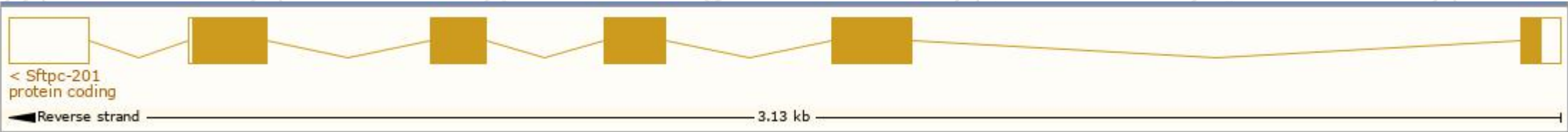
Transcript Information (Ensembl)



The gene has 2 transcripts, as shown below:

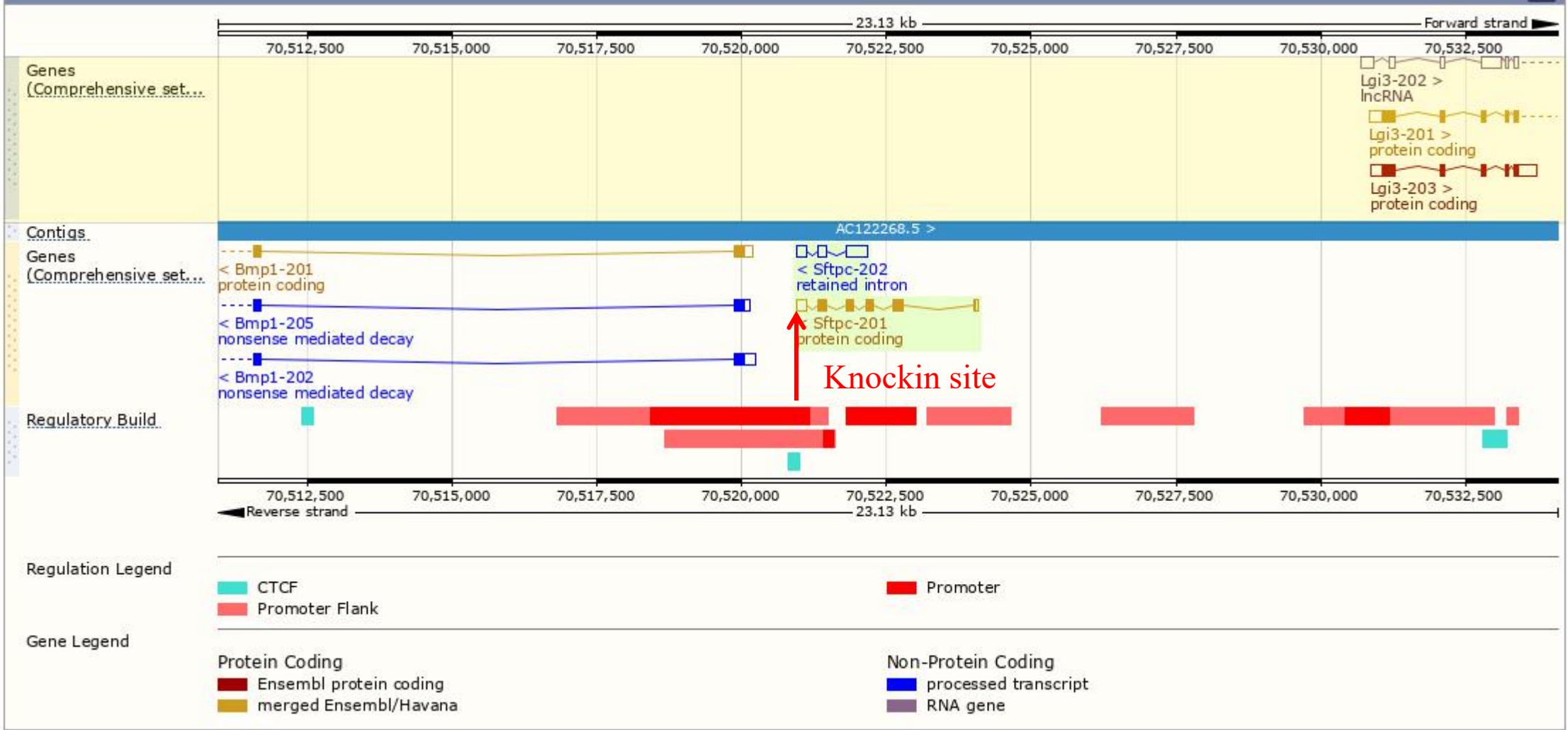
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000022692.5	Sftpc-201	792	193aa	Protein coding	CCDS27254	Q6P8P8	Ensembl Canonical Gencode basic APPRIS P1 TSL:1
ENSMUST000000227152.2	Sftpc-202	695	No protein	Retained intron		-	-

The strategy is based on *Sftpc*-201 transcript, which contains 6 exons, is 792 bps long, and encodes 193 amino acids.



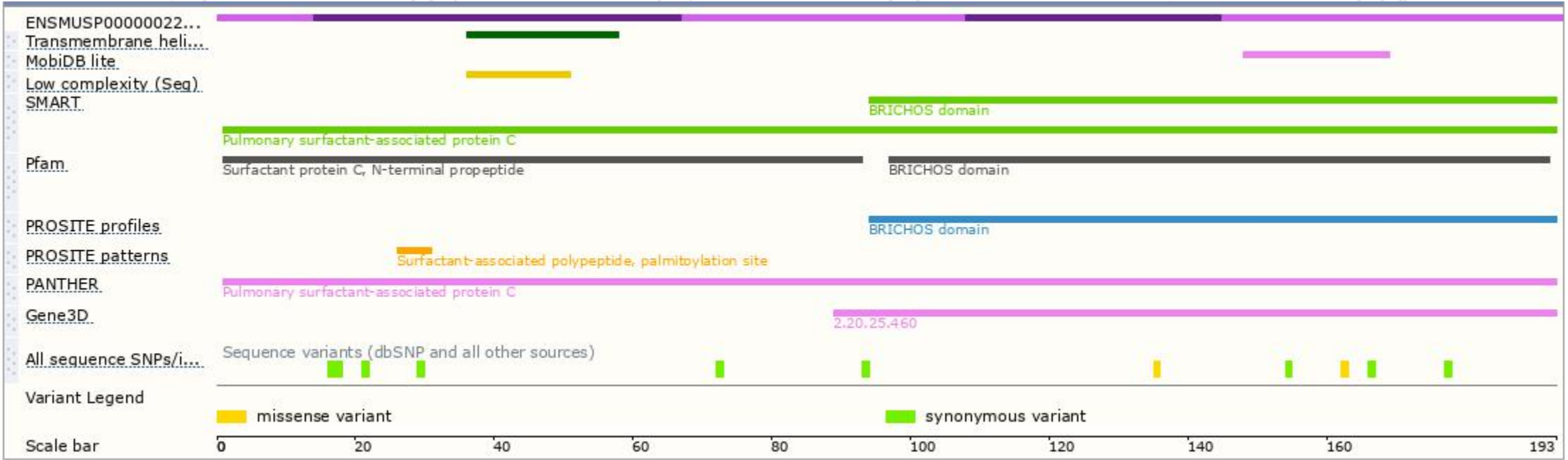


Genomic Information





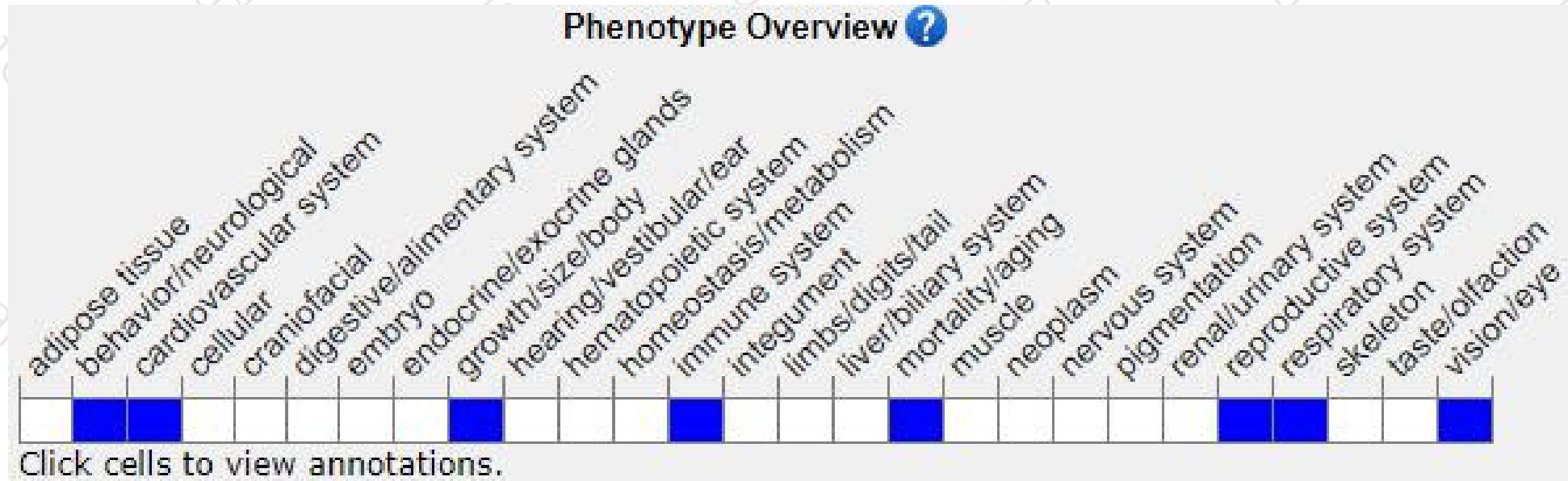
Protein Information





Mouse Phenotype Description (MGI)

<https://www.informatics.jax.org/marker/MGI:109517>



Mice homozygous for disruptions in this gene display respiratory abnormalities similar to emphysema.

Targeted Progress (from Jax)

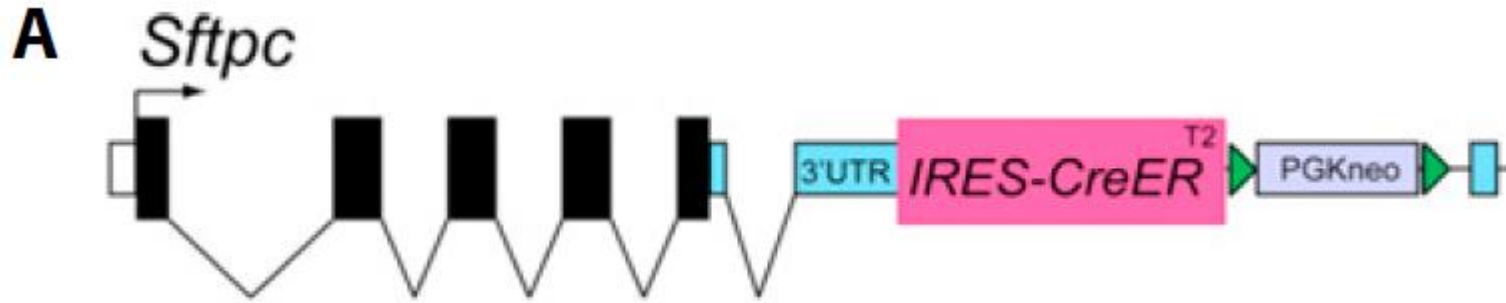


Fig. S4. (A) Schematic of *Sftpc*-CreER construct. The coding sequence and 3' UTR of *Sftpc* were retrieved from a BAC by recombineering into a vector upstream of a DT cassette for negative selection in ES cells. An *IRES-CreERT2* cassette and a PGKneo cassette flanked with FRT sites were recombined into the 3' UTR. (B

Materials and Methods

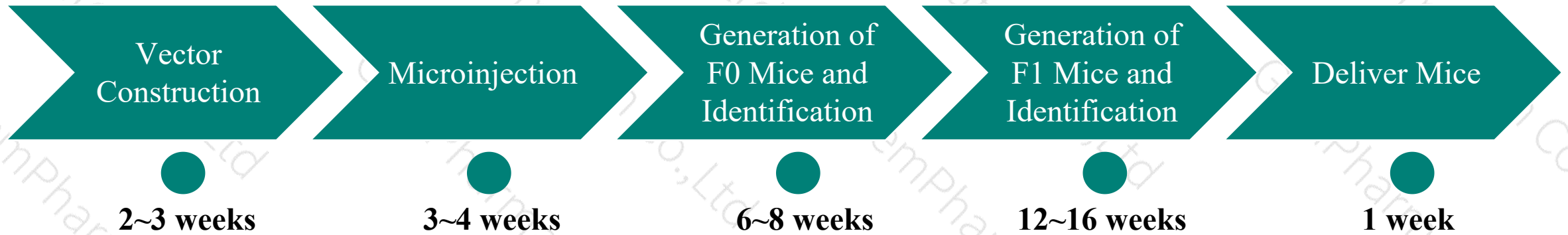
Mice. The *CC10-CreER* and *Rosa26R-CAG-farnesylated GFP* (*Rosa26R-fGFP*) mouse lines have been described previously (7). To generate *Sftpc^{tm1}(cre/ERT)Blh* (*Sftpc*-CreER) mice, the coding sequence and 3' UTR of *Sftpc* were retrieved from a BAC by recombineering into a vector upstream of a diphtheria toxin (DT) cassette for negative selection in ES cells. An *IRES-CreERT2* cassette and a PGKneo cassette flanked with FRT sites were recombined into the 3' UTR (Fig. S4). The construct was linearized and electroporated into 129S6/SvEvTac ES cells. Ten correctly targeted clones were identified by Southern blot and PCR, and ES cells from three clones were injected into C57BL/6 blastocysts. Mice heterozygous for *Sftpc*-CreER

<https://www.jax.org/strain/028054>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3323959/>



Work Flow





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