Pcdh15 Cas9-CKO Strategy

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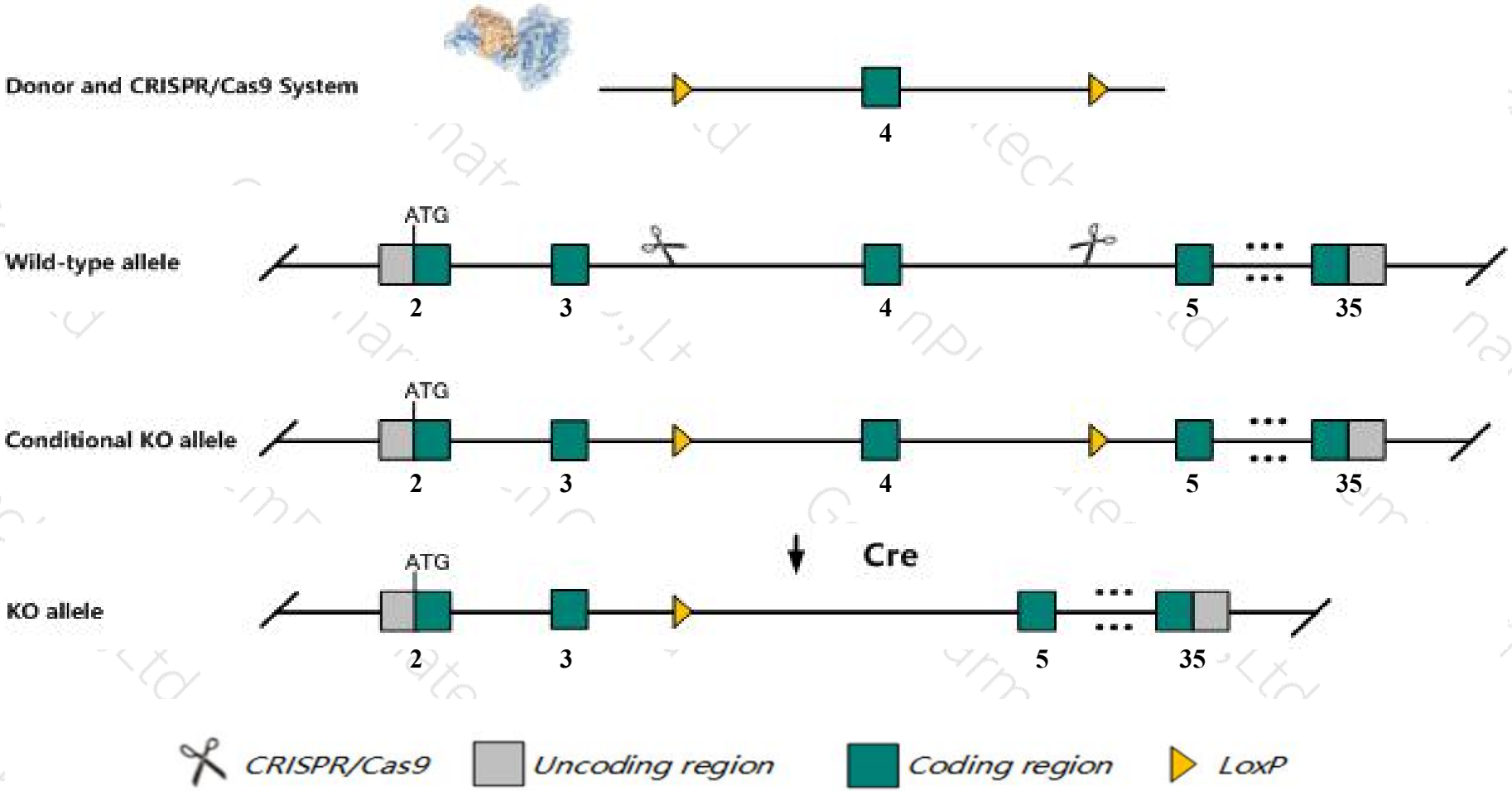
Project Overview

Project Name	***Pcdh15***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pcdh15* gene. The schematic diagram is as follows:



Technical routes

- The *Pcdh15* gene has 41 transcripts. According to the structure of *Pcdh15* gene, exon4 of *Pcdh15-234* (ENSMUST00000191854.5) transcript is recommended as the knockout region.The region contains 161bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Pcdh15* 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.

Notice

- According to the existing MGI data,Homozygotes for severe mutations exhibit circling, head-tossing, hyperactivity, impaired swimming and profound deafness. Mice have defects in cochlea and degeneration of hair cells, spiral ganglion cells and saccular macula. Females are poor mothers.

- The effect on transcript *Pcdh15*-207&221&239 is unknown.

- The *Pcdh15* gene is located on the Chr10. 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）

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Pcdh15 protocadherin 15 [*Mus musculus* (house mouse)]

Gene ID: 11994, updated on 1-Oct-2019

Summary

Official Symbol	Pcdh15 provided by MGI
Official Full Name	protocadherin 15 provided by MGI
Primary source	MGI:MGI:1891428
See related	Ensembl:ENSMUSG00000052613
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	av; roda; Ush1f; nmf19; Gm9815; BB078305
Expression	Biased expression in frontal lobe adult (RPKM 1.4), CNS E18 (RPKM 1.1) and 9 other tissues See more
Orthologs	human all

Genomic context

Location: 10 B5.3; 10 37.43 cM

See Pcdh15 in Genome Data Viewer

Exon count: 52

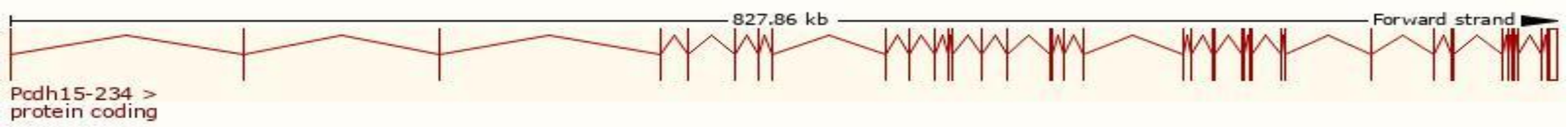
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	10	NC_000076.6 (73096277..74649831)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	10	NC_000076.5 (73284615..74112482)

Transcript information（Ensembl）

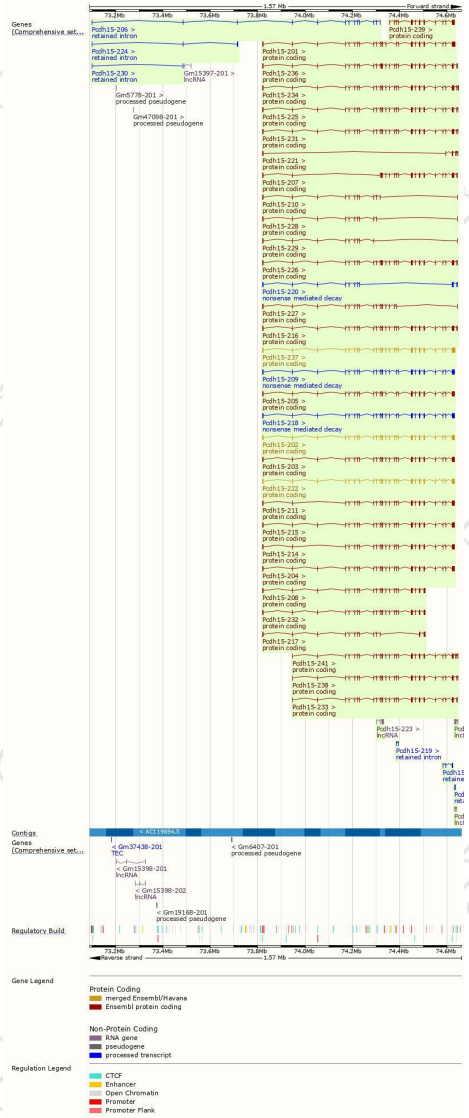
The gene has 41 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pcdh15-234	ENSMUST00000191854.5	9104	1677aa	Protein coding	CCDS56717	Q99PJ1	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-237	ENSMUST00000193361.5	7881	1943aa	Protein coding	CCDS35994	Q99PJ1	TSL:5 GENCODE basic APPRIS P3
Pcdh15-203	ENSMUST00000105424.9	6932	1938aa	Protein coding	CCDS56715	Q99PJ1	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-215	ENSMUST00000131321.8	6926	1936aa	Protein coding	CCDS56718	Q99PJ1	TSL:1 GENCODE basic APPRIS ALT2
Pcdh15-202	ENSMUST00000092420.12	6917	1933aa	Protein coding	CCDS48594	Q99PJ1	TSL:1 GENCODE basic APPRIS ALT2
Pcdh15-211	ENSMUST00000126920.8	6866	1916aa	Protein coding	CCDS56720	Q99PJ1	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-214	ENSMUST00000129404.8	6857	1913aa	Protein coding	CCDS56721	Q99PJ1	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-222	ENSMUST00000147189.8	6806	1896aa	Protein coding	CCDS48595	Q99PJ1	TSL:1 GENCODE basic APPRIS ALT2
Pcdh15-204	ENSMUST00000105426.9	6802	1936aa	Protein coding	CCDS56718	Q99PJ1	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-205	ENSMUST00000105429.9	6719	1867aa	Protein coding	CCDS56719	Q99PJ1	TSL:5 GENCODE basic
Pcdh15-216	ENSMUST00000131724.8	6322	1518aa	Protein coding	CCDS56716	Q99PJ1	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-226	ENSMUST00000151116.8	6287	1790aa	Protein coding	CCDS56711	Q99PJ1	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-231	ENSMUST00000177107.7	5696	1662aa	Protein coding	CCDS56713	Q99PJ1	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-232	ENSMUST00000177420.6	4878	1176aa	Protein coding	CCDS56712	Q99PJ1	TSL:1 GENCODE basic
Pcdh15-208	ENSMUST00000125006.8	4863	1171aa	Protein coding	CCDS56714	Q99PJ1	TSL:1 GENCODE basic
Pcdh15-201	ENSMUST00000064562.13	8830	1865aa	Protein coding	-	E9Q159	TSL:5 GENCODE basic
Pcdh15-236	ENSMUST00000193174.5	5936	1783aa	Protein coding	-	A0A0A6YY17	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-225	ENSMUST00000149977.8	5843	1675aa	Protein coding	-	A0A140T852	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-233	ENSMUST00000191709.5	5346	1781aa	Protein coding	-	Q99PJ1	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-207	ENSMUST00000124046.7	5336	1129aa	Protein coding	-	Q99PJ1	TSL:5 GENCODE basic
Pcdh15-241	ENSMUST00000195531.5	5241	1714aa	Protein coding	-	A0A0A6YXB5	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-238	ENSMUST00000193739.5	5034	1677aa	Protein coding	-	Q99PJ1	TSL:5 GENCODE basic APPRIS ALT2
Pcdh15-239	ENSMUST00000194315.1	4715	1333aa	Protein coding	-	A0A0A6YWL3	CDS 5' incomplete TSL:5
Pcdh15-227	ENSMUST00000152655.8	3860	981aa	Protein coding	-	Q99PJ1	TSL:1 GENCODE basic
Pcdh15-217	ENSMUST00000134009.8	3300	650aa	Protein coding	-	Q99PJ1	TSL:1 GENCODE basic
Pcdh15-210	ENSMUST00000125517.8	3074	719aa	Protein coding	-	Q99PJ1	TSL:1 GENCODE basic
Pcdh15-228	ENSMUST00000152819.8	2867	650aa	Protein coding	-	Q99PJ1	TSL:1 GENCODE basic
Pcdh15-229	ENSMUST00000155701.8	2754	334aa	Protein coding	-	Q99PJ1	TSL:1 GENCODE basic
Pcdh15-221	ENSMUST00000146682.7	1891	415aa	Protein coding	-	Q99PJ1	TSL:1 GENCODE basic
Pcdh15-209	ENSMUST00000125055.8	6852	684aa	Nonsense mediated decay	-	Q99PJ1	TSL:5
Pcdh15-218	ENSMUST00000136096.8	6846	684aa	Nonsense mediated decay	-	Q99PJ1	TSL:2
Pcdh15-220	ENSMUST00000144302.8	3315	326aa	Nonsense mediated decay	-	Q99PJ1	TSL:2
Pcdh15-240	ENSMUST00000194729.1	5272	No protein	Retained intron	-	-	TSLNA
Pcdh15-224	ENSMUST00000148572.1	2712	No protein	Retained intron	-	-	TSL:1
Pcdh15-206	ENSMUST00000123396.8	2436	No protein	Retained intron	-	-	TSL:1
Pcdh15-230	ENSMUST00000156999.1	1024	No protein	Retained intron	-	-	TSL:1
Pcdh15-213	ENSMUST00000128843.1	691	No protein	Retained intron	-	-	TSL:5
Pcdh15-219	ENSMUST00000139106.2	462	No protein	Retained intron	-	-	TSL:2
Pcdh15-212	ENSMUST00000127928.6	3404	No protein	lncRNA	-	-	TSL:1
Pcdh15-223	ENSMUST00000147455.1	2841	No protein	lncRNA	-	-	TSL:1
Pcdh15-235	ENSMUST00000192370.1	556	No protein	lncRNA	-	-	TSL:3

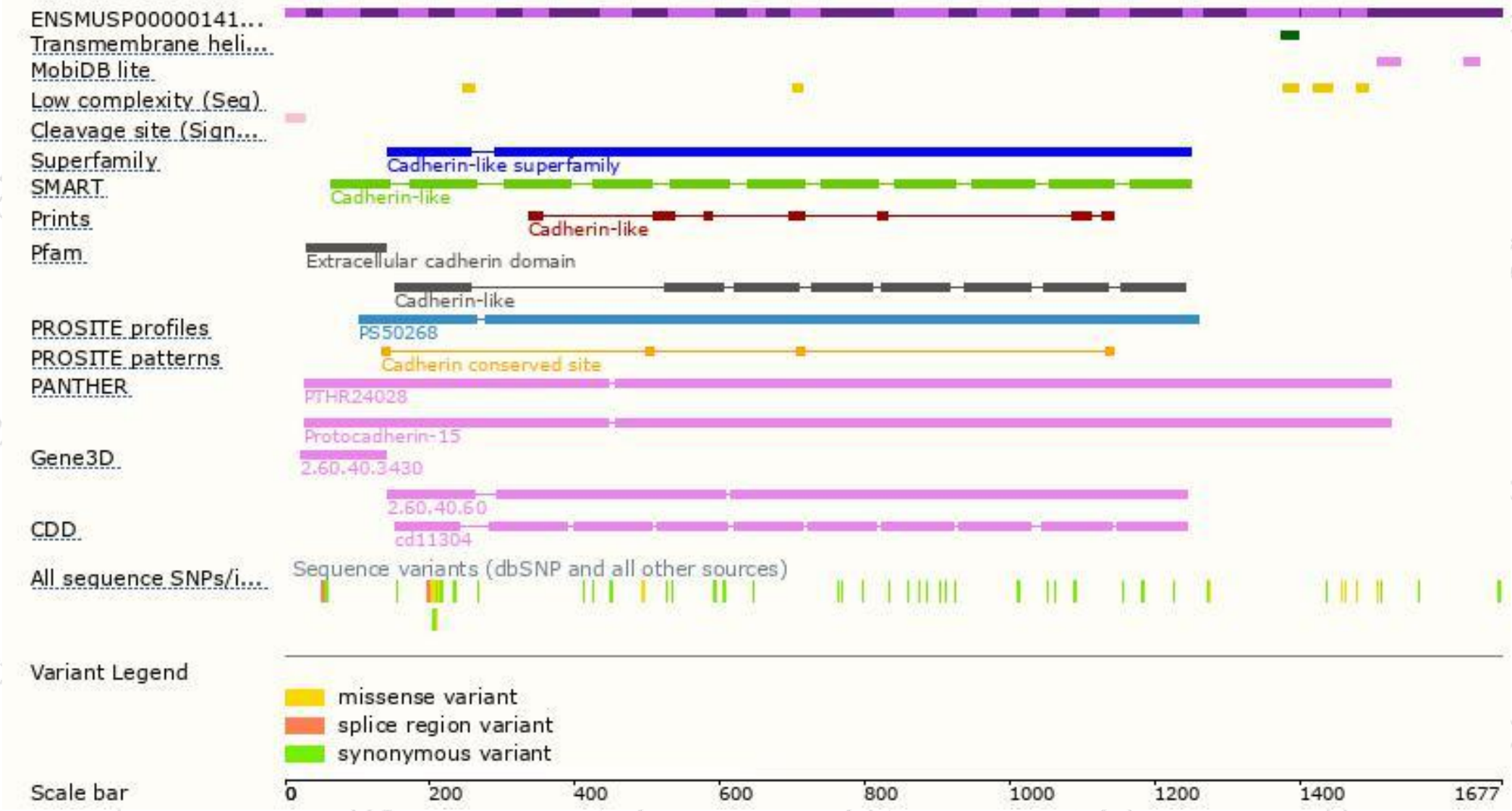
The strategy is based on the design of *Pcdh15-234* transcript,The transcription is shown below



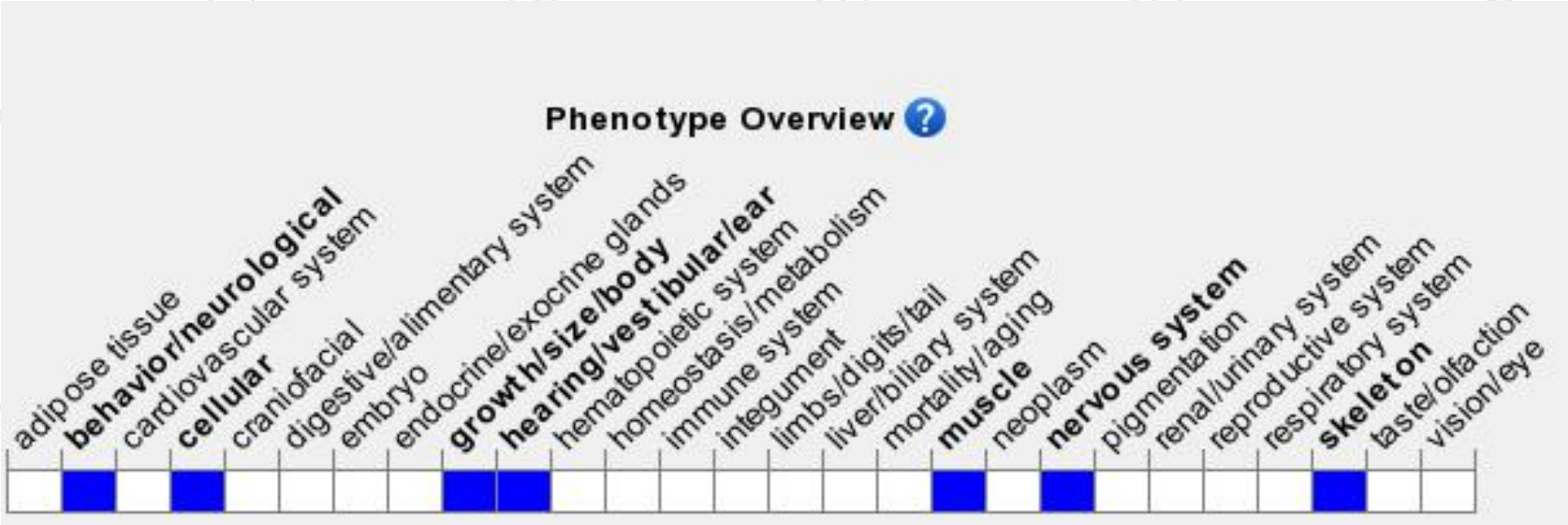
Genomic location distribution



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,Homozygotes for severe mutations exhibit circling, head-tossing, hyperactivity, impaired swimming and profound deafness. Mice have defects in cochlea and degeneration of hair cells, spiral ganglion cells and saccular macula. Females are poor mothers.

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If you have any questions, you are welcome to inquire.

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