

# ***Lama3 Cas9-CKO Strategy***

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**Reviewer:**

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# Project Overview

**Project Name**

***Lama3***

**Project type**

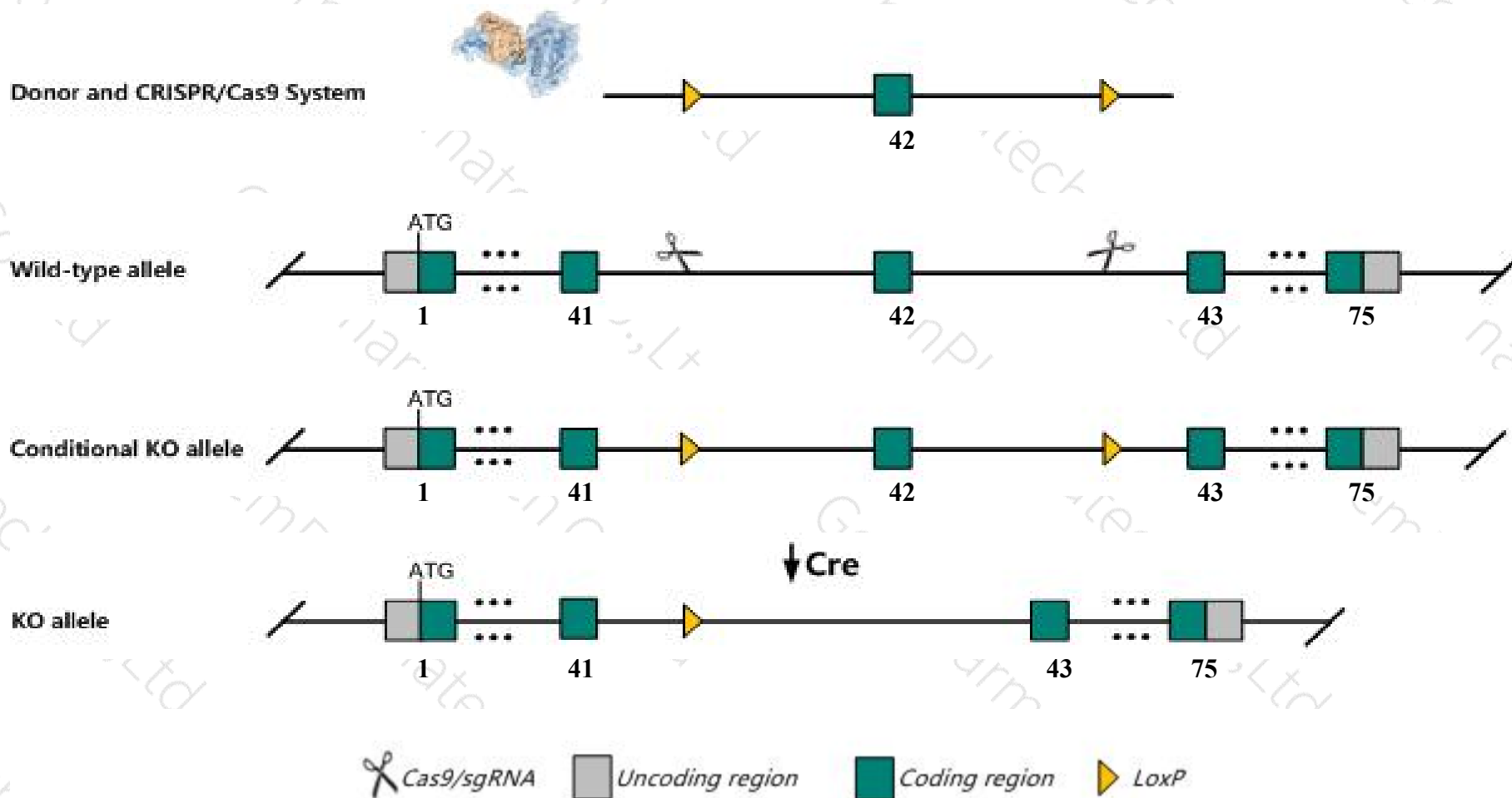
**Cas9-CKO**

**Strain background**

**C57BL/6J**

# Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lama3* gene. The schematic diagram is as follows:



# Technical routes

- The *Lama3* gene has 2 transcripts. According to the structure of *Lama3* gene, exon42 of *Lama3-201* (ENSMUST00000092070.12) transcript is recommended as the knockout region. The region contains 107bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Lama3* 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/6J 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/6J 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 targeted null mutation develop a lethal blistering phenotype similar to human junctional epidermolysis bullosa, and die 2-3 days after birth from a failure to thrive.
- According to references (Urich D, et al., Lung-specific loss of the laminin alpha3 subunit confers resistance to mechanical injury. J Cell Sci. 2011 Sep 1;124(Pt 17):2927-37), Exon 42 of *Lama3* gene is selected as the flox region in the strategy.
- The *Lama3* gene is located on the Chr18. 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)

## Lama3 laminin, alpha 3 [ *Mus musculus* (house mouse) ]

Gene ID: 16774, updated on 12-Aug-2019

### Summary

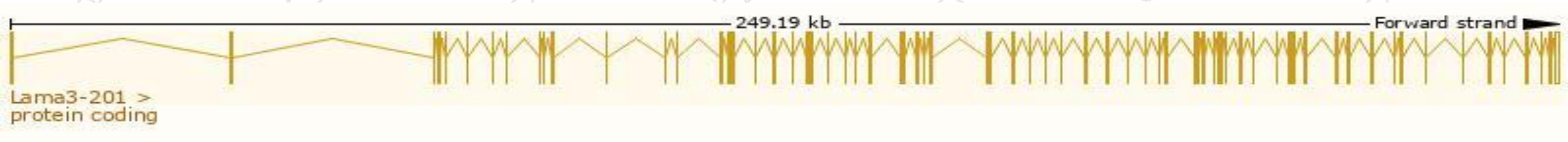
Official Symbol	Lama3 provided by <a href="#">MGI</a>
Official Full Name	laminin, alpha 3 provided by <a href="#">MGI</a>
Primary source	<a href="#">MGI:MGI:99909</a>
See related	<a href="#">Ensembl:ENSMUSG00000024421</a>
Gene type	protein coding
RefSeq status	VALIDATED
Organism	<a href="#">Mus musculus</a>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	[a]3B; Lama3B
Expression	Broad expression in lung adult (RPKM 9.8), colon adult (RPKM 3.3) and 15 other tissues <a href="#">See more</a>
Orthologs	<a href="#">human</a> <a href="#">all</a>

# Transcript information (Ensembl)

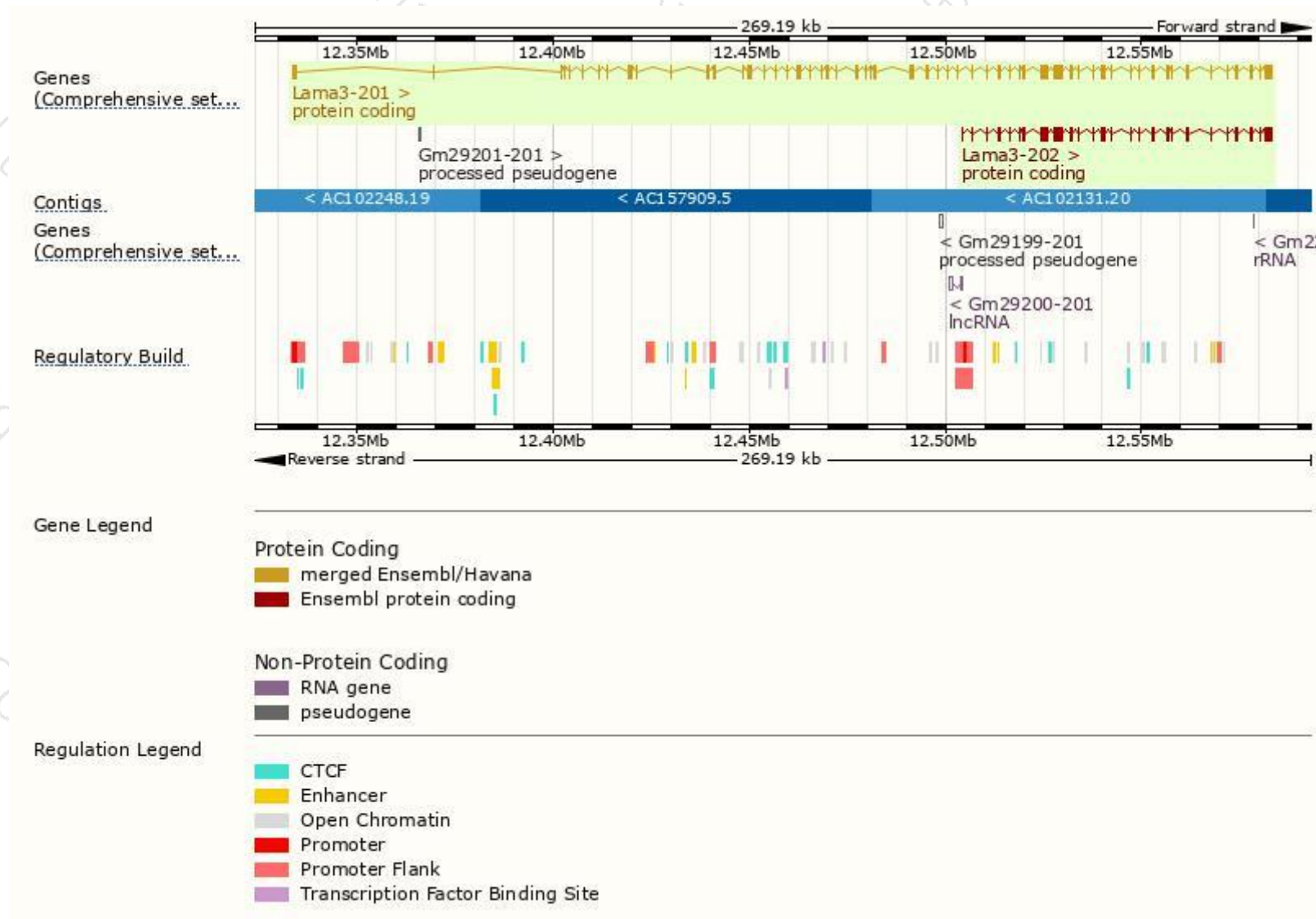
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lama3-201	<a href="#">ENSMUST00000092070.12</a>	10548	<a href="#">3330aa</a>	Protein coding	<a href="#">CCDS50222</a>	<a href="#">Q61789</a>	TSL:5 GENCODE basic APPRIS P1
Lama3-202	<a href="#">ENSMUST00000188815.1</a>	5551	<a href="#">1724aa</a>	Protein coding	<a href="#">CCDS84360</a>	<a href="#">Q61789</a>	TSL:1 GENCODE basic

The strategy is based on the design of *Lama3-201* transcript,The transcription is shown below

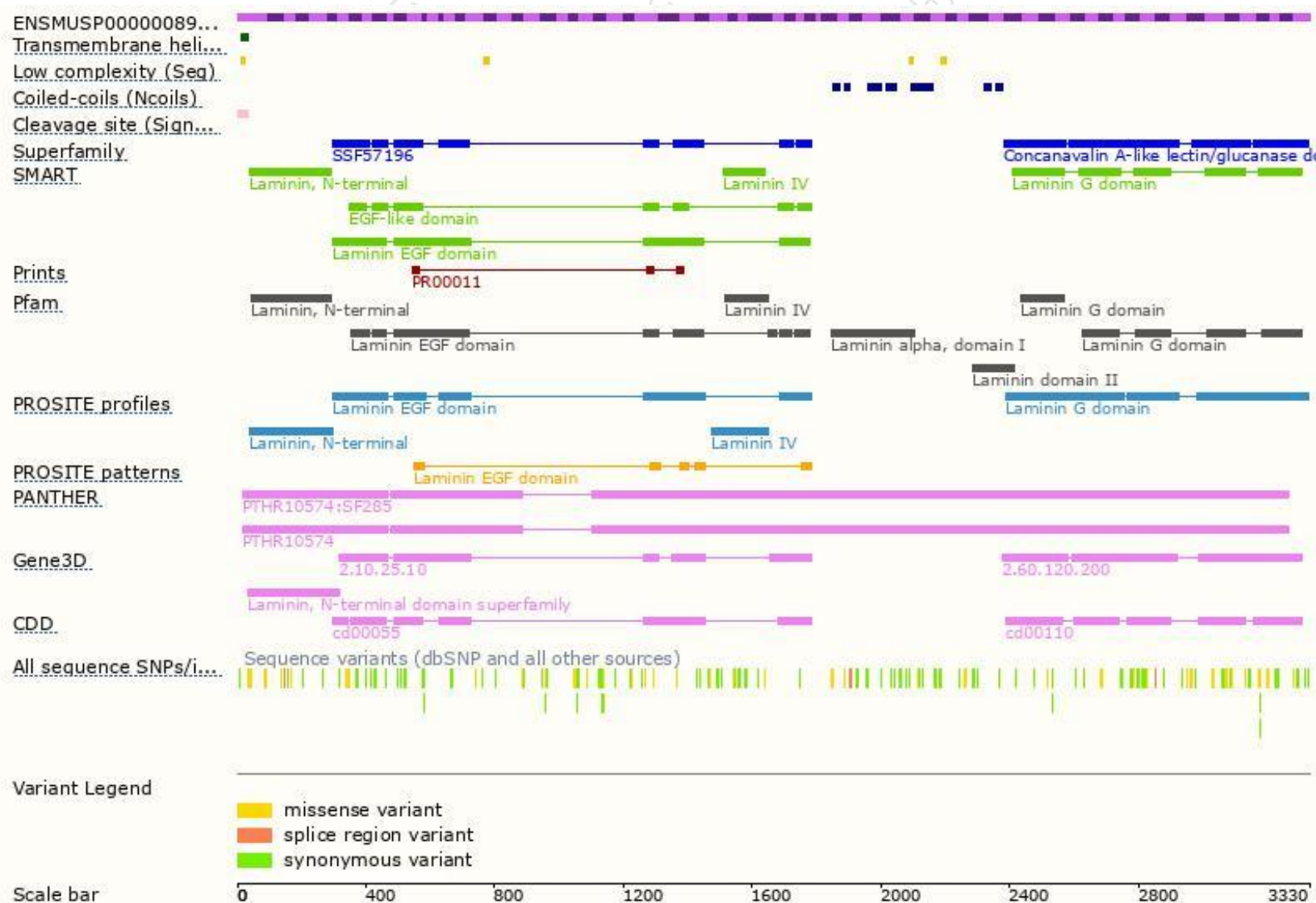


# Genomic location distribution

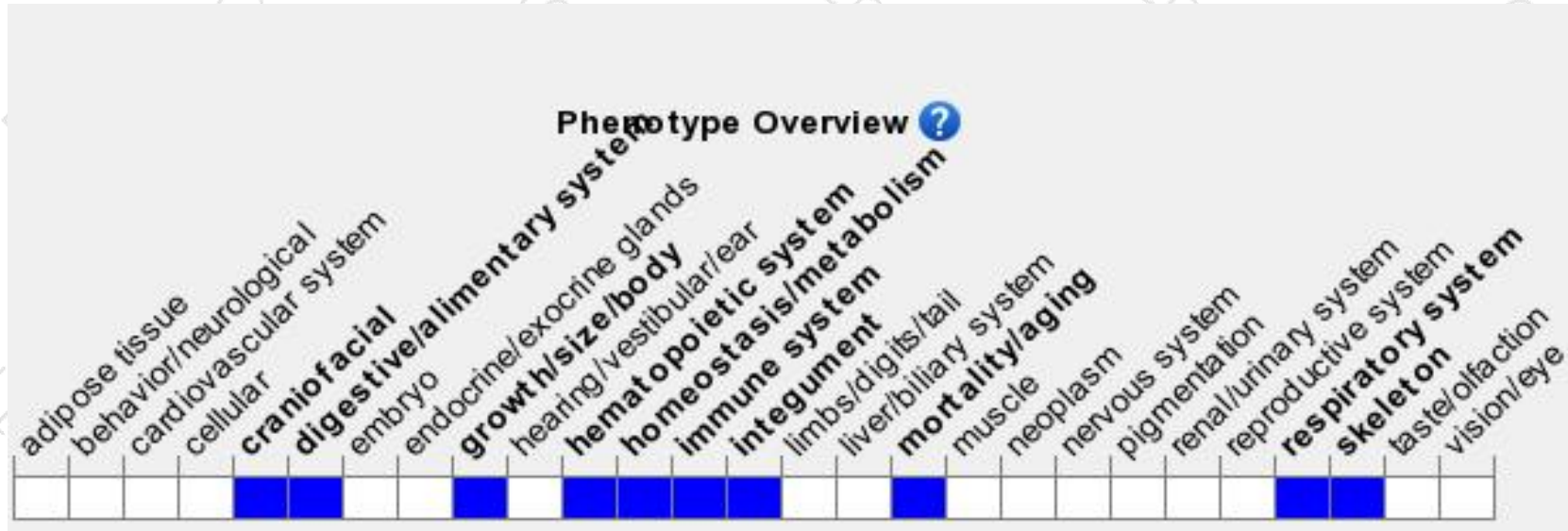




# Protein domain



# Mouse phenotype description(MGI)

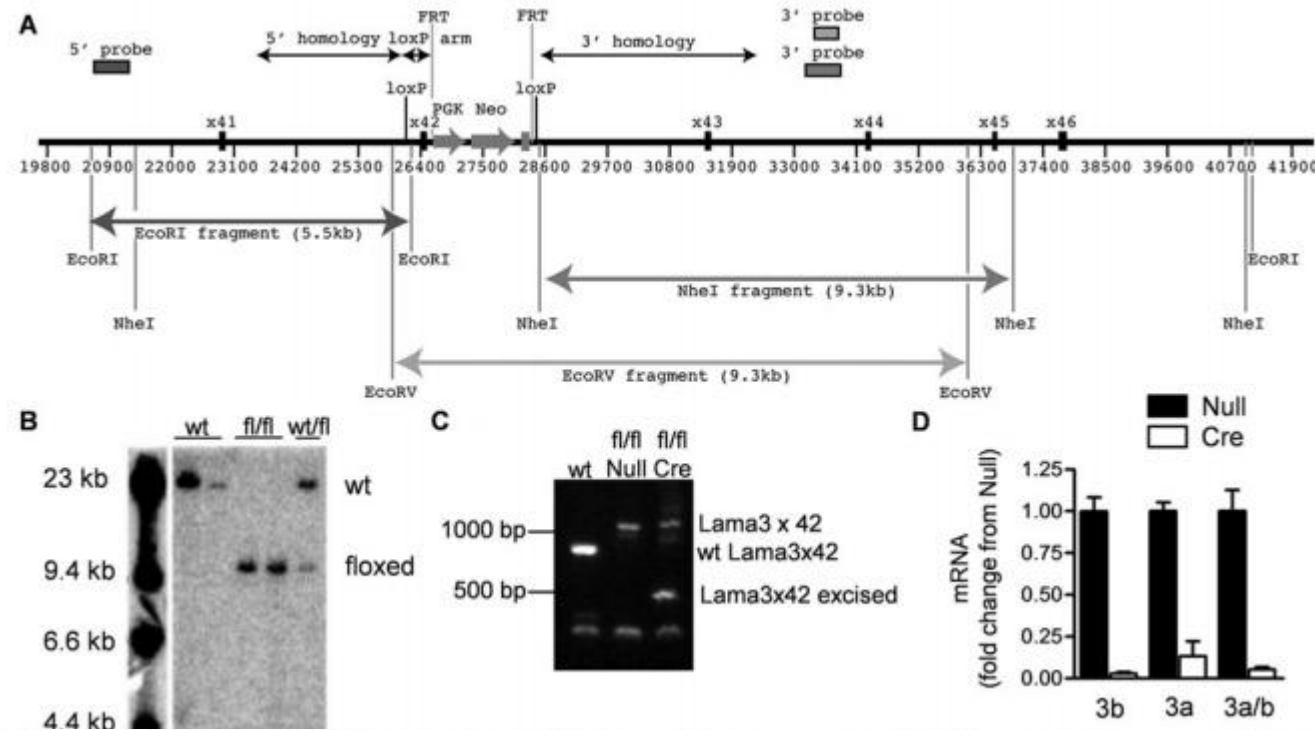


*Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).*

According to the existing MGI data, Mice homozygous for a targeted null mutation develop a lethal blistering phenotype similar to human junctional epidermolysis bullosa, and die 2-3 days after birth from a failure to thrive.



# References



**Fig. 1. Generation of the *Lama3*<sup>fl/fl</sup> mouse.** (A) Diagram of part of the vector that targets exon 42 of the *Lama3* mouse gene. (B) DNA isolated from tails of five littermates resulting from crossing animals exhibiting germline transmission of the floxed allele was digested with *EcoRV*, electrophoresed, transferred onto a nitrocellulose membrane and hybridized with a probe containing sequences 3' to the *loxP* sites. The expected size for the wild-type (wt) fragment is 26 kb and for the floxed fragment is 9.8 kb. (C) Genomic DNA was isolated from the lungs of null virus-infected wild-type mice (wt), null-virus-infected *Lama3*<sup>fl/fl</sup> mice (fl/fl Null) and Cre virus infected *Lama3*<sup>fl/fl</sup> mice (fl/fl Cre) and subjected to PCR using primers flanking the engineered region. (D) *Lama3*<sup>fl/fl</sup> mice were treated intratracheally with a null adenovirus (Null) or an adenovirus encoding Cre recombinase (Cre) and 60 days later alveolar type II cells were isolated from the mice from which RNA was isolated for measurement (qRT-PCR) of short regions of the *Lama3* gene specific to *Lama3a* (3a) or *Lama3b* (3b) transcripts or those common to *Lama3a* and *Lama3b* (3a/b) (also see supplementary material Fig. S1).

Urich D, et al., Lung-specific loss of the laminin alpha3 subunit confers resistance to mechanical injury. J Cell Sci. 2011 Sep 1;124(Pt 17):2927-37

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

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