

Ccl27a Cas9-CKO Strategy

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Project Overview

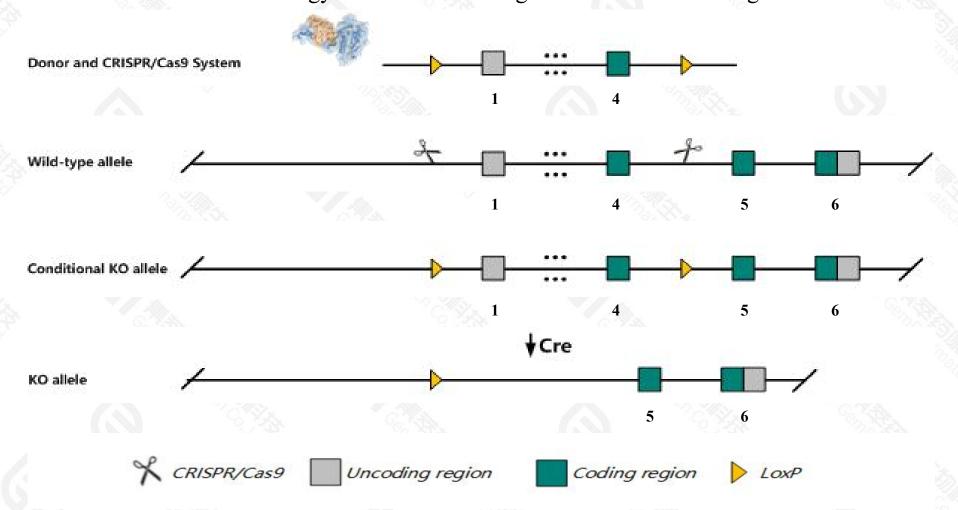


Project Name	Ccl27a		
Project type	Cas9-CKO		
Strain background	C57BL/6JGpt		

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Ccl27a gene. The schematic diagram is as follows:



Technical routes



- The *Ccl27a* gene has 19 transcripts. According to the structure of *Ccl27a* gene, exon1-exon4 of *Ccl27a-206*(ENSMUST00000108037.9) transcript is recommended as the knockout region. The region contains 205bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Ccl27a* 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



- > The *Ccl27a* gene is located on the Chr4. 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)



Ccl27a chemokine (C-C motif) ligand 27A [Mus musculus (house mouse)]

Gene ID: 20301, updated on 13-Mar-2020

Summary



Official Symbol Ccl27a provided by MGI

Official Full Name chemokine (C-C motif) ligand 27A provided by MGI

Primary source MGI:MGI:1343459

See related Ensembl: ENSMUSG00000073888

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 ALP, AW558992, CTACK, CTAK, Ccl27, ESkine, ILC, PESKY, Scya27, Scya27a

Expression Broad expression in testis adult (RPKM 85.9), cortex adult (RPKM 62.4) and 21 other tissuesSee more

Orthologs <u>human all</u>

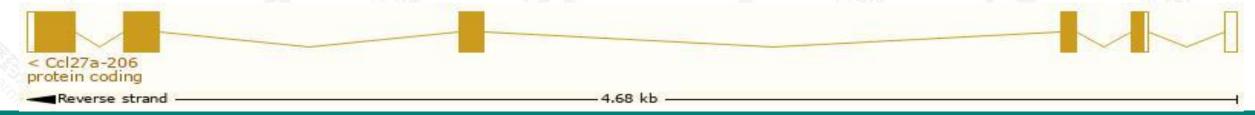
Transcript information (Ensembl)



The gene has 19 transcripts, all transcripts are shown below:

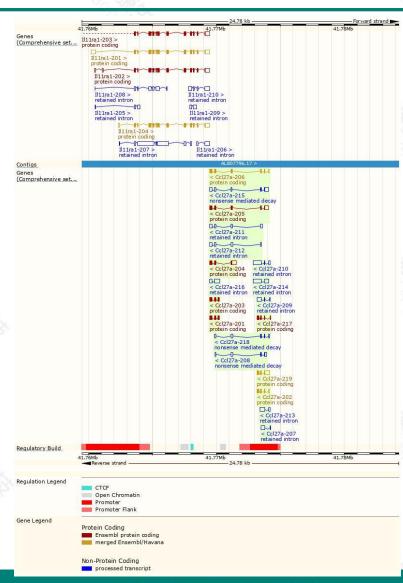
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ccl27a-205	ENSMUST00000108036.7	841	164aa	Protein coding	CCDS38721	A2AMS7	TSL:2 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT2
Ccl27a-204	ENSMUST00000108035.7	731	<u>127aa</u>	Protein coding	CCDS84714	A2AMS6	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALTZ
Ccl27a-219	ENSMUST00000173865.8	657	87aa	Protein coding	CCDS51144	A2AMS8	TSL:1 GENCODE basic
Ccl27a-206	ENSMUST00000108037.8	590	<u>164aa</u>	Protein coding	CCDS38721	A2AMS7	TSL:2 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT
Ccl27a-201	ENSMUST00000074387.3	405	<u>120aa</u>	Protein coding	CCDS18072	A0A0R4J0X6	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P3
cl27a-202	ENSMUST00000108032.2	350	87aa	Protein coding	CCDS51144	A2AMS8	TSL:1 GENCODE basic
cl27a-217	ENSMUST00000155240.1	343	87aa	Protein coding	CCDS51144	A2AMS8	TSL:2 GENCODE basic
cl27a-203	ENSMUST00000108033.7	428	<u>119aa</u>	Protein coding	2	A2AMS5	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS ALT
cl27a-215	ENSMUST00000151142.7	822	37aa	Nonsense mediated decay		A0A2I3BRY3	TSL:3
cl27a-208	ENSMUST00000131349.7	489	40aa	Nonsense mediated decay	-	A0A2I3BQE5	TSL:3
cl27a-218	ENSMUST00000155322.7	460	40aa	Nonsense mediated decay	2	A0A2I3BQE5	TSL:3
cl27a-214	ENSMUST00000144090.7	996	No protein	Retained intron	25	127	TSL:2
cl27a-210	ENSMUST00000133572.7	798	No protein	Retained intron	-		TSL:2
cl27a-216	ENSMUST00000151909.7	614	No protein	Retained intron	-	-	TSL:1
cl27a-211	ENSMUST00000136768.7	556 I	No protein	Retained intron	=	(5)	TSL:1
cl27a-212	ENSMUST00000138337.7	552	No protein	Retained intron	25	727	TSL:2
cl27a-213	ENSMUST00000143676.1	487 I	No protein	Retained intron	-	-	TSL:2
cl27a-209	ENSMUST00000133556.7	453	No protein	Retained intron	-	1-0	TSL:2
cl27a-207	ENSMUST00000126759.1	361 I	No protein	Retained intron	2	(4)	TSL:3

The strategy is based on the design of *Ccl27a-206* transcript, the transcription is shown below:



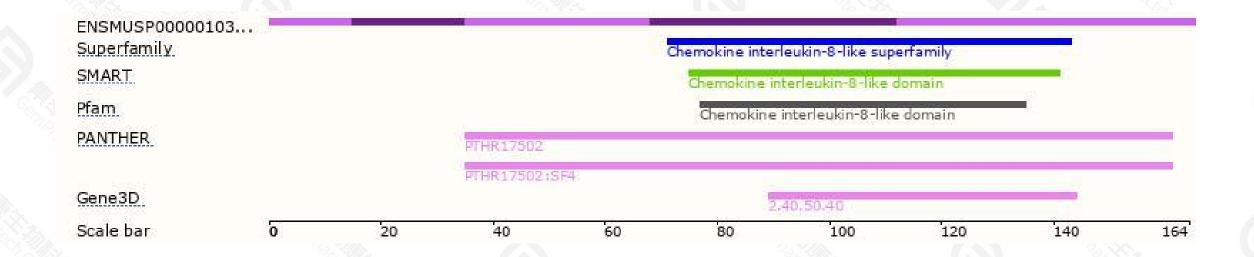
Genomic location distribution





Protein domain







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

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