Arraystar tRNA-Seq and tRNA Modification-Seq

From tRNA Expression & Modifications to Disease Mechanisms

Why Profile tRNAs?

Transfer RNAs (tRNAs) are ubiquitous and the most abundant small non-coding RNA molecules. As a fundamental component in translation, tRNAs serve as the physical link be-tween the mRNA coding and protein sequences. A wide variety of biological processes, such as cell proliferation [1], differentiation [1, 2] and apoptosis [3], are always accompanied with variation of tRNAs levels. Alterations of tRNA repertoire affect cell-fate choices during cell development (Fig. 1). Dysregulated tRNA repertoire can promote tumorigenesis and cancer progression [2, 4-11]. Additionally, various diseases show disruptions to the levels and distributions of tRNAs, such as type 2 diabetes mellitus [12], Huntington disease [13], and HIV infection [14]. Studying tRNA repertoire has become an important part of research of biological processes and human diseases.

Arraystar tRNA-Seq

- Overcome tRNA modification barriers for exceptional sequencing efficiency and accuracy

tRNAs undergo by far the greatest number of and the most chemically diverse post-transcriptional modifications, which are essential for tRNA stability, folding and decoding. As an amino acid carrier for peptide synthesis, tRNAs must be charged with amino acid. However, these aminoacylated termini and internal modifications impede the adaptor ligation and reverse transcription during tRNA-seq library preparation. Scientists at Arraystar have developed state of the art tRNA-seq methodologies that integrate modification removal and small RNA sequencing optimized for tRNA, ensuring the most reliable and accurate tRNA-seq data for tRNA study.

Highlights

- Efficiently remove internal and terminal modifications in the tRNA to reach exceptional tRNA-seq efficiency and accuracy
- Performance optimized tRNA sequencing methodologies with rigorous QC process
- Comprehensive tRNA transcriptome reference from authoritative databases
- Thorough tRNA annotation and bioinformatic analysis (Fig. 1,2)
- Data visualization with publication quality graphics
- Work well together with our broad portfolio of tRNA research technologies: tRF&tiRNA-seq, tRF&tiRNA PCR array, tRNA Repertoire PCR array and tRNA modification analysis.

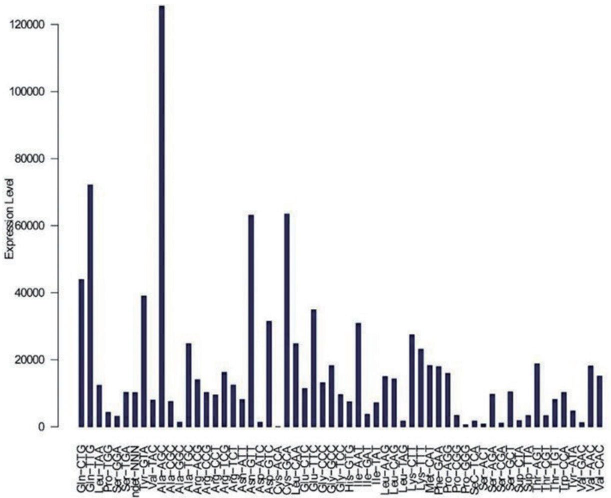
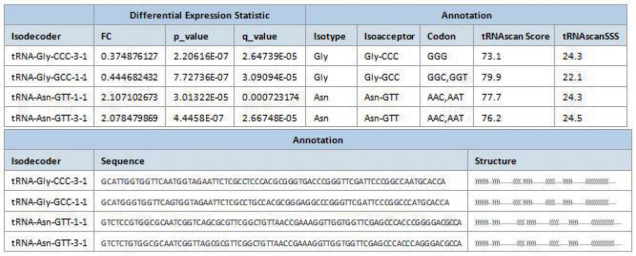


Figure 1. *Expression levels of different tRNA types grouped by anticodon.*



	Differential Expression Statistic			Annotation				
Isodecoder	FC	p_value	q_value	Isotype	Isoacceptor	Codon	tRNAscan Score	tRNAscanSSS
tRNA-Gly-CCC-3-1	0.374876127	2.20616E-07	2.64739E-05	Gly	Gly-CCC	GGG	73.1	24.3
tRNA-Gly-GCC-1-1	0.444682432	7.72736E-07	3.09094E-05	Gly	Gly-GCC	GGC,GGT	79.9	22.1
tRNA-Asn-GTT-1-1	2.107102673	3.01322E-05	0.000723174	Asn	Asn-GTT	AAC,AAT	77.7	24.3
tRNA-Asn-GTT-3-1	2.078479869	4.4458E-07	2.66748E-05	Asn	Asn-GTT	AAC,AAT	76.2	24.5

Annotation		
Isodecoder	Sequence	Structure
tRNA-Gly-CCC-3-1	GCATTGGTGGTTCAATGGTAGAATTCTCGCCTCCCACGCGGGTGACCCGGGTTCGATTCCCGGCCAATGCACCA	[illegible]
tRNA-Gly-GCC-1-1	GCATGGGTGGTTCAGTGGTAGAATTCTCGCCTGCCACGCGGGAGGCCCGGGTTCGATTCCCGGCCCATGCACCA	[illegible]
tRNA-Asn-GTT-1-1	GTCTCCGTGGCGCAATCGGTCAGCGCGTTCGGCTGTTAACCGAAAGGTTGGTGGTTCGAGCCCACCCGGGGACGCCA	[illegible]
tRNA-Asn-GTT-3-1	GTCTCTGTGGCGCAATCGGTTAGCGCGTTCGGCTGTTAACCGAAAGGTTGGTGGTTCGAGCCCACCCAGGGACGCCA	[illegible]

Figure 2. *tRNA expression profiling is annotated in detail.*

Why Profile tRNA Modifications?

tRNAs are the most heavily modified RNA species in cells. These chemical modifications regulate tRNA structure, stability, decoding fidelity, and translational efficiency. Aberrant tRNA modification patterns are closely associated with cancer, neurological disorders, and metabolic diseases (Fig. 3).

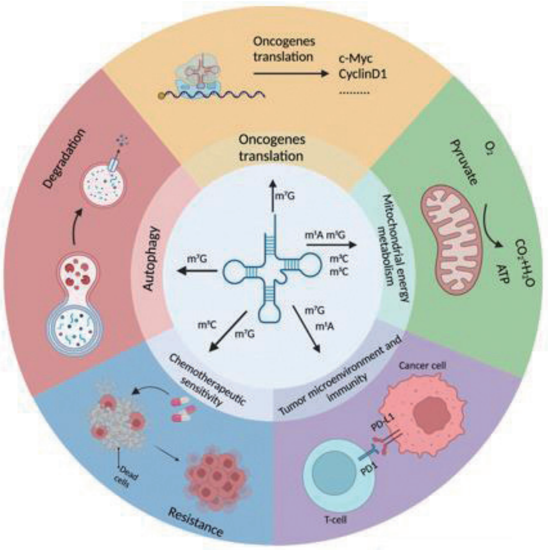


Figure 3. *tRNA modifications in diverse diseases.*

Arraystar tRNA Modification-Seq

- Simultaneously identify the exact sites of m1A, m3C, m1G and m2,2G modifications

tRNAs modified with multiple methylations (m1A, m3C, m1G, m2,2G) can be simultaneously and quantitatively profiled at single-base resolution by tRNA Modification-Seq (m1A/m3C/m1G/m2,2G). These modifications disrupt Watson–Crick base pairing and can induce mismatch mutations due to misincorporation by reverse transcription detectable by sequencing.

To identify modification sites and quantify the methylation in a tRNA, the highly optimized tRNA-seq is performed on the tRNA with and without demethylase treatment. The modification is detected by methylation induced mutation index (MI) at each base position compared with its demethylase-treated tRNA. Among m1A, m3C, m1G, and m2,2G modifications, the m1G and m2,2G are further distinguished by their known distinct base positions in mammalian tRNAs (m1G at 9 and 37; m2,2G at 26 tRNA base positions).

Highlights

- Multiple methylation modification detection. Simultaneously profile m1A, m3C, m1G and m2,2G modifications in tRNAs
- High efficiency methylation removal. Efficiently remove internal and terminal modifications in the demethylase treated tRNA.
- Single-base resolution. Identify the exact methylation modification sites by comparing demethylase-untreated tRNA with demethylase-treated tRNA side by side.
- Comprehensive tRNA epitranscriptomic analysis. Analyze modifications sites, methylation levels, and tRNA expression all at once (Fig. 4).
- High performance tRNA sequencing. Highly optimized tRNA-seq with rigorous QC process, overcoming difficulties of regular sequencing for tRNA.
- Integrated tRNA Solutions. Work well together with our broad portfolio of tRNA research technologies: tRNA PCR array, LC-MS tRNA modification analysis, tRF&tiRNA-seq, and tRF&tiRNA PCR array.

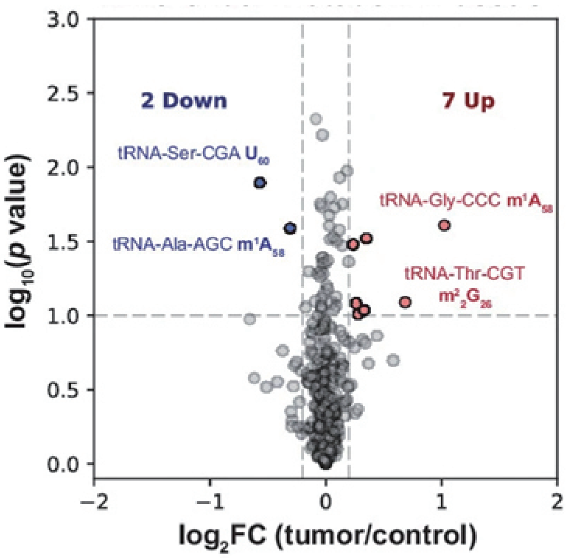


Figure 4. *Volcano plot showing differential tRNA methylation sites between two comparison groups.*

References (will be a link to save space)

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