Hectd4 Cas9-KO Strategy

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Project Overview

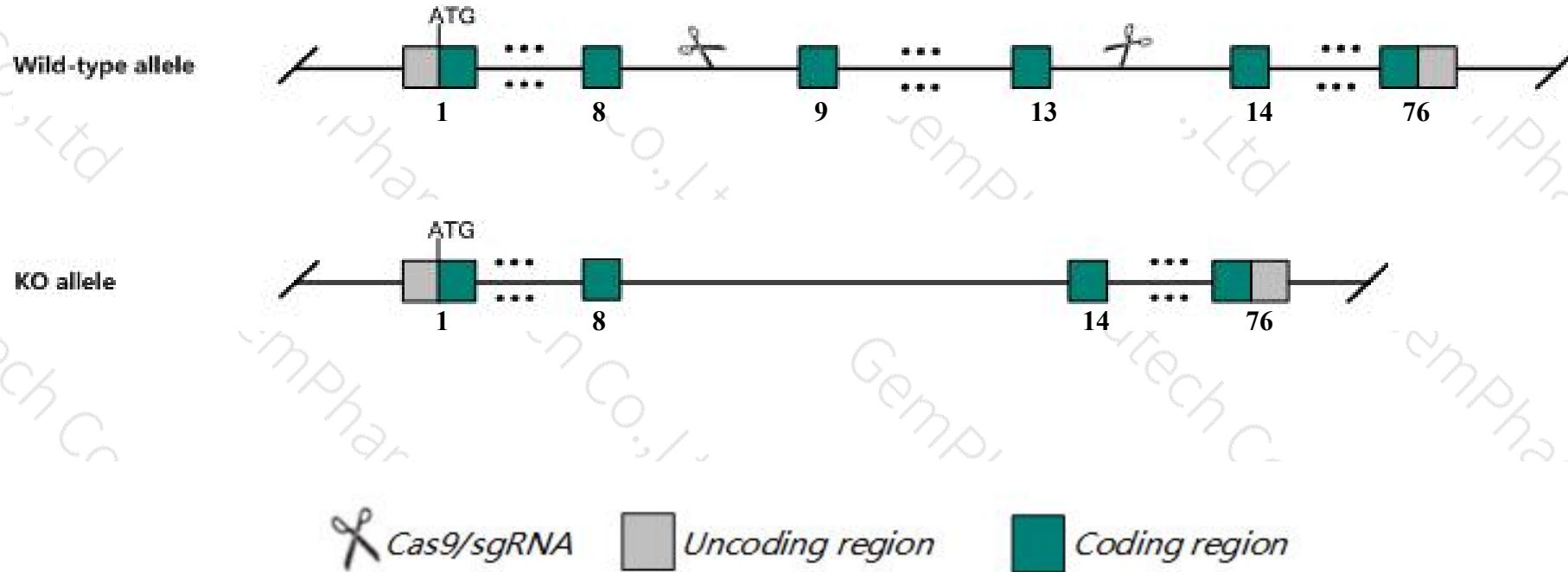|	
Project Name	***Hectd4***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Hectd4* gene. The schematic diagram is as follows:

Technical routes

➤ The *Hectd4* gene has 7 transcripts. According to the structure of *Hectd4* gene, exon9-exon13 of *Hectd4-201* (ENSMUST00000042614.12) transcript is recommended as the knockout region.The region contains 793bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Hectd4* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice

➢ The *Hectd4* gene is located on the Chr5. 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.

➢ The *Hectd4-202* and *Hectd4-205* transcripts are uncompleted, so the effect on them are unknown.

➢ 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）

Hectd4 HECT domain E3 ubiquitin protein ligase 4 [Mus musculus (house mouse)]
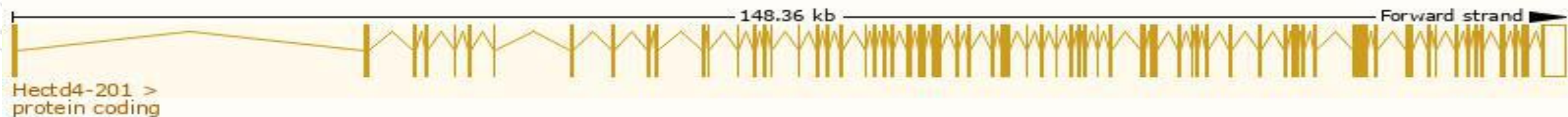
Gene ID: 269700, updated on 31-Jan-2019

▲Summary

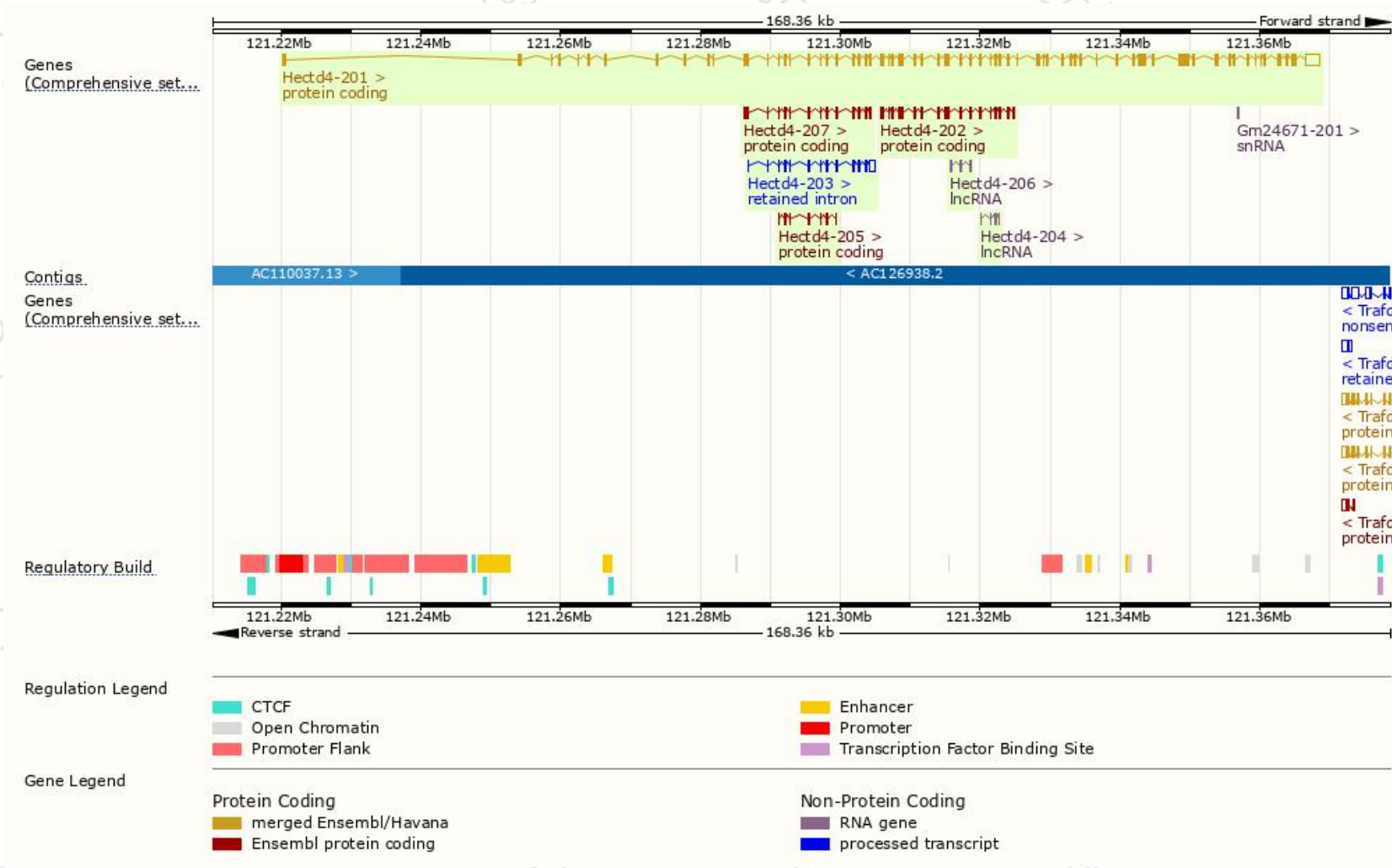|	
Official Symbol	Hectd4 provided by MGI
Official Full Name	HECT domain E3 ubiquitin protein ligase 4 provided by MGI
Primary source	MGI:MGI:3647820
See related	Ensembl:ENSMUSG00000042744
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	A130010L24, AU042671, BC019639, EG545802, Gm15800, mKIAA0614
Expression	Ubiquitous expression in cerebellum adult (RPKM 19.5), whole brain E14.5 (RPKM 18.1) and 28 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 7 transcripts,all transcripts are shown below:

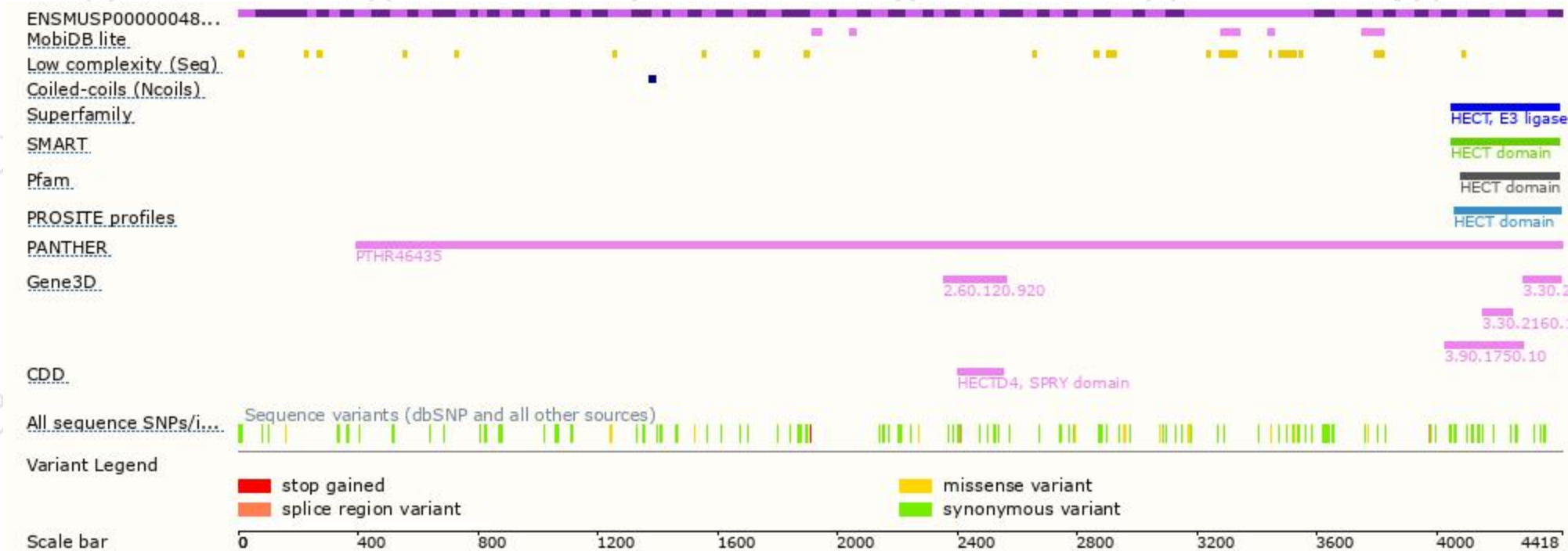| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Hectd4-201	ENSMUST00000042614.12	15482	4418aa	Protein coding	CCDS57377	E9Q2E4	TSL:5 GENCODE basic APPRIS P1
Hectd4-202	ENSMUST00000100769.2	3103	1014aa	Protein coding	-	Q6GQX8	CDS 5' incomplete TSL:1
Hectd4-207	ENSMUST00000201669.3	2035	678aa	Protein coding	-	A0A0J9YUS1	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Hectd4-205	ENSMUST00000148368.1	663	221aa	Protein coding	-	F6XXX5	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5
Hectd4-204	ENSMUST00000130655.1	458	No protein	Processed transcript	-	-	TSL:3
Hectd4-206	ENSMUST00000155743.1	375	No protein	Processed transcript	-	-	TSL:3
Hectd4-203	ENSMUST00000128514.1	2239	No protein	Retained intron	-	-	TSL:1

The strategy is based on the design of *Hectd4-201* transcript,The transcription is shown below

Genomic location distribution

Protein domain

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