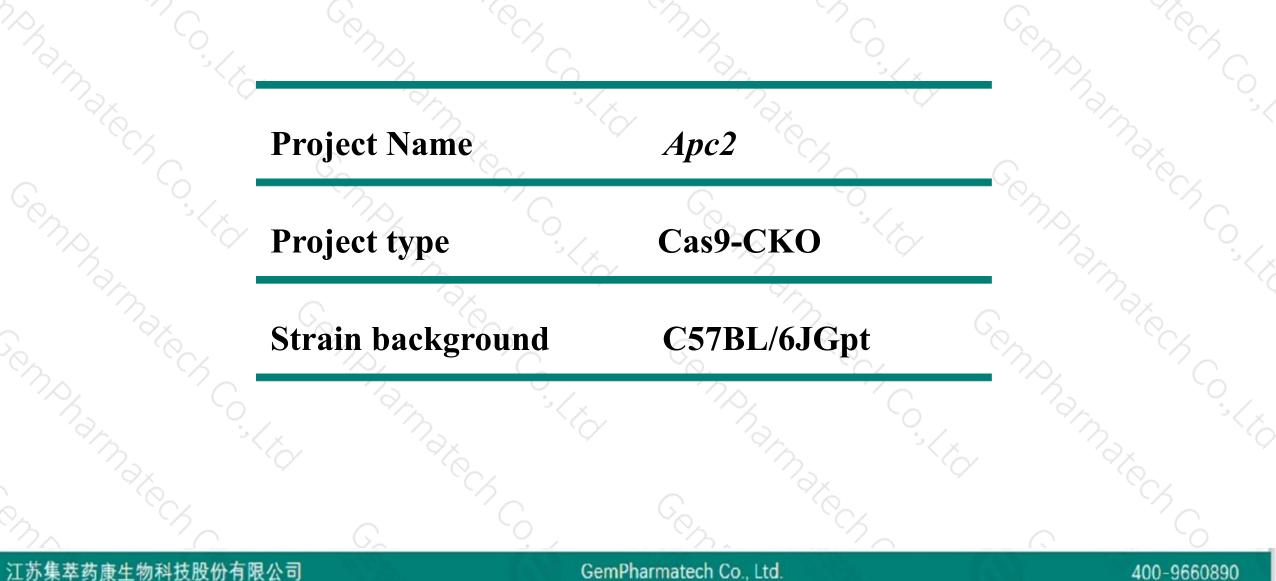


Apc2 Cas9-CKO Strategy

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Project Overview





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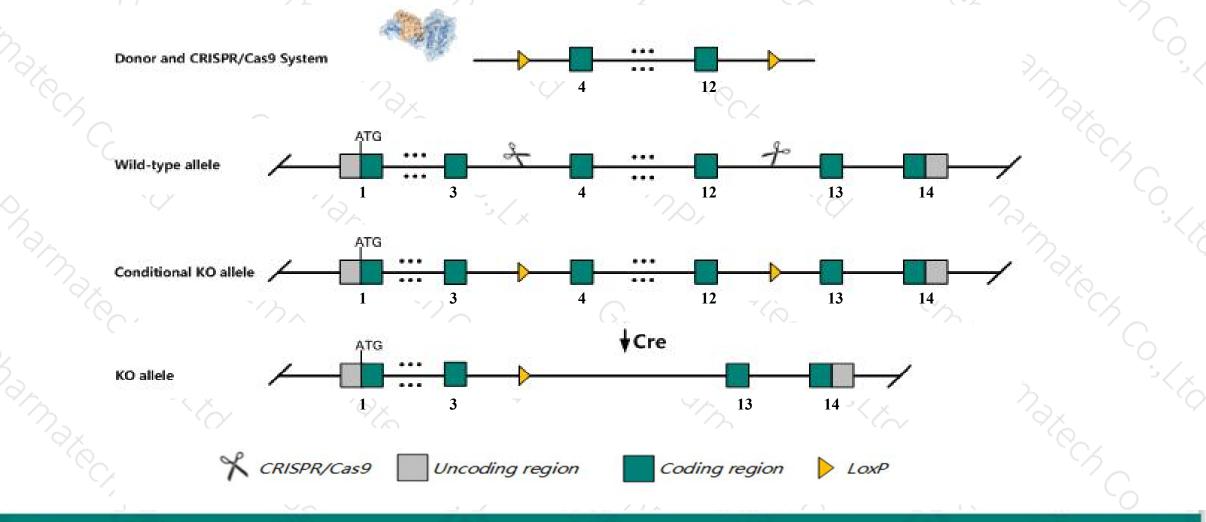
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Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the *Apc2* gene. The schematic diagram is as follows:



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The Apc2 gene has 6 transcripts. According to the structure of Apc2 gene, exon4-exon12 of Apc2-201 (ENSMUST0000020349.6) transcript is recommended as the knockout region. The region contains 1204bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Apc2* 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 null allele display gradual postnatal growth retardation, abnormal lamination of the cerebral cortex, hippocampus, olfactory bulb and cerebellum, impaired neuronal migration and impaired coordination.
- ➤ Transcript *Apc2*-204 may not be affected.
- ➤ The effect on transcript *Apc2*-203&205&206 is unknown.
- > The N-terminal of *Apc2* gene will remain several amino acids, it may remain the partial function of *Apc2* gene.
- The *Apc2* gene is located on the Chr10. 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.

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Gene information (NCBI)





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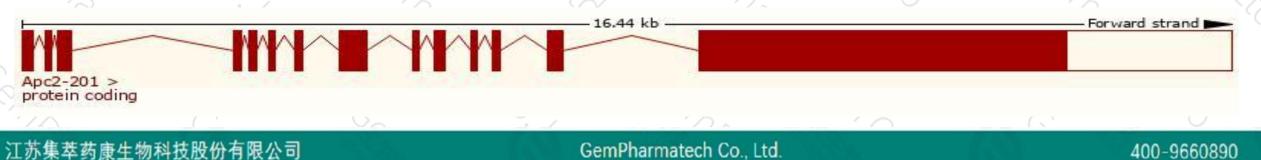
Transcript information (Ensembl)



The gene has 6 transcripts, all transcripts are shown below:

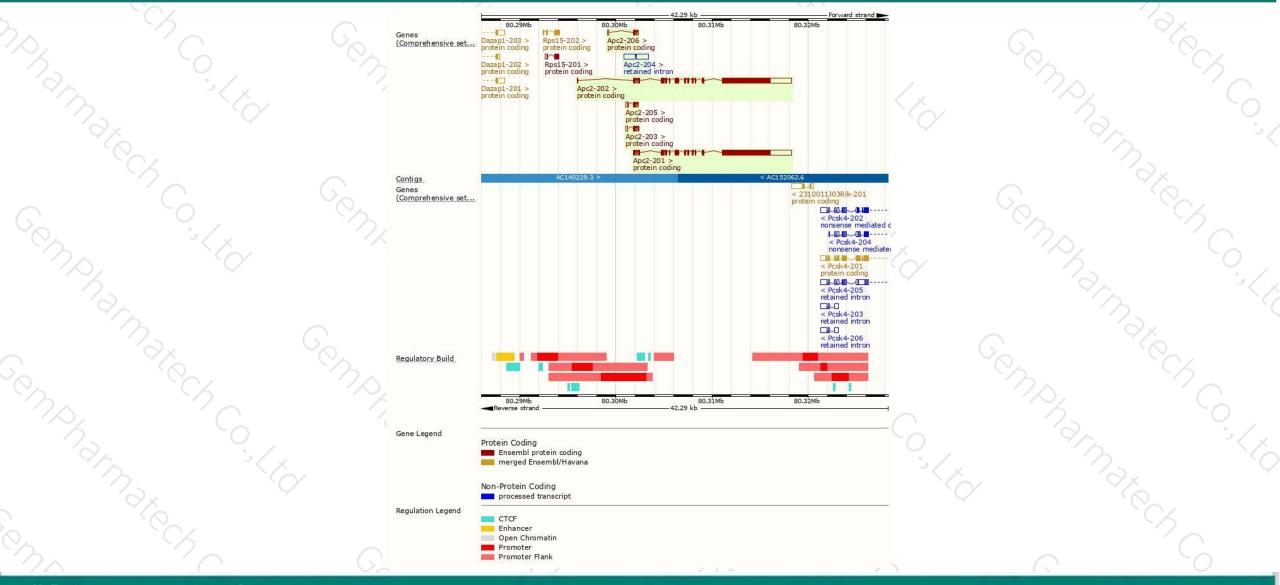
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Apc2-201	ENSMUST00000020349.6	9074	<u>2274aa</u>	Protein coding	CCDS24016	<u>G5E832</u>	TSL:5 GENCODE basic APPRIS P2
Apc2-202	ENSMUST00000105359.7	9173	<u>2303aa</u>	Protein coding	e .	D3YTR0	TSL:5 GENCODE basic APPRIS ALT2
Apc2-205	ENSMUST00000140828.7	566	<u>125aa</u>	Protein coding	5	<u>D3Z344</u>	CDS 3' incomplete TSL:1
Apc2-203	ENSMUST00000138909.7	558	<u>123aa</u>	Protein coding	-	D3Z3K9	CDS 3' incomplete TSL:3
Apc2-206	ENSMUST00000154212.7	486	<u>93aa</u>	Protein coding	2	D3YYQ9	CDS 3' incomplete TSL:2
Apc2-204	ENSMUST00000140658.1	2351	No protein	Retained intron	-	+3	TSL:1

The strategy is based on the design of Apc2-201 transcript, The transcription is shown below



Genomic location distribution





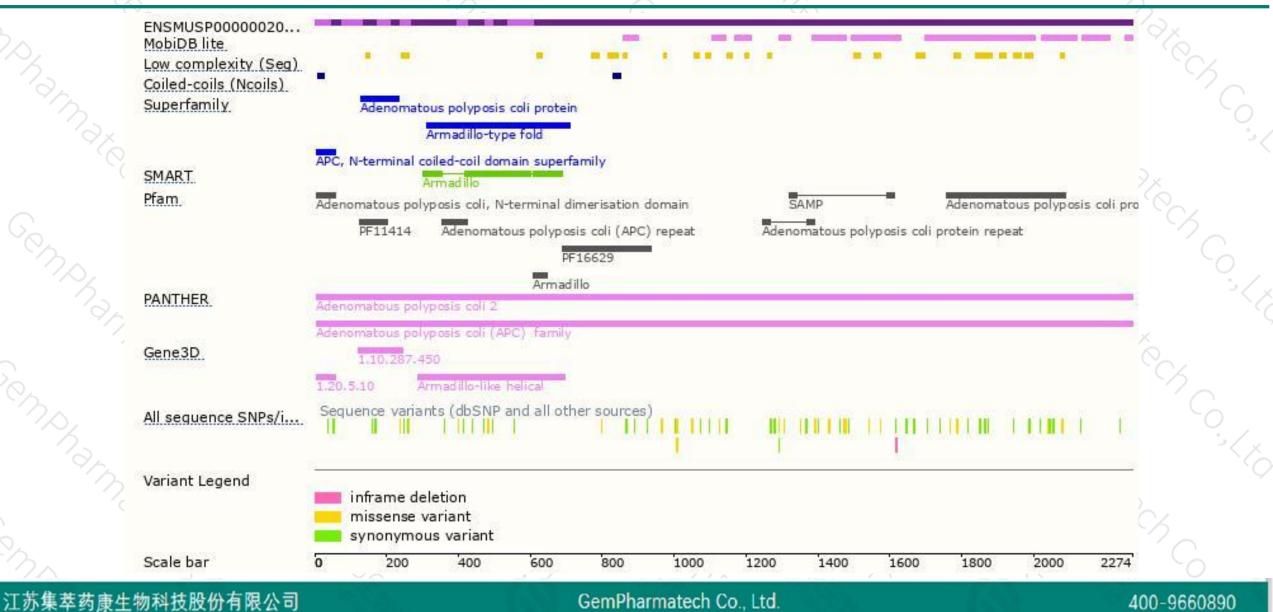
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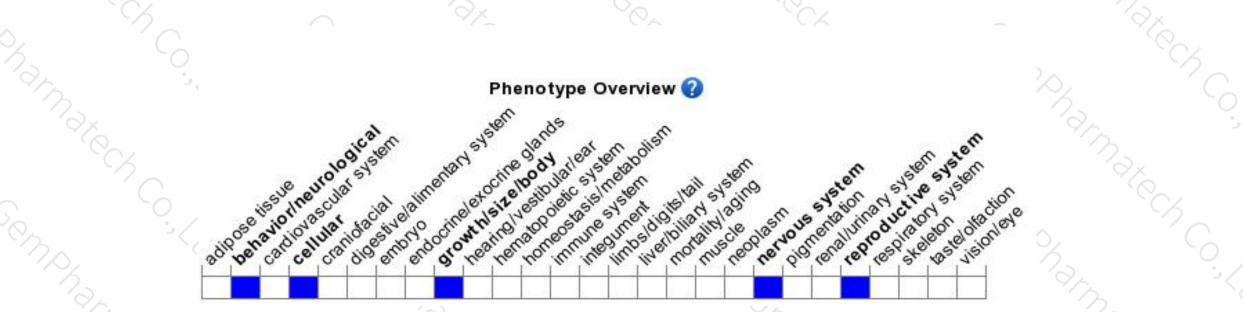
Protein domain





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 null allele display gradual postnatal growth retardation, abnormal lamination of the cerebral cortex, hippocampus, olfactory bulb and cerebellum, impaired neuronal migration and impaired coordination.

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



