

Ppp6c Cas9-CKO Strategy

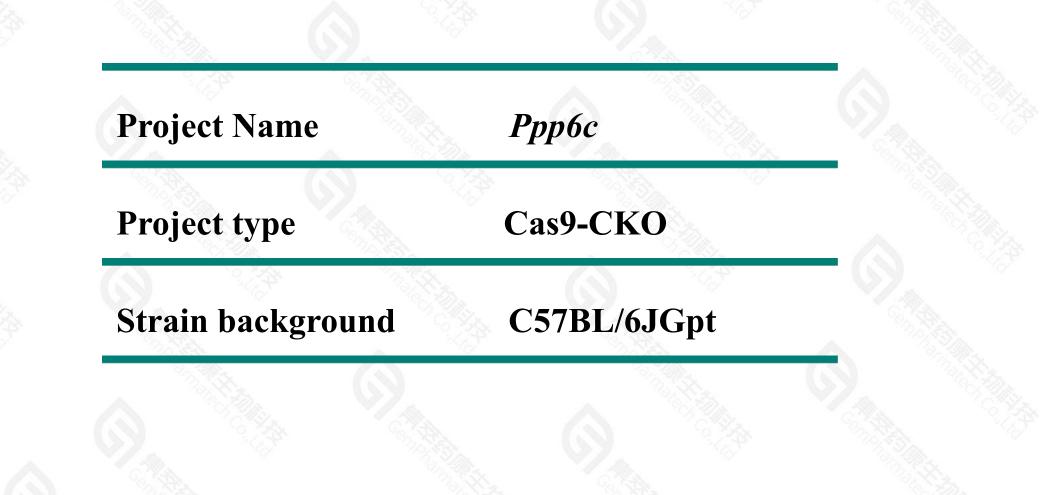
Designer: Ruirui Zhang

Reviewer: Xueting Zhang

Design Date: 2022/7/12

Project Overview



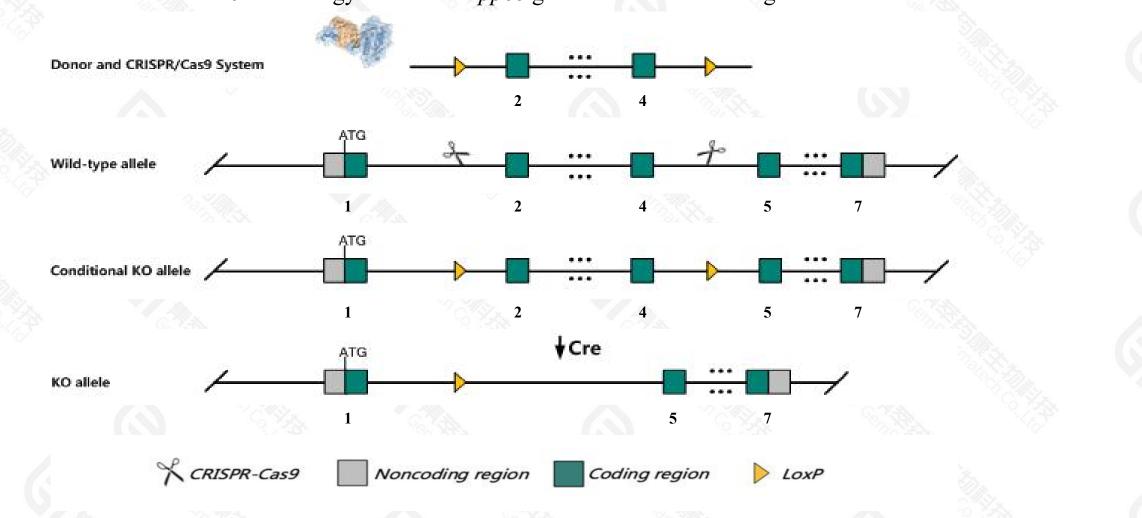


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Conditional Knockout strategy

This model will use CRISPR-Cas9 technology to edit the *Ppp6c* gene. The schematic diagram is as follows:



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Technical routes



The *Ppp6c* gene has 5 transcripts. According to the structure of *Ppp6c* gene, exon2-exon4 of *Ppp6c-201*(ENSMUST0000028087.6) transcript is recommended as the knockout region. The region contains 304bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR-Cas9 technology to modify *Ppp6c* 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.

 \succ 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.



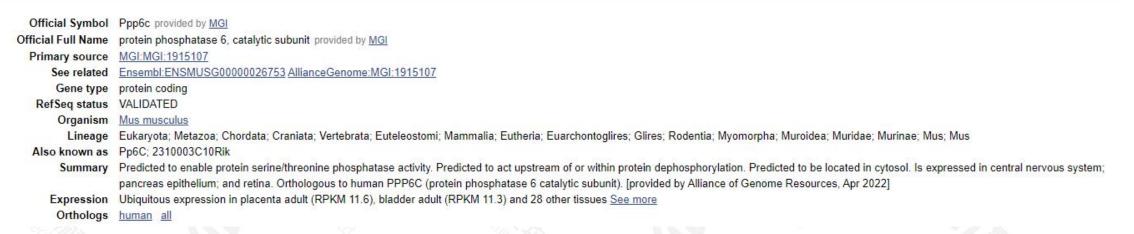
- \succ According to the existing MGI data,mice homozygous for a knock-out allele exhibit abnormal embryonic development and embryonic lethality. Mice homozygous for a conditional allele activated in skin cells exhibit increased susceptibility to chemically induced skin tumors with increased proliferative and inflammatory responses in the skin.
- > The *Ppp6c* gene is located on the Chr 2. 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.

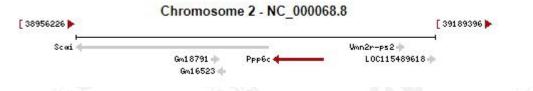
Gene information (NCBI)

Ppp6c protein phosphatase 6, catalytic subunit [Mus musculus (house mouse)]

Gene ID: 67857, updated on 29-May-2022

Summary





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400-9660890



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Transcript information (Ensembl)

The gene has 5 transcripts, all transcripts are shown below:

Name	Transcript ID		Protein	Biotype	CCDS	UniProt	Flags	
Ppp6c-201	ENSMUST0000028087.5	4115	<u>305aa</u>	Protein coding	CCDS16017	Q9CQR6	TSL:1 GENCODE basic APPRIS P1	
Ppp6c-205	ENSMUST00000204701.2	1495	<u>283aa</u>	Protein coding	(. .)	A0A0N4SVL9	TSL:5 GENCODE basic	
Ppp6c-204	ENSMUST00000204368.1	635	<u>83aa</u>	Protein coding	620	A0A0N4SW66	CDS 3' incomplete TSL:5	
Ppp6c-203	ENSMUST00000204257.2	1358	<u>81aa</u>	Nonsense mediated decay	1926	A0A0N4SVE2	TSL:5	
Ppp6c-202	ENSMUST00000143733.1	487	No protein	Processed transcript	1271	-	TSL:3	

The strategy is based on the design of *Ppp6c-201* transcript, the transcription is shown below:

< Ppp6c-201 protein coding

Reverse strand

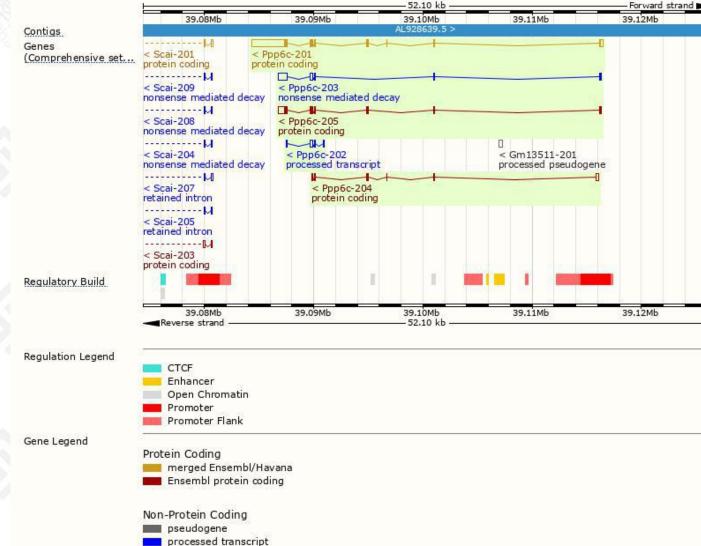
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Genomic location distribution





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Protein domain

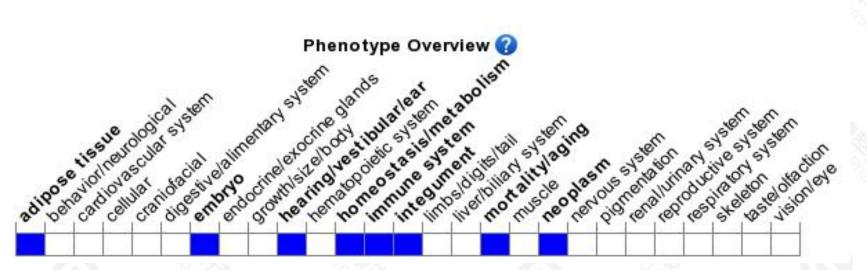


ENSMUSP00000028 Superfamily	SSF56 300											
SMART	Serine	/threonine-specific	protein phos	phatase/bis(5-n	ucleosyl)-tetrapho	sphatase		-				
Prints		Serine/three	nine-specific	protein phospha	tase/bis(5-nucleo	syl)-tetraphospha	tase					
<u>Pfam</u>		Calcineurin-l	ike phosphoe	sterase domain,	ApaH type							
PROSITE patterns	Serine/threonine-specific protein phosphatase/bis(5-nucleosyl)-tetraphosphatase											
PANTHER	PTHR45619 (5	9F50										
	PTHR45519											
Gene3D	Metallo-depen	dent phosphatase	-like					13				
CDD	cd07415											
All sequence SNPs/i	Sequence va	riants (dbSNP ar	nd all other s	sources)		11.5	U					
Variant Legend		e variant nous variant										
Scale bar	0	40	80	120	160	200	240	30				

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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 knock-out allele exhibit abnormal embryonic development and embryonic lethality. Mice homozygous for a conditional allele activated in skin cells exhibit increased susceptibility to chemically induced skin tumors with increased proliferative and inflammatory responses in the skin.

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If you have any questions, you are welcome to inquire. Tel: 400-9660890



