

Hectd1 Cas9-KO Strategy

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Project Overview

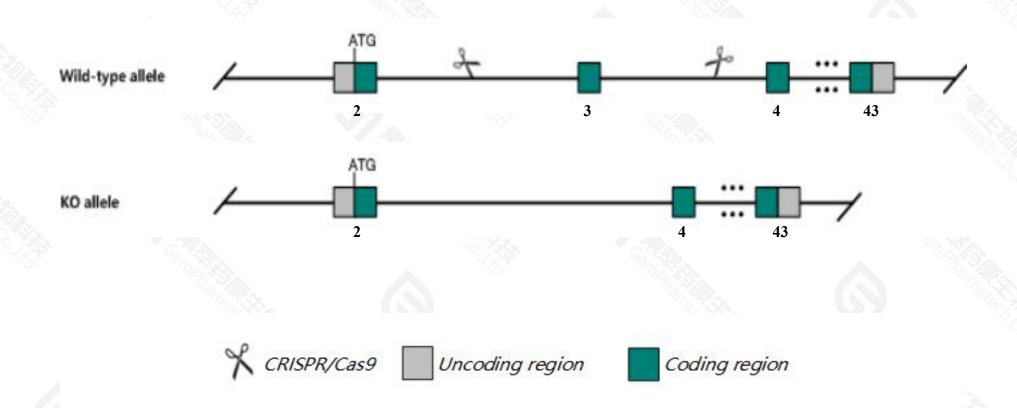


| Project Name | Hectd1 |
|-------------------|-------------|
| Project type | Cas9-KO |
| Strain background | C57BL/6JGpt |

Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Hectd1* gene. The schematic diagram is as follows:



Technical routes



- > The *Hectd1* gene has 5 transcripts. According to the structure of *Hectd1* gene, exon3 of *Hectd1*-201(ENSMUST00000042052.9) transcript is recommended as the knockout region. The region contains 235bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Hectd1* gene. The brief process is as follows: CRISPR/Cas9 system 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.

Notice



- > According to the existing MGI data, mice that are homozygous for either a gene trapped or an ENU-induced allele exhibit exencephaly associated with impaired head mesenchyme development and neural tube closure, and show eye and cranial vault dysplasia. Homozygotes for another ENU-induced allele show congenital cardiovascular defects.
- > The *Hectd1* gene is located on the Chr12. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



Hectd1 HECT domain E3 ubiquitin protein ligase 1 [Mus musculus (house mouse)]

Gene ID: 207304, updated on 26-Feb-2019

Summary



Official Symbol Hectd1 provided by MGI

Official Full Name HECT domain E3 ubiquitin protein ligase 1 provided by MGI

Primary source MGI:MGI:2384768

See related Ensembl:ENSMUSG00000035247

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 A630086P08Rik, Al844876, b2b327Clo, opm

Expression Ubiquitous expression in bladder adult (RPKM 17.4), liver E14 (RPKM 15.1) and 28 other tissuesSee more

Orthologs <u>human</u> all

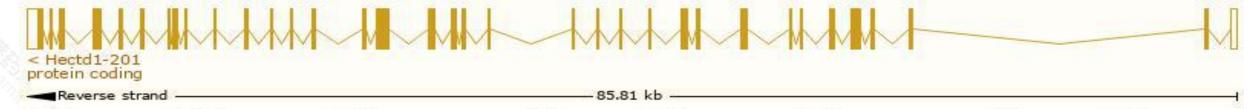
Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

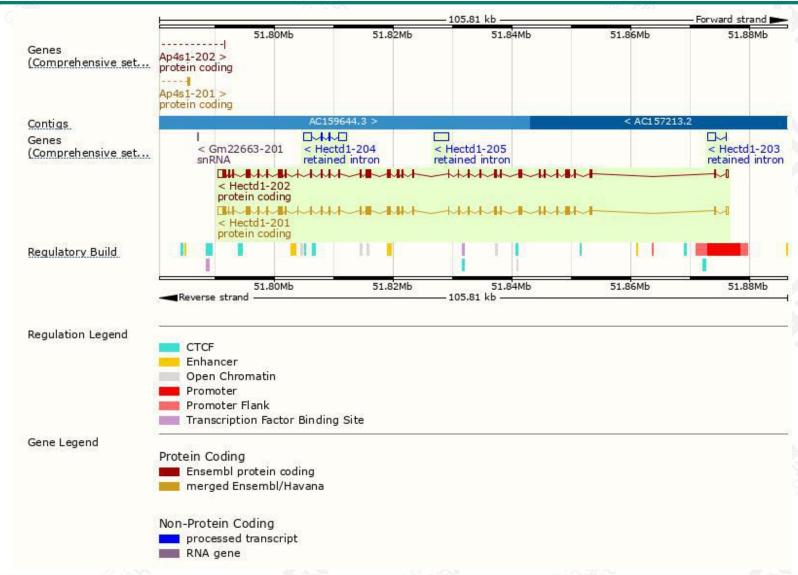
| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags | |
|------------|----------------------|------|---------------|-----------------|-----------|---------|-------------------------------|--|
| Hectd1-201 | ENSMUST00000042052.8 | 8988 | 2610aa | Protein coding | CCDS49064 | F8WIE5 | TSL:5 GENCODE basic APPRIS P1 | |
| Hectd1-202 | ENSMUST00000179265.7 | 9012 | <u>2618aa</u> | Protein coding | 678 | Q69ZR2 | TSL:5 GENCODE basic | |
| Hectd1-204 | ENSMUST00000218626.1 | 2895 | No protein | Retained intron | (SE) | - | TSL:1 | |
| Hectd1-205 | ENSMUST00000220098.1 | 2575 | No protein | Retained intron | 323 | - | TSL:NA | |
| Hectd1-203 | ENSMUST00000217804.1 | 1447 | No protein | Retained intron | (5) | | TSL:1 | |

The strategy is based on the design of *Hectd1-201* transcript, the transcription is shown below:



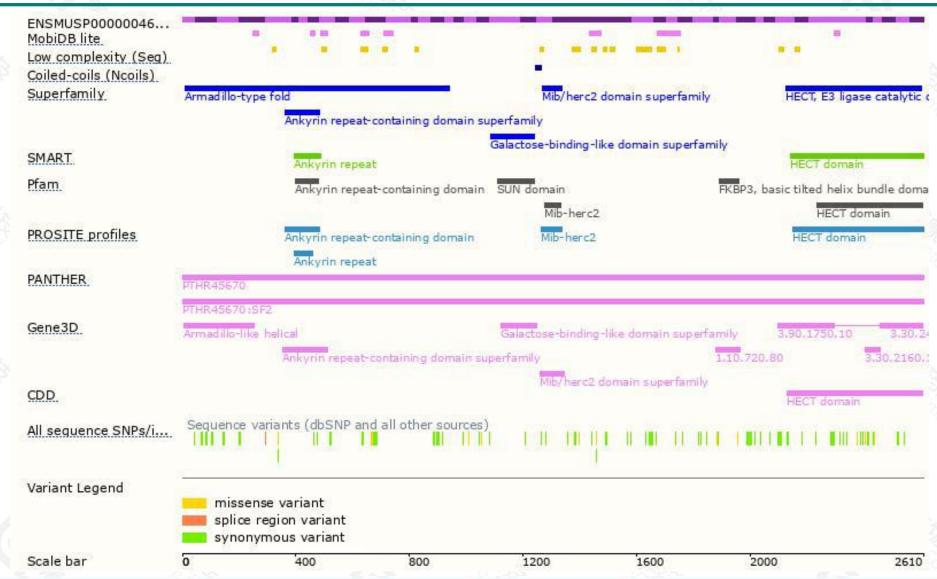
Genomic location distribution





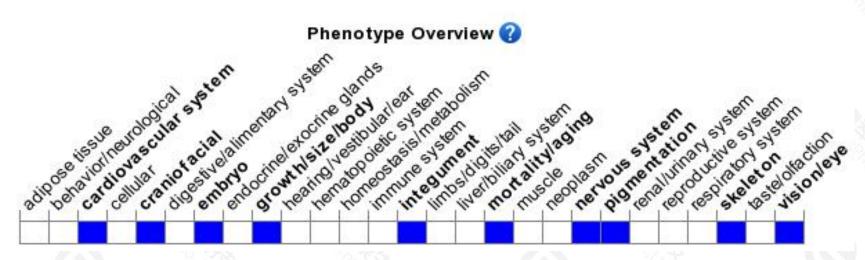
Protein domain





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, mice that are homozygous for either a gene trapped or an ENU-induced allele exhibit exencephaly associated with impaired head mesenchyme development and neural tube closure, and show eye and cranial vault dysplasia. Homozygotes for another ENU-induced allele show congenital cardiovascular defects.



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

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