# *Hars2* Cas9-KO Strategy

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# Project Overview

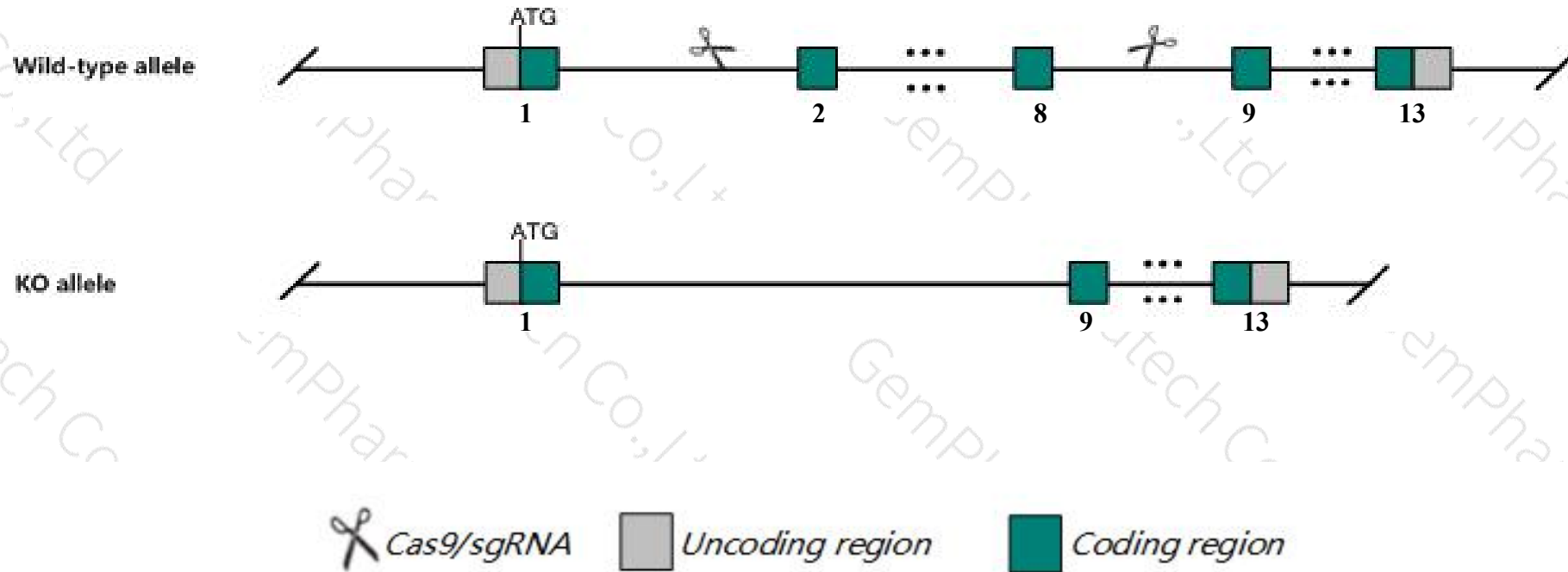| | |
|---|---|
| **Project Name** | *Hars2* |
| **Project type** | **Cas9-KO** |
| **Strain background** | **C57BL/6J** |

# Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Hars2* gene. The schematic diagram is as follows:

# Technical routes

➤ The *Hars2* gene has 7 transcripts. According to the structure of *Hars2* gene, exon2-exon8 of *Hars2-206* (ENSMUST00000152954.7) transcript is recommended as the knockout region.The region contains 721bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Hars2* 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 *Hars2* gene is located on the Chr18. 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）

## Hars2 histidyl-tRNA synthetase 2 [Mus musculus (house mouse)]

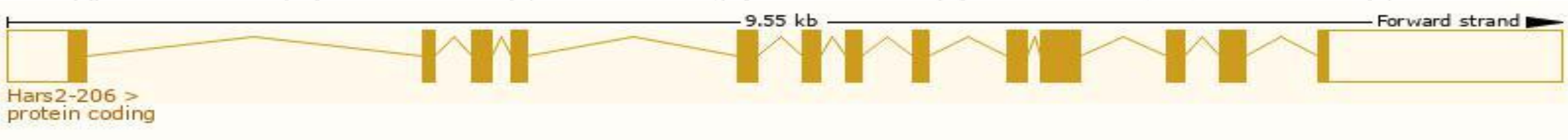Gene ID: 70791, updated on 31-Jan-2019

### Summary

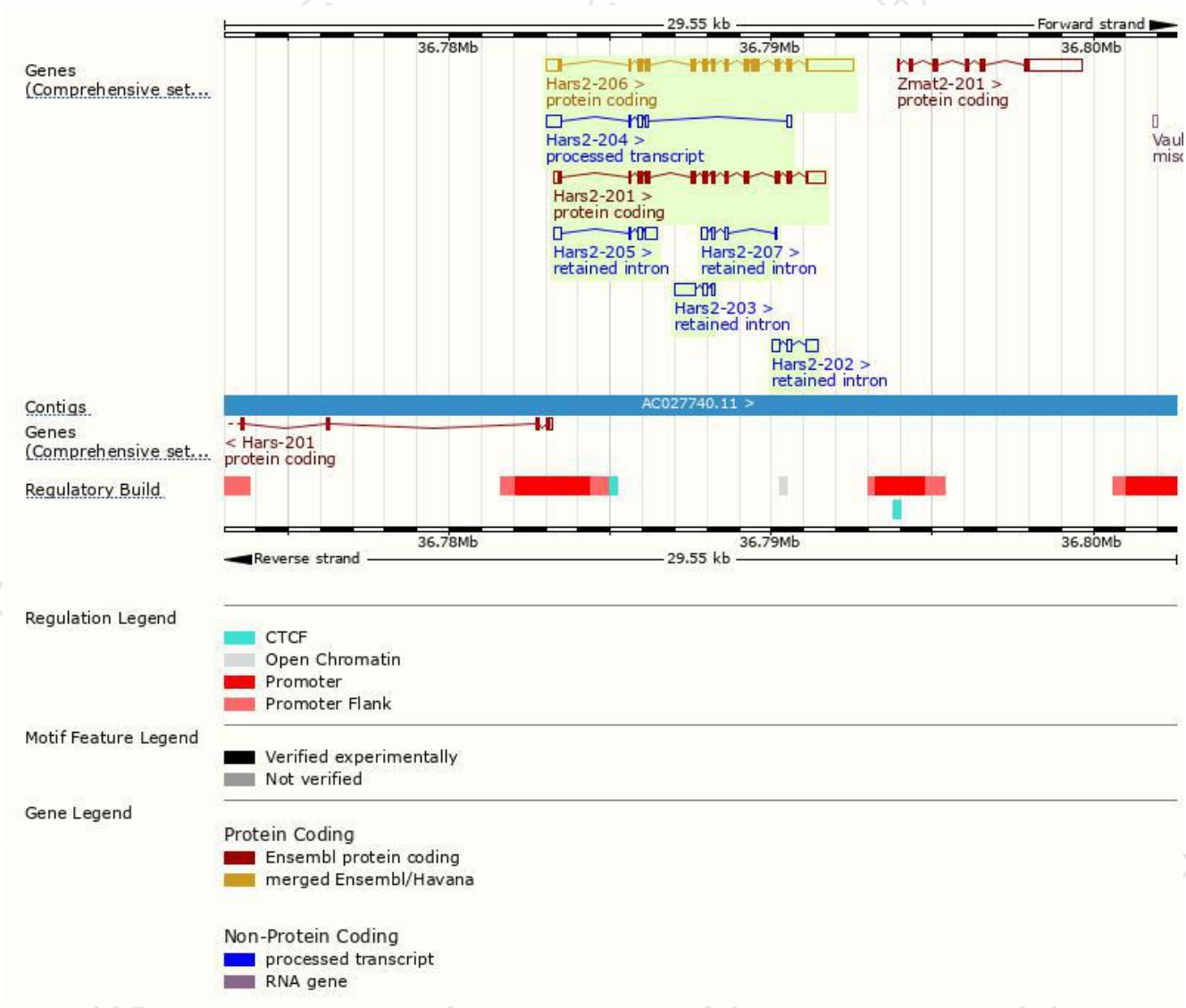| | |
|---|---|
| **Official Symbol** | Hars2 provided by MGI |
| **Official Full Name** | histidyl-tRNA synthetase 2 provided by MGI |
| **Primary source** | MGI:MGI:1918041 |
| **See related** | Ensembl:ENSMUSG00000019143 |
| **Gene type** | protein coding |
| **RefSeq status** | REVIEWED |
| **Organism** | Mus musculus |
| **Lineage** | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| **Also known as** | 4631412B19Rik, HARSR, HO3, Harsl |
| **Summary** | This gene encodes a putative member of the class II family of aminoacyl-tRNA synthetases. These enzymes play a critical role in protein biosynthesis by charging tRNAs with their cognate amino acids. This protein is encoded by the nuclear genome but is likely to be imported to the mitochondrion where it is thought to catalyze the ligation of histidine to tRNA molecules. Mutations in a similar gene in human have been associated with Perrault syndrome 2 (PRLTS2). [provided by RefSeq, Mar 2015] |
| **Expression** | Ubiquitous expression in CNS E11.5 (RPKM 11.8), CNS E14 (RPKM 10.4) 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 |
|---|---|---|---|---|---|---|---|
| Hars2-206 | ENSMUST00000152954.7 | 3336 | 505aa | Protein coding | CCDS29165 | Q99KK9 | TSL:1 GENCODE basic APPRIS P1 |
| Hars2-201 | ENSMUST00000019287.8 | 2002 | 424aa | Protein coding | CCDS84376 | G5E823 | TSL:1 GENCODE basic |
| Hars2-204 | ENSMUST00000134122.7 | 896 | No protein | Processed transcript | - | - | TSL:3 |
| Hars2-203 | ENSMUST00000131952.1 | 819 | No protein | Retained intron | - | - | TSL:5 |
| Hars2-205 | ENSMUST00000145876.1 | 772 | No protein | Retained intron | - | - | TSL:2 |
| Hars2-202 | ENSMUST00000124204.1 | 769 | No protein | Retained intron | - | - | TSL:1 |
| Hars2-207 | ENSMUST00000155842.1 | 406 | No protein | Retained intron | - | - | TSL:5 |

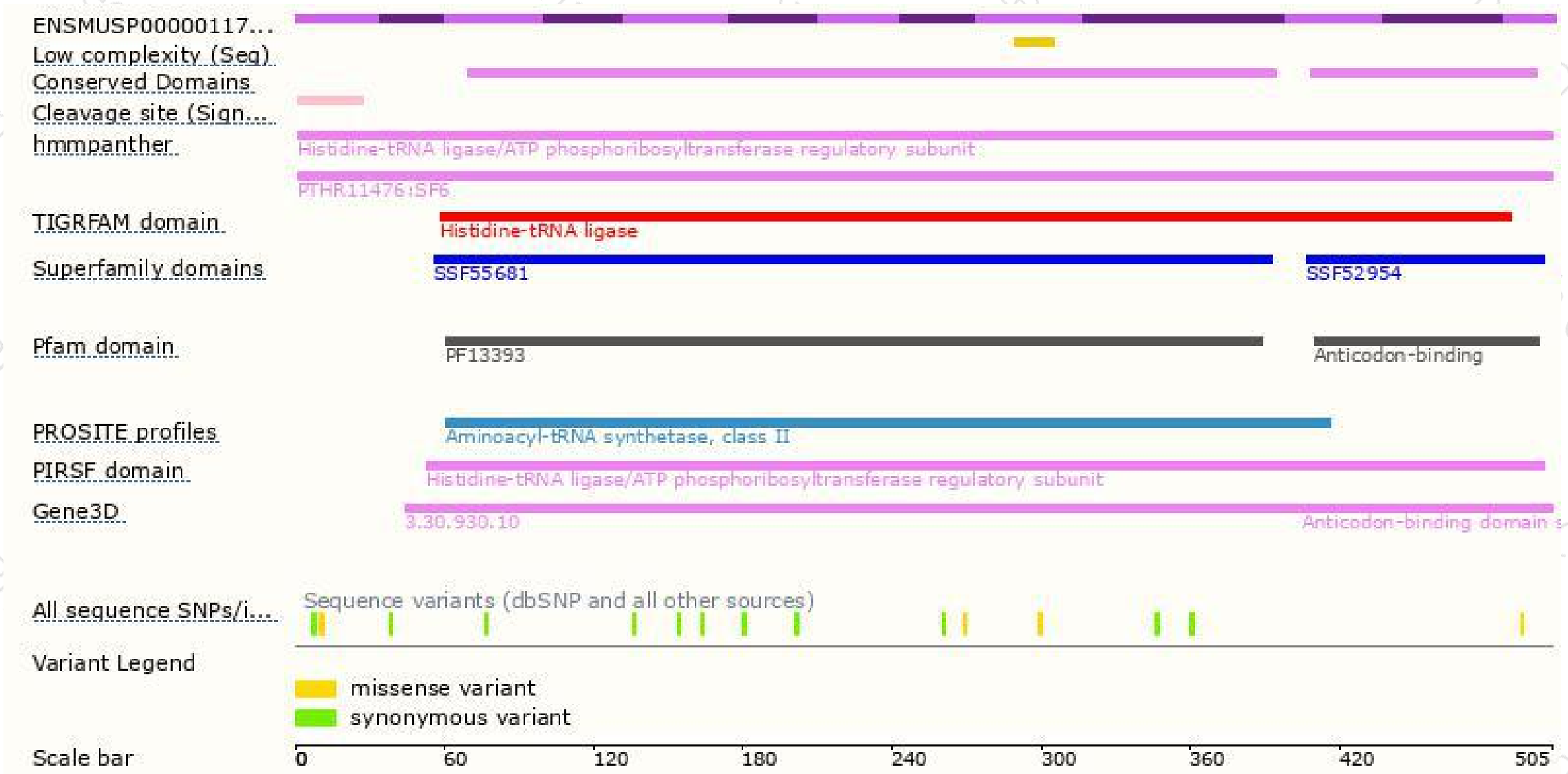The strategy is based on the design of *Hars2-206* transcript,The transcription is shown below

# Genomic location distribution

# Protein domain



| | |
|---|---|
| ENSMUSP00000117... | |
| Low complexity (Seg) | |
| Conserved Domains | |
| Cleavage site (Sign... | |
| hmmpanther | Histidine-tRNA ligase/ATP phosphoribosyltransferase regulatory subunit<br>PTHR11476:SF6 |
| TIGRFAM domain | Histidine-tRNA ligase |
| Superfamily domains | SSF55681  SSF52954 |
| Pfam domain | PF13393  Anticodon-binding |
| PROSITE profiles | Aminoacyl-tRNA synthetase, class II |
| PIRSF domain | Histidine-tRNA ligase/ATP phosphoribosyltransferase regulatory subunit |
| Gene3D | 3.30.930.10  Anticodon-binding domain s |
| All sequence SNPs/i... | Sequence variants (dbSNP and all other sources) |
| Variant Legend | ▮ missense variant<br>▮ synonymous variant |
| Scale bar | 0   60   120   180   240   300   360   420   505 |

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