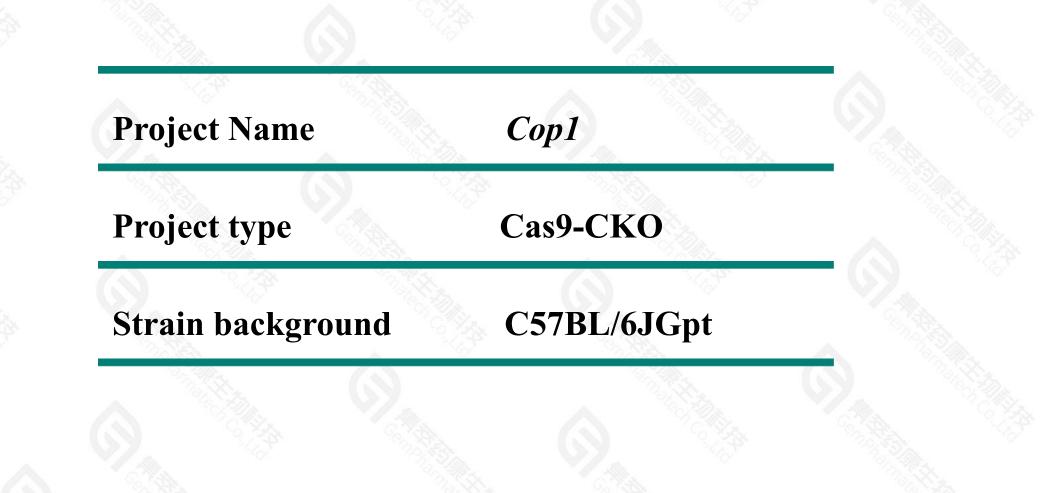


Cop1 Cas9-CKO Strategy

Designer: Daohua Xu

Project Overview





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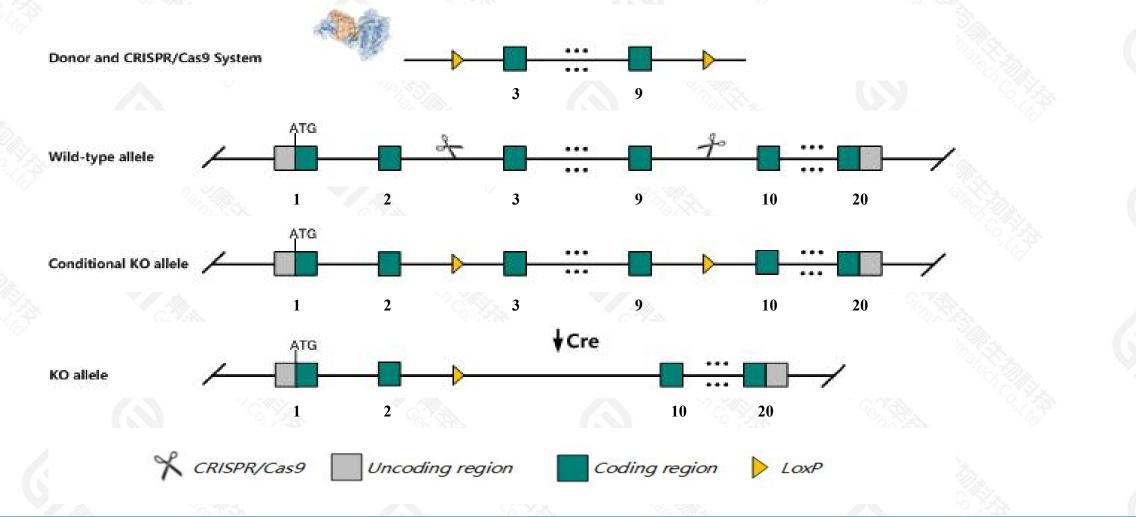
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Conditional Knockout strategy

「 集卒生 GemPharma

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This model will use CRISPR/Cas9 technology to edit the Cop1 gene. The schematic diagram is as follows:



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Technical routes



> The *Cop1* gene has 13 transcripts. According to the structure of *Cop1* gene, exon3-exon9 of *Cop1-*201(ENSMUST00000076894.11) transcript is recommended as the knockout region. The region contains 559bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Cop1* 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 conditional allele activated in prostate epithelial cells exhibit prostate gland hyperplasia and prostate intraepithelial neoplasia due to increased cell proliferation.
- > Transcript *Cop1-202* may not be affected.
- > The *Cop1* gene is located on the Chr1. 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)

Cop1 COP1, E3 ubiquitin ligase [Mus musculus (house mouse)]

Gene ID: 26374, updated on 17-Dec-2020

Summary

Official Symbol	Cop1 provided by MGI
Official Full Name	COP1, E3 ubiquitin ligase provided by <u>MGI</u>
Primary source	MGI:MGI:1347046
See related	Ensembl:ENSMUSG0000040782
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	AI316802, C80879, Co, Rfwd, Rfwd2
Expression	Ubiquitous expression in CNS E14 (RPKM 4.0), whole brain E14.5 (RPKM 4.0) and 28 other tissuesSee more
Orthologs	human all

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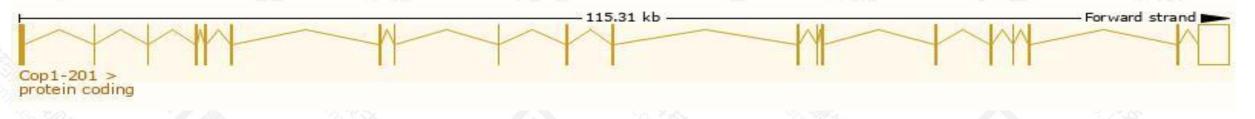
☆ ?

Transcript information (Ensembl)

Transcript ID CCDS UniProt Name bp Protein Biotype Flags ENSMUST00000076894.11 5116 733aa Protein coding TSL:1, GENCODE basic, APPRIS P1 Cop1-201 CCDS35745 Cop1-203 ENSMUST00000192215.6 1040 346aa Protein coding CDS 5' and 3' incomplete, TSL:5, Cop1-211 ENSMUST00000195044.2 745 Protein coding CDS 3' incomplete, TSL:3, 202aa _ Cop1-205 ENSMUST00000192762.6 680 227aa Protein coding CDS 5' and 3' incomplete, TSL:5, Cop1-202 ENSMUST00000192044.2 464 155aa Protein coding CDS 5' and 3' incomplete, TSL:3, Cop1-213 753 Nonsense mediated decay TSL:3. ENSMUST00000195800.6 159aa \sim Nonsense mediated decay CDS 5' incomplete, TSL:3, Cop1-212 ENSMUST00000195554.6 545 60aa -Cop1-209 ENSMUST00000194713.2 6999 No protein Retained intron TSL:NA, -Cop1-204 ENSMUST00000192597.2 Retained intron TSL:NA , 4961 No protein Cop1-206 ENSMUST00000192841.2 776 No protein Retained intron TSL:2 , 2 Cop1-208 ENSMUST00000194049.2 655 No protein Retained intron TSL:5, -Cop1-210 ENSMUST00000194798.2 473 No protein Retained intron TSL:3, Cop1-207 ENSMUST00000193776.2 392 No protein Retained intron TSL:2,

The gene has 13 transcripts, all transcripts are shown below:

The strategy is based on the design of Cop1-201 transcript, the transcription is shown below:

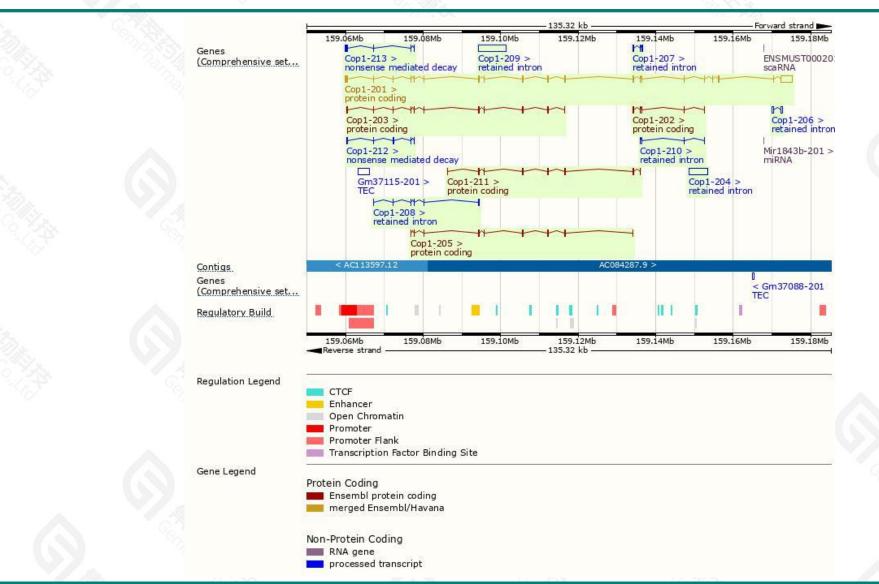


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Genomic location distribution





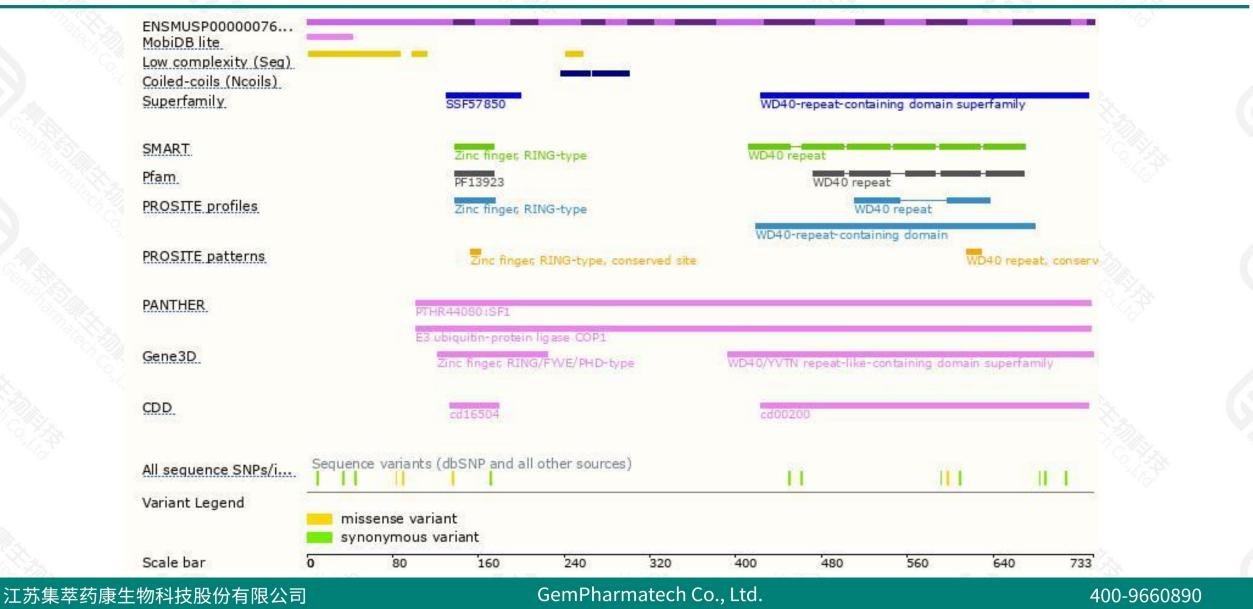
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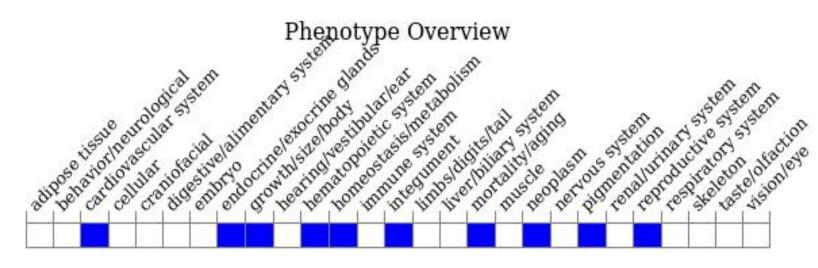
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Protein domain





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, mice homozygous for a conditional allele activated in prostate epithelial cells exhibit prostate gland hyperplasia and prostate intraepithelial neoplasia due to increased cell proliferation.

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



