

# **Naa60-p.D154fs cas9-ki(PM) Mouse Model Strategy**

## **-CRISPR/Cas9 technology**

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**Design Date: 2021-9-1**

# Project Overview

**Project Name**

Naa60-p.D154fs

**Project Type**

cas9-ki(PM)

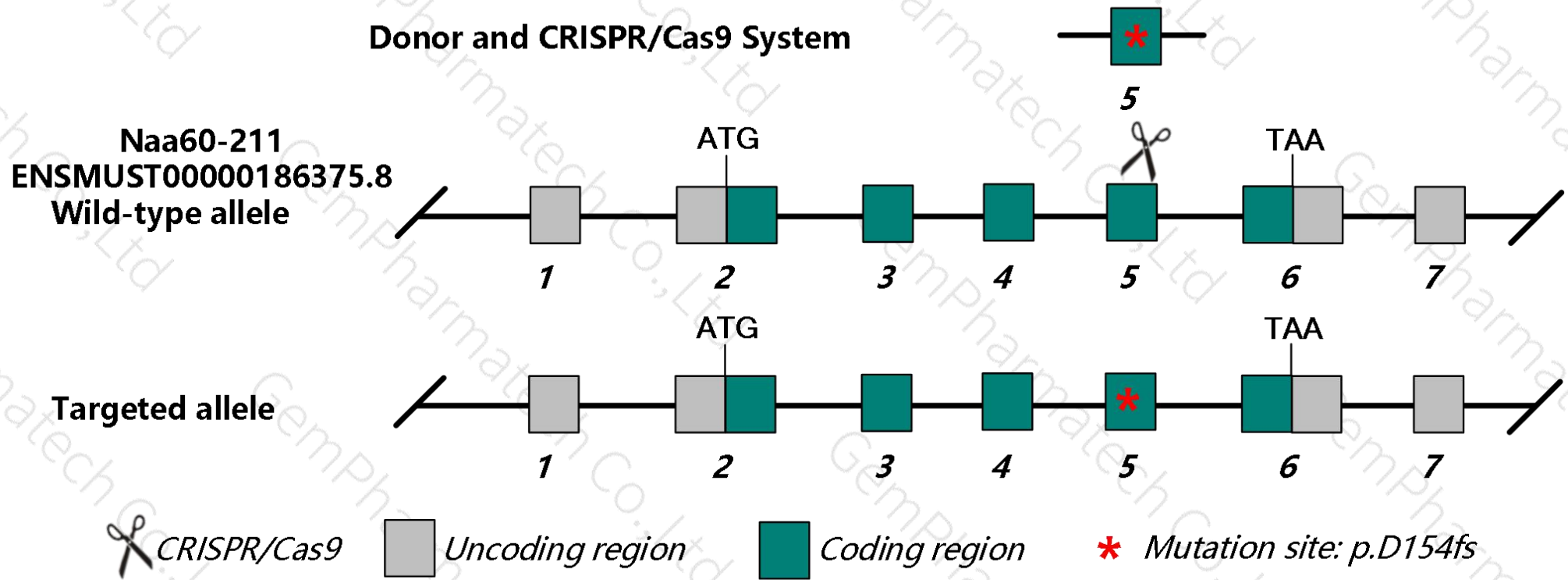
**Background**

C57BL/6JGpt

**Project cycle**

5-8months

This model uses CRISPR/Cas9 technology to edit the *Naa60* gene and the schematic diagram is as follow:



# A comparison of the aa homology of human and mouse *Naa60* gene

|                                    |       |                                                                                       |     |
|------------------------------------|-------|---------------------------------------------------------------------------------------|-----|
| Human NAA60-NM_001083601.3 protein | (1)   | MTEVVPSSALSEVSLRLLCHDDIDTVKHLCGDWFPIEYPDSWYRDITSNKKFFSLAATYRGAIVGMIVAEIKNRTKIHKEDGDIL | 85  |
| Mouse Naa60-NM_001290689.1 protein | (1)   | -----MIVAEIKNRTKIHKEDGDIL                                                             |     |
| Mouse Naa60-NM_029090.3 protein    | (1)   | MTEVVPSSALSEVSLRLLCHDDIDTVKHLCGDWFPIEYPDSWYRDITSNKKFFSLAATYRGAIVGMIVAEIKNRTKIHKEDGDIL |     |
| Consensus                          | (1)   | MTEVVPSSALSEVSLRLLCHDDIDTVKHLCGDWFPIEYPDSWYRDITSNKKFFSLAATYRGAIVGMIVAEIKNRTKIHKEDGDIL |     |
| Human NAA60-NM_001083601.3 protein | (86)  | ASNFSDVTQVAYILSLGVVKEFRKHGIGSLLESKLDHISTTAQDHCKAIYLVLTNTNNTAINFYENRDFKQHHYLPYYYSIRGV  | 170 |
| Mouse Naa60-NM_001290689.1 protein | (21)  | ASSFSVDTQVAYILSLGVVKEFRKHGIGSLLESKLDHISTTAQDHCKAIYLVLTNTNNTAINFYENRDFRQHHYLPYYYSIRGV  |     |
| Mouse Naa60-NM_029090.3 protein    | (86)  | ASSFSVDTQVAYILSLGVVKEFRKHGIGSLLESKLDHISTTAQDHCKAIYLVLTNTNNTAINFYENRDFRQHHYLPYYYSIRGV  |     |
| Consensus                          | (86)  | ASSFSVDTQVAYILSLGVVKEFRKHGIGSLLESKLDHISTTAQDHCKAIYLVLTNTNNTAINFYENRDFRQHHYLPYYYSIRGV  |     |
| Human NAA60-NM_001083601.3 protein | (171) | LKDGFTYVLYINGGHPPWTILDYIQHLGSALANLSPCSIPHRVYRQAHSLLCSFLPWSGISKSGIEYSRTM               | 242 |
| Mouse Naa60-NM_001290689.1 protein | (106) | LKDGFTYVLYINGGHPPWTILDYIQHLGSALANLSPCSIPHRIYRQAHSLLCSFLPWSISITKGGIEYSRTM              |     |
| Mouse Naa60-NM_029090.3 protein    | (171) | LKDGFTYVLYINGGHPPWTILDYIQHLGSALANLSPCSIPHRIYRQAHSLLCSFLPWSISITKGGIEYSRTM              |     |
| Consensus                          | (171) | LKDGFTYVLYINGGHPPWTILDYIQHLGSALANLSPCSIPHRIYRQAHSLLCSFLPWSISITKGGIEYSRTM              |     |

consensus positions: 100.0% identity positions: 70.2%

The 154th amino acid(D) of human *NAA60* gene corresponds to the 154th amino acid(D) of mouse *Naa60* gene after comparing homology of mouse *Naa60* gene and human *NAA60* gene.

# Technical Description

- The mouse *Naa60* gene has 11 transcripts. The human *NAA60* gene has 40 transcripts.
- According to the structure of *Naa60* gene and requirements of customer, the 154th amino acid(D) of human *NAA60*(NM\_001083601.3) gene corresponds to the 154th amino acid(D) of mouse *Naa60* gene after comparing homology of mouse *Naa60* gene and human *NAA60* gene. This project produced *Naa60*-p.D154fs point mutation on exon5 of the transcript of *Naa60*-211(ENSMUST00000186375.8, NM\_029090.3), the corresponding codon will be mutated to C by the GAC, produces frameshift mutation.
- The mouse *Naa60*-211 transcript contains 7 exons. The translation initiation site ATG is located at exon2, and the translation termination site TAA is located at exon6, encoding 242aa.
- In this project we use CRISPR/Cas9 technology to modify *Naa60* 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.

# Mutation Site

## Before mutation

|       |                            |            |            |             |            |            |            |             |            |            |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|-------|----------------------------|------------|------------|-------------|------------|------------|------------|-------------|------------|------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| +2    | S L L L E S L K D H I S T? |            |            |             |            |            |            |             |            |            |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 17901 | TGCTTGCTTC                 | TATGTTCTCT | TAAGTGCCT  | GCTGTGGGAA  | ACCTTCATCT | CCTTTCCCAG | GTTCCCTTTT | ACTAGAAAAGT | TTAAAGGATC | ATATTTCAAC |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|       | ACGAACGAAG                 | ATACAAGAGA | ATTGACGGGA | CGACACCCCT  | TGGAAGTAGA | GGAAAGGGTC | CAAGGGAAAA | TGATCTTTCA  | AATTTCTAG  | TATAAAGTTG |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| +2    | ? T                        | A          | Q          | D           | H          | C          | K          | A           | I          | Y          | L | H | V | L | T | T | N | N | T | A | I | N | F | Y | E | N | R | D | F | R | Q | H | H |
| 18001 | CACCGCCCAG                 | GACCACTGCA | AAGCCATCTA | CCTGTCATGTT | CTCACCACCA | ACAATACAGC | AATAAACTTC | TATGAAAACA  | GAGACTTTAG | GCAGCATCAC |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|       | GTGGCGGGTC                 | CTGGTGACGT | TTCGGTAGAT | GGACGTACAA  | GAGTGGTGGT | TGTTATGTCG | TTATTTGAAG | ATACTTTTGT  | CTCTGAAATC | CGTCGTAGTG |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| +2    | Y                          | L          | P          | Y           | Y          | Y          | S          | I           | R          | G          | V | L | K | D | G | F | T | Y | V | L | Y | I | N | G | G | H | P | P | W | T | I |   |   |
| 18101 | TATCTGCCCT                 | ACTACTACTC | CATTGAGGGG | GTCCTCAAAG  | ATGGCTTCAC | CTATGTCCTC | TACATCAATG | GCGGCCACCC  | TCCCTGGACC | ATTTTATATC |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|       | ATAGACGGGA                 | TGATGATGAG | GTAAGCTCCC | CAGGAGTTTC  | TACCGAAGTG | GATACAGGAG | ATGTAGTTAC | CGCCGGTGGG  | AGGGACCTGG | TAAAATATAG |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

## After mutation

|       |                                                                     |            |            |            |            |            |            |             |            |            |  |  |  |  |  |  |  |  |
|-------|---------------------------------------------------------------------|------------|------------|------------|------------|------------|------------|-------------|------------|------------|--|--|--|--|--|--|--|--|
| +2    | S L L L E S L K D H I S T?                                          |            |            |            |            |            |            |             |            |            |  |  |  |  |  |  |  |  |
| 17901 | TGCTTGCTTC                                                          | TATGTTCTCT | TAAGTGCCT  | GCTGTGGGAA | ACCTTCATCT | CCTTTCCCAG | GTTCCCTTTT | ACTAGAAAAGT | TTAAAGGATC | ATATTTCAAC |  |  |  |  |  |  |  |  |
|       | ACGAACGAAG                                                          | ATACAAGAGA | ATTGACGGGA | CGACACCCTT | TGGAAGTAGA | GGAAAGGGTC | CAAGGGAAAA | TGATCTTTCA  | AATTTCTAG  | TATAAAGTTG |  |  |  |  |  |  |  |  |
| +2    | ? T T A Q D H C K A I Y L H V L T T N N T A I N F Y E N R L A A S L |            |            |            |            |            |            |             |            |            |  |  |  |  |  |  |  |  |
| 18001 | CACCGCCCAG                                                          | GACCACTGCA | AAGCCATCTA | CCTGCATGTT | CTCACCACCA | ACAATACAGC | AATAAACTTC | TATGAAAACA  | GACTTTAGGC | AGCATCACTA |  |  |  |  |  |  |  |  |
|       | GTGGCGGGTC                                                          | CTGGTGACGT | TTCGGTAGAT | GGACGTACAA | GAGTGGTGGT | TGTTATGTCG | TTATTTGAAG | ATACTTTTGT  | CTGAAATCCG | TCGTAGTGAT |  |  |  |  |  |  |  |  |
| +2    | S A L L L L H S R G P Q R W L H L C P L H Q W R P P S L D H F       |            |            |            |            |            |            |             |            |            |  |  |  |  |  |  |  |  |
| 18101 | TCTGCCCTAC                                                          | TACTACTCCA | TTCGAGGGGT | CCTCAAAGAT | GGCTTCACCT | ATGTCCTCTA | CATCAATGGC | GGCCACCCTC  | CCTGGACCAT | TTTATATCCT |  |  |  |  |  |  |  |  |
|       | AGACGGGATG                                                          | ATGATGAGGT | AAGCTCCCCA | GGAGTTTCTA | CCGAAGTGGA | TACAGGAGAT | GTAGTTACCG | CCGGTGGGAG  | GGACCTGGTA | AAATATAGGA |  |  |  |  |  |  |  |  |

The yellow region is exon5 of *Naa60-211*, the red region represents the mutation site.

- One or Two synonymous mutations of amino acids will be introduced on exon5 of *Naa60*.
- This strategy had no effect on *Naa60*-208,209 transcripts.
- The mutation site is located in the intron of *Gm20695*-202 gene, and its effect is unknown.
- Mouse *Naa60* gene is located on Chr16. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr16, 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.

# Target gene

|                     |                                                                                                                                                                                                   |
|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gene name           | <i>Naa60</i>                                                                                                                                                                                      |
| Gene ID(NCBI)       | 74763                                                                                                                                                                                             |
| Gene link(NCBI)     | <a href="https://www.ncbi.nlm.nih.gov/gene/74763">https://www.ncbi.nlm.nih.gov/gene/74763</a>                                                                                                     |
| Gene link(Ensembl)  | <a href="http://www.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG0000005982;r=16:3690239-3722634">http://www.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG0000005982;r=16:3690239-3722634</a> |
| chromosome location | Chr16                                                                                                                                                                                             |

# Gene name and location (NCBI)

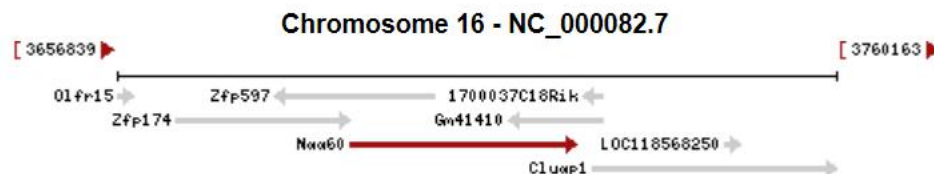
**Naa60** N(alpha)-acetyltransferase 60, NatF catalytic subunit [ *Mus musculus* (house mouse) ]

Gene ID: 74763, updated on 23-Jun-2021

[Download Datasets](#)

## Summary

|                           |                                                                                                                                                                           |
|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Official Symbol</b>    | Naa60 provided by <a href="#">MGI</a>                                                                                                                                     |
| <b>Official Full Name</b> | N(alpha)-acetyltransferase 60, NatF catalytic subunit provided by <a href="#">MGI</a>                                                                                     |
| <b>Primary source</b>     | <a href="#">MGI:MGI:1922013</a>                                                                                                                                           |
| <b>See related</b>        | <a href="#">Ensembl:ENSMUSG00000005982</a>                                                                                                                                |
| <b>Gene type</b>          | protein coding                                                                                                                                                            |
| <b>RefSeq status</b>      | VALIDATED                                                                                                                                                                 |
| <b>Organism</b>           | <a href="#">Mus musculus</a>                                                                                                                                              |
| <b>Lineage</b>            | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| <b>Also known as</b>      | Nat; HAT4; NatF; Nat15; AI315146; 1200013P24Rik                                                                                                                           |
| <b>Expression</b>         | Ubiquitous expression in adrenal adult (RPKM 39.1), mammary gland adult (RPKM 28.1) and 28 other tissues <a href="#">See more</a>                                         |
| <b>Orthologs</b>          | <a href="#">human</a> <a href="#">all</a>                                                                                                                                 |

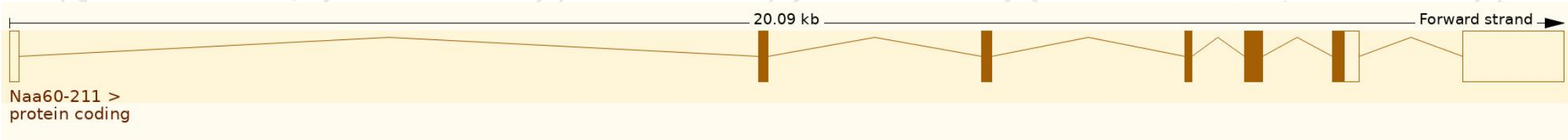


# Transcript information (Ensembl)

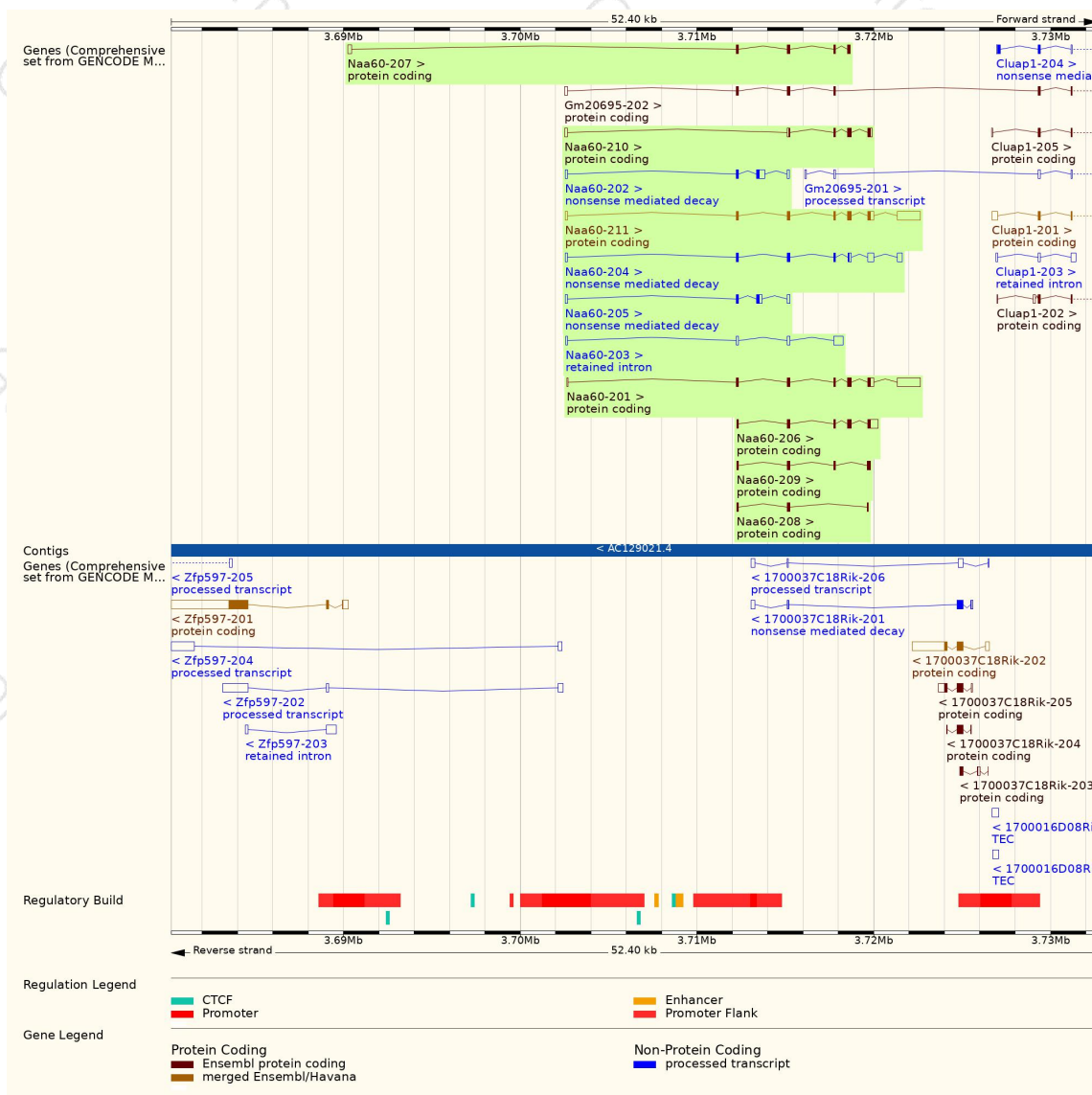
The gene has 11 transcripts, and all transcripts are shown below:

| Name      | Transcript ID                        | bp   | Protein               | Biotype                 | CCDS                      | UniProt Match          | Flags                         |
|-----------|--------------------------------------|------|-----------------------|-------------------------|---------------------------|------------------------|-------------------------------|
| Naa60-201 | <a href="#">ENSMUST00000115860.8</a> | 2303 | <a href="#">242aa</a> | Protein coding          | <a href="#">CCDS37239</a> | <a href="#">Q9DBU2</a> | GENCODE basic APPRIS P1 TSL:1 |
| Naa60-202 | <a href="#">ENSMUST00000135093.8</a> | 807  | <a href="#">79aa</a>  | Nonsense mediated decay | -                         | <a href="#">D6RDS3</a> | TSL:1                         |
| Naa60-203 | <a href="#">ENSMUST00000140832.2</a> | 869  | No protein            | Retained intron         | -                         | -                      | TSL:2                         |
| Naa60-204 | <a href="#">ENSMUST00000143537.9</a> | 1279 | <a href="#">133aa</a> | Nonsense mediated decay | -                         | <a href="#">H3BJZ6</a> | TSL:5                         |
| Naa60-205 | <a href="#">ENSMUST00000147949.8</a> | 653  | <a href="#">79aa</a>  | Nonsense mediated decay | -                         | <a href="#">D6RDS3</a> | TSL:2                         |
| Naa60-206 | <a href="#">ENSMUST00000150655.3</a> | 1166 | <a href="#">242aa</a> | Protein coding          | <a href="#">CCDS37239</a> | <a href="#">Q9DBU2</a> | GENCODE basic APPRIS P1 TSL:2 |
| Naa60-207 | <a href="#">ENSMUST00000151988.8</a> | 719  | <a href="#">168aa</a> | Protein coding          | -                         | <a href="#">D3YYN1</a> | TSL:3 CDS 3' incomplete       |
| Naa60-208 | <a href="#">ENSMUST00000175809.8</a> | 279  | <a href="#">92aa</a>  | Protein coding          | -                         | <a href="#">H3BLJ2</a> | GENCODE basic TSL:5           |
| Naa60-209 | <a href="#">ENSMUST00000176224.8</a> | 501  | <a href="#">166aa</a> | Protein coding          | -                         | <a href="#">H3BJW5</a> | GENCODE basic TSL:5           |
| Naa60-210 | <a href="#">ENSMUST00000176625.8</a> | 876  | <a href="#">177aa</a> | Protein coding          | <a href="#">CCDS70676</a> | <a href="#">H3BIY0</a> | GENCODE basic TSL:2           |
| Naa60-211 | <a href="#">ENSMUST00000186375.8</a> | 2350 | <a href="#">242aa</a> | Protein coding          | <a href="#">CCDS37239</a> | <a href="#">Q9DBU2</a> | GENCODE basic APPRIS P1 TSL:1 |

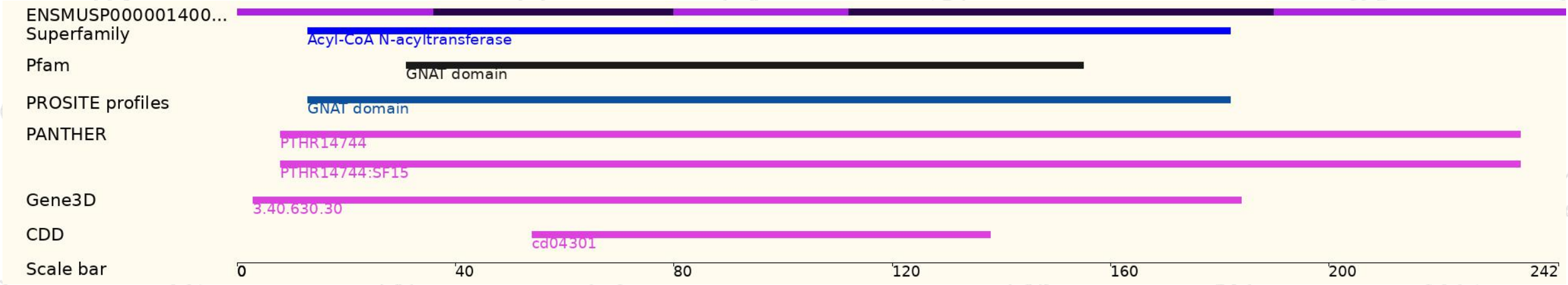
The strategy is based on the design of *Naa60-211* transcript, the transcription is shown below:



# Genomic location distribution



# Protein domain



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

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