# NuRNA™ Human tRNA Modification Enzymes PCR Array

Cat#: AS-NM-001

## Instruction Manual    Version 1.0

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# I. Introduction

## A. Overview

Transfer RNAs (tRNAs) are the key component for protein translation. Posttranscriptional modifications of tRNA are critical for all core aspects of tRNA function, such as folding, stability and decoding. These chemical modifications are dynamically regulated and catalyzed by tRNA modification enzymes. Recent discoveries have led to new appreciation of the key roles of tRNA modifications and tRNA modification enzymes as checkpoints for tRNA integrity and for integrating translation with other cellular functions such as transcription, primary metabolism and stress signaling[1]. Mutations in multiple tRNA modification proteins have been identified in patients and associated with diseases. For example, mutations in the enzymes responsible for methylthiolation of t6A can lead to type 2 diabetes[2]. To help easy and rapid profiling of tRNA modifiers, Arraystar has designed the first commercially available NuRNA™ Human tRNA Modification Enzymes PCR Array. The panel contains 85 validated or predicted tRNA modification enzymes or protein factors compiled from published studies and databases including UniProt and Modomics. The Array is a powerful tool for global survey of tRNA modification enzymes to analyze their roles in tRNA canonical functions in translation and non-canonical functions such as cellular metabolism and stress response.

## B. The functions of tRNA modifications

tRNAs are key adaptor molecules in the protein translation process. Chemical modifications are crucial for tRNA structure, function, and stability. To be fully active, tRNAs need to be extensively modified post-transcriptionally during their maturation. In general, hypo-modified tRNAs are targeted for degradation[3]. Specific modifications in the stem-loops are crucial for tRNA structure and stability, whereas modifications in the anticodon loop enhance the translation accuracy by preventing translational frameshifting. Modifications at position 34 in the anticodon typically increase the codon recognition diversity through codon-anticodon wobbling[4]. Furthermore, modifications at base 37 adjacent to the anticodon loop fine tune the stability of codon-anticodon interactions[1]. Modifications (*e.g.* pseudouridines) in the main body of the tRNA strengthen the binding affinity and rigidify the tRNA structure; whereas other modifications (*e.g.* dihydrouridines) maintain the flexibility of tRNA structure. In some cases, modifications serve as additional tRNA identity elements for accurate aminoacyl tRNA synthetase recognition. Post-transcriptional addition of a guanosine (G) at the 5'-end of tRNA$^{His}$ is critical for specific histidine charging by histidinyl-tRNA synthetase[5]. In addition, certain tRNA modifications affect the translation of only a defined subset of transcripts enriched with certain types of codons. These transcripts could be collectively linked to a common cellular pathway[6].

More than 50 different chemical modifications have been described affecting different positions in eukaryotic tRNAs. In recent years, human enzymes catalyzing these modifications and their biological roles have started to be documented (**Figure 1**). A link between tRNA modifications and human diseases is becoming increasingly clear.
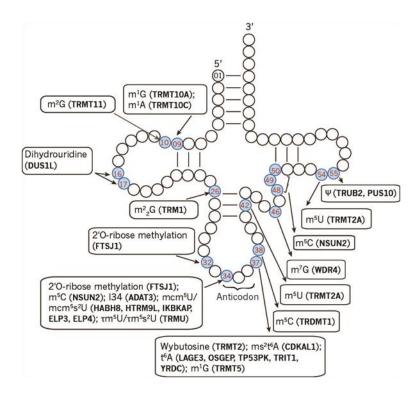
**Figure 1**. tRNA modifications and tRNA modification enzymes (in parentheses) in human.

# C. tRNA modifications and diseases

## ■ Neurological Disorders

Several mutations in tRNA modification enzymes have been associated with human intellectual disability. For example, human FtsJ RNA methyltransferase homolog 1 (FTSJ1) methylates tRNA$^{Leu}$, tRNA$^{Phe}$, and tRNA$^{Trp}$ at positions 32 and 34. FTSJ1 mutations is associated with non-syndromic X-linked mental retardation. Additionally, genetic variations in FTSJ1 are strongly correlated with cognitive functions[7]. Human tRNA methyltransferase 1 (TRM1) modifies tRNAs at position 26 with dimethyl guanosines (m$^2_2$G). A homozygous frameshift mutation that inactivates this gene has been reported as a novel marker for recessive cognitive disorders[8]. NOP2/Sun RNA methyltransferase family member 2 (NSUN2) catalyzes the formation of 5-methylcytosine (m5C) at position 34 of tRNALeu(CCA) and also positions 48, 49, and 50 on several other tRNAs. NSUN2 mutations are associated with autosomal-recessive intellectual disability[9, 10]. Mutations in human tRNA modification enzymes such as WD repeat domain 4 (WDR4) and adenosine deaminase acting on tRNA 3 (ADAT3) are linked to neurological disorders[11, 12].

## ■ Cancers

tRNA modifications haven been directly linked to skin, breast, bladder, and colorectal cancers. NSUN2 is expressed at low levels in normal tissues, but it is abundant in a range of human and mice tumor types, including squamous cell carcinoma, colorectal cancer, and breast cancer. NSUN2 knockdown reduces the growth of human squamous cell carcinoma in xenograft model[13]. Human tRNA methyltransferase homolog 12 (TRMT12) catalyzes the formation of wybutosine at position 37 on tRNA$^{Phe}$. TRMT12 gene is amplified in several breast cancer cell lines and overexpressed in 26 out of 30 analyzed breast cancer tumors[14]. Human RNA (guanine-9-) methyltransferase

domain containing 2 (HRG9MTD2) is responsible for m1G9 modification of several tRNAs. HRG9MTD2 is among the few genes differentially expressed between early-onset and late-onset colorectal cancer patients[15].

### ■ Type 2 diabetes

tRNA modification enzymes are associated with metabolic disorders including type-2 diabetes. Mutations in the CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*) gene are frequently associated with an increased risk for developing type 2 diabetes mellitus in humans and mice[2, 16, 17]. CDKAL1 catalyses 2-methylthio-*N6*-threonylcarbamoyl-adenosine ($ms^2t^6A$) modification of A37 in tRNA$^{Lys}$(UUU), which is crucial for codon–anticodon interaction and for preventing translational misreading. *Cdkal1*-deficient mouse β-cells display a significantly reduced incorporation of Lys residues, an indication of misreading Lys codons AAA or AAG, and altered glucose-induced proinsulin biosynthesis and folding.

## D. Product summary

NuRNA™ Human tRNA Modification Enzymes PCR Array profiles 85 critical enzymes and protein factors involved in tRNA modifications. All the enzymes/proteins are comprehensively collected based on research publications and from the most updated authoritative databases including UniProt and Modomics.
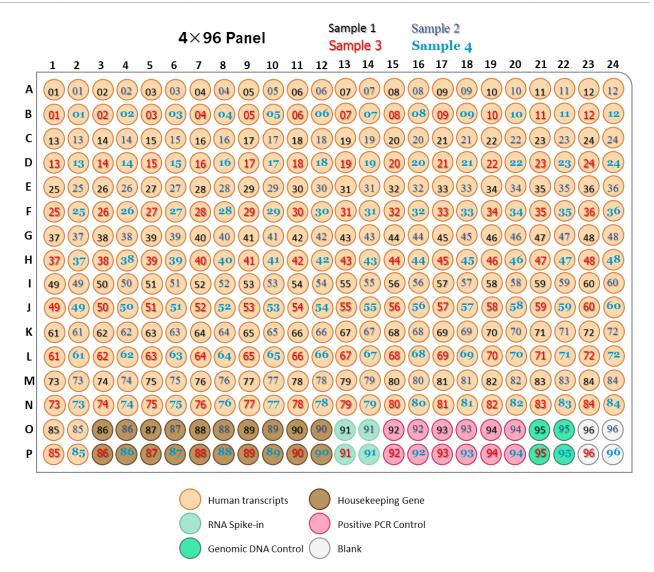
■ **Kit Contents**

**Table 1** List of human tRNA modification enzymes and controls

| 01 | ADAT1 | 25 | LAGE3 | 49 | SSB | 73 | TRMT61A |
|----|-------|----|-------|----|-----|----|---------|
| 02 | ADAT2 | 26 | LCMT2 | 50 | TARBP1 | 74 | TRMT61B |
| 03 | ADAT3 | 27 | METTL1 | 51 | THUMPD1 | 75 | TRMU |
| 04 | ALKBH1 | 28 | METTL2A | 52 | THUMPD2 | 76 | TRUB1 |
| 05 | ALKBH8 | 29 | METTL2B | 53 | THUMPD3 | 77 | TRUB2 |
| 06 | C9orf64 | 30 | MOCS3 | 54 | TP53RK | 78 | TYW1 |
| 07 | CDK5RAP1 | 31 | MTO1 | 55 | TPRKB | 79 | TYW1B |
| 08 | CDKAL1 | 32 | NAT10 | 56 | TRDMT1 | 80 | TYW3 |
| 09 | CDKL1 | 33 | NFS1 | 57 | TRIT1 | 81 | TYW5 |
| 10 | CTU1 | 34 | NSUN2 | 58 | TRMO | 82 | UBA5 |
| 11 | CTU2 | 35 | NSUN6 | 59 | TRMT1 | 83 | URM1 |
| 12 | DUS1L | 36 | OSGEP | 60 | TRMT10A | 84 | WDR4 |
| 13 | DUS2 | 37 | OSGEPL1 | 61 | TRMT10B | 85 | YRDC |
| 14 | DUS3L | 38 | PUS1 | 62 | TRMT10C | 86 | GAPDH |
| 15 | DUS4L | 39 | PUS10 | 63 | TRMT11 | 87 | ACTB |
| 16 | ELP3 | 40 | PUS3 | 64 | TRMT112 | 88 | B2M |
| 17 | ELP4 | 41 | PUS7L | 65 | TRMT12 | 89 | Gusb |
| 18 | FBLL1 | 42 | PUSL1 | 66 | TRMT13 | 90 | Hsp90ab1 |
| 19 | FTSJ1 | 43 | QTRT1 | 67 | TRMT1L | 91 | RNA Spike-in |
| 20 | GTPBP3 | 44 | QTRT2 | 68 | TRMT2A | 92 | PPC |
| 21 | HSD17B10 | 45 | RPUSD1 | 69 | TRMT2B | 93 | PPC |
| 22 | IKBKAP | 46 | RPUSD2 | 70 | TRMT44 | 94 | PPC |
| 23 | KIAA0391 | 47 | RPUSD3 | 71 | TRMT5 | 95 | GDC |
| 24 | KIAA1456 | 48 | RPUSD4 | 72 | TRMT6 | 96 | Blank |

■ **Description of the control assays**

NuRNA™ Human tRNA Modification Enzymes PCR Array includes a series of external and internal controls as described below.

- **HK** (Housekeeping Genes; Internal Controls): 5 human housekeeping genes GAPDH, ACTB, B2M, Gusb, and Hsp90ab1 are included as the internal qPCR normalization references. Arraystar PCR system provides multiple reference genes selected among commonly used reference genes by using a stringent bioinformatic algorithm, which offers the flexibility of choosing the most valid reference gene(s) for qPCR normalization for your sample types.

- **RNA Spike-in** (External Control): One External RNA Spike-in Mix is added in the RNA sample prior to the first strand cDNA synthesis. The RNA Spike-in control assay indicates the overall success and the efficiency of the reaction beginning from the cDNA synthesis to the final qPCR. Any problem(s) in these steps will result in a failed or compromised RNA Spike-in outcome. RNA Spike-in assay results for samples are compared and outliers or failed reactions may be identified and excluded from further data analysis.

- **PPC** (Positive PCR control)**:** 3 replicates of one artificial DNAs and the PCR primer pairs to indicate the qPCR amplification efficiency. A Ct value greater than 25 is an indication of low qPCR amplification efficiency. More importantly, the PPC are used as inter-plate calibrator (IPC) between PCR plate runs to give the same Ct value for the calibrator, thereby reducing run-to-run variance. Inter-plate calibration (IPC) can easily be performed with the data analysis software avaliable on our website (www.arraystar.com).

- **GDC** (Genomic DNA Control): The control assay consists of PCR primers for an untranscribed genomic region. Non-RT sample or RNA sample are added during the qPCR Process. The Ct values should be greater than 35. A positive GDC signal indicates the array result may have been compromised with genomic DNA contamination.

- **Blank (Blank Control):** The background reading from the SYBR Green Master Mix.

### ■ Shipping and Storage

Arraystar PCR Arrays are shipped at ambient temperature, on ice, or on dry ice depending on the destination and accompanying products. Store at –20°C upon receipt. The contents are stable for at least 6 months.
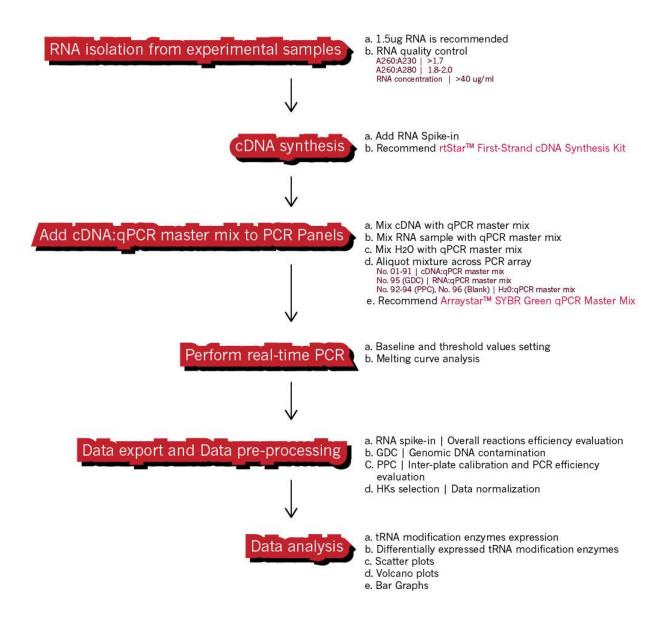
### ■ Additional Required Equipment

- Thermal cycler
- Real time qPCR instrument, compatible with 384-well format

### ■ Additional Required Reagents

- rtStar™ First-Strand cDNA Synthesis Kit (Cat# AS-FS-001)
- Arraystar SYBR® Green qPCR Master Mix(ROX+) (AS-MR-006-5)
- Nuclease-free PCR-grade water

# E. Protocol overview

**RNA isolation from experimental samples**
a. 1.5ug RNA is recommended
b. RNA quality control
  A260:A230 | >1.7
  A260:A280 | 1.8-2.0
  RNA concentration | >40 ug/ml

**cDNA synthesis**
a. Add RNA Spike-in
b. Recommend rtStar™ First-Strand cDNA Synthesis Kit

**Add cDNA:qPCR master mix to PCR Panels**
a. Mix cDNA with qPCR master mix
b. Mix RNA sample with qPCR master mix
c. Mix H2O with qPCR master mix
d. Aliquot mixture across PCR array
  No. 01-91 | cDNA:qPCR master mix
  No. 95 (GDC) | RNA:qPCR master mix
  No. 92-94 (PPC), No. 96 (Blank) | H2O:qPCR master mix
e. Recommend Arraystar™ SYBR Green qPCR Master Mix

**Perform real-time PCR**
a. Baseline and threshold values setting
b. Melting curve analysis

**Data export and Data pre-processing**
a. RNA spike-in | Overall reactions efficiency evaluation
b. GDC | Genomic DNA contamination
C. PPC | Inter-plate calibration and PCR efficiency evaluation
d. HKs selection | Data normalization

**Data analysis**
a. tRNA modification enzymes expression
b. Differentially expressed tRNA modification enzymes
c. Scatter plots
d. Volcano plots
e. Bar Graphs

# II. Protocol

**IMPORTANT:** Please read through this entire protocol before your first experiment. Prepare a workspace and use labwares free of DNA, RNA, and nuclease contamination. Wear gloves while performing this protocol.

## A. RNA sample preparation and quality control

For best results from the PCR array, RNA samples must meet the QC standards of integrity and purity from protein, organics, genomic DNA contamination and excessive RNA degradation. You can measure the RNA concentration and purity by UV spectrophotometry. You can also check 18S and 28S ribosomal RNA as an indicator of RNA integrity by denaturing gel electrophoresis or by using an Agilent BioAnalyzer.

- A260:A230 ratio greater than 1.7.
- A260:A280 ratio between 1.8 and 2.0.
- Total RNA concentration greater than 40 ng/µL

Eliminating genomic DNA contamination is essential for accurate gene expression profiling by qPCR, which is particularly important for genes at low expression levels. Due to the presence of pseudogenes, even cross-intron primers are not a guarantee of avoiding amplification from contaminating genomic DNA. The Genomic DNA Control (GDC) in the PCR Array specifically detects potential genomic DNA contamination. A Ct for GDC less than 35 indicates the presence of genomic DNA contamination that may compromise the qPCR results.

## B. First-strand cDNA synthesis

Use the same amount of total RNA in this reaction for every sample. High quality cDNA synthesis is vital for the following qPCR performance. We highly recommend using rtStar™ First-Strand cDNA Synthesis Kit (Cat# AS-FS-001), which is specifically optimized for and fully compatible with the Arraystar PCR Arrays.

1.  Prepare reagents

    Gently thaw all of the kit components except for Reverse Transcriptase, and immediately place on ice. Mix by vortexing. Spin down all reagents.

    **NOTE:** *The first time to use this kit, please reconstitute the RNA Spike-in by adding 200 µL Nuclease-free Water to the tube. Mix by vortexing and spin down. Leave on ice for 20 ~ 30 min to fully dissolve the RNA Spike-in. Vortex again, then spin down.*

2.  Combine Annealing Mix according to Table

    If performing first-strand cDNA synthesis on multiple RNA samples, it is recommended to prepare an Annealing Mix of the Primer, dNTP Mix and RNA Spike-in (in the proportion indicated in Table). 10% excess volume for pipetting losses is recommended.

| | |
|---|---|
| Oligo(dT)18, or Random Primers | 1.0 µL |
| dNTP Mix | 1.0 µL |
| RNA Spike-in | 2.0 µL |
| Template Total RNA | 10.0 µL |
| Total volume | 14.0 µl |

3. Incubate in a thermal cycler at 65°C for 5 min, and then immediately place on ice for at least 1 min. Collect the contents of the tube by brief centrifugation.

4. Combine cDNA Synthesis Mix

   cDNA Synthesis Mix is recommended to prepare for multiple RNA samples. It includes the components in the following table. 10% excess volume for pipetting losses is recommended.

| | |
|---|---|
| 5 × RT Reaction buffer | 4.0 µL |
| RNase Inhibitor | 1.0 µL |
| Reverse Transcriptase | 1.0 µL |
| Total volume | 6.0 µL |

5. Add cDNA Synthesis Mix to the tube from STEP 3. Vortex the sample briefly to mix, and collect by brief centrifugation. Incubate at 25°C for 10 min, followed by 30 min at 45°C.

6. Terminate the reactions at 85°C for 5 min. Chill on ice.

7. OPTIONAL. To check the synthesized cDNA quality, reconstitute the RNA Spike-in qPCR Primer Mix in 100 µl nuclease-free water. Use 1 µl RNA Spike-in qPCR Primer Mix with 2 µl cDNA, 5 µl SYBR Green Master Mix, and 2 µl Nuclease-free Water. Run the PCR program described in "Running Real-Time PCR Detection" below. A *Ct* value < 30 for the RNA Spike-in indicates a successful cDNA synthesis.

   **NOTE:** *The cDNA synthesis product can proceed directly to PCR or can be stored at -20°C.*

# C. Perform qPCR for the PCR array

   **NOTE:** *One PCR panel can run up to 4 samples as depicted in the plate layout in Figure 2. The fellow protocol is written for one sample.*

1. Dilute the cDNA in Nuclease-free Water. If 1.5 µg input RNA is used with rtStar™ First-Strand cDNA Synthesis Kit (Cat#AS-FS-001), the dilution factor is 1:20. Mix well and spin down. The diluted cDNA is used as the qPCR template in the wells for tRNA Modification Enzyme Transcript assays, Internal Control References, and Spike-in External Controls.

2. For GDC Controls, combine 1.5 µL NRT (no RT) sample or 1.5 µL RNA sample, 7.5 µL SYBR Green Master Mix, and 6 µL Nuclease-free Water. Mix well and spin down.

3. For Blank Controls, combine 25 µL SYBR Green Master Mix and 25 µL Nuclease-free Water. Mix well and spin down.

4. Use Arraystar SYBR Green Real-Time Quantitative PCR Master Mix to prepare the qPCR Master Mix. There are total of 96 wells of PCR reaction per sample. Some extra amount is included for consumption by the liquid dispensing operation. Prepare the cocktail according to the following table.

| | |
|---|---|
| SYBR Green Master Mix | 500 µL |
| diluted cDNA template | 400 µL |

| | |
|---|---|
| ddH$_2$O | 100 µL |
| total volume | 1000 µL |

5.  Loading the PCR Array plate.

    **NOTE:** *The fellow operations are written for "Sample1" in Figure 2 as an example. For other samples on the panel, it is very important to load the reagents to the corresponding well number and color coding corroding to Figure 2 and Table 1.*

    a.  Carefully remove the plate seal from the PCR Array;

    b.  Add 10 µL of the cocktail from STEP 4 to each PCR Array plate well (except Well number 92 to 96, i.e. Well positions O15, O17, O19, O21, O23);

    c.  Add10 µL GDC Mixture aliquot from STEP 2 into the No.95 (well O21) to detect genomic DNA contamination.

    d.  Add 10 µL Blank Mixture aliquot from STEP 3 into the No.92 (well O15), No.93 (well O17), No.94 (well O19) and No.96 (well O23).

    e.  Carefully but tightly seal the PCR Array plate with the optical adhesive cover. Be sure that no bubbles appear in any of the wells. To remove bubbles, tap the plate gently on the bench top or centrifuge the plate briefly.

    f.  Keep the plate on ice while setting up the PCR program described in "Running Real-Time PCR Detection" below.

6.  Running Real-Time PCR

| Cycles | Temperature | Time |
|---|---|---|
| 1 | 95 ℃ | 10 minutes |
| 40 | 95 ℃ | 10 seconds |
| | 60 ℃ | 1 minutes |
| Melting curve analysis | | |

# D. Data pre-processing and data analysis

Pre-process the qPCR run data according to the qPCR instrument manufacturer's instructions. If using baseline and threshold method for Ct calculations, such as with ABI 7900HT, the optimal baseline and threshold manual settings applied consistently across all assays on the plate are preferred over the software automatic settings for better reliability and accuracy.

Inspect the melting curve analysis of the post-PCR products to verify the amplification specificity. If the melting curve has multiple peaks or poor peak morphology deviated from that of the known single amplicon product, it may indicate the presence of non-specific off-target amplification or primer dimers, which will compromise the

quantification. In such cases, gel electrophoresis or DNA fragment analysis can be performed to verify whether the product is from a single correct amplicon.

Export the raw Ct values to Excel. Free, easy-to-use data analysis software is available online, please refer to www.arraystar.com for detailed instruction. The data analysis procedures include:

## ■ Data pre-processing

1. Set all Ct values ≥ 35 or N/A (not detected) to 35. From this point on, any Ct value equal to 35 is considered a negative result.

2. Examine the Ct values of the Genomic DNA Control (GDC) wells. If the value is greater than 35, no genomic DNA contamination is detected and no action is needed. If the value is less than 35, genomic DNA contamination is evident and the result may be compromised.

3. Before initiating the data analysis, the RNA Spike-in wells are compared. Outlier samples (Ct >25) may be identified and considered for exclusion in the further data analysis.

4. Inter-plate calibration (IPC) can be performed using the PPC assay replicates either with the data analysis software or manually. For each plate, verify that the replicates have Ct standard deviation ≤0.5. If this is not the case, exclude the outlier if it can be identified. Calculate the average of the replicates for each plate, the overall average (average of IPC values from all plates). The calibration factor for each plate is calculated as the difference between the plate average and the overall average:

$$calibration\ factor = IPC(plate\ n) - IPC(overall)$$

The Ct value is corrected with the calibration factor as

$$Ct_{RNA} = Ct_{RNA}(Raw\ value, plate\ n) - IPC(plate\ n) + IPC(overall)$$

or

$$Ct_{RNA} = Ct_{RNA}(Raw\ value, plate\ n) - calibration\ factor$$

|  | Plate 1 | Plate 2 | Plate 3 |
|---|---|---|---|
| ELP3 | 20.26 | 20.83 | 20.44 |
| IPC Plate average | 16.70 | 16.70 | 16.40 |
| IPC overall average | 16.60 | 16.60 | 16.60 |
| Calibration factor | 0.10 | 0.10 | -0.20 |
| ELP3 (Calibrated) | 20.16 | 20.73 | 20.64 |

5. Calculate the ΔCt for each tRNA in the plate.

$$\Delta Ct_{RNA} = Ct_{RNA} - average(Ct_{HKs})$$

Where average (Ct $_{HKs}$) is the average of the Ct values derived from the multiple housekeeping genes. Three most stably expressed housekeeping genes were selected from abroad range of samples by our stringent algorithm that evaluates the optimal properties and the number for endogenous controls.

## ■ Data analysis

1. Calculate the ΔΔCt for each tRNA

$$\Delta\Delta Ct = \Delta Ct(sample\ 1) - \Delta Ct(sample\ 2),\ \text{between samples}$$

or

$$\Delta\Delta Ct = \Delta Ct(group\ 1) - \Delta Ct(group\ 2),\ \text{between groups}$$

2. Calculate the fold changes for each gene from sample 1 to sample 2 or group 1 to group 2 as following:

$$Fold\ Change = 2^{-\Delta\Delta Ct}$$

> **NOTE:** *By convention, if the fold change is greater than 1, the result is reported as a fold up-regulation. If the fold change is less than 1, its negative inverse is reported as a fold down-regulation.*

3. When comparing profiling differences between two groups (such as disease versus control) with biological replicates, the statistical significance of the difference can be estimated as p-value by *t*-test. RNAs having fold changes ≥ 2 and *p*-values ≤ 0.05 are selected as the significantly differentially expressed RNAs.

> **NOTE:** Fold change is related to biological effect size. Ranking by fold change is preferred over *p*-value. qPCR as commonly used in confirmation has a limit of quantification of 0.5 ΔCt, which is equivalent to approximately 2 fold change.
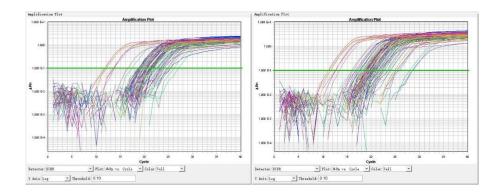
4. Other analyses such as scatter plots, volcano plots, list of differentially expressed genes and bar graph of expression differences for the tRNA modification enzymes are performed and included in the standard analysis package.

# III. Quality Control and Sample Data

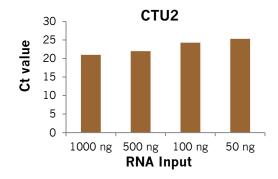## A. NuRNA™ Human tRNA Modification Enzymes PCR Array validation
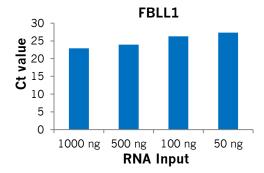
### ■ Validation qPCR

The performance of NuRNA™ Human tRNA Modification Enzymes Panel was tested using a cohort of pancreatic carcinoma and para-carcinoma tissues. The extracted RNA samples were converted to cDNA using rtStar™ First-Strand cDNA Synthesis Kit (Cat#AS-FS-001). The cDNA were profiled using the PCR array according to aforementioned protocol without modification. The results are charted on the real-time amplification plots for the entire plate for the cell lines.
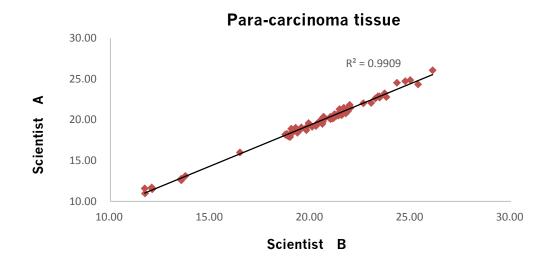


### ■ Sensitivity Test

The decreasing input amounts of total RNAs from human glioblastoma cell lines were converted to cDNAs and profiled by the PCR arrays. The Ct values were determined using the software default automatic baseline and Ct settings. CTU2 and FBLL1 transcripts were detected at Ct values of 25.4 and 27.5 respectively, with the input RNA amount as low as 50 ng.
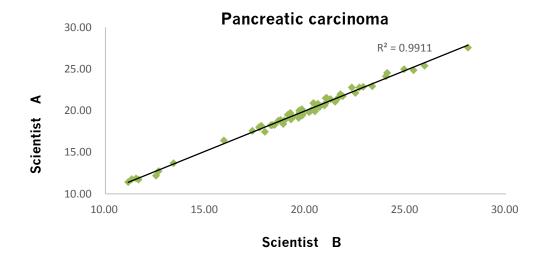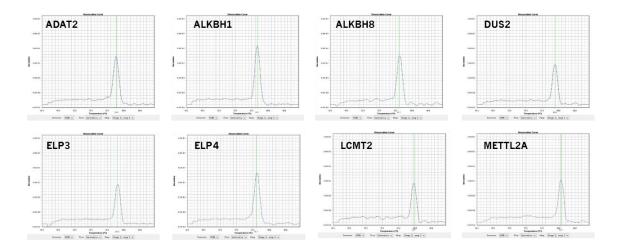
## ■ Reproducibility Test

Two independent runs of NuRNA™ Human tRNA Modification Enzymes PCR Array were conducted by two different scientists A and B at two different times using two different cells. The results demonstrate a high degree of reproducibility with correlation $R^2>0.98$.

### Para-carcinoma tissue

$R^2 = 0.9909$

Scientist A (y-axis)

Scientist B (x-axis)

### Pancreatic carcinoma

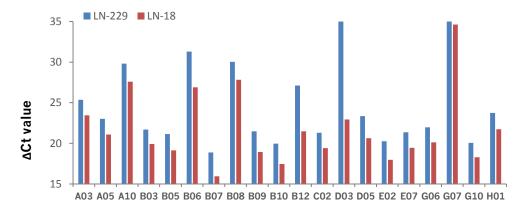$R^2 = 0.9911$

Scientist A (y-axis)

Scientist B (x-axis)

## ■ Specificity Test

The amplification products of transcripts of ADAT2, ALKBH1, ALKBH8, DUS2, ELP3, ELP4, LCMT2, and METTL2A were analyzed by melting curves, all of which had single sharp melting peaks. The results demonstrate the high amplification specificities for the transcripts with the assays on the array.

## B. Sample data: Analysis of human tRNA modification enzyme transcripts levels in cell lines

The sample data were generated from RNAs extracted from two glioblastoma cell lines LN-229 and LN-18. The normalization was carried out using the average of the housekeeping genes. ΔCt for the gene transcripts (well positions) in LN-229 vs. LN-18 cells are graphed in the bar chart below.



# IV. Troubleshooting

| Problem | Possible solution |
|---------|-------------------|
| qPCR background too high | • Reduce the amount of cDNA used in the SYBR Green Master Mix. |
| No qPCR signals | • Inspect if the Internal Controls have valid qPCR signal<br>• Set SYBR Green as the Detector's Reporter Dye<br>• Use more cDNA in the Master Mix<br>• Lower the annealing temperature in Protocol STEP C.6 from 60°C to 50°C. |
| Baseline and threshold settings | • Follow the instructions of the qPCR system manufacturer.<br>• Contact their technical support as necessary. |

# V. References

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# VI. Technical Support

For additional information, manual download or technical assistance, please visit our website at www.arraystar.com, or contact us at:

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# VII. Terms and Conditions

By purchasing and using any part of the NuRNA™ Human tRNA Modification Enzymes PCR Array, you agree to accept the following terms and conditions.

### ■ Product Use Limitation

Except as otherwise agreed in writing, all products are sold to end-users for research purposes only, and not for human or animal therapeutic or diagnostic use. We do not submit our products for regulatory review by any government body or other organization for clinical, therapeutic or diagnostic use. You are solely responsible for the way you use the products in compliance with applicable laws, regulations, and governmental policies.

The purchase of Product does not grant any right to use such Product in the practice of any methods covered by Arraystar intellectual property rights. You may not perform compositional, structural, functional or other analysis of our products, or undertake deconvolution or reverse engineering with respect to our products.

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Arraystar warrants that the Product will meet the specifications stated on the technical data sheet for that product, and agrees to replace the product free of charge if the product does not conform to the specifications. Notice for non-conformity and request for replacement must be given within 30 days of receipt of Products. In consideration of the above warranty by Arraystar, the buyer agrees to and accepts the following conditions:

That the buyer's sole remedy shall be to obtain replacement of the product from Arraystar; and Arraystar Inc. shall not be responsible for replacing Product that has been improperly stored, handled, or used by buyer or End-User.

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