Efemp2 Cas9-CKO Strategy

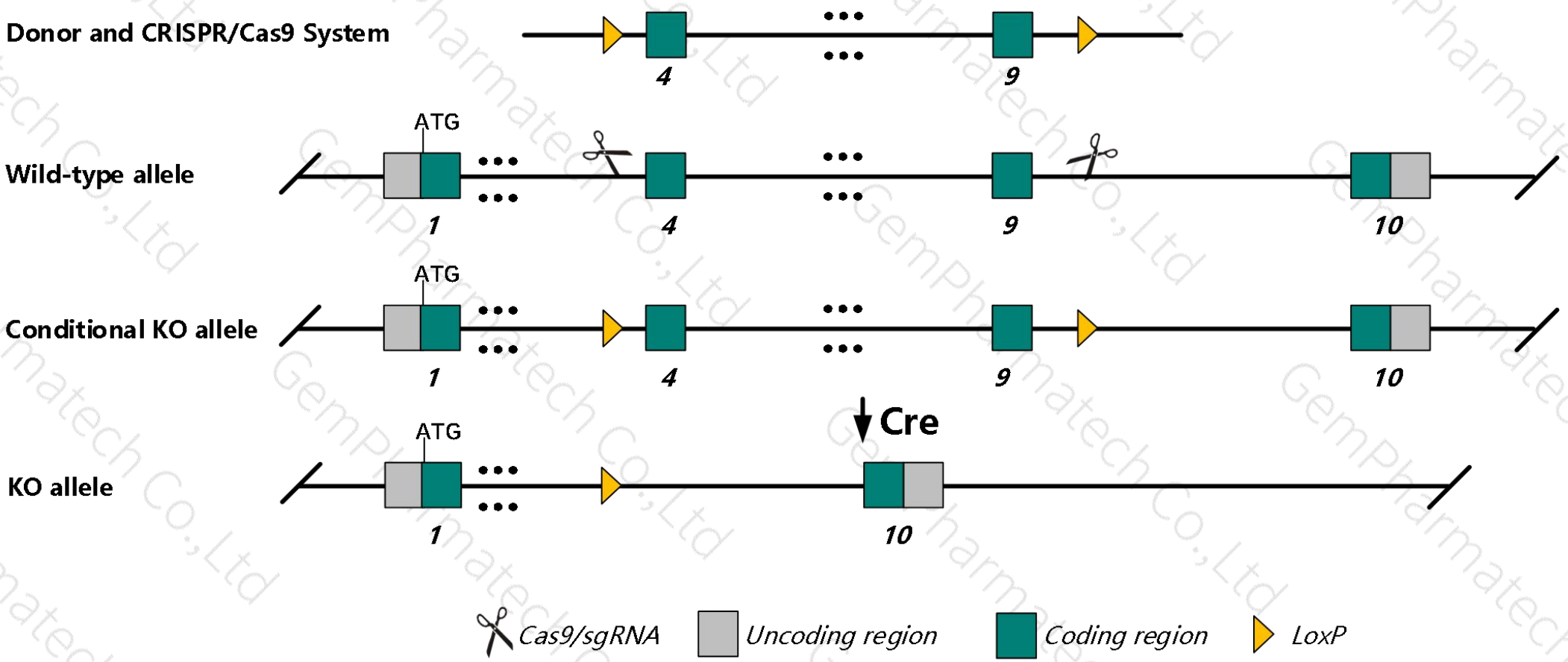
Designer:Xiaojing Li
Reviewer:JiaYu
Design Date:2020-3-9

Project Overview

Project Name	***Efemp2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Efemp2* gene. The schematic diagram is as follows:



Technical routes

- The *Efemp2* gene has 17 transcripts. According to the structure of *Efemp2* gene, exon4-exon9 of *Efemp2-201* (ENSMUST00000070118.13) transcript is recommended as the knockout region.The region contains 803bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Efemp2* gene. The brief process is as follows:CRISPR/Cas9 system 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 will be 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.

Notice

➢ According to the existing MGI data,Homozygous mutation of this gene results in perinatal lethality with abnormal artery and lung morphology and defects in vascular, pulmonary, and hypodermal elastic fibers. Some alleles of Mus81 also affect expression of this gene.

➢ The *Efemp2* gene is located on the Chr19. 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）

集萃药康 GemPharmatech

Efemp2 epidermal growth factor-containing fibulin-like extracellular matrix protein 2 [*Mus musculus* (house mouse)]

Gene ID: 58859, updated on 28-Oct-2019

Summary

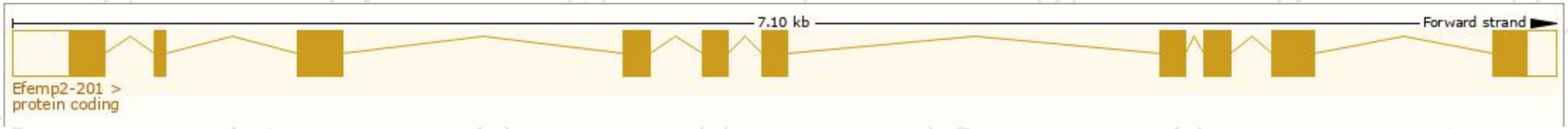
Official Symbol	Efemp2 provided by MGI
Official Full Name	epidermal growth factor-containing fibulin-like extracellular matrix protein 2 provided by MGI
Primary source	MGI:MGI:1891209
See related	Ensembl:ENSMUSG00000024909
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	MBP1; Fbln4; 0610011K11Rik
Expression	Broad expression in limb E14.5 (RPKM 51.1), ovary adult (RPKM 49.4) and 27 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

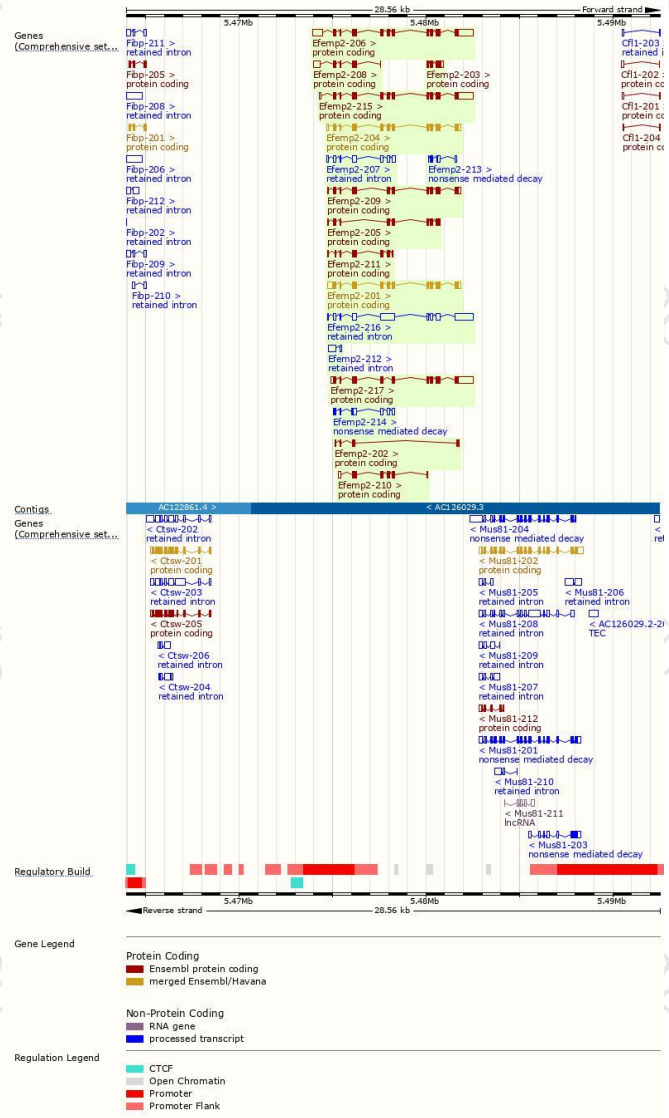
The gene has 17 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Efemp2-206	ENSMUST00000166303.8	2634	443aa	Protein coding	CCDS29466	E9Q8E0 Q542X5	TSL:5 GENCODE basic APPRIS P1
Efemp2-201	ENSMUST00000070118.13	1781	462aa	Protein coding	CCDS50358	G5E8D6	TSL:1 GENCODE basic
Efemp2-204	ENSMUST00000165485.7	1557	443aa	Protein coding	CCDS29466	Q542X5 Q9WVJ9	TSL:1 GENCODE basic APPRIS P1
Efemp2-215	ENSMUST00000235523.1	2215	455aa	Protein coding	-	-	GENCODE basic
Efemp2-217	ENSMUST00000236518.1	2154	404aa	Protein coding	-	-	GENCODE basic
Efemp2-209	ENSMUST00000167371.7	1382	402aa	Protein coding	-	E9Q3N9	TSL:5 GENCODE basic
Efemp2-205	ENSMUST00000166253.7	882	280aa	Protein coding	-	E9Q3F3	CDS 3' incomplete TSL:5
Efemp2-210	ENSMUST00000167827.1	769	191aa	Protein coding	-	E9Q2T8	CDS 3' incomplete TSL:5
Efemp2-208	ENSMUST00000167304.7	742	128aa	Protein coding	-	E9Q1U0	CDS 3' incomplete TSL:5
Efemp2-211	ENSMUST00000167855.7	607	153aa	Protein coding	-	E9Q2A0	CDS 3' incomplete TSL:3
Efemp2-203	ENSMUST00000164388.1	603	157aa	Protein coding	-	F6Z1C2	CDS 5' incomplete TSL:5
Efemp2-202	ENSMUST00000164204.1	362	120aa	Protein coding	-	F7AYH6	CDS 5' incomplete TSL:5
Efemp2-214	ENSMUST00000169943.7	773	82aa	Nonsense mediated decay	-	E9PYW6	TSL:5
Efemp2-213	ENSMUST00000168330.1	406	70aa	Nonsense mediated decay	-	F6R0V7	CDS 5' incomplete TSL:5
Efemp2-216	ENSMUST00000236109.1	2556	No protein	Retained intron	-	-	
Efemp2-207	ENSMUST00000166558.7	848	No protein	Retained intron	-	-	TSL:2
Efemp2-212	ENSMUST00000167920.1	463	No protein	Retained intron	-	-	TSL:2

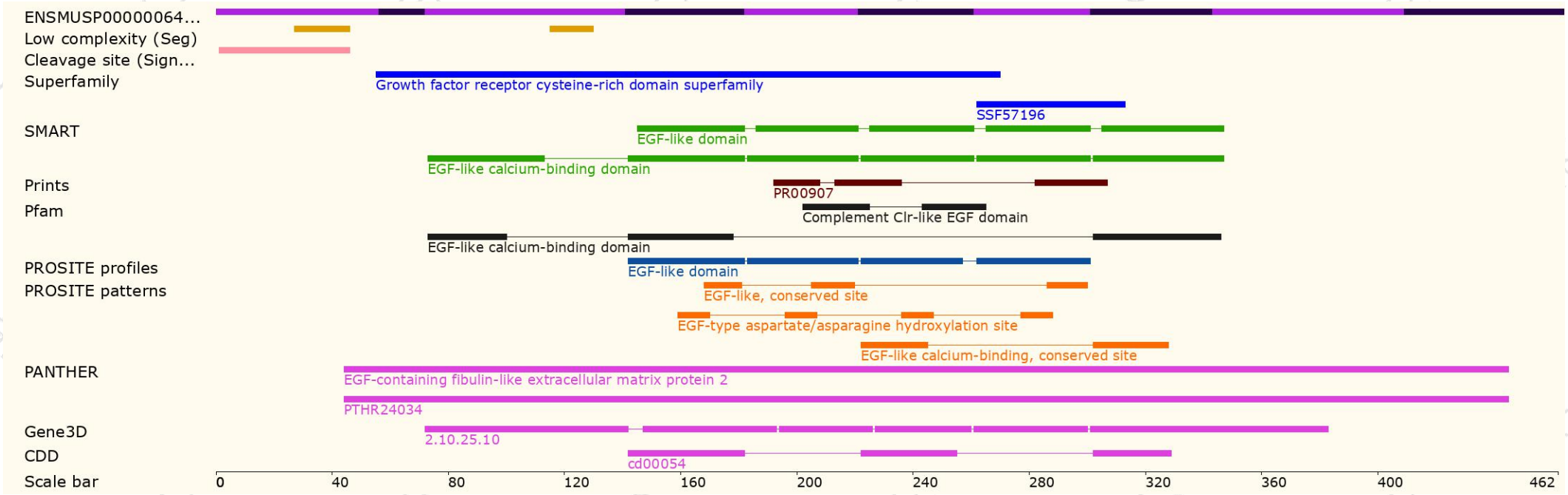
The strategy is based on the design of *Efemp2-201* transcript,The transcription is shown below



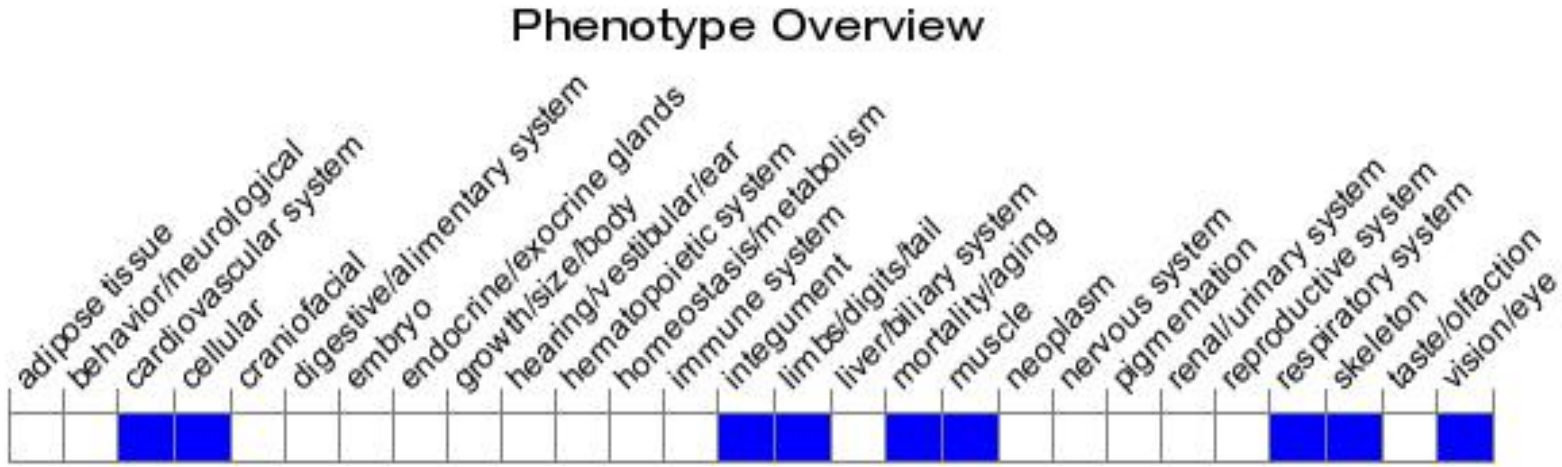
Genomic location distribution



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,Homozygous mutation of this gene results in perinatal lethality with abnormal artery and lung morphology and defects in vascular, pulmonary, and hypodermal elastic fibers. Some alleles of Mus81 also affect expression of this gene.

集萃药康
GemPharmatech

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd