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Apc Cas9-CKO Strategy

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Reviewer: Yanhua Shen

Design Date: 2022-11-15

Overview

Target Gene Name

- Apc

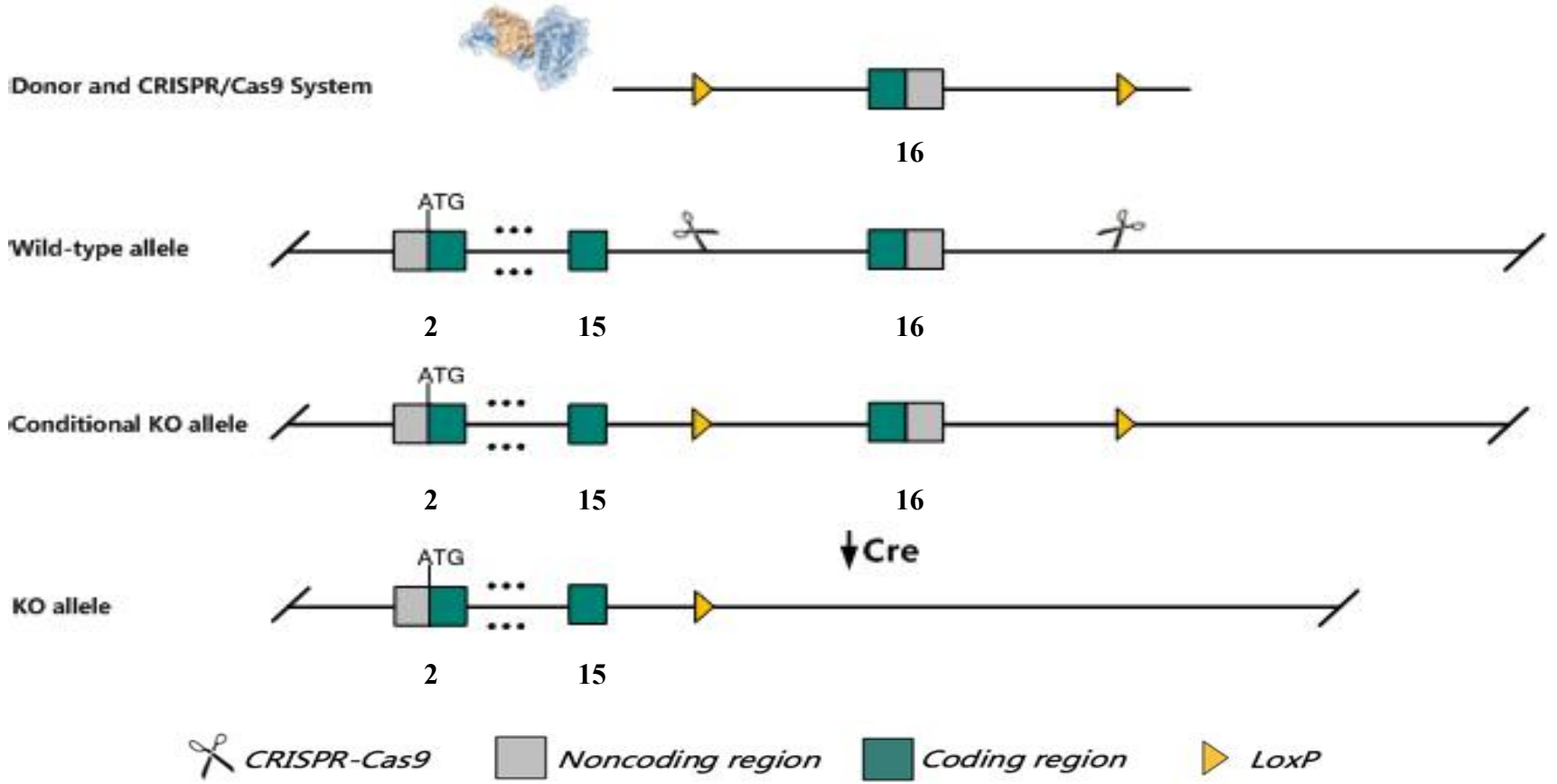
Project Type

- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy



Schematic representation of CRISPR-Cas9 engineering used to edit the *Apc* gene.

Technical Information

- The *Apc* gene has 9 transcripts. According to the structure of *Apc* gene, exon16 of *Apc-202* (ENSMUST00000079362.13) transcript is recommended as the knockout region. The region contains most of coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Apc* 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 on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- 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.

Gene Information

Apc APC, WNT signaling pathway regulator [Mus musculus (house mouse)]

Gene ID: 11789, updated on 12-Jul-2022

Summary

Official Symbol	Apc provided by MGI
Official Full Name	APC, WNT signaling pathway regulator provided by MGI
Primary source	MGI:MGI:88039
See related	Ensembl:ENSMUSG00000005871
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	AI047805, AU020952, AW124434, CC1, Min, mAPC
Expression	Broad expression in frontal lobe adult (RPKM 29.9), CNS E18 (RPKM 22.5) and 18 other tissues See more
Orthologs	human all

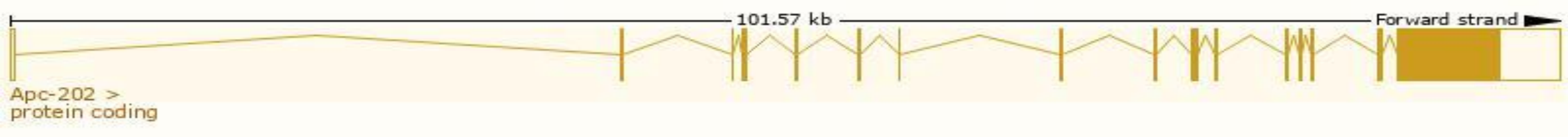
Source: https://www.ncbi.nlm.nih.gov/

Transcript Information

The gene has 9 transcripts, all transcripts are shown below:

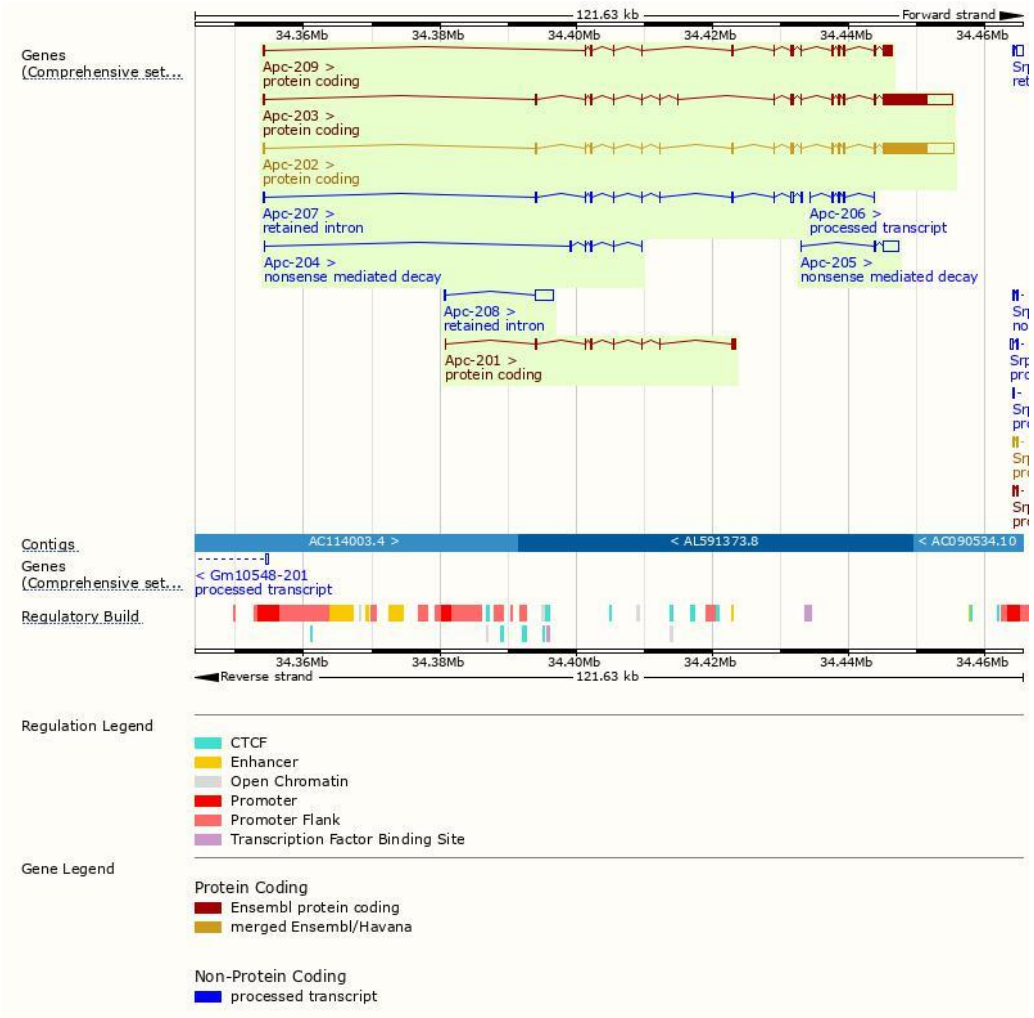
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Apc-202	ENSMUST00000079362.13	12811	2842aa	Protein coding	CCDS29125		TSL:1 , GENCODE basic , APPRIS P2 ,
Apc-203	ENSMUST00000115781.10	12346	2808aa	Protein coding	-		TSL:5 , GENCODE basic , APPRIS ALT2 ,
Apc-209	ENSMUST00000171187.8	3588	1133aa	Protein coding	-		CDS 3' incomplete , TSL:5 ,
Apc-201	ENSMUST00000066133.7	1185	324aa	Protein coding	-		TSL:1 , GENCODE basic ,
Apc-205	ENSMUST00000165590.2	2633	51aa	Nonsense mediated decay	-		CDS 5' incomplete , TSL:5 ,
Apc-204	ENSMUST00000163295.2	608	21aa	Nonsense mediated decay	-		CDS 5' incomplete , TSL:5 ,
Apc-206	ENSMUST00000167136.2	435	No protein	Processed transcript	-		TSL:3 ,
Apc-208	ENSMUST00000170195.2	2732	No protein	Retained intron	-		TSL:1 ,
Apc-207	ENSMUST00000170023.8	1825	No protein	Retained intron	-		TSL:1 ,

The strategy is based on the design of *Apc-202* transcript, the transcription is shown below:



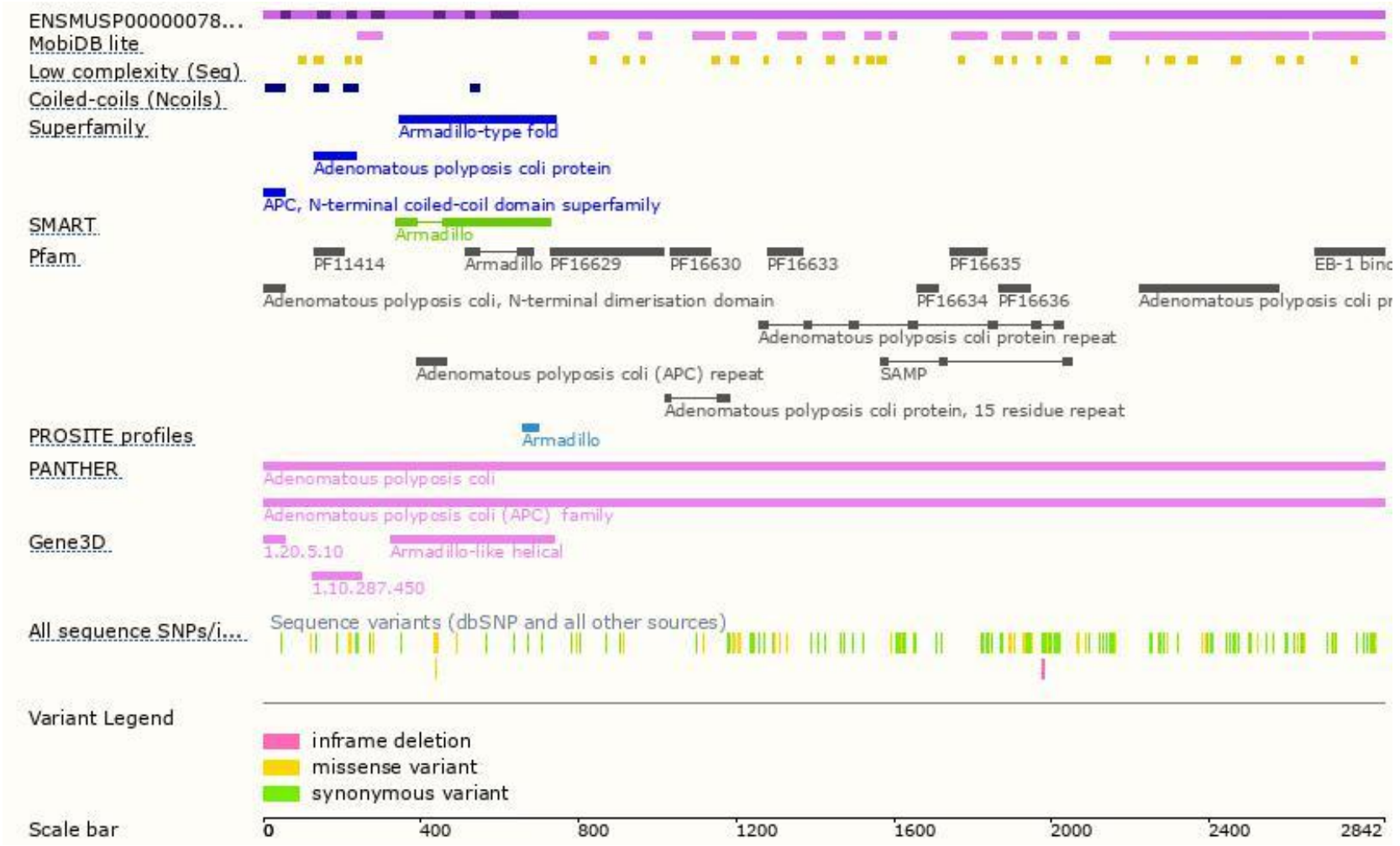
Source: https://www.ensembl.org

Genomic Information



Source: : https://www.ensembl.org

Protein Information



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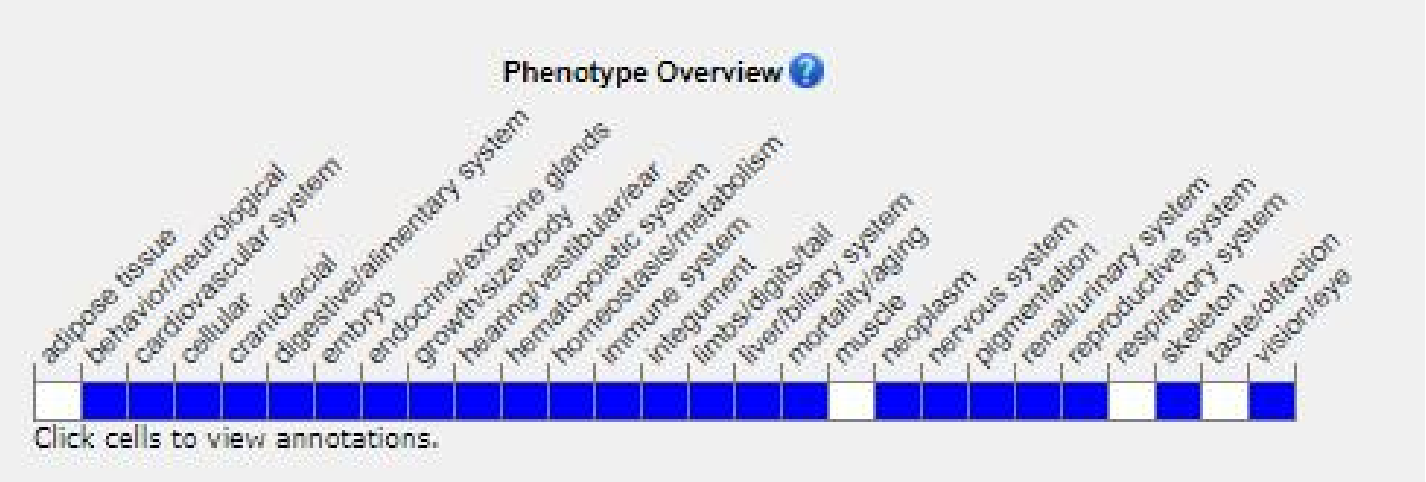
Source: : https://www.ensembl.org

Mouse Phenotype Information (MGI)



- Most targeted and hypomorphic heterozygous mutants develop intestinal polyps and colorectal cancer, associated with anemia from intestinal bleeding. Homozygotes are embryonic lethal. Homozygotes for a mild alleles survive and have less extreme tumor incidence.

Source: https://www.informatics.jax.org

Important Information

- According to the existing MGI data,most targeted and hypomorphic heterozygous mutants develop intestinal polyps and colorectal cancer, associated with anemia from intestinal bleeding. Homozygotes are embryonic lethal. Homozygotes for a mild alleles survive and have less extreme tumor incidence.
- The effect of this strategy on *Apc*-201 transcript is unknown.
- *Apc* is located on Chr18. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is 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 the existing technology level.