Cdh5-P2A-CreERT2 Mouse Model Strategy

-CRISPR/Cas9 technology

Designer **Ruirui Zhang**

Reviewer **Jia Yu**

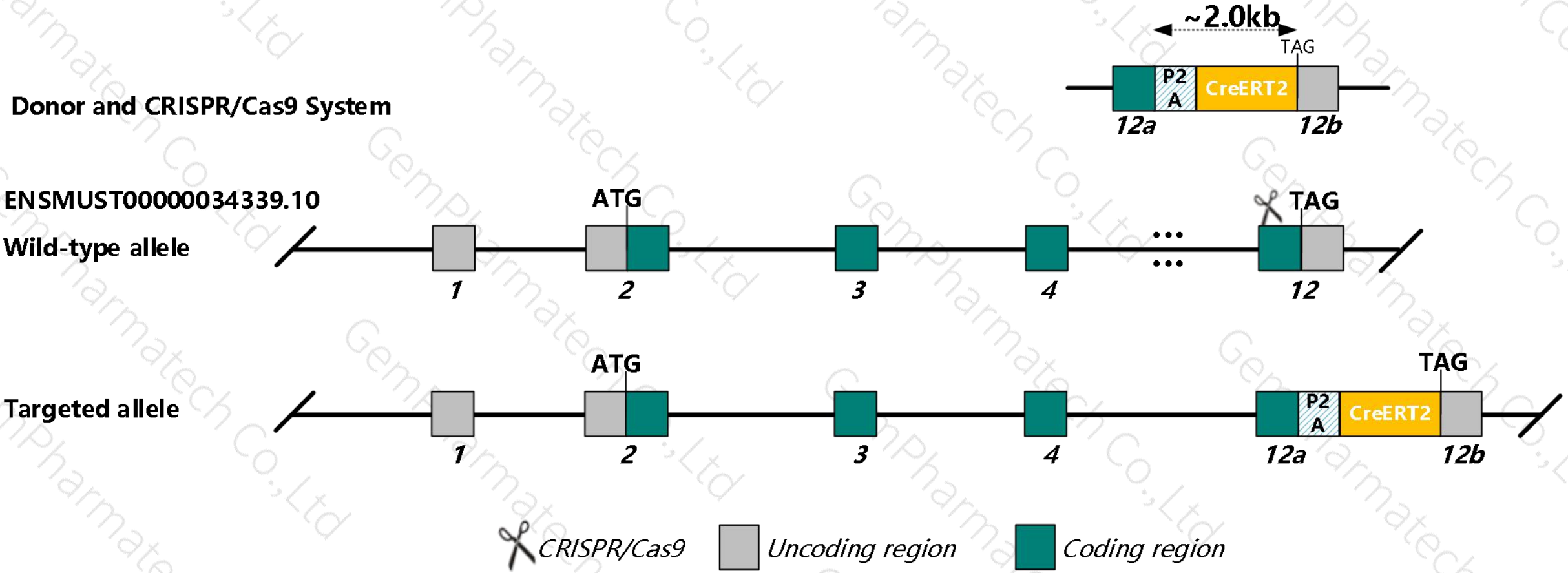
Date **2021-9-15**

Project Overview

Project Name	Cdh5-P2A-CreERT2
Project Type	Cas9-KI
Background	C57BL/6JGpt

Strategy

This model uses CRISPR/Cas9 technology to edit the *Cdh5* gene and the schematic diagram is as follow:



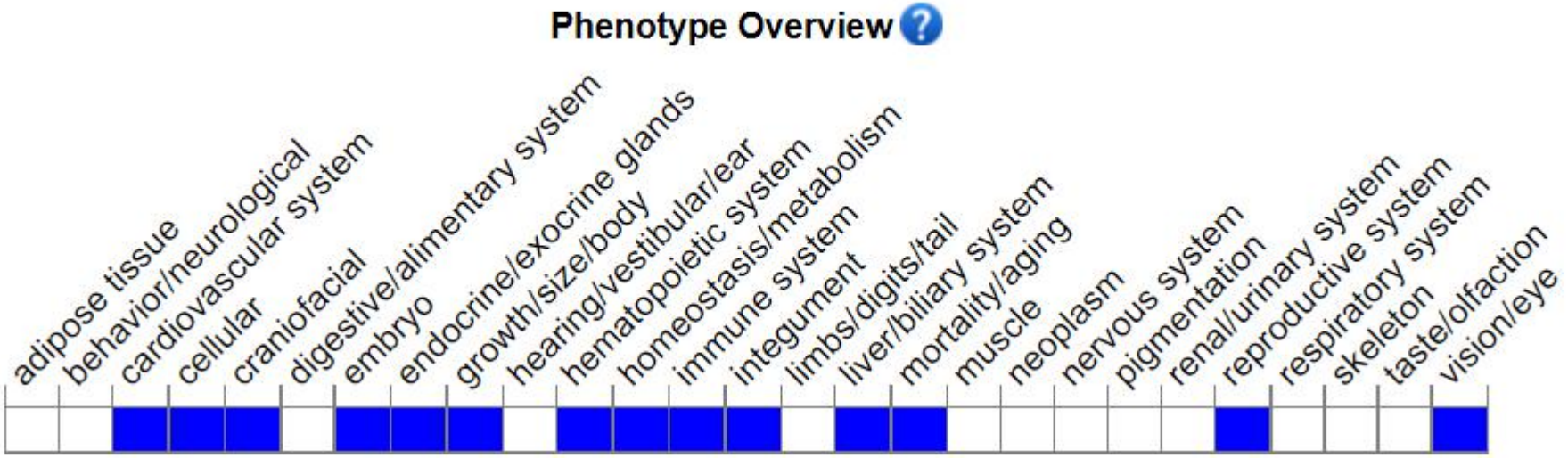
Technical Description

- According to the data of Ensembl, mouse *Cdh5* gene has 2 transcripts. The targeting vector contained P2A-CreERT2 element was inserted into the translation stop codon of *Cdh5* gene(*Cdh5*-201, ENSMUST00000034339.10), and the expression of foreign CreERT2 was depend on the regulation of endogenous *Cdh5*.
- *Cdh5*-201 has 12 exons,the translation start codon ATG is located in exon2, and the translation stop codon TAG is located in exon12, which encodes 784 amino acids.
- In this project, *Cdh5* gene will be modified by CRISPR/Cas9 technology.The brief process is as follows: the donor vector and gRNA were constructed in vitro, CRISPR/Cas9 system were microinjected into the fertilized eggs of C57BL/6JGpt mice, and obtained positive F0 generation mice. The F0 positive mice were mated with C57BL/6JGpt mice,the pups will be genotyped by PCR, followed by sequence analysis.

Notice

- Acroding to the data from MGI, Homozygous inactivation or cytosolic truncation of this gene causes embryonic growth retardation, abnormal somite and heart development, impaired remodeling and maturation of endothelial cells, increased endothelial apoptosis and severe vascular defects leading to embryonic death at midgestation.
- The coding region of exon12 will be introduced 1~2 synonymous mutations.
- The CreERT2 may be mainly expressed in endothelial cell, lymphatic endothelial cell
- The *Cdh5* and CreERT2 gene linked by P2A will expressed under the regulation of endogenous *Cdh5,* and protein products will be divided into two protein molecules. The anterior protein will carry the peptide translated by the P2A sequence.
- The structure of *Cdh5*-202 is incomplete, and the influence is unknown.
- Mouse *Cdh5* gene is located on Chr 8. Please take the loci in consideration when breeding this knockin mice with other gene modified strains, if the other gene is also on the same chromosome, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Inserting exogenous genes into the coding and non-coding regions may affect the regulation of the endogenous gene. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Phenotype Overview(MGI)



http://www.informatics.jax.org/marker/MGI:105057

Homozygous inactivation or cytosolic truncation of this gene causes embryonic growth retardation, abnormal somite and heart development, impaired remodeling and maturation of endothelial cells, increased endothelial apoptosis and severe vascular defects leading to embryonic death at midgestation.

Gene name and location （NCBI)

集萃药康 GemPharmatech

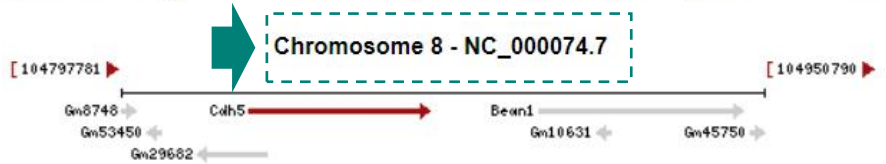
Cdh5 cadherin 5 [*Mus musculus* (house mouse)]

Gene ID: 12562, updated on 7-Sep-2021

Download Datasets

Summary

Official Symbol	Cdh5 provided by MGI
Official Full Name	cadherin 5 provided by MGI
Primary source	MGI:MGI:105057
See related	Ensembl:ENSMUSG00000031871
Gene type	protein coding
RefSeq 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	VE; 7B4; Vec; VE-C; VECD; Cd144; VEcad; VE-Cad; AA408225
Summary	This gene encodes a member of the cadherin family of calcium-dependent glycoproteins that mediate cell adhesion and regulate many morphogenetic events during development. The encoded preproprotein is further processed to generate a mature protein. Mice lacking the encoded protein die in utero due to vascular insufficiency, caused by increased endothelial apoptosis. Multiple distinct genes of the cadherin family, including this gene, are found on chromosome 8. [provided by RefSeq, Oct 2015]
Expression	Biased expression in lung adult (RPKM 243.6), subcutaneous fat pad adult (RPKM 72.5) and 8 other tissues See more
Orthologs	human all



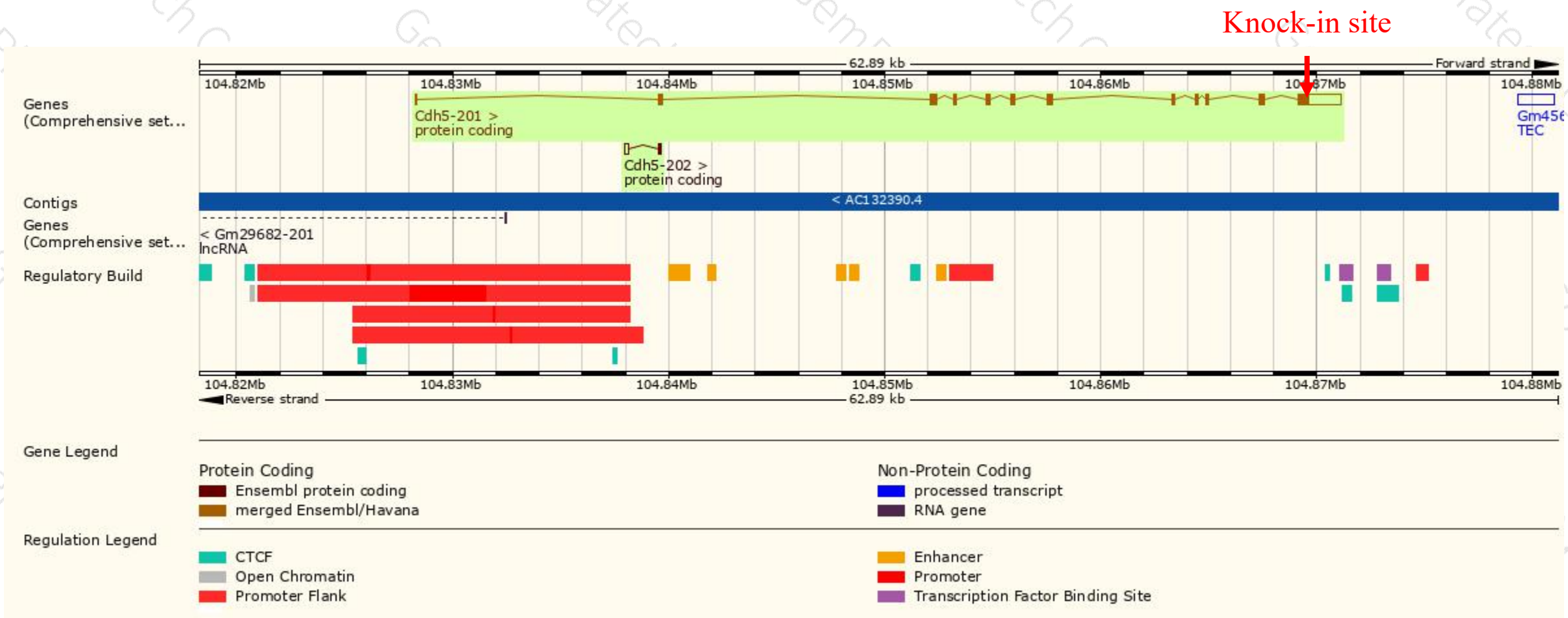
Transcript information （Ensembl）

The gene has 2 transcripts, and all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match ▲	Flags
Cdh5-202	ENSMUST00000209911.2	346	39aa	Protein coding	-	A0A1B0GQW9	TSL:3 CDS 3' incomplete
Cdh5-201	ENSMUST00000034339.10	3995	784aa	Protein coding	CCDS22572	P55284	GENCODE basic APPRIS P1 TSL:1

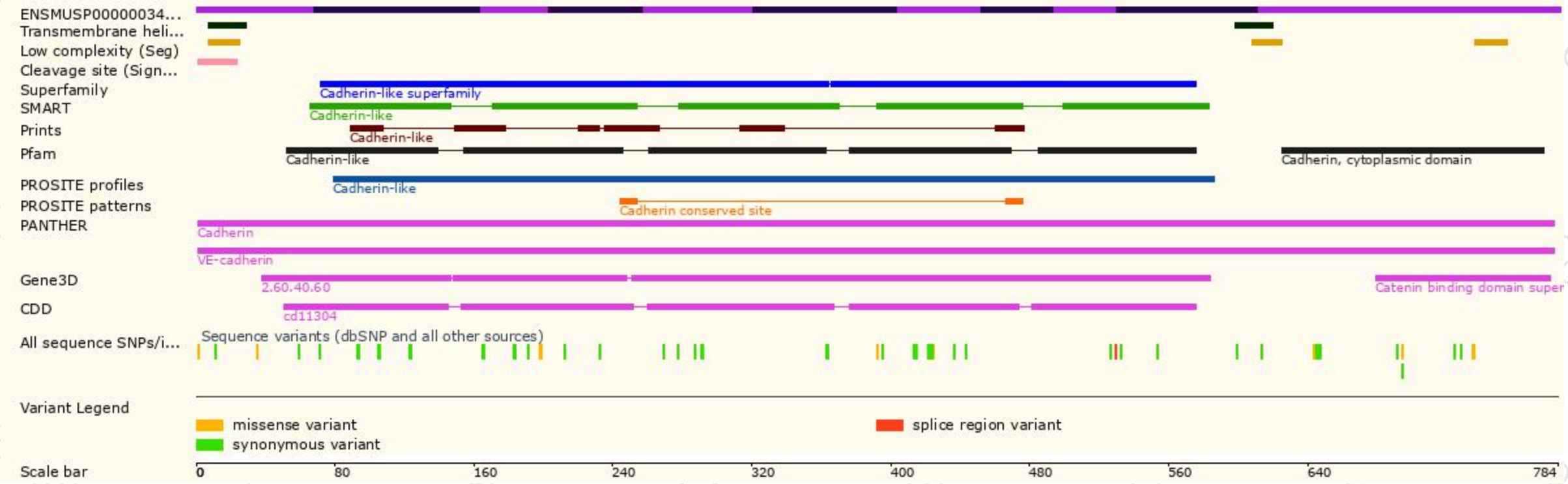
The strategy is based on the design of *Cdh5-201* transcript, the transcription is shown below:

Genomic location distribution



Protein domain

Protein domains for ENSMUSP00000034339.9



If you have any questions, please feel free to contact us.
Tel: 025-5864 1534

集萃药康生物科技
GemPharmatech Co.,Ltd

服务号

订阅号