

***H11-Cdh16-iCre-*ployA** Cas9-KI Strategy**

Designer:

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Design Date:

2019-8-15

Reviewer

Huimin Su

Project Overview

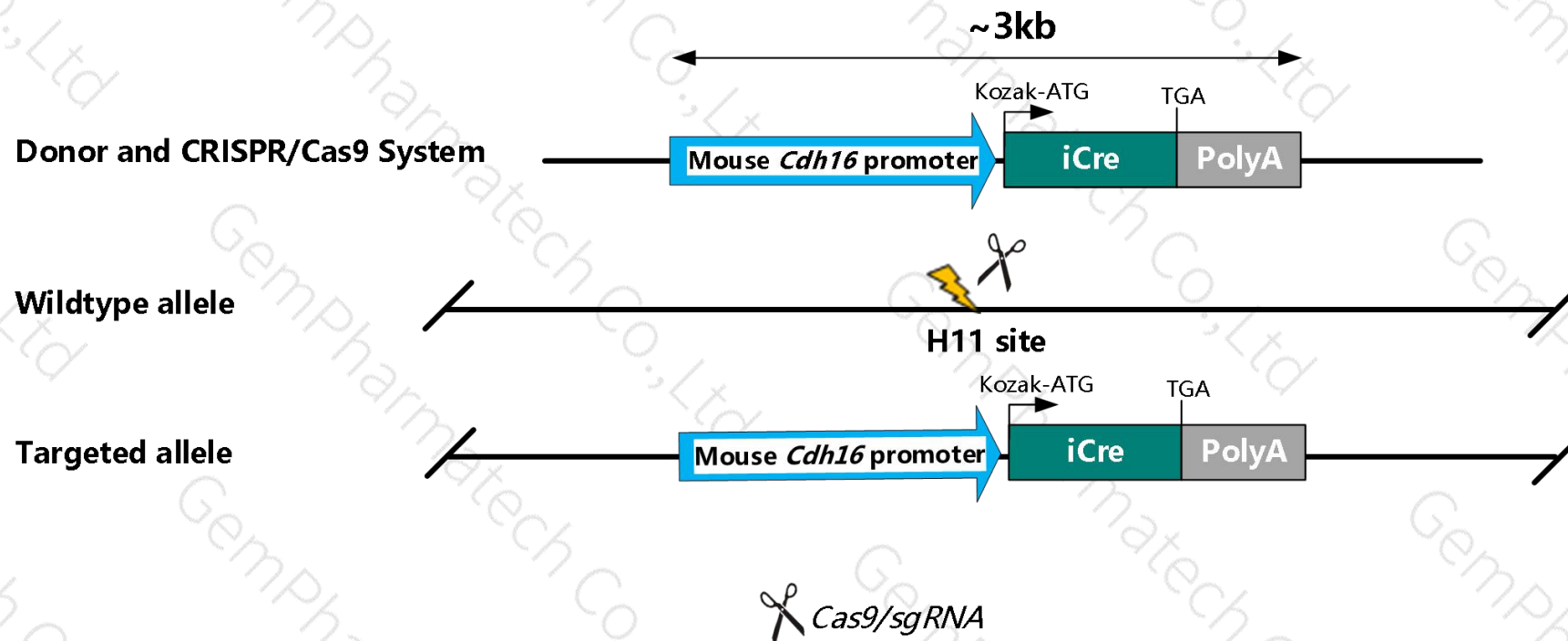
Project Name	<i>H11-Cdh16-iCre-<i>ployA</i></i>
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Project type	Cas9-KI
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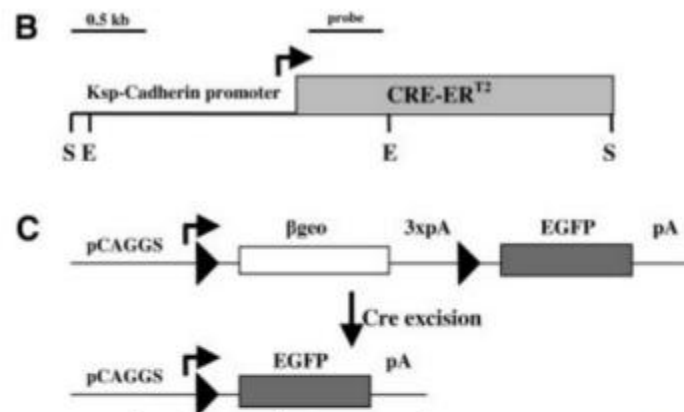
Strain background	C57BL/6J
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Knockin strategy

The *Cdh16-iCre-loyA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:



Summary of mouse *Cdh16* promoter^[1]



protein is not expressed, leaving floxed transgenes intact. **B:** Schematic representation of the *KspCad-CreER^{T2}* construct. The tamoxifen-inducible CreER^{T2} is placed under the control of regulatory elements of the *Ksp-cadherin* gene. Restriction sites used to linearize the DNA construct and for Southern blot analysis are shown: S, SalI and E, EcoRI. The probe used for genotyping by Southern blotting is shown. **C:** The Z/EG reporter gene (Novak *et al.*, 2000). In the absence of active Cre recombinase there is expression from the β-geo-cassette, resulting in β-galactosidase expression. Deletion of the floxed β-geo cassette by Cre-mediated recombination results in expression of EGFP. pCAGGS, modified chicken β-actin promoter; β-geo, β-geo cassette (that is, lacZ/neomycin-resistance fusion gene); pA, polyadenylation sequence; EGFP, enhanced green fluorescence protein; ►, loxP site. Adapted from Novak *et al.* (2000).

A 3606-bp promoter fragment of the murine Ksp-cadherin gene was amplified by PCR using *PfuTurbo* DNA polymerase (Stratagene, La Jolla, CA) and the primers KspF1 5'-CCACACACATAAAGGGAAACTGAGG-3' and KspR1 5'-CAAGAGCTCTCAGGCACTCACCTT-3'. In a nested PCR reaction, a 1681-bp promoter fragment was generated with the primers KspF3 5'-GGGAGCCCTTGAGACCTAGT-3' and KspR2 5'-ACAACTGCAGTCTCCCTTGGTCCAGT-TTCCAG-3' (with attached *Pst*I site in bold). The 1681-bp PCR product was digested with *Pst*I and *Hind*III and the resulting 1.4-kb *Hind*III-*Pst*I fragment (containing bp 2430-3825 of GenBank Access. No. AF118228) was

- 1、Irma S. Lantinga-van Leeuwen, et.al. (2006). Transgenic Mice Expressing Tamoxifen-Inducible Cre for Somatic Gene Modification in Renal Epithelial Cells. *Genesis*. 44:225-232

The promoter Sequence of Mouse *Cdh16*(1397bp)

AAGCTTGCTCTGCCATGGGAAGGTCCCCAAACCTGAAAAAGAAACCCAGGCGCCTTGGGCAAACGGCTTAGCCTCTCTGTACCCCAGAGGGGTAT
GACAAGGGGGACAGTTCCCTCTTCAGAATGAGTGGGTGGGATAGCAGCGATATGTGCTCACAGAAGTTCTGGGTGCTGGTTAGTAGGCACTCAGTAT
GTGTGAGACCTCCTTCAACAACCCACATATAGCACTAACCTAGGCTCAGTGCCTCAGTGATCCTAAAACAGACACTCAGCTTGTCCCAGTCCCGCT
TCCATCTGGAATGCTGACCAGATATACCATGAGCAGTCCTCGGGGGAGACCCCAAACATGGAAGGAAAGTGAGAGGAGGGAGACCAGGGTCTTC
ATACTCTGTACCCTCATCTGAGATTCCCAGAAAACAAGGTTTCTGCTGGGTCTGCCTTTCAGGCCTTACAGGGGGAGGTTGAGGAGGCCCTGGTCAG
CCCTGTAACCTCCCTCAAAGTCACCTGAGCTGGCTCGGTCTTCTCTTTTCTGCTTAGAGACATGAGACAGGTCAGAGCCTCACCTTATCTGTGTTCA
GGAGACCATGTGAGGCTAGGAGAGCTTCAGGGGGACCTCTAGGCTTCTGTCCCACCCACTGGTCTCACTGATGAATGTTCTAGAGCTCCGAACACA
CACTTGGGGTCAGCTGCCCTTTGGGTACACTCCCAGTCCTTTCCTTAGCTCCACGGGCCACTTGGGGCAGGACAAGGTCATAGCCTACTCAGAGG
AACTCCGAAGCTAATACGTGAGAAACAAGCTGGTGTCTTCTGGGCATCCGTAGAGGCAGTTCATGGTATGGAGTGGGGGAGCCTAATCCAGCCTGT
GAATGTAAGGGCTTTCCTGAAGAAGGGGTTATTAGTGCTTGTTGCTAAAGCGTAAGCAGGTACTTACTGTCCTGTAGAAAGGTGGGAAGAGCATTC
CAGGCACAAGGAACAATATCTGGAGAGATCTGGCTACAGATGCCACTGCACAGAGGAGAAAGTGGGAGCCAAGTCTGAACACACACACACACACA
CACACACACACACACAGAAGTTATGTCTAAGCCCAGAGGGCCAGCAAGTGCTCATTGGGCTGTGTCAAGGGGGCAGTGACAGACCAAGAGCTGC
CCACCTCCTAGGGCTGGCAGTCACGGATGCTGAGCAGATCTGGCTCTCCAAAGTCAATAAGTAACTTGGGGGGACTAGGCGGGGCCAGGCCTGCT
CCTGTGGGCCCCGGTGGCATTTCCTCACTCCTGAGCAAGCACGGCCAGACCGCCTACCTGCTCAAGTGTCCACCTTGCCTCGCCCCACCTAAGCCAA
ATTGCCAGAGCTCCCTGAAGAAGGATTCCTTCTCCTGGAACTGGACCAAGGGAGA

We choose 1397bp fragment of *Cdh16* as *Cdh16* promoter to drive Cre expression according to the reference^[1] described

- The *Cdh16* gene has 13 transcripts. According to the structure of *Cdh16* gene, *Cdh16-204*(ENSMUST00000211903.1) is selected for presentation of the recommended strategy.
- *Cdh16-204* gene has 17 exons, with the ATG start codon in exon2 and TGA stop codon in exon17.
- The *Cdh16* promoter is from article, the length is about 1.4kb.
- H11, located on mouse chromosome 11, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene.
- In this study, the *Cdh16-iCre-plotA* gene fragment was inserted into H11 site of mice by CRISPR/Cas9 technology. The brief process is as follows: the donor vector and sgRNA were constructed in vitro, Cas9, donor and sgRNA were microinjected into the fertilized eggs of C57BL/6J mice, and F0 generation mice were obtained. The F0 positive mice were mated with C57BL/6J mice by PCR, sequencing, and southern blot, then the stable inheritance of F1 positive mice model was obtained.

- H11 is located on Chr11. Please take the loci in consideration when breeding the Knock-in mice with other gene modified (e.g., iCre) strains, if the other gene is also on Chr11, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

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

Gene ID: 12556, updated on 12-Aug-2019

Summary

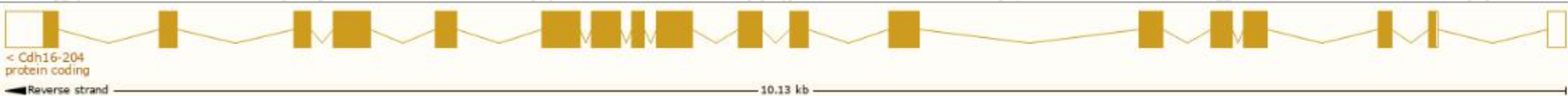
Official Symbol	Cdh16 provided by MGI
Official Full Name	cadherin 16 provided by MGI
Primary source	MGI:MGI:106671
See related	Ensembl:ENSMUSG00000031881
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Restricted expression toward kidney adult (RPKM 275.2) See more
Orthologs	human all

Transcript information (Ensembl)

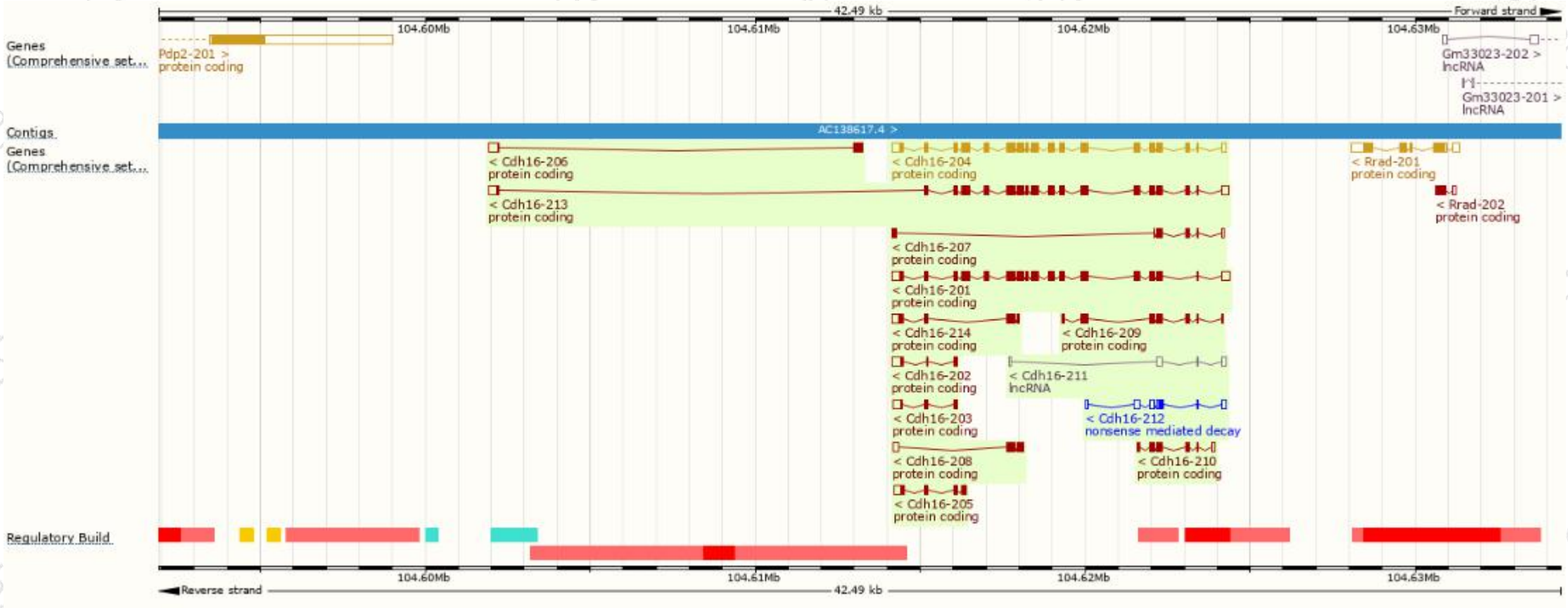
The gene has 13 transcripts, and the transcript is shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cdh16-213	ENSMUST00000212882.1	2923	815aa	Protein coding	CCDS85595	Q8C730	TSL:1 GENCODE basic APPRIS ALT2
Cdh16-201	ENSMUST00000163783.3	2910	800aa	Protein coding	CCDS85594	Q3TPA4	TSL:1 GENCODE basic APPRIS ALT2
Cdh16-204	ENSMUST00000211903.1	2874	830aa	Protein coding	CCDS22583	O88338 Q546A8	TSL:1 GENCODE basic APPRIS P3
Cdh16-214	ENSMUST00000213033.1	733	179aa	Protein coding	-	A0A1D5RMA8	CDS 5' incomplete TSL:5
Cdh16-209	ENSMUST00000212447.1	720	223aa	Protein coding	-	A0A1D5RMM2	CDS 3' incomplete TSL:5
Cdh16-205	ENSMUST00000212045.1	646	149aa	Protein coding	-	A0A1D5RM27	CDS 5' incomplete TSL:3
Cdh16-210	ENSMUST00000212662.1	632	163aa	Protein coding	-	A0A1D5RMI6	CDS 3' incomplete TSL:3
Cdh16-208	ENSMUST00000212420.1	594	142aa	Protein coding	-	A0A1D5RMB7	CDS 5' incomplete TSL:5
Cdh16-206	ENSMUST00000212318.1	586	98aa	Protein coding	-	A0A1D5RLE7	CDS 5' incomplete TSL:5
Cdh16-203	ENSMUST00000211889.1	519	93aa	Protein coding	-	A0A1D5RLS8	CDS 5' incomplete TSL:5
Cdh16-207	ENSMUST00000212324.1	506	141aa	Protein coding	-	A0A1D5RLG9	CDS 3' incomplete TSL:5
Cdh16-202	ENSMUST00000211849.1	474	75aa	Protein coding	-	A0A1D5RL88	CDS 5' incomplete TSL:3
Cdh16-212	ENSMUST00000212748.1	730	58aa	Nonsense mediated decay	-	A0A1D5RMKQ	TSL:5
Cdh16-211	ENSMUST00000212689.1	421	No protein	lncRNA	-	-	TSL:5

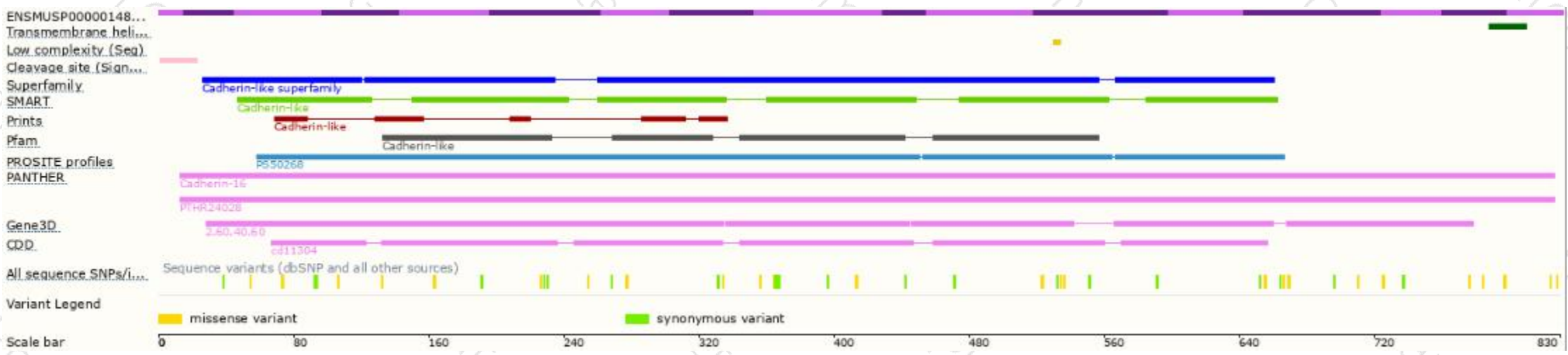
The strategy is based on the design of *Cdh16-204* transcript, The transcription is shown below



Genomic location distribution



Protein domain



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



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