Lars2 Cas9-CKO Strategy

Designer: Yanhua Shen

Reviewer： Xueting Zhang

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Project Overview

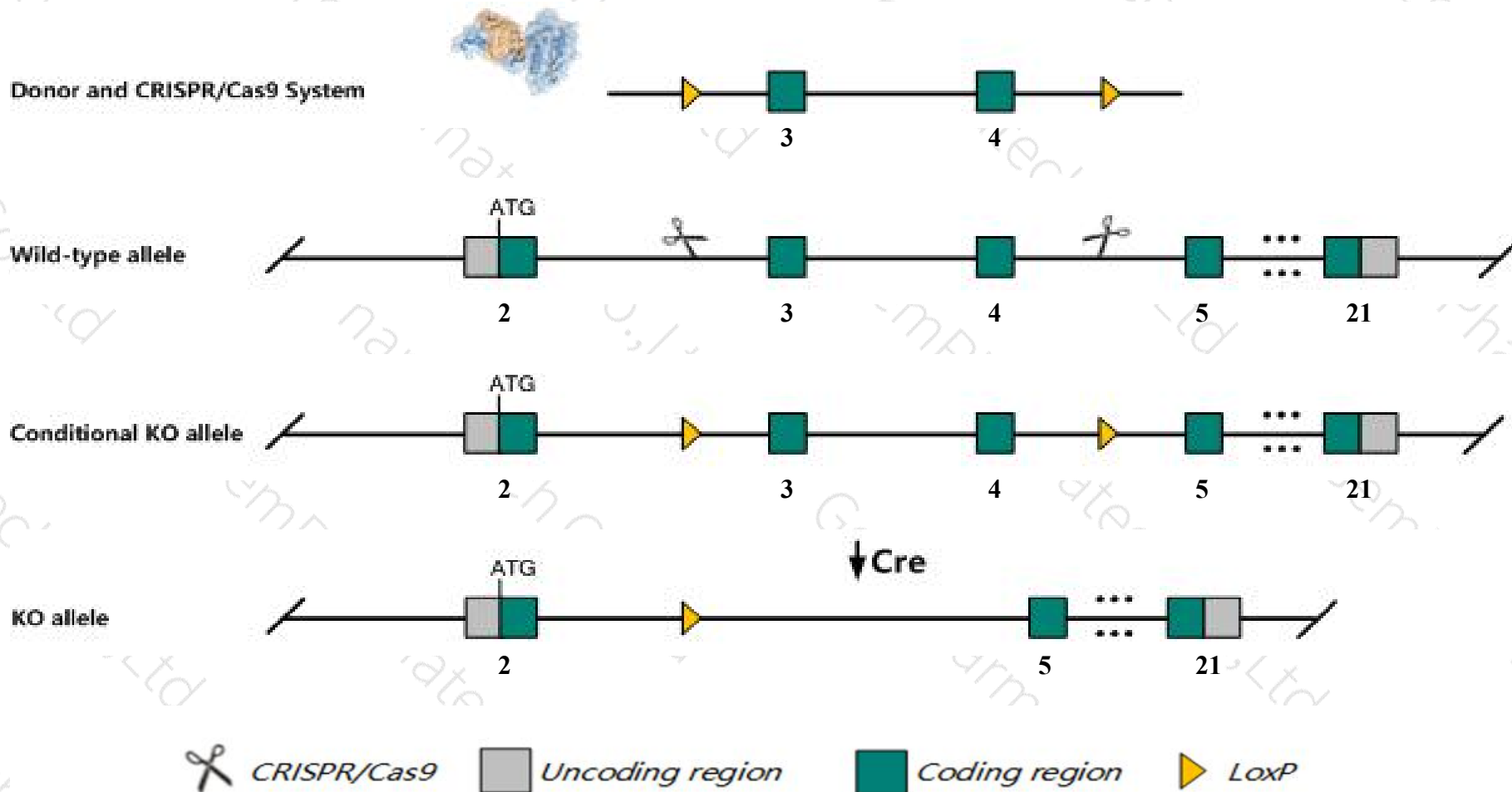|	
Project Name	*Lars2*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Lars2* gene. The schematic diagram is as follows:

Technical routes

➤ The *Lars2* gene has 9 transcripts. According to the structure of *Lars2* gene, exon3-exon4 of *Lars2-201* (ENSMUST00000038863.8) transcript is recommended as the knockout region.The region contains Ens 221bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Lars2* 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.

➤ The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

➢ Transcripts 202,203,204,205,207 may not be affected. The effect of transcripts 206,208 is unknown.

➢ The *Lars2* gene is located on the Chr9. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

Lars2 leucyl-tRNA synthetase, mitochondrial [Mus musculus (house mouse)]
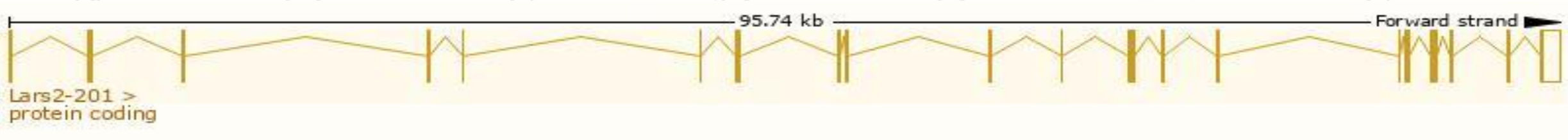
Gene ID: 102436, updated on 13-Mar-2020

▲Summary

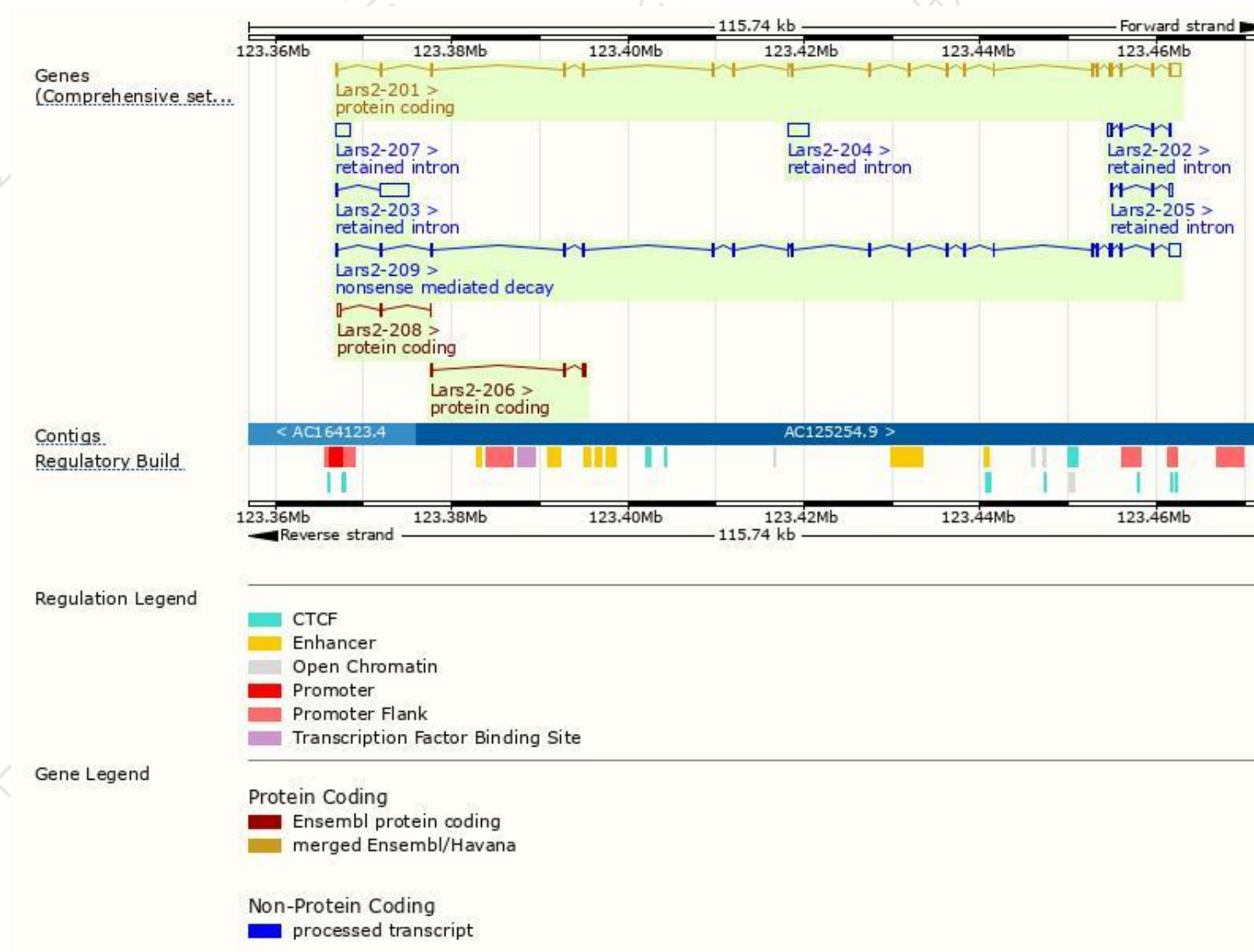|	
Official Symbol	Lars2 provided by MGI
Official Full Name	leucyl-tRNA synthetase, mitochondrial provided by MGI
Primary source	MGI:MGI:2142973
See related	Ensembl:ENSMUSG00000035202
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AI035546, Kiaa0028, LEURS
Expression	Ubiquitous expression in cortex adult (RPKM 1105.1), placenta adult (RPKM 827.6) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 9 transcripts,all transcripts are shown below:

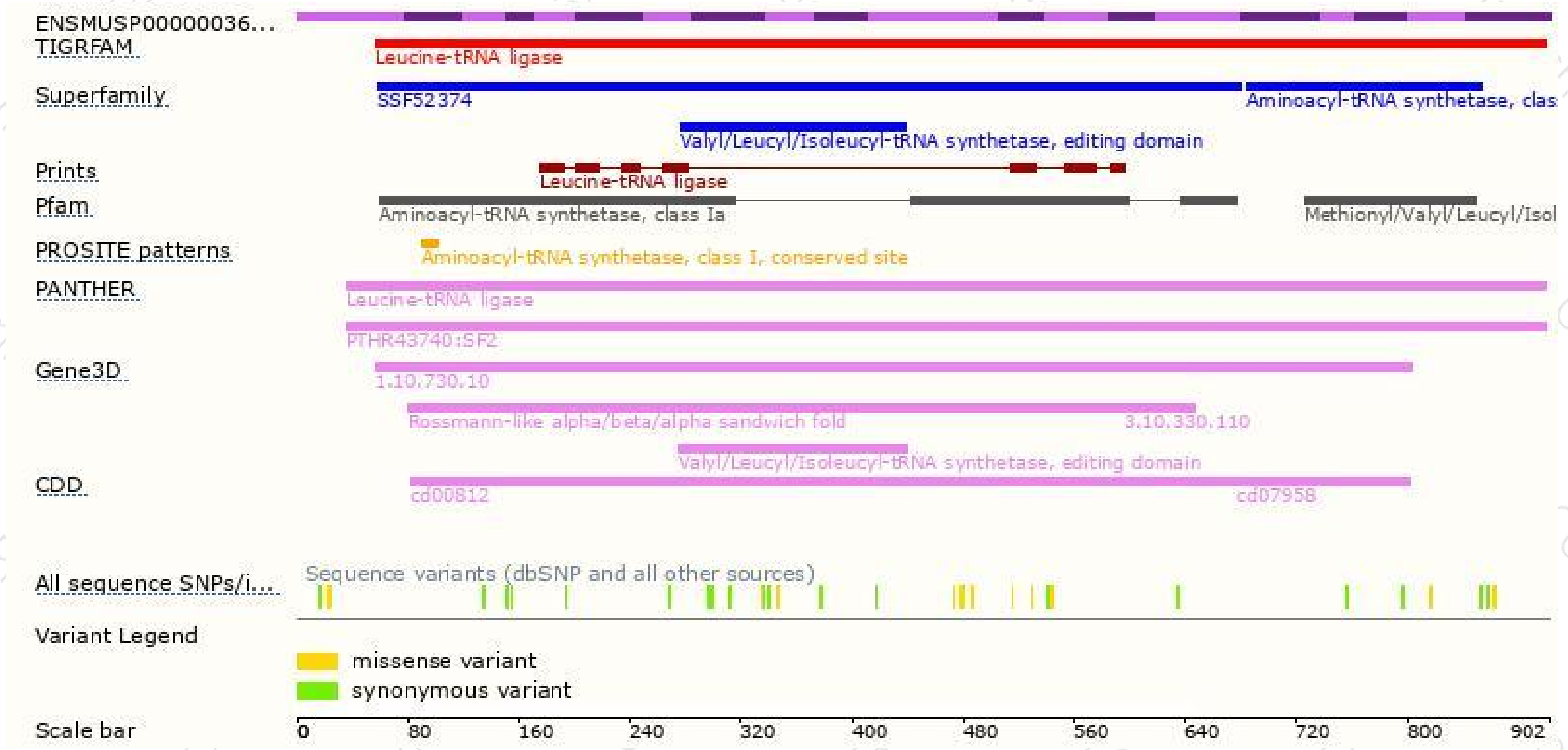| Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Lars2-201	ENSMUST00000038863.8	3894	902aa	Protein coding	CCDS23659	Q8VDC0	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Lars2-208	ENSMUST00000216843.1	720	88aa	Protein coding	-	A0A1L1SUS6	CDS 3' incomplete TSL:5
Lars2-206	ENSMUST00000215464.1	554	121aa	Protein coding	-	A0A1L1SQK2	CDS 5' incomplete TSL:3
Lars2-209	ENSMUST00000217116.1	3756	495aa	Nonsense mediated decay	-	A0A1L1SSW1	TSL:5
Lars2-203	ENSMUST00000214074.1	3415	No protein	Retained intron	-	-	TSL:1
Lars2-204	ENSMUST00000214557.1	2441	No protein	Retained intron	-	-	TSL:NA
Lars2-207	ENSMUST00000215674.1	1598	No protein	Retained intron	-	-	TSL:NA
Lars2-205	ENSMUST00000215087.1	889	No protein	Retained intron	-	-	TSL:3
Lars2-202	ENSMUST00000213711.1	787	No protein	Retained intron	-	-	TSL:5

The strategy is based on the design of *Lars2-201* transcript,the transcription is shown below:

Genomic location distribution

Protein domain

ENSMUSP00000036...			
TIGRFAM	Leucine-tRNA ligase		
Superfamily	SSF52374	Aminoacyl-tRNA synthetase, clas
	Valyl/Leucyl/Isoleucyl-tRNA synthetase, editing domain		
Prints	Leucine-tRNA ligase		
Pfam	Aminoacyl-tRNA synthetase, class Ia	Methionyl/Valyl/Leucyl/Isol
PROSITE patterns	Aminoacyl-tRNA synthetase, class I, conserved site		
PANTHER	Leucine-tRNA ligase		
	PTHR43740:SF2		
Gene3D	1.10.730.10		
	Rossmann-like alpha/beta/alpha sandwich fold	3.10.330.110	
	Valyl/Leucyl/Isoleucyl-tRNA synthetase, editing domain		
CDD	cd00812	cd07958	

All sequence SNPs/i... — Sequence variants (dbSNP and all other sources)

Variant Legend

- missense variant (yellow)
- synonymous variant (green)

Scale bar: 0 80 160 240 320 400 480 560 640 720 800 902

If you have any questions, you are welcome to inquire.
Tel: 400-9660890

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