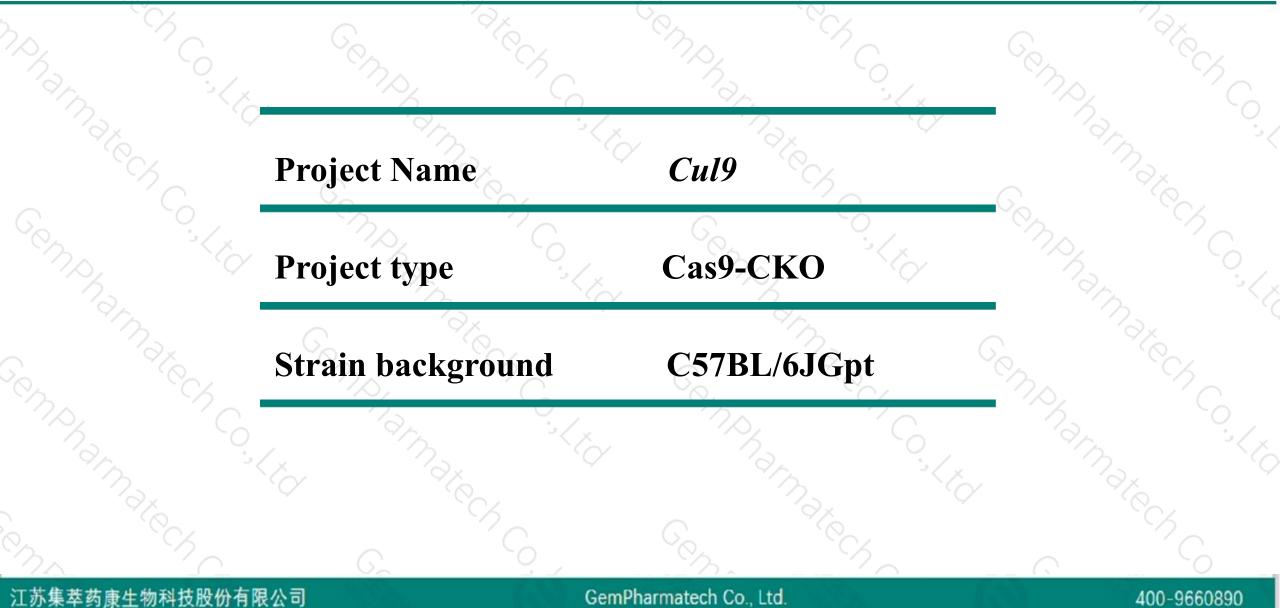


Cul9 Cas9-CKO Strategy

Designer: Yanhua Shen Reviewer: Xueting Zhang Design Date: 2019-08-30

Project Overview



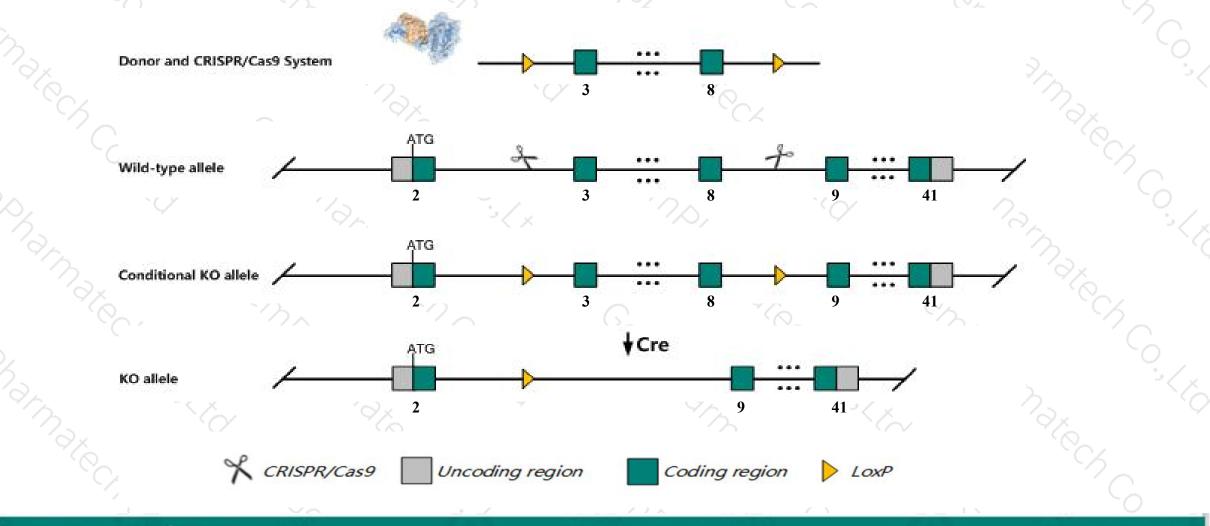


Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the Cul9 gene. The schematic diagram is as follows:



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The Cul9 gene has 11 transcripts. According to the structure of Cul9 gene, exon3-exon8 of Cul9-204 (ENSMUST00000182485.7) transcript is recommended as the knockout region. The region contains 1591bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Cul9* 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.



- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit decreased body weight, increased incidence of tumors, and decreased cellular sensitivity to radiation-induced apoptosis.
- > Some amino acids will remain at the N-terminus and some functions may be retained.
- ➤ The transcripts 205, 206, 207, 208, 209, 211 are unaffected.
- > The flox region coincides with Gm26904-201 and the effect on this gene is unknown.
- The Cul9 gene is located on the Chr17. 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)



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Cul9 cullin 9 [Mus musculus (house mouse)]

Gene ID: 78309, updated on 13-Aug-2019

- Summary

Official Symbol	Cul9 provided by MGI
Official Full Name	cullin 9 provided by MGI
Primary source	MGI:MGI:1925559
See related	Ensembl:ENSMUSG0000040327
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	Parc; Cul-9; mKIAA0708; 1810035l07Rik
Expression	Ubiquitous expression in ovary adult (RPKM 12.3), adrenal adult (RPKM 12.3) and 24 other tissues See more
Orthologs	human all

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Transcript information (Ensembl)



The gene has 11 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Cul9-204	ENSMUST00000182485.7	7848	<u>2530aa</u>	Protein coding	CCDS37636	<u>S4R1Y1</u>	TSL:5 GENCODE basic APPRIS P2	
Cul9-201	ENSMUST00000066026.7	7818	<u>2520aa</u>	Protein coding	-	E9QP09	TSL:5 GENCODE basic APPRIS ALT2	
Cul9-202	ENSMUST00000182315.7	8451	No protein	Retained intron	-	8 2 3	TSL:2	
Cul9-205	ENSMUST00000182530.7	4151	No protein	Retained intron	-	323	TSL:5	
Cul9-206	ENSMUST00000182668.1	4109	No protein	Retained intron	5	1.7	TSL:2	
Cul9-209	ENSMUST00000183078.7	3070	No protein	Retained intron	÷	19 4 0	TSL:1	
Cul9-207	ENSMUST00000182799.1	1673	No protein	Retained intron	-	(a 2)	TSL:1	
Cul9-210	ENSMUST00000183163.1	1017	No protein	Retained intron	-	100	TSL:3	
Cul9-208	ENSMUST00000183016.7	791	No protein	Retained intron		1.7	TSL:2	
Cul9-203	ENSMUST00000182451.1	700	No protein	Retained intron	-		TSL:2	
Cul9-211	ENSMUST00000183312.1	500	No protein	Retained intron	-	640	TSL:NA	
	1112		11	/	2		on Vision	

The strategy is based on the design of Cul9-204 transcript, The transcription is shown below

-45.78 kb -

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< Cul9-204 protein coding

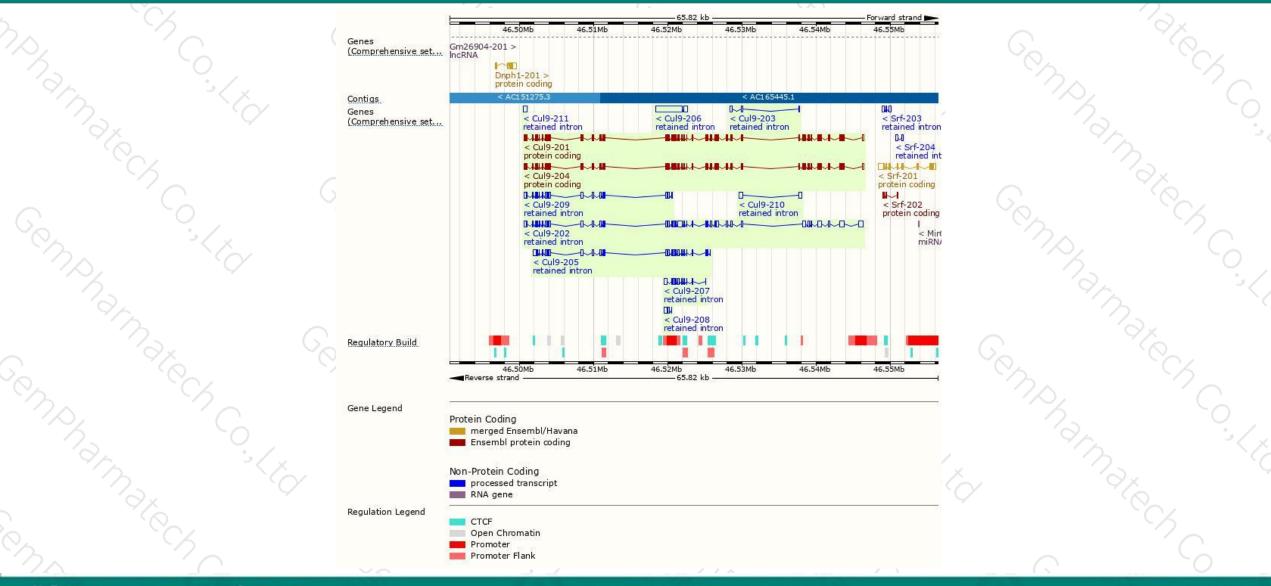
Reverse strand

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Genomic location distribution





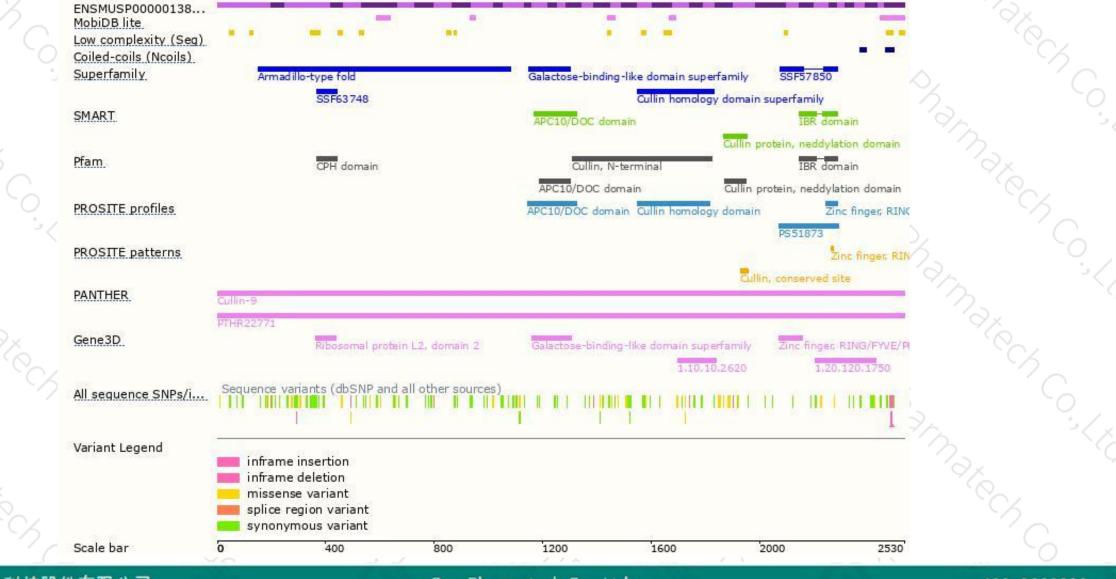
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Protein domain





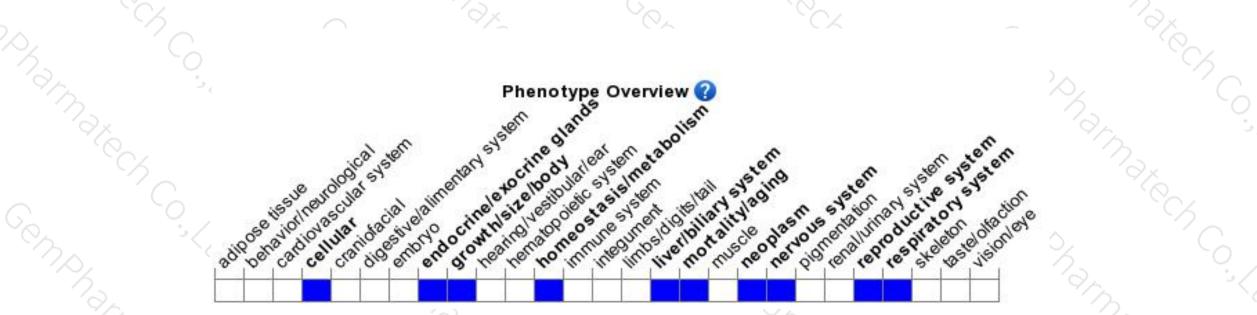
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Mouse phenotype description(MGI)





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 knock-out allele exhibit decreased body weight, increased incidence of tumors, and decreased cellular sensitivity to radiation-induced apoptosis.



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



