

***Cdh16-CreERT2-P2A* Cas9-KI Strategy**

Designer:

Xueting Zhang

Reviewer:

Yanhua Shen

Design Date:

2018/8/28

Project Overview

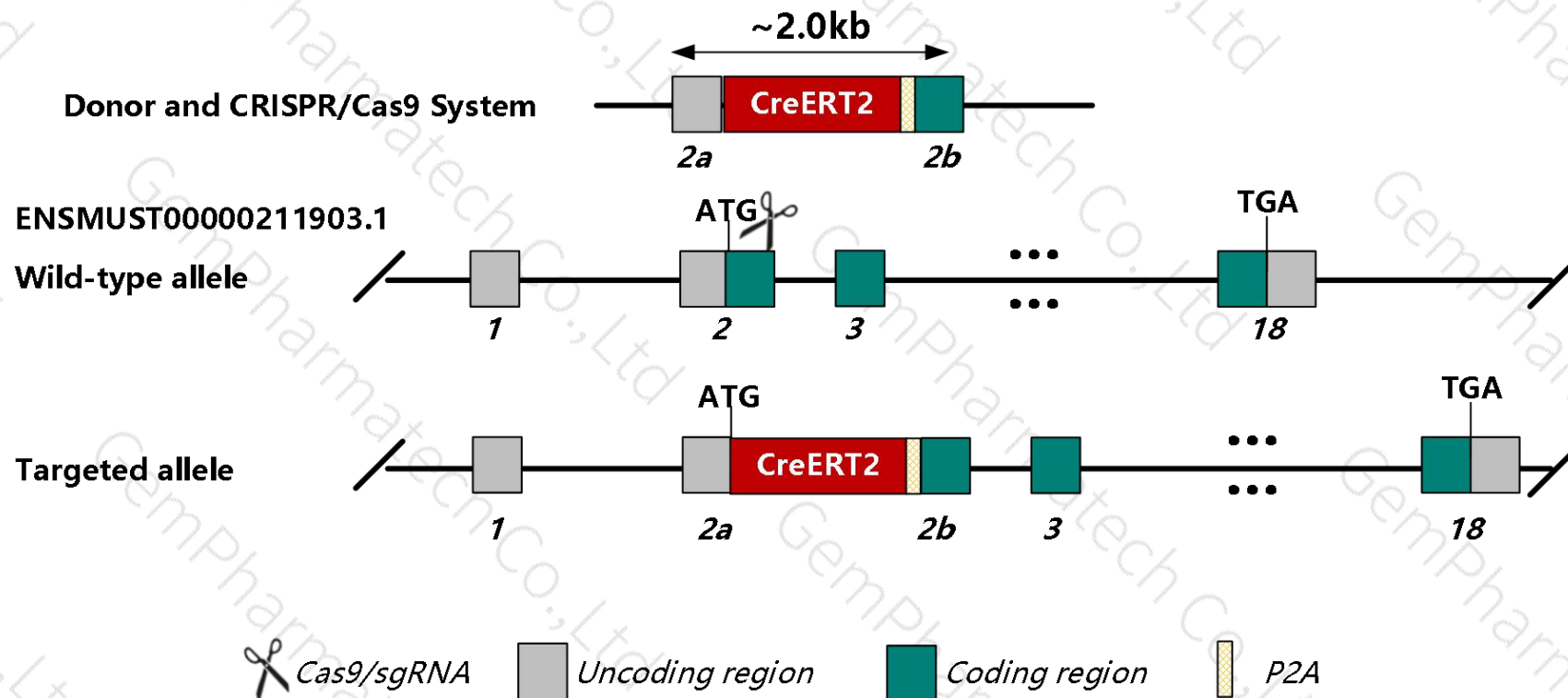
Project Name	<i>Cdh16-CreERT2-P2A</i>
---------------------	---------------------------------

Project type	Cas9-KI
---------------------	----------------

Strain background	C57BL/6JGpt
--------------------------	--------------------

Knockin strategy

This model will use CRISPR/Cas9 technology to edit the *Cdh16* gene. The schematic diagram is as follows:



- The *Cdh16* gene has 14 transcripts. According to the structure of *Cdh16* gene, *Cdh16*-204(ENSMUST00000211903.1) is selected for presentation of the recommended strategy.
- *Cdh16*-204 gene has 18 exons, with the ATG start codon in exon2 and TGA stop codon in exon18.
- We make *Cdh16*-*CreERT2*-*P2A* knockin mice via CRISPR/Cas9 system. Cas9 mRNA, sgRNA and donor will be co-injected into zygotes. sgRNA direct Cas9 endonuclease cleavage near translational start coding of *Cdh16* gene, and create a DSB(double-strand break). Such breaks will be repaired, and result in the insertion of *CreERT2*-*P2A* after translational start coding of *Cdh16* gene by homologous recombination. The pups will be genotyped by PCR, followed by sequence analysis.

- The expression of two genes linked with P2A peptide is driven by the same promoter, and the fused protein will be cleaved into two proteins folding independently, while the former will carry the P2A-translated polypeptide.
- The insertion of *CreERT2* may affect the 5-terminal regulation of the *Cdh16* gene.
- There will be 1 to 2 amino acid synonymous mutation in exon2 of *Cdh16* gene in this strategy.
- The *Cdh16* gene is located on the Chr8. If the knock in 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 gene transcription and translation processes, all risks cannot be predicted under existing information.

Gene information (NCBI)

Cdh16 cadherin 16 [*Mus musculus* (house mouse)]

Gene ID: 12556, updated on 12-Aug-2018

Summary

- Official Symbol

Cdh16 provided by [MGI](#)
- Official Full Name

cadherin 16 provided by [MGI](#)
- Primary source

[MGI:MGI:106671](#)
- See related

[Ensembl:ENSMUSG000000031881](#) [Vega:OTTMUSG000000061956](#)
- 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
- Expression

Restricted expression toward kidney adult (RPKM 275.2) [See more](#)
- Orthologs

[human](#) [all](#)

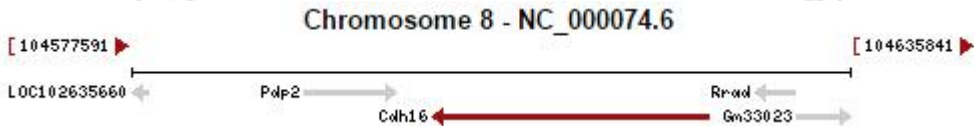
Genomic context

Location: 8 D3; 8 53.04 cM

See Cdh16 in [Genome Data Viewer](#)

Exon count: 19

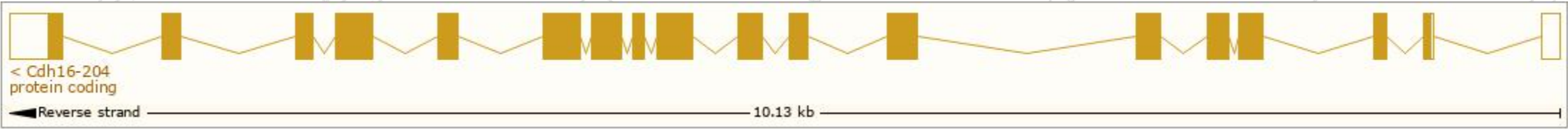
Annotation release	Status	Assembly	Chr	Location
106	current	GRCm38.p4 (GCF_000001635.24)	8	NC_000074.6 (104601915..104624396, complement)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	8	NC_000074.5 (107138047..107148161, complement)



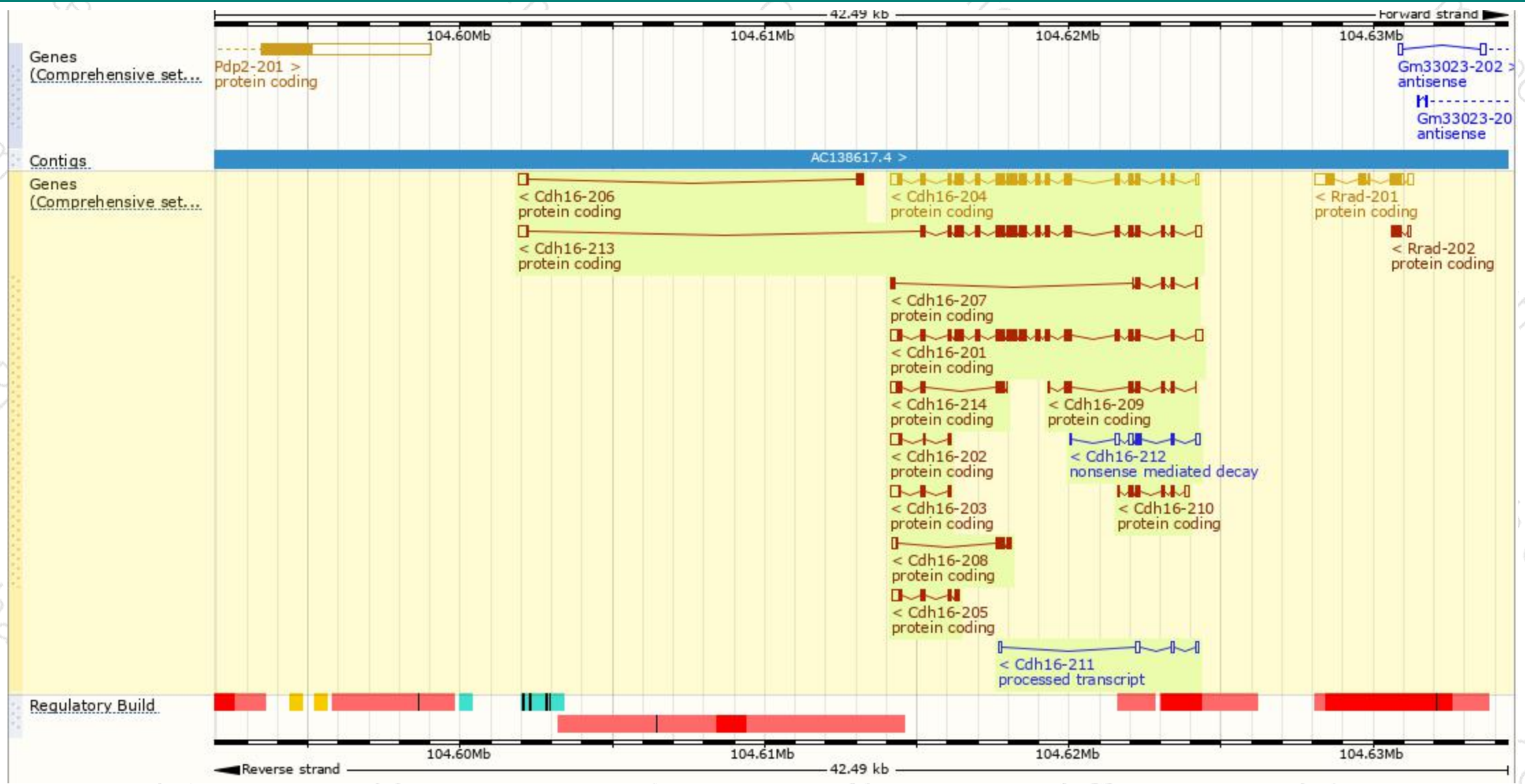
Transcript information (Ensembl)

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Cdh16-214	ENSMUST00000213033.1	733	179aa	Protein coding	-	A0A1D5RMA8	-	CDS 5' incomplete TSL:5
Cdh16-213	ENSMUST00000212882.1	2923	815aa	Protein coding	CCDS85595	Q8C730	NM_001252628 NP_001239557	TSL:1 GENCODE basic APPRIS ALT2
Cdh16-212	ENSMUST00000212748.1	730	58aa	Nonsense mediated decay	-	A0A1D5RMK0	-	TSL:5
Cdh16-211	ENSMUST00000212689.1	421	No protein	Processed transcript	-	-	-	TSL:5
Cdh16-210	ENSMUST00000212662.1	632	163aa	Protein coding	-	A0A1D5RMI6	-	CDS 3' incomplete TSL:3
Cdh16-209	ENSMUST00000212447.1	720	223aa	Protein coding	-	A0A1D5RMM2	-	CDS 3' incomplete TSL:5
Cdh16-208	ENSMUST00000212420.1	594	142aa	Protein coding	-	A0A1D5RMB7	-	CDS 5' incomplete TSL:5
Cdh16-207	ENSMUST00000212324.1	506	141aa	Protein coding	-	A0A1D5RLG9	-	CDS 3' incomplete TSL:5
Cdh16-206	ENSMUST00000212318.1	586	98aa	Protein coding	-	A0A1D5RLE7	-	CDS 5' incomplete TSL:5
Cdh16-205	ENSMUST00000212045.1	646	149aa	Protein coding	-	A0A1D5RM27	-	CDS 5' incomplete TSL:3
Cdh16-204	ENSMUST00000211903.1	2874	830aa	Protein coding	CCDS22583	O88338 Q546A8	NM_007663 NP_031689	TSL:1 GENCODE basic APPRIS P3
Cdh16-203	ENSMUST00000211889.1	519	93aa	Protein coding	-	A0A1D5RLS8	-	CDS 5' incomplete TSL:5
Cdh16-202	ENSMUST00000211849.1	474	75aa	Protein coding	-	A0A1D5RL88	-	CDS 5' incomplete TSL:3
Cdh16-201	ENSMUST00000163783.3	2910	800aa	Protein coding	CCDS85594	Q3TPA4	NM_001252627 NP_001239556	TSL:1 GENCODE basic APPRIS ALT2

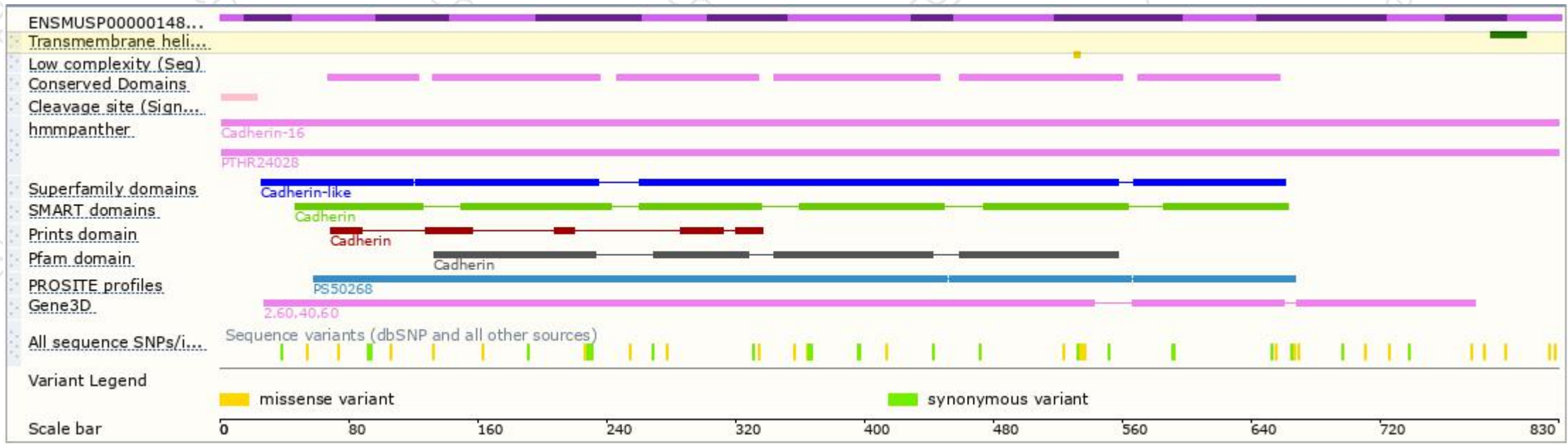
The strategy is based on the design of *Cdh16-204* transcript,the transcription is shown below:



Genomic location distribution



Protein domain



If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534



集萃药康生物科技
GemPharmatech Co.,Ltd

