

# Cdh1 Cas9-CKO Strategy

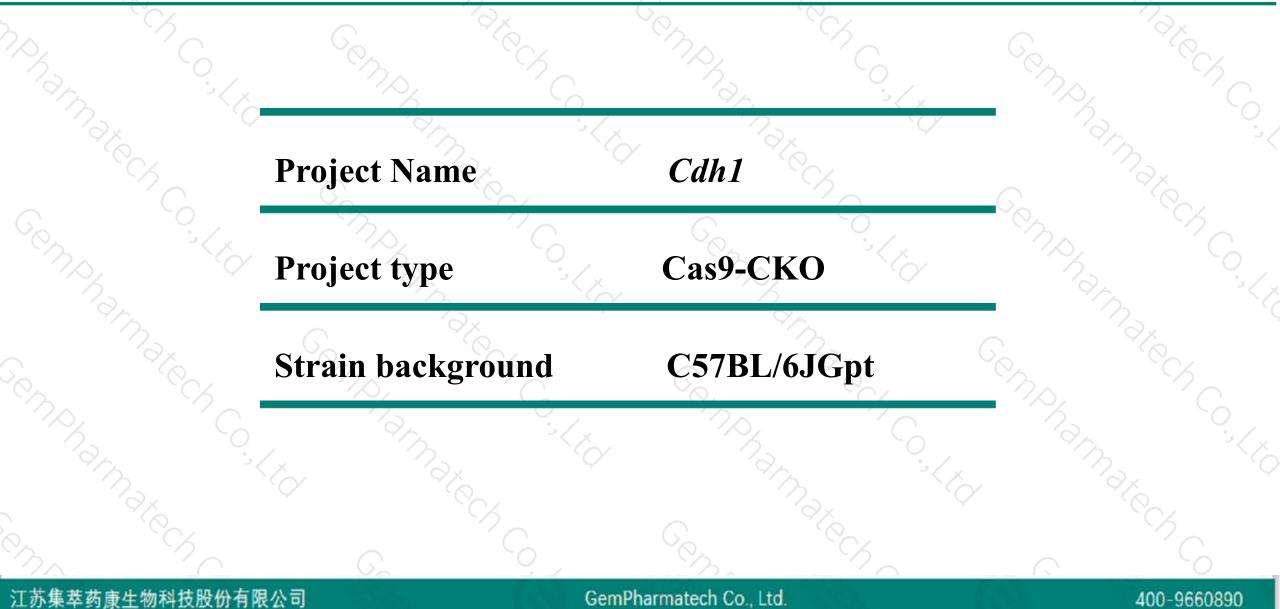
Designer: Reviewer:

Design Date:

Huan Wang Huan Fan 2019-12-25

# **Project Overview**



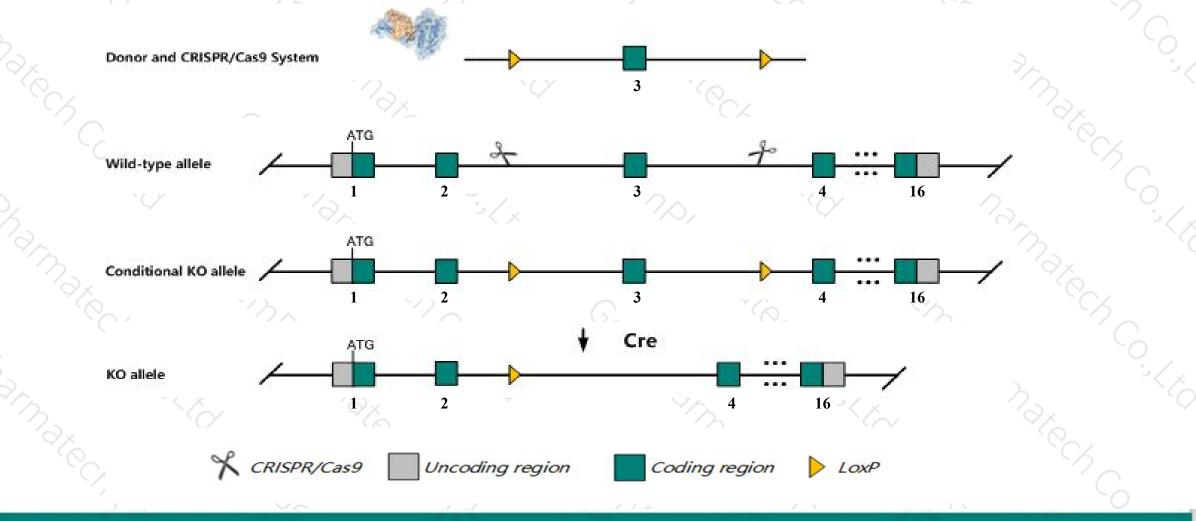


# **Conditional Knockout strategy**



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Cdh1* gene. The schematic diagram is as follows:



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The *Cdh1* gene has 3 transcripts. According to the structure of *Cdh1* gene, exon3 of *Cdh1-201* (ENSMUST0000000312.11) transcript is recommended as the knockout region. The region contains 224bp coding sequence.
Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Cdh1* 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, In mutant homozygotes, adhesive cells of the morula dissociate shortly after initial compaction, probably due to depletion of maternal protein. Mutant embryos fail to form a trophectodermal epithelium or blastocyst cavity, and die near implantation time.
- The Cdh1 gene is located on the Chr8. 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)



~ 1

### Cdh1 cadherin 1 [Mus musculus (house mouse)]

Gene ID: 12550, updated on 2-Apr-2019

### Summary

- Official Symbol Cdh1 provided by MGI Official Full Name cadherin 1 provided byMGI Primary source MGI:MGI:88354 See related Ensembl:ENSMUSG0000000303 Gene type protein coding RefSeg status REVIEWED Organism Mus musculus Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus Also known as AA960649, ARC-1, E-cad, Ecad, L-CAM, UVO, Um Summary This gene encodes E-cadherin, a calcium-dependent cell adhesion molecule that functions in the establishment and maintenance of epithelial cell morphology during embryongenesis and adulthood. The encoded preproprotein undergoes proteolytic processing to generate a mature protein. Targeted mutations disrupting binding of calcium to the encoded protein in mice cause death in utero due to failed blastocyst and trophectoderm formation. This gene is located adjacent to a related cadherin gene on chromosome 8. [provided by RefSeq, Oct 2015]
  - Expression Broad expression in colon adult (RPKM 139.2), large intestine adult (RPKM 100.0) and 15 other tissues See more

Orthologs human all

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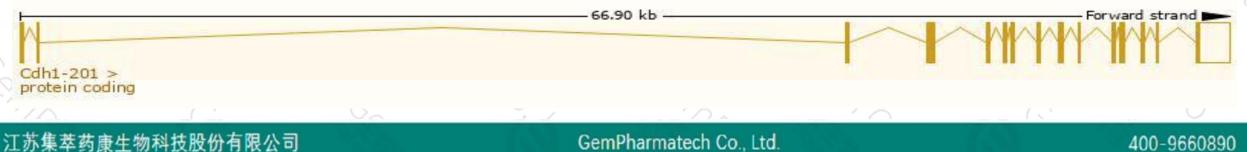
# **Transcript information (Ensembl)**



The gene has 3 transcripts, all transcripts are shown below:

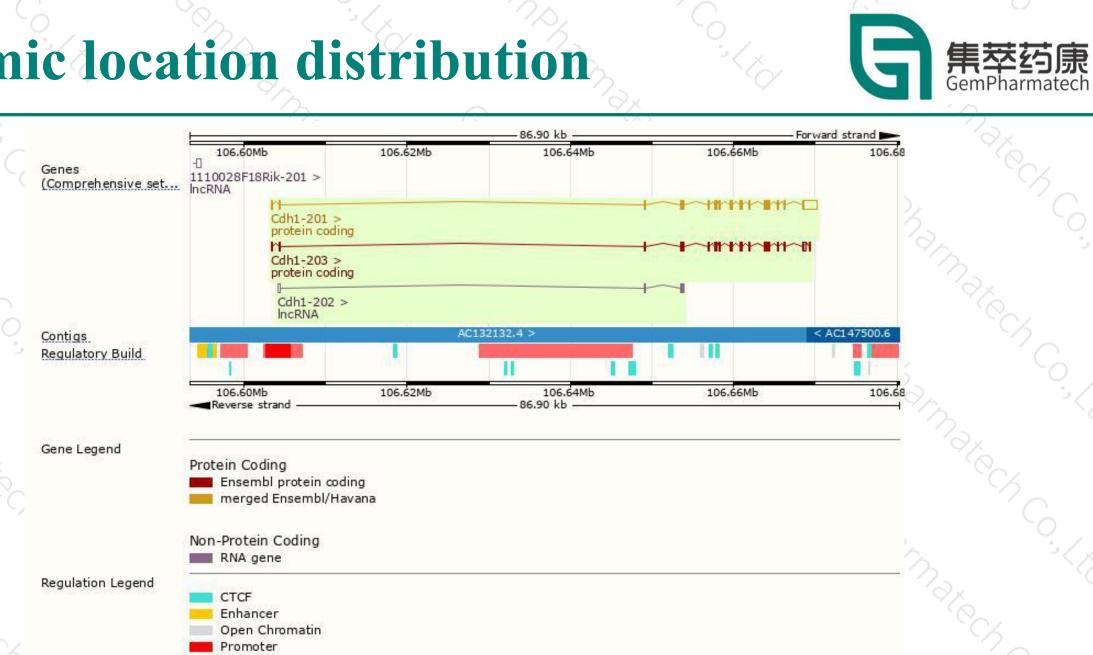
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cdh1-201	ENSMUST0000000312.11	4430	<u>884aa</u>	Protein coding	CCDS22638	A0A0R4IZW5	TSL:1 GENCODE basic APPRIS P1
Cdh1-203	ENSMUST00000167688.1	3203	<u>884aa</u>	Protein coding	CCDS22638	A0A0R4IZW5	TSL:5 GENCODE basic APPRIS P1
Cdh1-202	ENSMUST00000136580.1	789	No protein	IncRNA	(23)	2	TSL:2

The strategy is based on the design of *Cdh1-201* transcript, The transcription is shown below



## **Genomic location distribution**

Promoter Flank



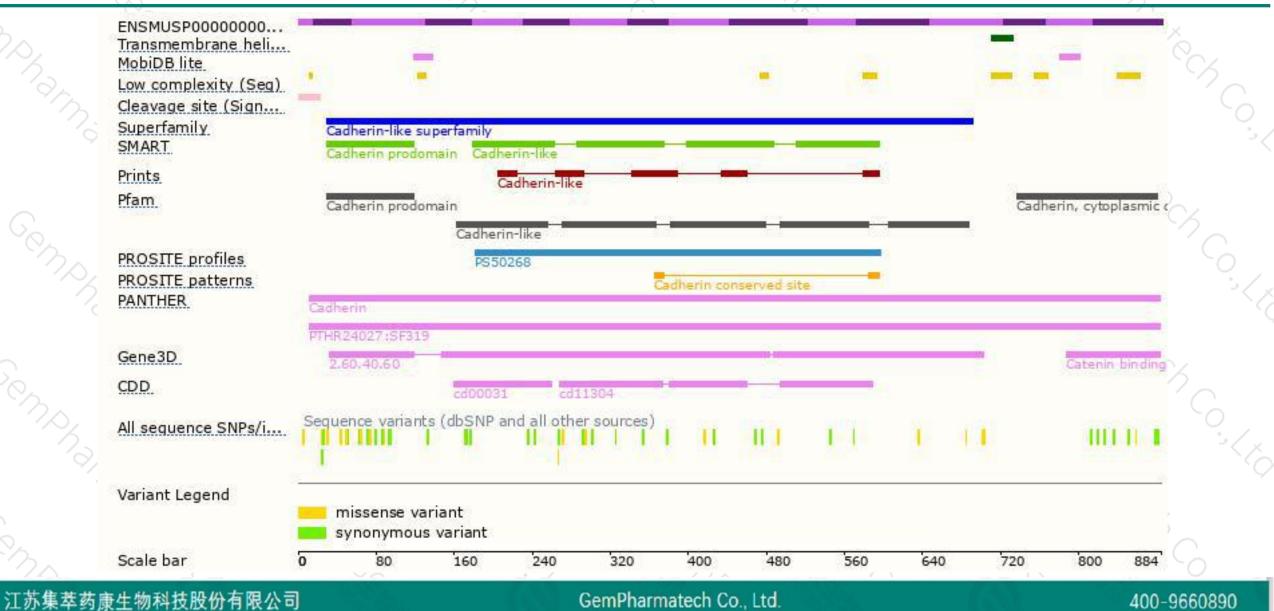
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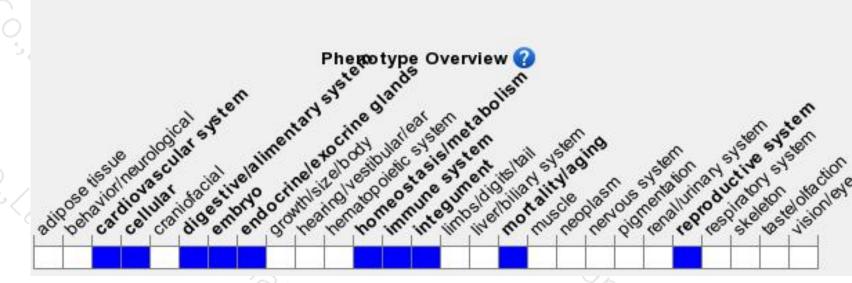
## **Protein domain**





# 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, In mutant homozygotes, adhesive cells of the morula dissociate shortly after initial compaction, probably due to depletion of maternal protein. Mutant embryos fail to form a trophectodermal epithelium or blastocyst cavity, and die near implantation time.

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



