Egfl7 Cas9-KO Strategy

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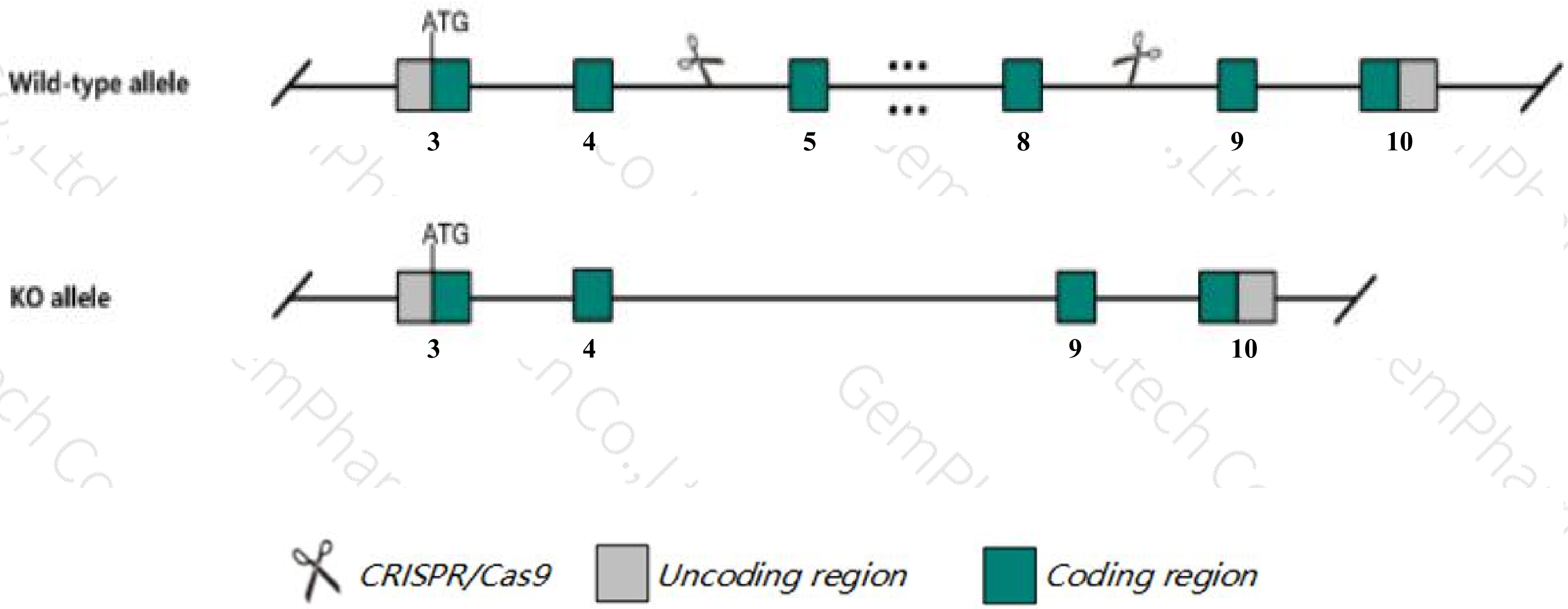
Project Overview

Project Name	*Egfl7*
Project type	Cas9-KO
Strain background	C57BL/6JGpt

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Egfl7* gene. The schematic diagram is as follows:



Technical routes

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➢ The *Egfl7* gene has 23 transcripts. According to the structure of *Egfl7* gene, exon5-exon8 of *Egfl7-220*(ENSMUST00000238951.1) transcript is recommended as the knockout region.The region contains 442bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Egfl7* 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,mice homozygous for a null allele exhibit some embryonic lethality with absent heartbeat and systemic edema, delayed vasculogenesis, decreased angiogenesis, abnormal adult vasculature, and low-level hypoxia. Mice homozygous for a null allele that does not disrupt Mirn126 expression are normal.

➢ The KO region contains functional region of the Egfl7 gene.Knockout the region may affect the function of Mir126a,Mir126b gene.

➢ The Intron8 is only 389bp,loxp insertion may affect mRNA splicing.

➢ The *Egfl7* 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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Egfl7 EGF-like domain 7 [Mus musculus (house mouse)]

Gene ID: 353156, updated on 13-Mar-2020

Summary

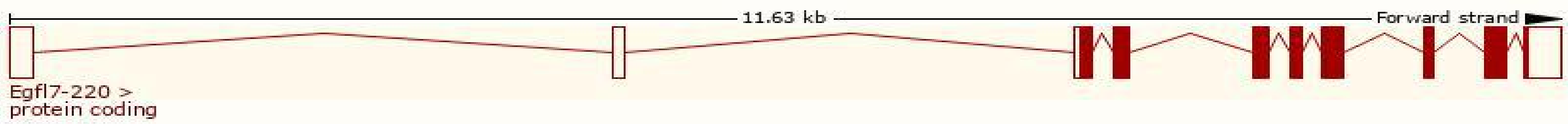
Official Symbol	Egfl7 provided by MGI
Official Full Name	EGF-like domain 7 provided byMGI
Primary source	MGI:MGI:2449923
See related	Ensembl:ENSMUSG00000026921
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	VE-statin, Zneu1
Expression	Biased expression in lung adult (RPKM 371.9), subcutaneous fat pad adult (RPKM 89.0) and 13 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

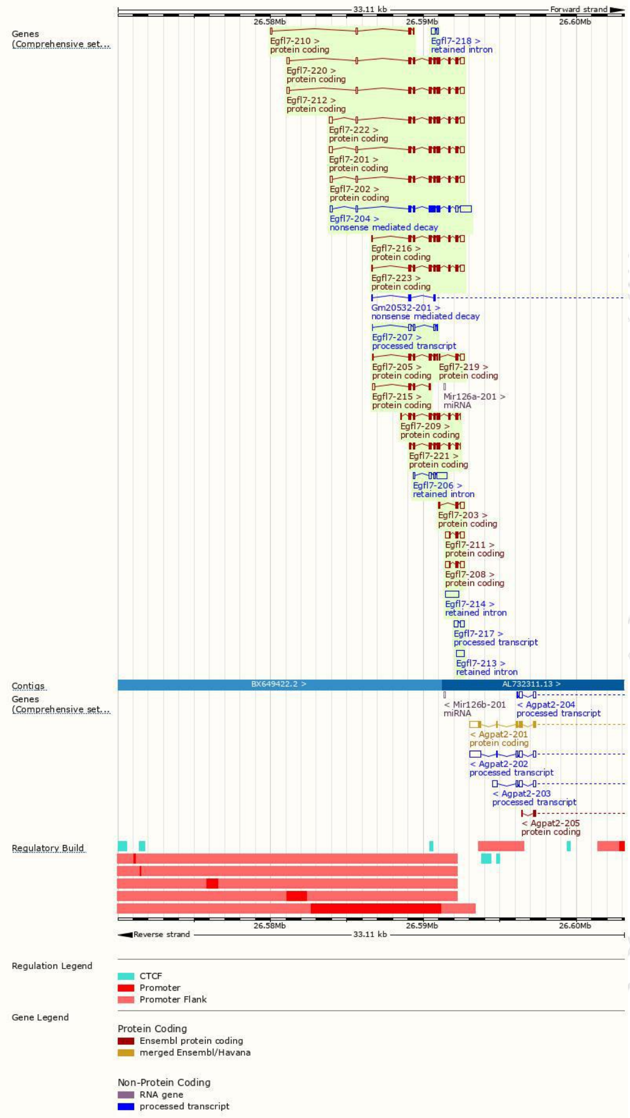
The gene has 23 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Egfl7-220	ENSMUST00000238951.1	1389	278aa	Protein coding	CCDS15807	-	GENCODE basic APPRIS P4
Egfl7-222	ENSMUST00000238983.1	1388	278aa	Protein coding	CCDS15807	-	GENCODE basic APPRIS P4
Egfl7-216	ENSMUST00000174211.7	1163	278aa	Protein coding	CCDS15807	Q3UTJ7	TSL:1 GENCODE basic APPRIS P4
Egfl7-221	ENSMUST00000238966.1	798	265aa	Protein coding	CCDS15808	-	GENCODE basic APPRIS ALT2
Egfl7-212	ENSMUST00000166920.9	1391	275aa	Protein coding	-	Q3UTJ7 Q9QXT5	TSL:1 GENCODE basic APPRIS ALT2
Egfl7-202	ENSMUST00000102907.12	1372	275aa	Protein coding	-	Q3UTJ7 Q9QXT5	TSL:1 GENCODE basic APPRIS ALT2
Egfl7-201	ENSMUST00000100290.12	1348	262aa	Protein coding	-	A0A0R4J189	TSL:1 GENCODE basic APPRIS ALT2
Egfl7-223	ENSMUST00000239075.1	1163	275aa	Protein coding	-	Q9QXT5	GENCODE basic APPRIS ALT2
Egfl7-209	ENSMUST00000150404.9	892	275aa	Protein coding	-	Q3UTJ7 Q9QXT5	TSL:5 GENCODE basic APPRIS ALT2
Egfl7-211	ENSMUST00000152988.8	723	79aa	Protein coding	-	G3UY71	TSL:2 GENCODE basic
Egfl7-208	ENSMUST00000149789.1	683	66aa	Protein coding	-	G3UWX8	TSL:3 GENCODE basic
Egfl7-205	ENSMUST00000145575.9	541	151aa	Protein coding	-	F6W8P4	CDS 3' incomplete TSL:2
Egfl7-203	ENSMUST00000131112.1	521	71aa	Protein coding	-	F7BU34	CDS 5' incomplete TSL:2
Egfl7-215	ENSMUST00000173920.8	484	103aa	Protein coding	-	G3UZH9	CDS 3' incomplete TSL:3
Egfl7-219	ENSMUST00000174656.1	424	50aa	Protein coding	-	G3UYR2	CDS 5' incomplete TSL:3
Egfl7-210	ENSMUST00000152713.8	367	46aa	Protein coding	-	F6RMR6	CDS 3' incomplete TSL:3
Egfl7-204	ENSMUST00000139801.8	1865	238aa	Nonsense mediated decay	-	E9Q4M7	TSL:5
Egfl7-217	ENSMUST00000174465.1	491	No protein	Processed transcript	-	-	TSL:2
Egfl7-207	ENSMUST00000149460.7	369	No protein	Processed transcript	-	-	TSL:5
Egfl7-206	ENSMUST00000147351.2	989	No protein	Retained intron	-	-	TSL:2
Egfl7-214	ENSMUST00000173777.1	845	No protein	Retained intron	-	-	TSL:NA
Egfl7-213	ENSMUST00000172683.1	536	No protein	Retained intron	-	-	TSL:NA
Egfl7-218	ENSMUST00000174572.1	307	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Egfl7-220* transcript,the transcription is shown below:



Genomic location distribution



Protein domain

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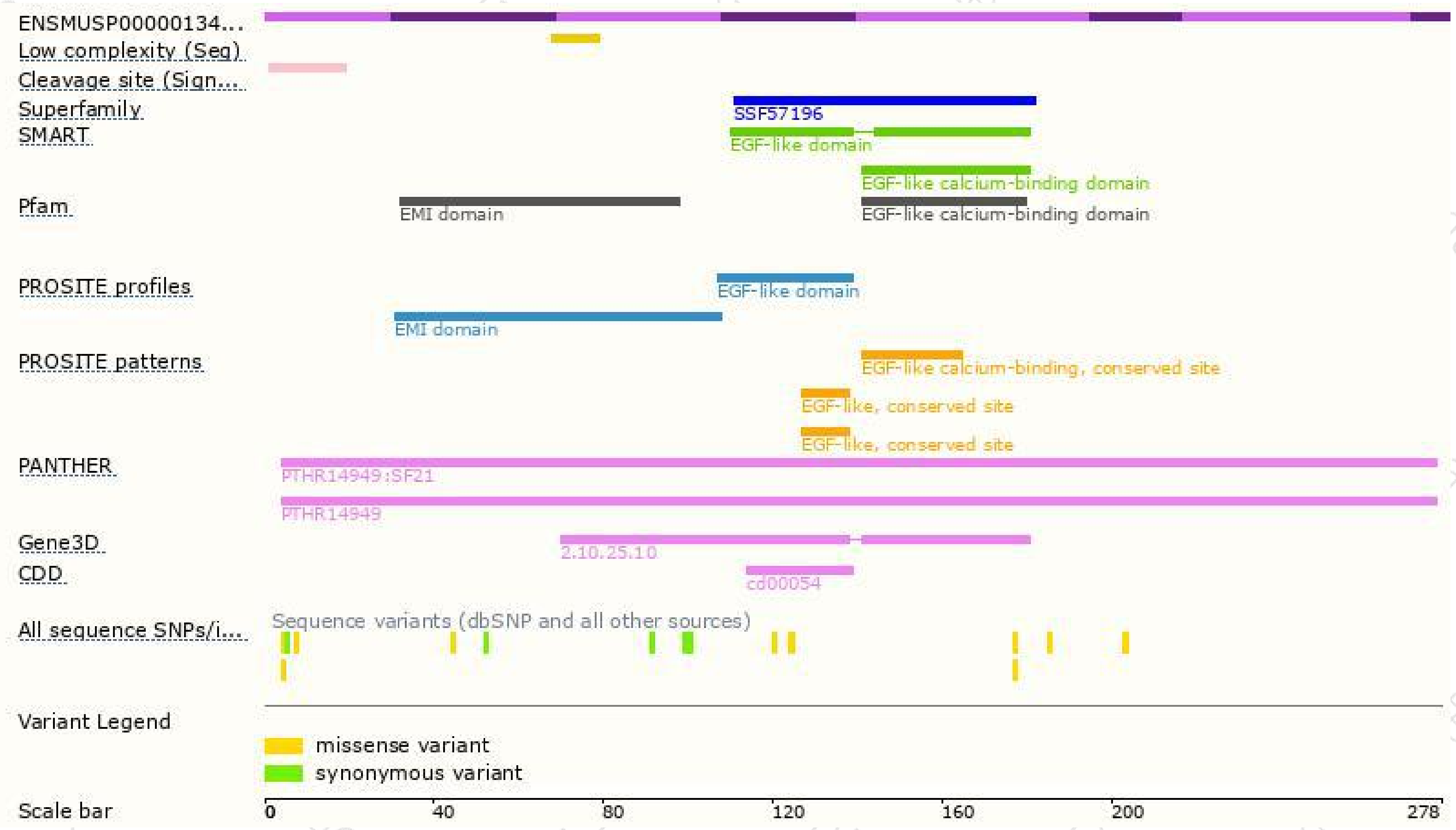
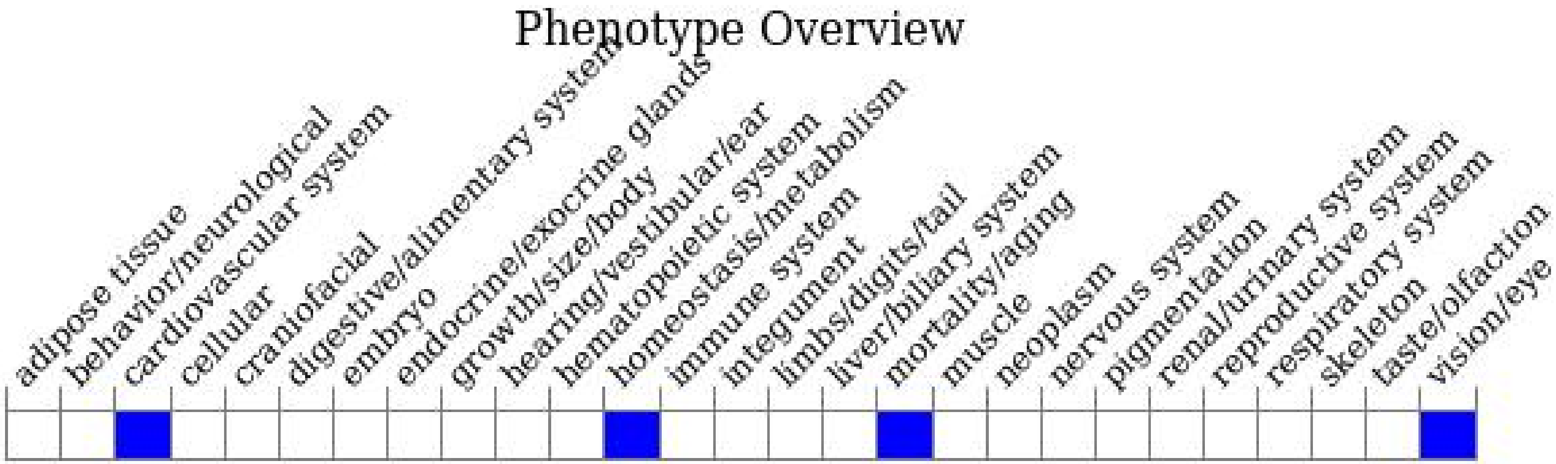


Mouse phenotype description(MGI)



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 null allele exhibit some embryonic lethality with absent heartbeat and systemic edema, delayed vasculogenesis, decreased angiogenesis, abnormal adult vasculature, and low-level hypoxia. Mice homozygous for a null allele that does not disrupt Mirn126 expression are normal.

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