# Profiling the new life of tRNAs with
# tRNA PCR arrays
## From passive translators to active regulators

## Highlights

- Profile tRNA isodecoders and isoacceptors for all anticodons cataloged in GtRNAdb and mitotRNAdb.
- Comprehensive collection of real and predicted tRNA genes with high tRNAScan score > 50.
- tRNA demethylation pretreatment to overcome tRNA modifications blocking cDNA copying in qPCR.
- Lab validated optimal primer design.
- Gold standard qPCR quantification accuracy.
- Rapid results in hours with the free automatic analysis tool provided.

## Introduction

tRNAs are thought of as abundant, ubiquitous, passive mRNA decoders and protein translators. Latest scientific advances have found new tRNA functions that can profoundly impact the biology and diseases (Fig. 1,2).

The tRNA pool, composed of tRNA isoacceptor families for each amino acid, is an important source of insider information in the study of codon usage, protein translation efficiency and accuracy, biological processes and human diseases. Also a codon may be decoded by a near-cognate tRNA with a single mismatch. The cognate:near-cognate tRNA ratios in the tRNA pool are dynamically regulated to affect translation efficiency, fidelity, and transcript stability. Under various conditions, tRNA pools can alter in ways to maintain homeostasis or to favor a particular gene expression program [1] (Fig. 2).

tRNAs with the same anticodon but having different body sequences are "isodecoders". Yet, the highly conserved tRNA isodecoder sequences can exhibit different tissue-specific expression and perform significantly different functions. For example, one of five mouse tRNA-Arg/UCU isodecoders is a central nervous system specific isodecoder. It has new non-canonical functions of maintaining cell homeostasis and preventing neurodegeneration not present in the "housekeeping" tRNA isodecoders [2] (Fig. 3).

Additionally, the abundance in biofluids and the tight association of perturbed tRNA repertoires with diseases can make tRNAs an excellent class of biomarker.
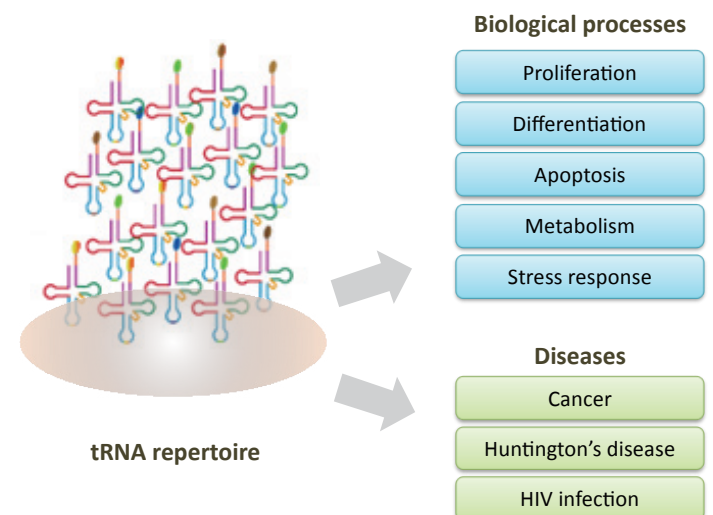
| Classical understanding | Latest advances |
| --- | --- |
| □ Ordinary, passive RNAs in protein translation<br>□ Abundant, ubiquitous | □ Cell, tissue, and disease specific expression<br>□ Profound impacts on biological processes and diseases<br>□ tRNAs and fragments are the most abundant biomarkers in biofluids |

**Fig. 1.** tRNAs have many new found biological and disease functions beyond canonical roles in protein translation.



**tRNA repertoire**

**Biological processes**
- Proliferation
- Differentiation
- Apoptosis
- Metabolism
- Stress response

**Diseases**
- Cancer
- Huntington's disease
- HIV infection

**Fig. 2.** The dynamics of tRNA repertoire has profound impacts on biological processes and diseases.

### CNS-specific isodecoder: Prevention of neurodegeneration

```
GTCTCTGTGGCGCAATGGACGAGCGCGCTGGACTTCTAATCCAGAGGTTCTGGGTTCGAGTCCCGGCAGAGATG

GGCTCTGTGGCGCAATGGA:TAGCGCATTGGACTTCTAATTCAAAGGTTGTGGGTTCGAATCCCACCAGAGTCG
GGCTCCGTGGCGCAATGGA:TAGCGCATTGGACTTCTAATTCAAAGGTTCCGGGTTCGAGTCCCGGCGGAGTCG
GGCTCTGTGGCGCAATGGA:TAGCGCATTGGACTTCTAATTCAAAGGTTGCGGGTTCGAGTCCCGCCAGAGTCG
GGCTCTGTGGCGCAATGGA:TAGCGCATTGGACTTCTAATTCAAAGGTTGTGGGTTCGAGTCCCACCAGAGTCG
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### "Housekeeping" isodecoders

**Fig. 3.** The CNS-specific tRNA-Arg-TCT isodecoder (top) has new functions in cell homeostasis and prevention of neurodegeneration, compared with the "housekeeping" isodecoders (bottom) [2].

# Arraystar nrStar™ tRNA PCR Arrays [3]

## Isoacceptors and isodecoders

nrStar™ tRNA PCR Arrays profile 185 Human/Mouse tRNAs, covering all the nuclear anticodon catalogued in GtRNAdb, which include the real and computationally predicted tRNAs (high tRNAScan score > 50 for human tRNAs). The expression analysis detects tRNAs at isoacceptor and isodecoder detail levels resolvable by PCR (Table 1):

- **Isoacceptor level** detects collectively the total level of the isoaccepters having the same codon, which is excellent for tRNA pool and repertoire analysis.

- **Isodecoder level** detects individual tRNA isodecoders, which has the resolving power to study isodecoder-specific functions beyond "housekeeping".

The arrays include all anticodons to construct expressional information for both cognate and near-cognate tRNAs and to dissect their ratio changes under biological or disease conditions.

## tRNA demethylation to gain high signal sensitivity

tRNAs are most heavily modified with chemical groups. Many of the modifications can block the reverse transcriptase during the first strand cDNA synthesis for qPCR. In fact, such difficulty had long been a big hurdle for tRNA profiling. Arraystar tRNA PCR arrays use rtStar™ tRNA-optimized First-Strand cDNA Synthesis Kit to enzymatically remove m1A, m1G and m3C, the major modifications blocking the cDNA copying. The tRNA pretreatment dramatically increases the PCR signals by a magnitude (Fig. 4). Many differentially expressed tRNAs hidden without pretreatment can now be clearly detected.

## Automatic analysis and annotation

By importing the tRNA PCR array data into the downloadable software analysis tool [3], the results are automatically and instantly analyzed, annotated, and graphically presented (Table 1, Fig. 5).
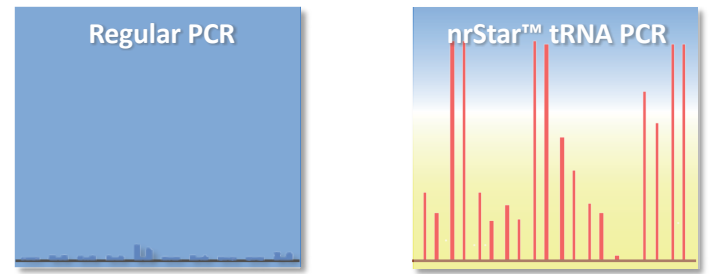


**Fig. 4.** Arraystar nrStar™ tRNA PCR with tRNA demethylation (right) gains a magnitude of signal sensitivity, compared with regular PCR severely blocked by heavy tRNA modifications (left).
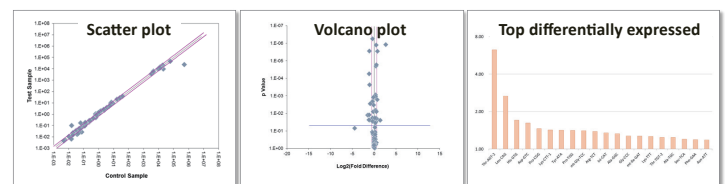


**Fig. 5.** nrStar™ Human tRNA PCR Array data are automatically analyzed to generate annotated results and graphic presentations.

| Arraystar tRNA PCR Products | Catalog |
|---|---|
| nrStar™ Human tRNA PCR Array V2.0 | AS-NR-001H-1 |
| nrStar™ Human tRNA PCR Array V2.0 (Roche Light Cycler 480) | AS-NR-001H-1-R |
| nrStar™ Mouse tRNA PCR Array | AS-NR-001M-1 |
| nrStar™ Mouse tRNA PCR Array (Roche Light Cycler 480) | AS-NR-001M-1-R |
| rtStar™ tRNA-optimized First-Strand cDNA Synthesis Kit | AS-FS-004 |
| Arraystar SYBR® Green qPCR Master Mix(ROX+), 25mL | AS-MR-006-25 |
| Arraystar SYBR® Green qPCR Master Mix(ROX-), 25mL | AS-MR-005-25 |

## References

[1] Gavin H. et al. (2014) Molecular Cell Biology [PMID: 29018283]

[2] Ishimura R. et al. (2014) Science [PMID: 25061210]

[3] https://www.arraystar.com/nrstar-trna-pcr-array/

Table 1. Ala-tRNA isoaccetors and isodecoders

| Primer | Anticodon | tRNAs | Level | Profile | FC | p |
|---|---|---|---|---|---|---|
| Ala-AGC-1 | Ala-AGC | Ala-AGC-1-1/ Ala-AGC-2-1~2/ Ala-AGC-3-1/ Ala-AGC-4-1/ Ala-AGC-5-1/ Ala-AGC-6-1/ Ala-AGC-7-1/ Ala-AGC-8-1~2/ Ala-AGC-10-1/ Ala-AGC-11-1/ Ala-AGC-12-1~3/ Ala-AGC-13-1~2/ Ala-AGC-14-1/ Ala-AGC-15-1 | Isoacceptor | tRNA Pool/Repertoire | -1.95 | 0.18 |
| Ala-AGC-2 | | Ala-AGC-3-1 | Isodecoder | Differential isodecoder | -4.97 | 0.04 |
| Ala-AGC-3 | | Ala-AGC-9-1~2 | Isodecoder | Differential isodecoder | 1.98 | 0.53 |
| Ala-AGC-4 | | Ala-AGC-10-1 | Isodecoder | Differential isodecoder | 1.12 | 0.79 |
| Ala-CGC-1 | Ala-CGC | Ala-CGC-1-1/ Ala-CGC-2-1/ Ala-CGC-3-1 | Isoacceptor | tRNA Pool/Repertoire | -3.47 | 0.05 |
| Ala-CGC-2 | | Ala-CGC-3-1 | Isodecoder | Differential isodecoder | -3.25 | 0.03 |
| Ala-CGC-3 | | Ala-CGC-1-1/ Ala-CGC-2-1 | Isodecoder | Differential isodecoder | -3.67 | 0.17 |