

# ***Htra1-p.Q318H* Mouse Model Strategy**

## **-CRISPR/Cas9 technology**

<b>Designer</b>	<b>Ruirui Zhang</b>
<b>Reviewer</b>	<b>Zihe Cui</b>
<b>Date</b>	<b>2021-9-14</b>

# Project Overview

**Project Name**

Htra1-p.Q318H

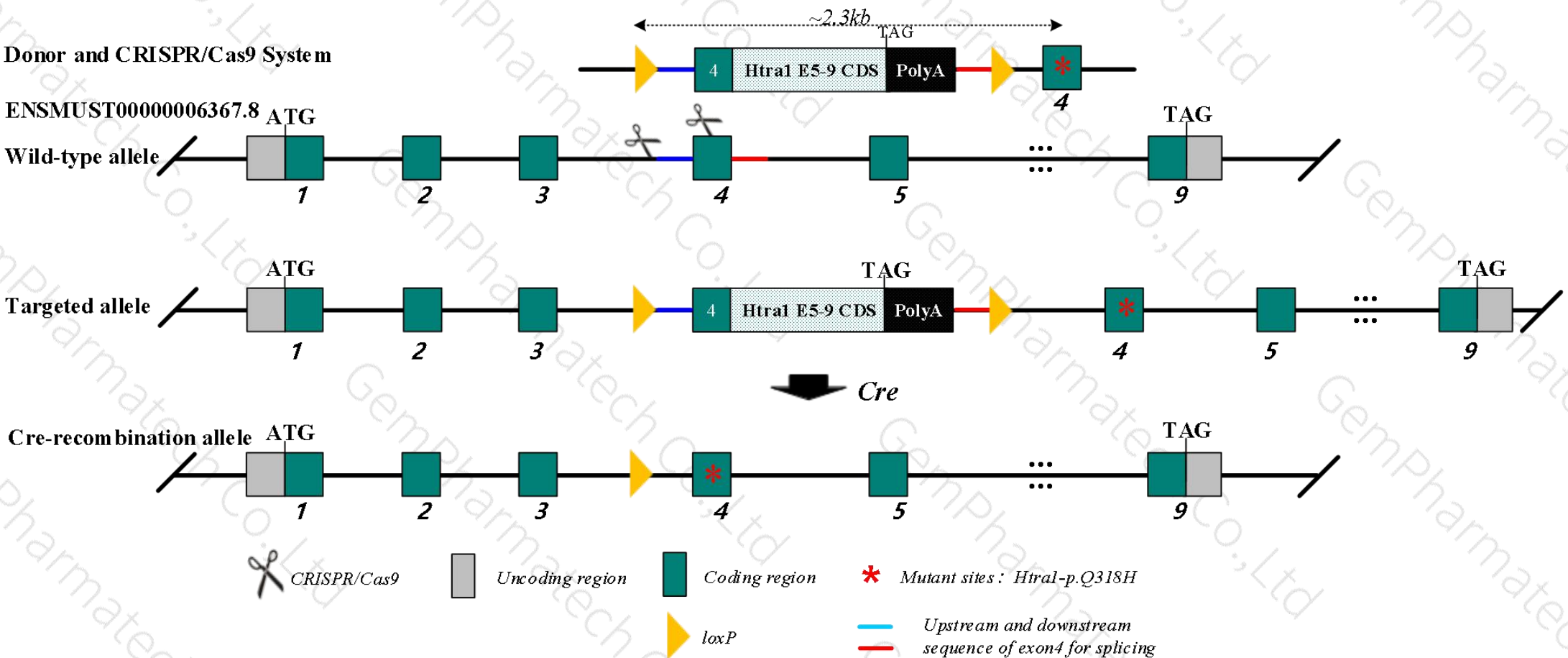
**Project Type**

Cas9-KI

**Background**

C57BL/6JGpt

This model uses CRISPR/Cas9 technology to edit the *Htra1* gene and the schematic diagram is as follow:



# Technical Description

- According to the data of Ensembl, mouse *Htra1* gene has 5 transcripts.
- The mouse model will introduce *p.Q318H* point mutation in exon4 of *Htra1*-201 (ENSMUST000000006367.8), the 318<sup>th</sup> amino acid of *Htra1* is mutated from Q(Gln) to H(His), and the loxP-flanked *Htra1* exon4-9 CDS with polyA signal will be inserted into intron3-4, when the Cre recombinase was expressed in the target cells, these flanking elements will be deleted for following expression of *Q318H*.
- *Htra1*-201 has 9 exons, the translation start codon ATG is located in exon1, and the translation stop codon TAG is located in exon9, which encodes 480 amino acids.
- In this project, *Htra1* gene will be modified by CRISPR/Cas9 technology. The brief process is as follows: the donor vector and gRNA were constructed in vitro, Cas9, donor and gRNA 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.

- According to the data of MGI, Mice homozygous for a knock-out allele exhibit normal retinal morphology. Mice homozygous for a different allele exhibit increased bone volume and increased trabecular bone thickness without body weight gain, and the lethality of Q318H mutation is unknown.
- Intron3-4 of *Htr1* gene contains repeat structure, mutations may be introduced in this region during targeting.
- In addition to the target mutation of p.Q318H, it may be necessary to introduce 1~2 amino acid synonymous mutations on both endogenous and exogenous exon4 respectively.
- Mouse *Htr1* gene is located on Chr7. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr7, 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.



# Mutation Site

## Before mutation

AGGGGTGTCC ACCCCAAGCT CAGCTCCTAG GGACCGGGAC TGTCCTTTGT ATTTGTATTA TTCAGCTTGC TAAGCCACAG CTCCGTTTGT GCTTACAGGG  
TCCCCACAGG TGGGGTTCGA GTCGAGGATC CCTGGCCCTG ACAGGAAACA TAAACATAAT AAGTCGAACG ATTCGGTGTC GAGGCAAACA CGAATGTCCC  
?G K L P V L L L G R S S E L R P G E F V V A I G S P F S L Q N T V T  
AAAGCTGCCA GTCCTGCTGC TCGGCCGCTC CTCAGAGCTG AGACCTGGAG AATTTGTAGT TGCCATTGGA AGCCCCTTTT CTCTTCAAAA CACAGTCACC  
TTTCGACGGT CAGGACGACG AGCCGGCGAG GAGTCTCGAC TCTGGACCTC TTAAACATCA ACGGTAACCT TCGGGGAAAA GAGAAGTTTT GTGTCAGTGG  
T G I V S T T Q R G G K E L G L R N S D M D Y I Q T D A I I N  
ACTGGGATCG TCAGCACCAC CCAGCGAGGC GGCAAAGAGC TGGGACTTCG GAACTCCGAT ATGGACTACA TTCAGACAGA CGCTATCATC AATGTGAGCC  
TGACCCTAGC AGTCGTGGTG GGTCGCTCCG CCGTTTCTCG ACCCTGAAGC CTTGAGGCTA TACCTGATGT AAGTCTGTCT GCGATAGTAG TTACACTCGG

## After mutation

AGGGGTGTCC ACCCCAAGCT CAGCTCCTAG GGACCGGGAC TGTCCTTTGT ATTTGTATTA TTCAGCTTGC TAAGCCACAG CTCCGTTTGT GCTTACAGGG  
TCCCCACAGG TGGGGTTCGA GTCGAGGATC CCTGGCCCTG ACAGGAAACA TAAACATAAT AAGTCGAACG ATTCGGTGTC GAGGCAAACA CGAATGTCCC  
G K L P V L L L G R S S E L R P G E F V V A I G S P F S L Q N T V T  
AAAGCTGCCA GTCCTGCTGC TCGGCCGCTC CTCAGAGCTG AGACCTGGAG AATTTGTAGT TGCCATTGGA AGCCCCTTTT CTCTTCAAAA CACAGTCACC  
TTTCGACGGT CAGGACGACG AGCCGGCGAG GAGTCTCGAC TCTGGACCTC TTAAACATCA ACGGTAACCT TCGGGGAAAA GAGAAGTTTT GTGTCAGTGG  
T G I V S T T Q R G G K E L G L R N S D M D Y I H T D A I I N  
ACTGGGATCG TCAGCACCAC CCAGCGAGGC GGCAAAGAGC TGGGACTTCG GAACTCCGAT ATGGACTACA TTCACACAGA CGCTATCATC AATGTGAGCC  
TGACCCTAGC AGTCGTGGTG GGTCGCTCCG CCGTTTCTCG ACCCTGAAGC CTTGAGGCTA TACCTGATGT AAGTCTGTCT GCGATAGTAG TTACACTCGG

The yellow region is exon4 of *Htra1-201*, and the red region represents the p.Q318H mutation site.

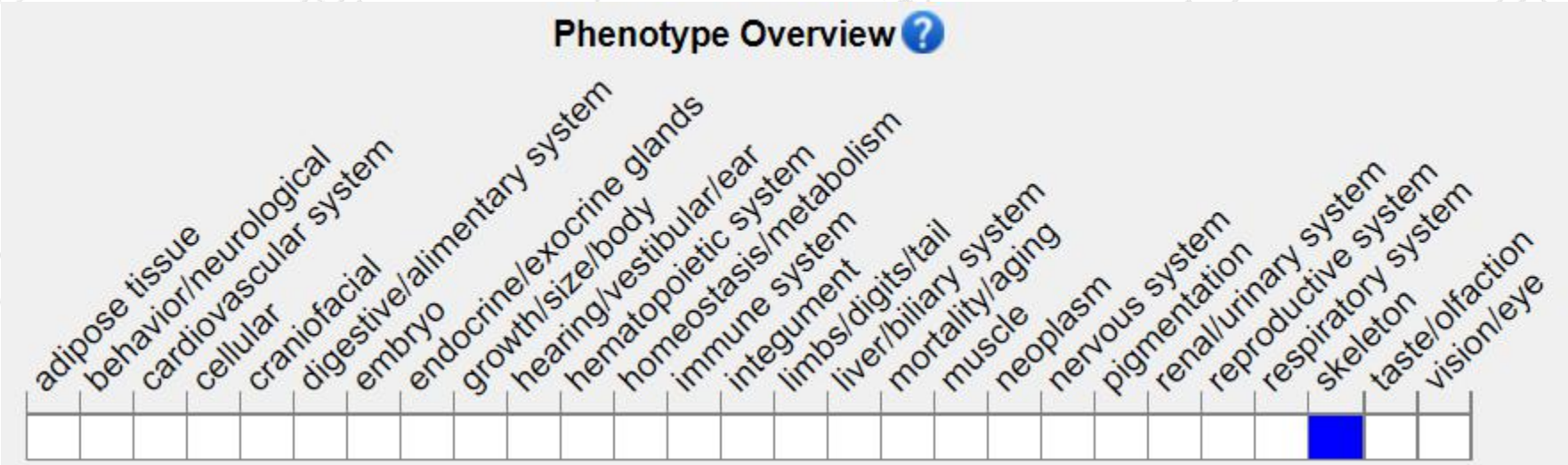
# *Htra1* exon4-9 CDS Sequence(666nt)

GGAAAGCTGCCAGTCCTGCTGCTCGGCCGCTCCTCAGAGCTGAGACCTGGAGAATTTGTAGTTGCCATTGGAAGCCCCTTTTCTCTTCAAAACACA  
GTCACCACTGGGATCGTCAGCACCACCCAGCGAGGCGGCAAAGAGCTGGGACTTCGGAACTCCGATATGGACTACATTCAGACAGACGCTATCATC  
AATTATGGAAATTCCGGAGGCCCCGTTAGTAAACCTGGATGGCGAGGTGATTGGGATTAACACCTTGAAGGTGACGGCGGGCATCTCCTTCGCAATT  
CCATCCGATAAGATAAAAAAGTTCTTGACAGAGTCCCACGATCGACAGGCCAAAGGGAAAGCTGTCACCAAGAAGAAGTATATTGGGATCCGAATG  
ATGTCGCTCACATCTAGCAAAGCCAAAGAGCTGAAGGACCGTCACCGAGACTTCCCGGATGTGCTCTCTGGGGCATATATCATTGAAGTCATTCCT  
GACACCCCGGCAGAAGCCGGAGGGCTCAAGGAAAATGACGTCATCATCAGCATCAACGGACAGTCTGTGGTCACTGCCAATGACGTCAGCGATGTC  
ATCAAAAAGGAGAACACCCTGAACATGGTTGTCCGCAGGGGCAATGAAGACATTGTGATTACCGTGATTCCTGAAGAAATCGACCCCTAG



# Mouse phenotype description(MGI)

<http://www.informatics.jax.org/marker/MGI:1929076>



Mice homozygous for a knock-out allele exhibit normal retinal morphology. Mice homozygous for a different allele exhibit increased bone volume and increased trabecular bone thickness without body weight gain.



# Gene name and location (NCBI)

**Htra1** HtrA serine peptidase 1 [ *Mus musculus* (house mouse) ]

Gene ID: 56213, updated on 29-Jun-2021

 Download Datasets

## Summary

**Official Symbol** Htra1 provided by MGI

**Official Full Name** HtrA serine peptidase 1 provided by MGI

**Primary source** [MGI:MGI:1929076](#)

**See related** [Ensembl:ENSMUSG00000006205](#)

**Gene type** protein coding

**RefSeq status** VALIDATED

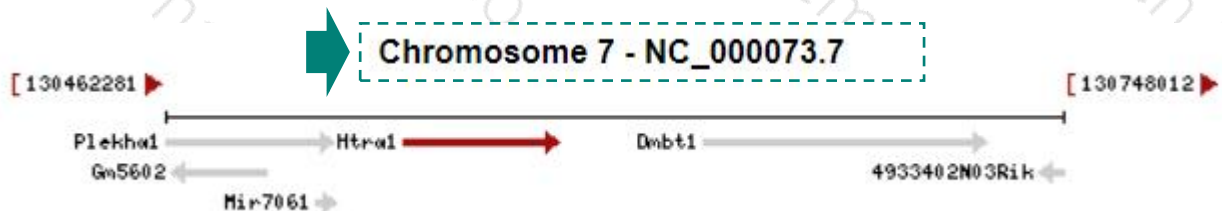
**Organism** [Mus musculus](#)

**Lineage** Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus

**Also known as** L5; Pr; RS; Htr; L56; HTRA; Prss11; RSP11; A1429470

**Expression** Biased expression in ovary adult (RPKM 297.0), mammary gland adult (RPKM 75.1) and 14 other tissues [See more](#)

**Orthologs** [human](#) [all](#)

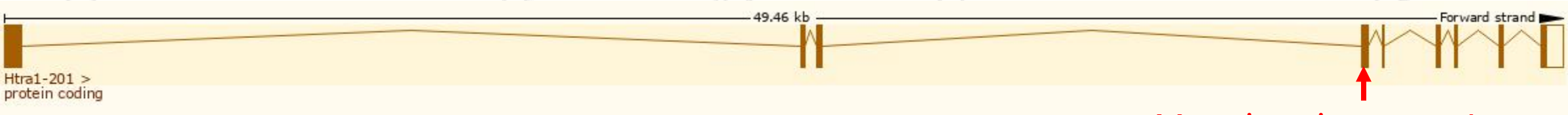


# Transcript information (Ensembl)

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

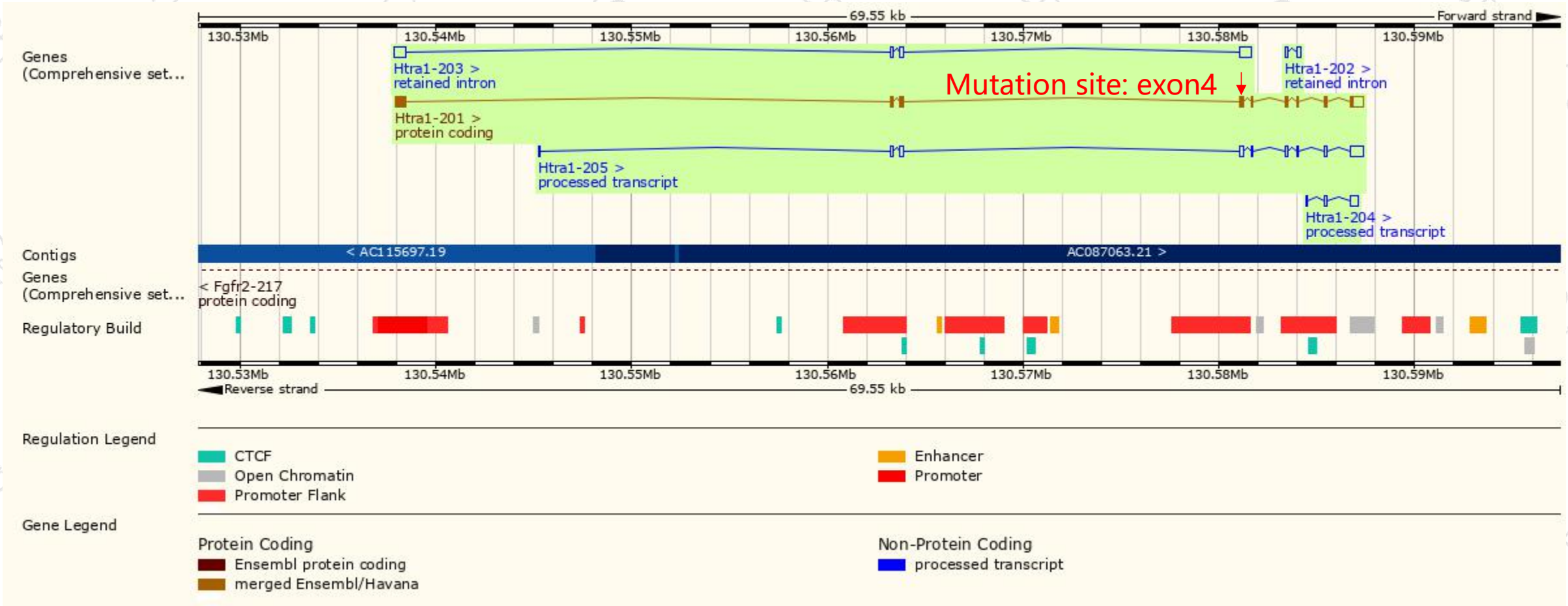
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt Match	Flags
Htra1-201	<a href="#">ENSMUST00000006367.8</a>	2041	<a href="#">480aa</a>	Protein coding	<a href="#">CCDS21908</a>	<a href="#">Q9R118</a>	GENCODE basic APPRIS P1 TSL:1
Htra1-205	<a href="#">ENSMUST00000153290.8</a>	1606	No protein	Processed transcript	-	-	TSL:1
Htra1-204	<a href="#">ENSMUST00000150905.2</a>	582	No protein	Processed transcript	-	-	TSL:3
Htra1-203	<a href="#">ENSMUST00000150717.8</a>	1572	No protein	Retained intron	-	-	TSL:5
Htra1-202	<a href="#">ENSMUST00000140741.2</a>	309	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Htra1-201* transcript, the transcription is shown below:



Mutation site: exon4

# Genomic location distribution





## Protein domains for ENSMUSP00000006367.8





If you have any questions, please feel free to contact us.

Tel: 025-5864 1534



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