

Non-coding RNA and Epitranscriptomic Solutions



| Circular RNA Arrays

Accurately profile circular RNAs by highly specific circular junction probe design

| LncRNA Arrays

Overcome the limitations of RNA-seq for lncRNAs often at low abundance

| Small RNA Arrays

Accurately profile miRNA, pre-miRNA, tRNA, tsRNA, and snoRNA simultaneously

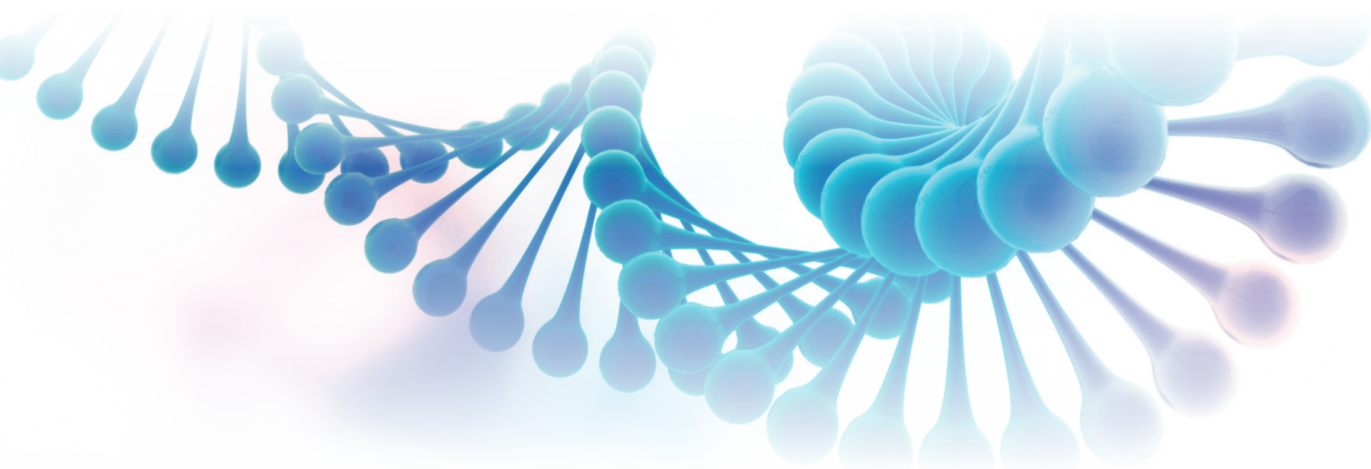
| Epitranscriptomic Arrays

Quantify the percentage of m6A modifications at the transcript specific level

| m6A Single Nucleotide Arrays

Locate and quantify the exact m6A site at single nucleotide resolution

Eqpvcev"wu



Go Beyond RNA

With the discoveries of novel gene regulatory activities beyond coding mRNAs and encoded proteins, a pursuit to uncover pivotal roles of ncRNAs has attracted intense scientific interest with alluring opportunities of finding new disease regulators, biomarkers, and therapeutic targets. Arraystar is taking the lead in supporting scientific endeavor into the once hidden yet exciting world of non-coding RNAs: lncRNAs, circular RNAs, microRNAs, tRNAs, tRFs, tiRNAs, snoRNAs and more.

As a newly unveiled layer of gene regulation, epitranscriptomic modifications of coding mRNAs and non-coding RNAs determine the molecular fates and profoundly impact cellular processes. Arraystar is in the best position to help your research into this omics dimension of not only mRNA, but also long and small ncRNA epitranscriptomics.

Our innovative products, in-depth expertise, and strong bioinformatics in these areas have supported 1700+ high impact publications since our 2009 inception. Arraystar will continue to take the industry lead to provide the best-in-class technologies and services for non-coding RNA and epitranscriptomic research, to explore new horizon, to open new opportunities, and to accelerate and advance your science of groundbreaking discoveries.

Page **02** | Circular RNA Arrays

Page **06** | LncRNA Arrays

Page **10** | Small RNA Arrays

Page **13** | Epitranscriptomics Arrays

Page **15** | m6A Single Nucleotide Arrays

Arraystar Circular RNA Microarrays

Accurately profile circular RNAs by highly specific circular junction probe design

Highlights

- The only commercially available circRNA microarrays.** Specifically designed for circRNA expression profiling. CircRNAs were comprehensively curated from the landmark publications and multiple data sources with stringent collection pipelines to produce the best circular RNA array contents.
- Highly specific circular RNA signal detection.** Samples are treated with RNase R to specifically remove linear RNA in the total RNA samples. Circular junction sequence specific array probes ensure the most specific, accurate and reliable circRNA profiling, even in the presence of linear counterparts.
- Detailed Annotation.** In addition to standard microarray data analysis, circRNAs specific information is further annotated with the target sites of conserved miRNAs with good mirSVR scores, to unravel their functional roles as miRNA sponges.
- The preferred choice over RNA-sequencing, as RNA-seq is currently inadequate for such task due to the particular properties of circular RNA.

Why Study Circular RNAs?

Circular RNA (circRNA) is a novel type of non-coding RNA covalently circularized in a closed loop, produced by RNA back splicing process. circRNAs are not known to translate proteins. With their extensive complementarity to linear RNA counterparts, stability against nucleases, resistance to miRNA-targeted degradation, high expression levels, enrichment in cytoplasm, and large number of miRNA binding sites,

circRNAs have been increasingly recognized as exceptionally effective natural miRNA sponges and competing endogenous RNAs (ceRNAs) in gene regulation. Some intronic circular RNAs (ciRNAs) have been shown to enhance the host gene transcription [6]. Additionally, the tissue/developmental-stage-specific expression and long half-lives constitute an enormous advantage as a novel class of biomarkers.

To facilitate the analysis of circRNAs, Arraystar has pioneered the circRNA microarrays for human and mouse to systematically profile circRNAs under physiological and disease conditions.

circRNAs as microRNA Sponges

Circular RNAs can have multiple microRNA binding sites. For example, the physical interactions of ciRS-7 with miR-7 and gene silencing complex have been demonstrated by Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation (PAR-CLIP) with AGO2 in the presence of miR-7, and by biotinylated miR-7 capture (Fig 1), showing ciRS-7 biochemically as a microRNA sponge.

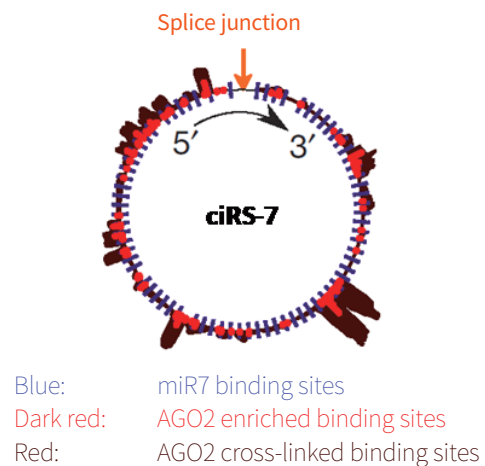


Figure 1. ciRS-7, short for "circular RNA Sponge for miR7", has more than 70 copies of predicted miR-7 binding sites. It interacts with miR-7 and the catalytic component of RNA-induced silencing complex AGO2.

circRNAs and RNA Binding Proteins

Circular RNAs may partner with very diverse RNA binding proteins (RBP) to perform wide range of molecular functions

(Fig. 2). Circular RNAs may bind RBPs for subcellular delivery/localization (Fig. 3), sponge RBPs just like miRNAs, assemble different sets of RBP complexes, or act as an allosteric co-factor for enzymatic RBPs [8]. Antisense circular RNAs may form direct base pairing with mRNAs to regulate the activity.

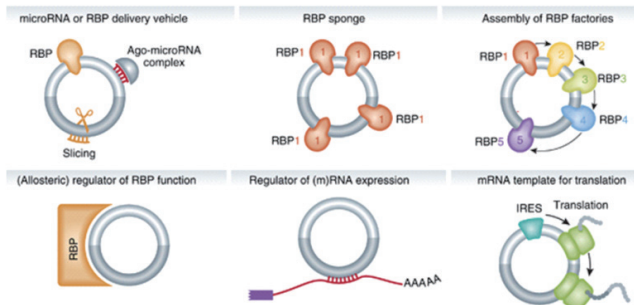


Figure 2. Interplay of circular RNA with RNA binding proteins [8].

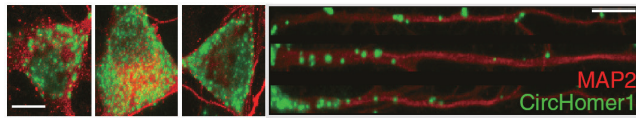


Figure 3. Dynamic subcellular localization of circular RNAs in neuronal cell body and dendrites to regulate neuronal synaptic connections [7].

CircRNAs in Biology and Disease

MicroRNAs may regulate up to 1/3 of all gene expression. circRNA regulation of microRNA activities can lead to biological phenotypes (Fig. 4). Disproportionately large number of circRNA targets are protein kinases, which are signal transduction mediators. Aberrant circRNA expression is involved in human diseases such as cancer, Alzheimer's disease and atherosclerosis. The higher specificity and stability of circRNA in diseases are desired properties in biomarker applications.

CircRNAs Are Stable due to Resistance to Exonuclease Degradation

Circular RNAs lack exposed 5' and 3' termini and are resistant to exonuclease degradation. Thus, circRNAs are stable and have much longer half-lives than their linear RNA counterparts (Fig. 5). The elevated abundance contributes to functioning as microRNA sponges. It also presents a good opportunity for biomarker applications.

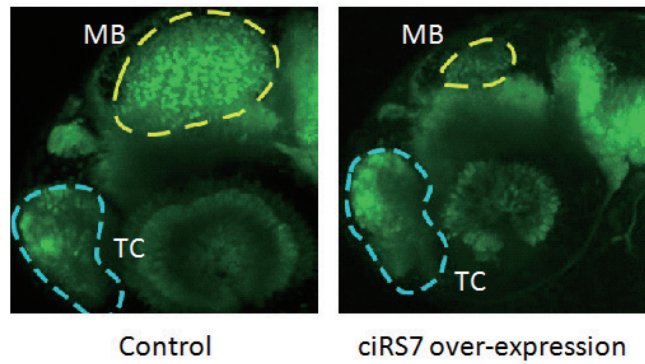


Figure 4. *ciRS-7* overexpression in embryonic brain leads to profound reduction of mid brain (MB) size (right), compared to the normal control (left). Co-overexpression of miR-7 ameliorates the effect. The telencephalons (TC) are relatively unaffected.

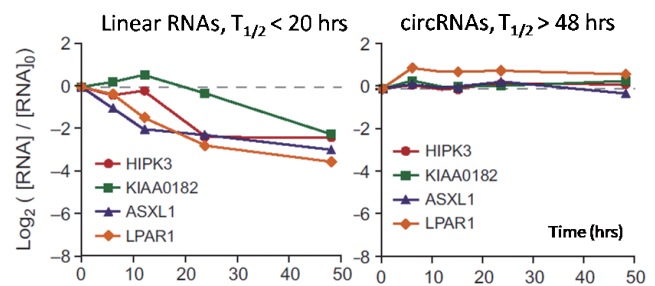


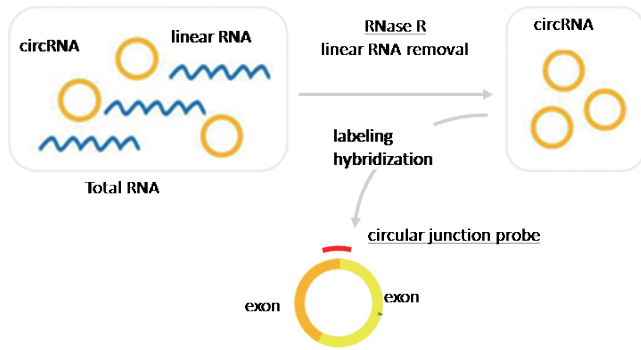
Figure 5. Circular RNAs are stable and have half-lives more than 48 hours (right), compared to their linear RNA counterparts of less than 20 hours (left).

Arraystar Circular RNA Microarrays

Arraystar circRNA Microarrays were the first and are currently the only commercially available technology for sensitive and reliable circRNA expression profiling. The microarrays use RNase R linear RNA removal and circular junction probes to achieve high specificity for circRNAs, even in the predominant presence of linear RNAs (Fig. 6, top). The profiling is complete with comprehensive, systematic and detailed annotation of circRNAs, including miRNA binding sites as microRNA sponges, to gain insight into circular RNA biology (Fig. 6, bottom).

Why Use Microarray Over RNA Sequencing for Circular RNA Profiling?

Circular RNAs as a population are typically present at much lower levels, at about 5~10% of linear RNAs. The cross circular junction sequences are even lower. At a typical RNA-seq depth, less than 5% of circRNAs (red circle) may be reliably quantified



circRNA	linear RNA	miRNA	Binding Alignment	mirSVR score	PhastCons
circRNA-000482	NM_001286646	miR-340	3' UUGUCAGAGUACCAAGAUUUU 5' miR-340 UUCUGUUGUUAUACUUAUUU cIRS-9	-1.295	0.7719
		miR-125-3p	3' cGAGGGU-UUUGGAGUGGAC 5' miR-125a-3p cAUCAUAAUAGUUCACUGG cIRS-9	-1.141	0.7597
		miR-499-5p	3' UUGUGUGUACUUCAGAGAUU 5' miR-217 GUUGUGUUAUAGUUCUUAU cIRS-9	-1.143	0.7314
		miR-217	3' UGUGUUCAGAGGACU-ACGUCAU 5' miR-217 UCAGUUCUUGAGUGUUAUUCAGU cIRS-9	-1.131	0.7481

Figure 6. Overview of circRNA microarray workflow (top) and detailed annotation of circRNA (bottom).

(Fig. 7). Even at great sequencing depth of > 300 mil at high costs, the accuracy gains are only modest.

In practice, generic RNA-seq, mostly intended for mRNAs, are inadequate or simply unavailable as a provided service for circular RNA profiling. Circular RNA sequencing requires very deep sequencing depth and paired-end chemistry. Read mapping and data analysis require specialized database, de novo transcript assembly, special algorithms and complex computational pipeline. circRNA annotations such as microRNA binding as sponges are typically not included. Novel circular RNA discovery, a consideration of using RNA-seq, is actually not available for the above reasons.

On the other hand, circular RNA microarrays use circular junction probes, combined with enzymatic linear RNA removal, to interrogate circular RNAs highly specifically. Array hybridization is relatively independent of other high abundance RNAs. High sensitivity at one transcript per cell can be achieved. Overall, microarray is more efficient and robust in sample labeling than RNA sequencing library prep. At present, it is a mature technology that outperforms RNA-seq in circular RNA profiling.

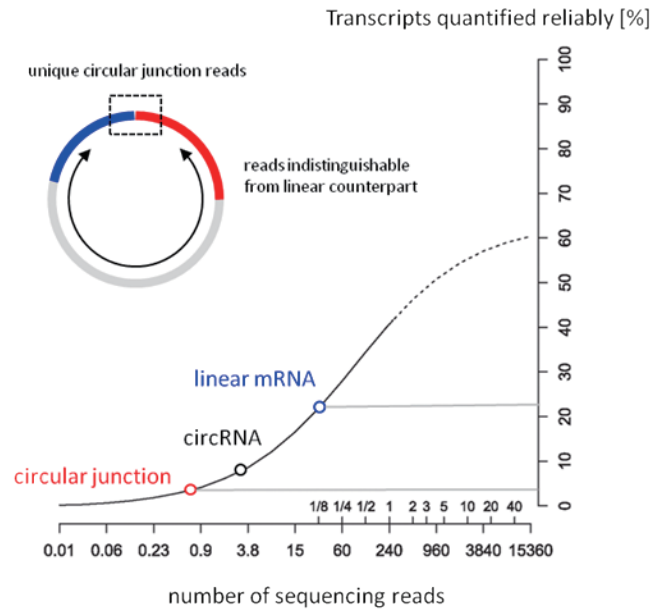


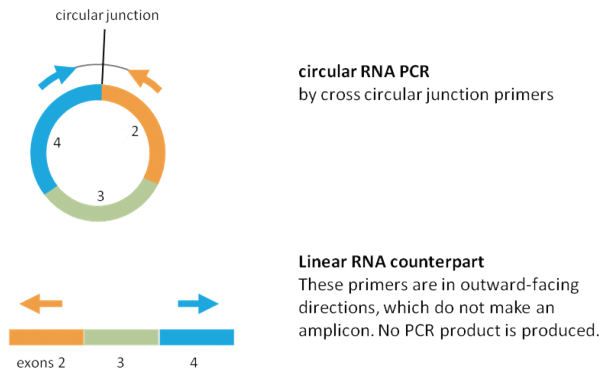
Figure 7. RNA-seq quantification reliability vs read depth. Typical RNA-seq has a depth of < 30 mil reads for mRNAs (blue circle), which is < 0.5 mil for cross circular junction reads (red circle). Less than 5% circular junctions can be reliably quantified. Adopted from [9].

Arraystar Circular RNA Microarray Specifications

	Human	Mouse
Total number of unique circRNAs	13,617	14,236
Probe Length	60nt	
Probe Region	Circular junctions of circRNAs	
Probe Specificity	Transcript specific	
CircRNA Enrichment	RNase R treatment	
Labeling Method	Labeling by random priming	
Salzman' s circRNAs (2013)	8,529	
Memczak' s circRNAs (2013)	1,601	1,750
Zhang' s ciRNAs (2013)	93	
Zhang' s circRNAs (2014)	4,980	
Jeck' s circRNAs (2013)	3,769	
Guo' s circRNAs (2014)	5,536	570
You' s circRNAs (2015)	13,300	
Array Format	8x15K	

Circular RNA Research Roadmap

The differentially expressed circRNA candidates screened by circRNA microarrays are typically confirmed by an independent method such as by qPCR (Fig. 8). The validated circRNAs are further studied for biological functions, molecular mechanisms in gene regulation and biomarker applications (Fig. 9).



- circRNAs do not have poly(A). Random primers, not oligo-dT, must be used in the first strand cDNA synthesis by reverse transcription.
- PCR by cross circular junction primers
- Parallel assays with and without RNase R treatment
- Sequencing confirmation of the circular junction

Figure 8. qPCR validation of differentially expressed circRNAs screened by circRNA microarray. The concordance between qPCR and microarray for the differentially expressed circRNA is related to the magnitude of change (FC), p-value, as well as the abundance level.

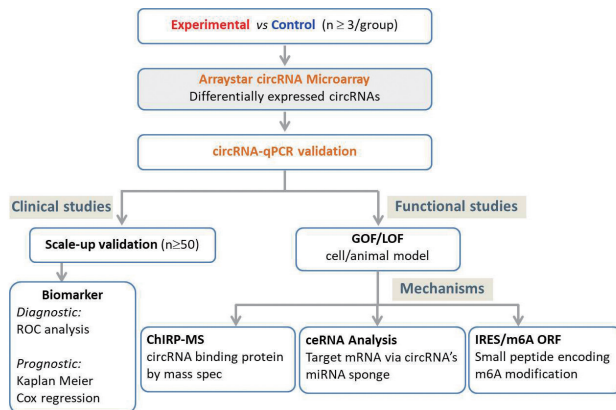


Figure 9. Roadmap of circRNA expression profiling and follow-up studies.

References

1. Guo, J. U., V. Agarwal, et al. (2014). Genome Biol 15(7): 409.
2. Jeck, W. R., J. A. Sorrentino, et al. (2013). RNA 19(2): 141-157.
3. Memczak, S., M. Jens, et al. (2013). Nature 495(7441): 333-338.
4. Salzman, J., R. E. Chen, et al. (2013). PLoS Genet 9(9): e1003777.
5. Zhang, X. O., H. B. Wang, et al. (2014). Cell 159(1): 134-147.
6. Zhang, Y., X. O. Zhang, et al. (2013). Mol Cell 51(6): 792-806.
7. You X. et. al. (2015) Nat Neurosci. 2015 Apr;18(4):603-10
8. Hentze and Preiss (2013) EMBO J 3;32(7):923-5.

Arraystar LncRNA Microarrays

Overcome the limitations of RNA-seq for lncRNAs often at low abundance

Highlights

- Most sensitive and best technology for lncRNA profiling, superior to RNA-seq
- Comprehensive and robust full-length lncRNA* collection curated from all major latest databases and landmark publications
- Systematic and specialized lncRNA annotation, including genomic context, epigenomic context*, completeness*, subcellular localization**, miRNA recognition site...
- Unambiguous, reliable and accurate detection and quantification of lncRNA transcript isoforms otherwise difficult by RNA-seq
- Simultaneous lncRNA and mRNA profiling on the same array for co-expressional and correlational expression and regulation

*Applicable to Human V5.0 ** Applicable to Human V5.0 and Mouse V4.0

Why Study LncRNAs?

LncRNAs are a major RNA class in the transcriptome [1]. These noncoding RNAs are transcribed from genomic sites either in association with a protein coding gene nearby or in the intergenic regions as lincRNAs (Fig. 1), with functions in gene expression regulation by multiple mechanisms, either in cis or in trans, at transcriptional or post-transcriptional levels (Fig. 2). LncRNAs are a key player in a wide range of biological systems and diseases. Cutting edge lncRNA science has resolved many long standing mysteries in, for example, chromosomal inactivation, developmental and differentiation programming, and diseases of unknown etiology. In general, lncRNAs exhibit more restricted cell type-specific expression compared to mRNAs, making lncRNAs a class of higher specificity biomarker.

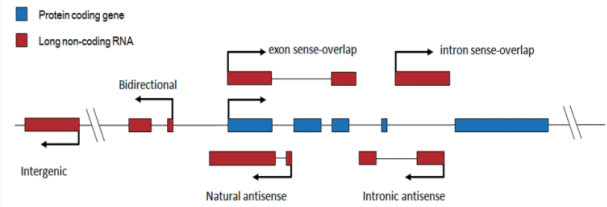


Figure 1. lncRNA classification based on genomic contexts with the closest protein coding gene.

Arraystar LncRNA Microarrays

Arraystar LncRNA Arrays are designed to systematically profile lncRNAs along with the entire set of protein-coding mRNAs, providing the most current, robust, full length lncRNA collections, complete with major upgrades in scientific annotation and analyses.

Consolidated, comprehensive, robust, most up-to-date full-length lncRNA contents*

Arraystar maintains high quality proprietary transcriptome and lncRNA databases that extensively collect lncRNAs through all major external data sources, knowledge-based mining of scientific publications, and our lncRNA collection pipelines. Especially, we place premium attention on full-length lncRNAs collection. Full length lncRNAs as annotated or experimentally supported in the public databases are compiled with high priority. The lncRNAs in Arraystar proprietary transcriptome databases and published lncRNA studies are carefully assessed by supporting evidence for their sequence completeness: 5'ends by host gene histone marks [3-5], CAGE clus-ter [6-9], and DNA hypersensitivity (DHS) [5] data; 3'ends by poly(A)-position profiling (3P-Seq) [10]. Additionally, lncRNA candidates are evaluated for protein coding potentials by a combination of prediction methods [11-13]. Only the lncRNAs that pass these assessments are curated into the full length lncRNA collections (Fig. 2).

For the total of 39,317 lncRNAs on Arraystar Human LncRNA Array V5.0, we further place the lncRNA collection in two tiers: 8,393 Gold Standard lncRNAs and 30,924 Reliable lncRNAs. The Gold Standard lncRNAs are well annotated and experimentally supported genuine lncRNAs. The Reliable

lncRNAs are the comprehensive yet highly reliable lncRNA collection tier, which are the lncRNAs remained from the Gold Standard lncRNA collection.

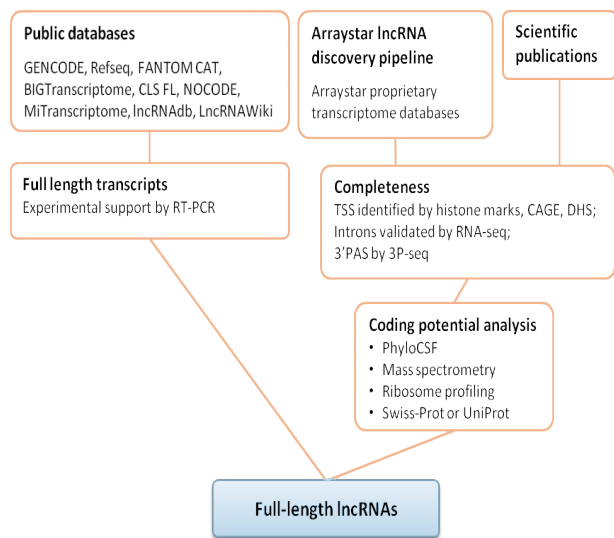


Figure 2. Comprehensive and robust collection of full-length lncRNAs from all major sources.

Towards systematic and functional annotation of lncRNAs

The Arraystar lncRNA microarray package includes systematic and detailed lncRNA annotations, subclassification, and analyses, providing genomic information, epigenomic context, subclassification, transcript model completeness, subcellular localization, miRNA recognition, conservation, tissue/cell specificity, and small peptide coding potentials, biological process, and disease association, to gain insight into the complex biological functions of the lncRNAs. lncRNAs with reported biological processes or associated with human diseases are researched, annotated and cross referenced. This rich source of information helps to unravel functional roles and molecular mechanisms of the lncRNAs.

Epigenomic context* lncRNAs can be transcribed in and regulated by a promoter or enhancer region with characteristic promoter or enhancer epigenetic marks [5]. Many active promoter and enhancer regions are themselves transcription units, capable of generating functionally active noncoding RNAs for these cis-regulatory DNA elements. The lncRNAs are thus classified into promoter-lncRNAs (p-lncRNA) and enhancer-lncRNAs (e-lncRNA) based on the epigenomic context (e.g. DNase I hypersensitive sites). The p-lncRNAs are further grouped into intergenic and divergent p-lncRNAs based on their genomic context (Fig. 3). p-lncRNAs are often positively

correlated with transcription of their protein-coding genes under the same promoters. e-lncRNAs often trap TF proteins to the local sites, modify the local chromatin environment, and organize three-dimensional nuclear topology domains for correct activation of the target gene program.

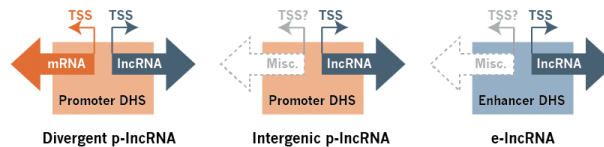


Figure 3. Promoter and enhancer lncRNA categories based on the epigenomic and genomic context. lncRNAs are classified into inter-genic p-lncRNA, divergent p-lncRNA, e-lncRNA, and other, based on their TSS and DNase I hypersensitive sites (DHS) in the promoter (marked by H3K4me3), enhancer (marked by H3K4me1, H3K27ac and H3K9ac), or dyadic regulatory (enhancer-promoter alternating states) regions.

Subcellular localization** The molecular functions of lncRNAs are tightly coupled with their subcellular localization [14-16]. For example, lncRNAs localized in the nucleus or chromatin often regulate the gene expression by epigenetic modification and transcription. lncRNAs in the cytoplasm are more likely involved in translation regulation or miRNA sponging such as competing endogenous RNAs (ceRNA)[17-20].

miRNA recognition site Predicted or experimentally identified microRNA sites on the lncRNAs are annotated to indicate potential post-transcriptional regulatory functions in the miRNA regulatory network, such as acting as competing endogenous RNAs (ceRNA).

Tissue specific lncRNAs* The function of a lncRNA can be directly or indirectly related to and indicated by the tissue or cell type in which it is specifically expressed. In Human lncRNA Microarray, 6,059 cell lineage and cancer associated lncRNAs are annotated.

Disease-associated lncRNAs* lncRNAs known to be associated with diseases, such as cataloged in lncRNADisease [29, 30], are annotated for clinical and translational investigations.

*Applicable to Human V5.0 ** Applicable to Human V5.0 and Mouse V4.0

Why Use Microarray Over RNA Sequencing for lncRNA Profiling?

lncRNAs often express and function at low abundance, buried in other classes of abundant RNAs (Fig. 4A). There are serious

limitations of RNA-seq for lncRNA profiling.

LncRNA quantification. For mere detection of the presence of a lncRNA, a few reproducible sequencing reads should suffice. But for quantification, at least hundreds read counts are required to reliably represent the RNA level [33] (Fig. 4A). LncRNAs are generally ~10X less abundant than mRNA [34]. RNA-seq quantification at these low lncRNA levels is unacceptably poor and not nearly sufficient for differential expression analysis [2, 35] (Fig. 4C, 4D). Even if the sequencing coverage is increased to an unaffordably deep coverage (dotted curve, several hundred times the normal RNA-seq coverage at 20 mil), a large proportion (40%) of transcripts can never be reliably quantified [35] (Fig.4B). Additionally, FPKM (Fragments Per Kilobase of transcript per Million mapped reads) calculation in RNA-seq depends on accurate lncRNA transcript model lengths, many of which still lack completeness in lncRNA annotation [1]. In contrast, LncRNA Microarray oligo probes hybridize the target RNA at high affinity, independent of other abundant RNAs. The microarrays are highly sensitive and accurate even for low abundance lncRNAs [37] (Fig. 4D).

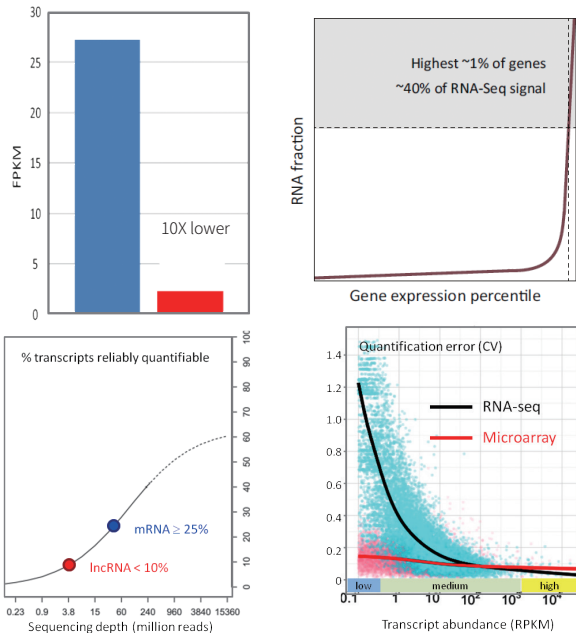


Figure 4. (A) The median lncRNA expression level is approximately 10X lower than that of mRNAs (based on GENCODE data)[34]. (B) Top 1% of the highest expressed genes, such as housekeeping genes, occupy ~40% of RNA-seq signal. Lowly expressed lncRNAs receive very little sequencing coverage [35]. (C) In a typical mRNA-seq depth at 40 million reads, < 10% lncRNAs can be reliably quantified [36]. (D) While quantitative error becomes unacceptably high for RNA-seq when the RNA level is low, microarray continues to perform very well [37].

LncRNA transcript isoforms. LncRNAs often have multiple transcript isoforms and function differently in complex genomic and regulatory relationships with their target mRNA genes. Profiling lncRNAs at transcript-specific level is important. However, RNA-seq coverage for the splice profiles is weak and non-uniform, particularly for non-predominant isoforms[2] (Fig. 5). Even at saturating coverage, accurate reconstruction of transcript isoform is inherently challenging due to the missing connectivity information with the short reads in distant exons on the same RNA fragment [2]. Arraystar LncRNA Microarrays use "transcript-specific" probes that hybridize to the splice junctions or exon sequences that are unique to each transcript isoform from the same gene, which is unambiguous and highly accurate in isoform detection and quantification (Fig.6).

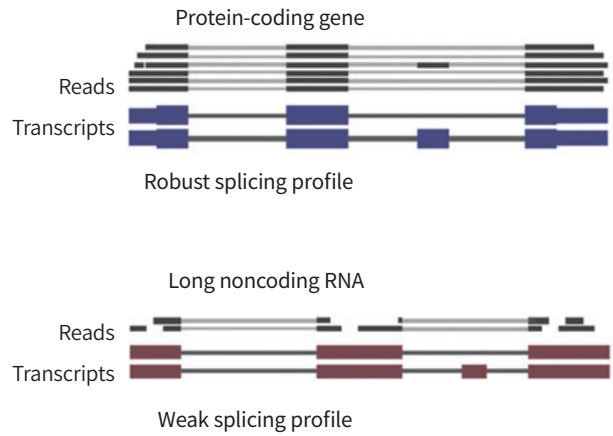


Figure 5. Compared with better expressed mRNAs, lowly expressed lncRNA isoforms cannot be adequately covered by short RNA-seq reads to reconstruct the exon models nor their quantification [2].

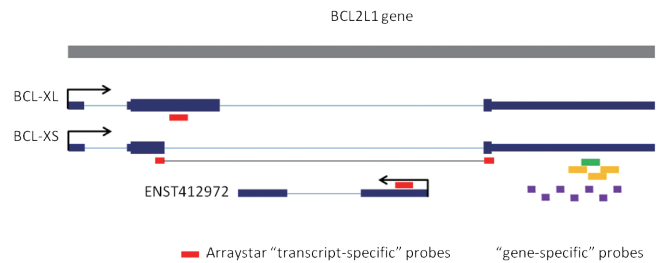


Figure 6. Arraystar LncRNA Microarray transcript-specific probes unambiguously and accurately detect and quantify transcript isoforms BCL-XL, BCL-XS, and ENST412972 having distinct oncogenic functions. The "Gene-specific" probes not designed for lncRNA isoforms cannot make such distinction. The arrows indicate the transcription direction.

LncRNA annotation and analysis. Unlike well established and curated protein coding genes, RNA-seq raw data are still in need of wellresourced and consolidated reference bases for mapping and annotation, which are not readily publically available. Arraystar Microarray lncRNA contents are based on the foundation of high quality proprietary Arraystar lncRNA transcriptome databases that extensively collect lncRNAs through all major public databases and repositories, knowledge-based mining of scientific publications, and our lncRNA collection pipelines. The microarray annotation and analyses are, rich, detailed, and comprehensive, unrivaled by any other profiling platforms.

LncRNA Microarray vs RNA-seq for lncRNA profiling

LncRNA Microarray	RNA-Seq
High sensitivity and quantification accuracy for lncRNAs as low as 1 transcript/cell.	Most lncRNAs at low levels cannot be accurately and reliably quantified.
Natively specific for RNA strandedness for both sense and antisense lncRNAs.	Stranded RNA-sequencing library prep required.
Unambiguous and specific lncRNA isoform detection/quantification.	Poor sensitivity and accuracy for lncRNA isoforms.
Arraystar LncRNA Microarray premium lncRNA collection, annotation and analyses. Entire coding mRNA gene set also included.	Public lncRNA reference databases can be deficient. Systematic lncRNA annotation and analyses are not readily available for the RNA-seq data.

Arraystar LncRNA Microarray Specifications

	Human V5.0	Mouse V4.0	Rat V3.0
Total number of distinct probes	60,491	60,641	38,352
Probe length	60 nt		
Probe selection region	Specific exon or splice junction along the entire length of the transcript		
Probe specificity	Transcript-specific		
mRNAs	21,174	22,692	27,770
lncRNAs	40,173	37,949	10,582
Gold Standard lncRNAs	8,393		
Reliable lncRNAs	30,924		
mRNA sources	Refseq, UCSC, GENECODE, FANTOM5, CAT	Refseq, Known Gene, GENECODE	Refseq, Ensembl
lncRNA sources: Databases and Literatures (up to 2018)	FANTOM5 CAT (v1), GENECODE (v29), RefSeq (2018.11), BIGTranscriptome (v1), knownGene (2018.11), lncRNAdb, lncRNAWiki, RNAdb, NRED, CLS FL, NONCODE (v5), MiTranscriptome (v2)	GENECODE (VM19), RefSeq, KnownGene, GenBank	Refseq, Ensembl
Array format	8 × 60K	8 × 60K	4 × 44K

LncRNA Research Roadmap

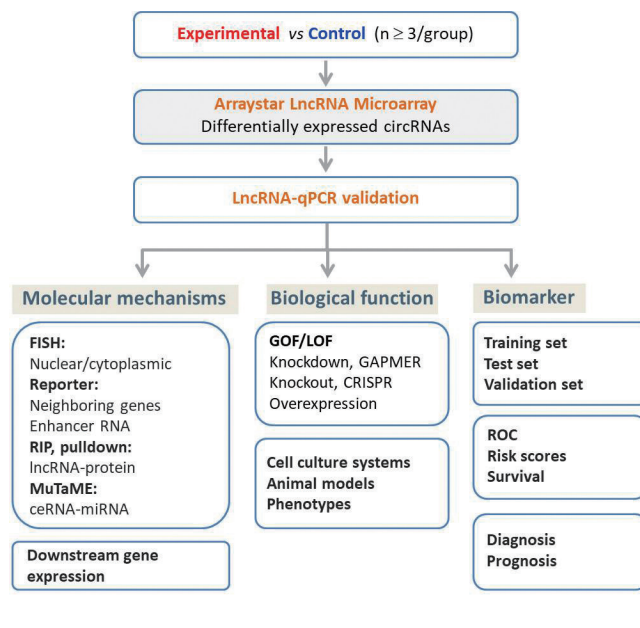


Figure 7. LncRNA research roadmap for studying regulatory molecular mechanisms, biological functions, and biomarker development.

References

1. Uszczynska-Ratajczak, B., et al. (2018) *Nat Rev Genet*. [PMID: 29795125]
3. Guttman, M., et al. (2009) *Nature*. [PMID: 19182780]
4. Khalil, A. M., et al. (2009) *Proc Natl Acad Sci U S A*. [PMID: 19571010]
5. Hon, C. C., et al. (2017) *Nature*. [PMID: 28241135]
6. Lagarde, J., et al. (2017) *Nat Genet*. [PMID: 29106417]
7. Marques, A. C., et al. (2013) *Genome Biol*. [PMID: 24289259]
8. Alam, T., et al. (2014) *PLoS One*. [PMID: 25275320]
9. Mele, M., et al. (2017) *Genome Res*. [PMID: 27927715]
10. You, B. H., et al. (2017) *Genome Res*. [PMID: 28396519]
11. Wang, L., et al. (2013) *Nucleic Acids Res*. [PMID: 23335781]
12. Kong, L., et al. (2007) *Nucleic Acids Res*. [PMID: 17631615]
13. Lin, M. F., et al. (2011) *Bioinformatics*. [PMID: 21685081]
14. Kaewsapsak, P., et al. (2017) *Elife*. [PMID: 29239719]
15. Mas-Ponte, D., et al. (2017) *RNA*. [PMID: 28386015]
16. Benoit Bouvrette, L. P., et al. (2018) *RNA*. [PMID: 29079635]
17. Salmena, L., et al. (2011) *Cell*. [PMID: 21802130]
18. Tay, Y., et al. (2011) *Cell*. [PMID: 22000013]
19. Chan, J. J. and Tay, Y. (2018) *Int J Mol Sci*. [PMID: 29702599]
20. Tay, Y., et al. (2014) *Nature*. [PMID: 24429633]
29. Bao, Z., et al. (2019) *Nucleic Acids Res*. [PMID: 30285109]
30. Chen, G., et al. (2013) *Nucleic Acids Res*. [PMID: 23175614]
33. Anders, S. and Huber, W. (2010) *Genome Biol*. [PMID: 20979621]
34. Derrien, T., et al. (2012) *Genome Res*. [PMID: 22955988]
35. Ira W. Deveson., et al. (2017) *Trends in Genetics*. [PMID: 28535931]
36. Labaj, P. P., et al. (2011) *Bioinformatics*. [PMID: 21685096]
37. Zhang, X., et al. (2010) *Endocrinology*. [PMID: 20032057]

Arraystar Small RNA Microarrays

Accurately profile miRNAs, pre-miRNAs, tRNAs, tsRNAs, and snoRNAs simultaneously

Highlights

- Simultaneously profile the major small RNA classes: miRNA, pre-miRNA, tRNA, tsRNA, and snoRNA
- Raise the bar of small RNA profiling to high sensitivity, specificity and accuracy by direct end-labeling and smart probe design.
- Direct and simplified procedures to overcome biases from RNA modifications, RNA fold hindrance, reverse transcription blocks, PCR amplifications, and analysis inaccuracy in small RNA-seq.
- Required RNA sample amounts starting as low as 100 ng, opening up many research opportunities.
- Tolerant for RNA samples at lower qualities: e.g. degraded RNAs, serum/plasma/biofluid RNAs, FFPE RNAs.

Biomarker Potentials of tRNAs and tsRNAs

Small RNAs such as microRNAs have been popularly explored as biomarkers. tRNA and tsRNA populations are now emerging as new classes of biomarkers with greater potentials, owing to their many desired characteristics. The high stability and abundance of tRNA and tsRNA in body fluids (Fig. 1)[1-6], the involvement in pathological processes, the demonstrated differential expression in solid tumors and hematological malignancies, and their power to discriminate cancer patients from healthy controls open the prospect for development of tRNAs and tsRNA-based biomarker tests. For example, the tRF profiles have been shown to discriminate triple-negative, triple positive breast cancer cells from the normal controls in unsupervised clustering [7] (Fig. 2). The ratio of tsRNAs has also been demonstrated as a good indicator of cancer progression-

free survival and a candidate prognostic marker [4].

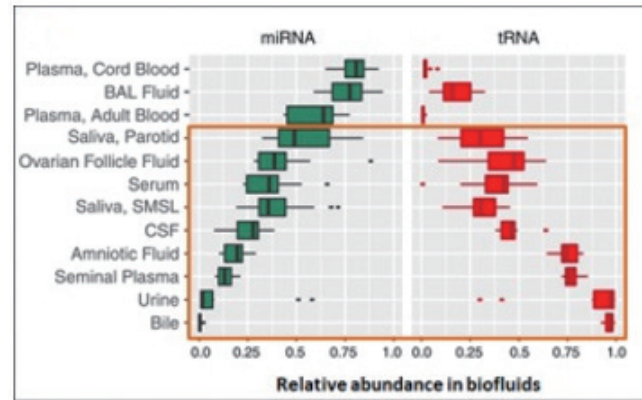


Figure 1. Relative proportions of miRNA vs tRNA in biofluids, where many biofluids have tRNA contents much higher than miRNA [1,2].

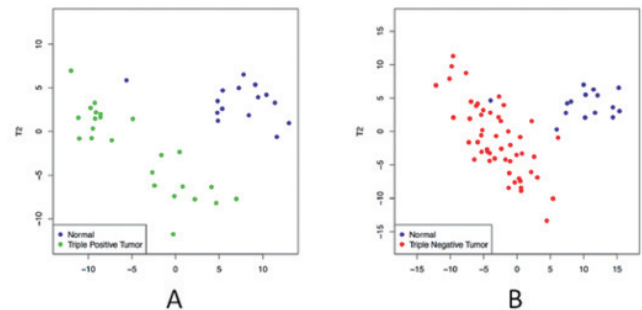


Figure 2. tRF profiles discriminate triple-positive (A), triple-negative breast cancer cells (B) from the normal controls in unsupervised clustering[7].

Arraystar Small RNA Array, with the low requirements of RNA amount and quality, opens up opportunities for tRNA/tsRNA biomarker research projects where the samples are rare or of limited supply.

Arraystar Small RNA Microarrays

Arraystar Small RNA Array, combining direct end-labeling and smart probe design microarray technologies, is designed as a practical and effective solution to profile the full spectrum of small RNAs at high sensitivity and accuracy yet at much less input RNA amounts.

Raising the bar of small RNA profiling for high sensitivity, specificity and accuracy

By end-direct labeling, the small RNAs are ligated with pCp-Cy3 onto the 3'-ends by T4 RNA ligase, and one RNA molecule is labeled with one Cy3 label. This method eliminates biases from cDNA synthesis by reverse transcription due to RNA modification interference and RNA folding hindrance as in small RNA-seq; avoids distortions from PCR amplification cycles as in required small RNA-seq library amplification; uses DMSO to reduce the RNA structure and sequence context differences among small RNAs. All these help to preserve the fidelity of native small RNA levels and achieve the unbiased high quantification accuracy better than RNA-seq or even qPCR.

The smart probe design incorporates 5'-hairpin structure and normalized sequence targeting region to specifically distinguish small RNAs with only 1~2 nucleotide differences. Moreover, the high affinity probe hybridization ensures very high sensitivity even for small RNAs at low abundance.

Low RNA sample amount requirements

Arraystar Small RNA Microarray requires as little as 100 ng total RNA, which is magnitudes lower than what small RNA-seq requires. As its direct end labeling chemistry does not require RNA pretreatments that often cause RNA loss, the microarray significantly reduces the demand for input RNA amounts especially for heavily modified RNA biotypes (e.g. tRNA and tsRNA). The low sample amount requirement opens up opportunities for research projects where the samples are rare or of limited supply.

Tolerant for RNA samples at lower qualities

The direct end-labeling is relatively insensitive to nucleotide damage in the substrate RNA sequence as it does not rely on cDNA copying by reverse transcription. Furthermore, whereas the microarray probes are unaffected by unrelated sequence presence, RNA fragments from the abundant rRNAs in degraded RNA samples can contaminate small RNAs in the size range, depressing small RNA coverage in small RNA-seq.

For these reasons, Small RNA Array is particularly advantageous for preserved or chemically treated samples or degraded samples. e.g. serum/plasma/biofluid/FFPE RNAs.

Simultaneous profiling of multiple small RNA classes

Profiling different small RNA classes by sequencing requires

separate sequencing methods and experiments: miRNA-seq, tRNA-seq, tsRNA-seq, and regular RNA-seq for longer snoRNA and small RNA precursors. Arraystar Small RNA microarrays use unified labeling chemistry to hybridize to the probes in one array for all major small RNA classes.

Rich Small RNA Analyses and Annotations

For each small RNA class, the data analyses include profiling measurement values, statistical computations, informative annotations, and publication quality graphics.

Differential expression analysis (tsRNA and tRNA as examples)

tsRNA					
tsRNA_type	tsRNA-sequence	tsRNA-length	tsRNA-precursor	Level	Molecular mechanism
3' tiRNA	ATTCAAAGGTTCCGGG TTCGAGTCCCAGCGGA GTCGCCA	39	tRNA-Arg- TCT-1	Potential	Cytotoxicity to neurons
3' tiRNA	ATGCCGAGGTTGTGAG TTCAAGCCTCACCTGG AGCACCA	39	tRNA-Ile- TAT-3	Potential	Cytotoxicity to neurons

tsRNA_type: tsRNA type (tRF-5, tRF-3, tRF-1, 5-Leader, 5-tiRNA, 3-tiRNA, and i-tRF).

tsRNA-sequence: tsRNA sequence.

tsRNA-length: tsRNA length.

tsRNA-precursor: Symbol for the tsRNA precursor.

Level: Confidence level for the tsRNA

Functional - Documented with characterized biological functions or disease association;

Reliable - Recorded in tRFdb or reported by literatures, but without further studies;

Potential - Predicted by Arraystar based on RNA fragment lengths and cleavage positions in the tRNA.

Mechanism: The molecular mechanism of tsRNA.

Sequence: Sequence of the tRNA isodecoder.

Gene name: Gene name of the isodecoder tRNA.

GenomeLocus: Genome locus of the tRNA isodecoder.

tRNA promoter Locus: Genome locus of the tRNA isodecoder promoter. TRNA promoter - tRNA promoters which include a tRNA gene plus 100 base pairs of upstream sequence. (PMC6108506).

pre-tRNA locus: Genome locus of the tRNA isodecoder precursor. pre-tRNA - precursor tRNA which include a tRNA gene plus 100 base pairs of upstream sequence and a 3'trailer.

tRNA neighboring gene: The nearest gene name of the tRNA isodecoder.

tRNA					
tRNA-Sequence	Gene name	GenomeLocus	tRNA promoter locus	pre-tRNA locus	tRNA neighbouring gene
GGGGTAT AGCTCAG.....	tRNA-Ala- AGC-1-1	chr6: 28795963- 28796035-	chr6: 28795963- 28796135-	chr6: 28795952- 28796135-	XXbac- BPG308K3.5
GGGGAATT AGCTCAA.....	tRNA-Ala- AGC-10-1	chr6: 26687256- 26687329+	chr6: 26687156- 26687329+	chr6: 26687156- 26687342+	RP11-457M11.7

Hierarchical clustering

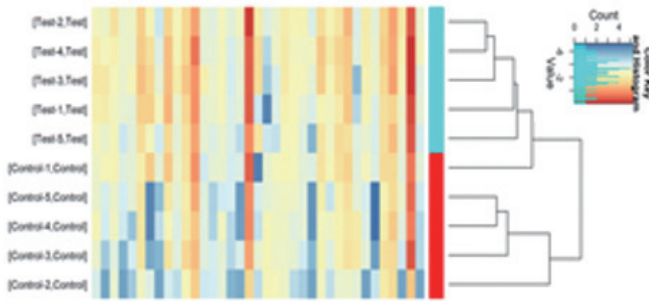


Figure 4. Hierarchical clustering heatmap of differentially expressed miRNAs.

Arraystar Small RNA Microarray Specifications

	Human	Mouse	Rat
Total probes	14,707	14,895	14,238
miRNAs	2,627 (1,318 5-p-miRNAs; 1,309 3-p-miRNAs)	1,949 (966 5-p-miRNAs; 983 3-p-miRNAs)	749 (355 5-p-miRNAs; 394 3-p-miRNAs)
tsRNAs	4,254	1,809	1,135
pre-miRNAs	1,745	1,122	448
mature tRNAs	346	270	197
snoRNAs	955	1,323	1,486
Small RNA sources	miRNA: miRBase tsRNA: tRFdb, MINTbase, GtRNADB pre-miRNA: miRBase tRNA: GtRNADB, ENSEMBL snoRNA: ENSEMBL Scientific publications		
Array Format	8 x 15K		

References

1. Godoy P.M. et al. (2018) Cell Rep [PMID: 30380423]
2. Schageman J. et al. (2013) Biomed Res Int [PMID: 24205503]
3. Quek C. et al. (2017) RNA Biol [PMID: 28005467]
4. Olvedy M. et al. (2016) Oncotarget [PMID: 27015120]
5. Dhahbi J.M. et al. (2013) BMC Genomics 14:298 [PMID: 23638709]
6. Schageman J. et al. (2013) Biomed Res Int 2013:253957 [PMID: 24205503]
7. Telonis A.G. et al. (2015) Oncotarget 6(28):24797-822 [PMID: 26325506]

Arraystar Epitranscriptomic Microarrays

Quantify the percentage of m6A/m5C modifications at the transcript specific level

Highlights

Arraystar Epitranscriptomic Microarrays have unique advantages over MeRIP-seq (Table 1).

- ▶ A single Epitranscriptomic Microarray to simultaneously profile what gene transcripts are modified, differential modification between conditions, and very importantly, the percentage of modification for each transcript
- ▶ Excellent coverage for coding and noncoding RNA classes, even for lncRNAs and circRNAs that are difficult by MeRIP-seq
- ▶ rRNA depletion not required. Faster, simpler than MeRIP-seq
- ▶ Low demand for sample amounts, starting from as little as 1 µg total RNA
- ▶ Suitable for more sample types, such as degraded FFPE, and serum/plasma/whole blood samples

Table 1.	Epitranscriptomic Microarray	MeRIP-seq
RNA amount	≥ 1µg total RNA	≥ 120 µg total RNA
%Modification	Yes	No
RNA biotypes	mRNA, lncRNA, circRNA, pre-miRNA, pri-miRNA, snoRNA, snRNA	Poly(A+) mRNA and lncRNA
Transcript isoform-specific	Excellent	Poor
RNA sample sources	Cell lines, tissues, low or degraded samples (FFPE, serum, plasma)	Cell lines, tissues in large quantity
mRNA isolation or rRNA removal	Not required	Required (large RNA amount scale-up)

Why Quantify RNA Modifications at the Transcript Specific Level?

RNA modifications, such as m6A, m1A, m5C, and pseudouridine, together form the epitranscriptome and collectively encode a new layer of gene expression regulation. m6A, the most abundant internal modification in mRNAs and lncRNAs, impacts all aspects of post-transcriptional mRNA/lncRNA metabolism and functions [1]. In addition to mRNA, m6A also functions in noncoding RNAs, such as cap-independent translation initiation of circRNA[2] and pri-miRNA processing[3]

The potential effects of RNA modifications depend on not only which gene transcripts, but also the percentage of transcripts that are modified. However, current transcriptome-wide RNA modification profiling methods deal mostly with mapping the modification sites but are unable to quantify the percentage of modified RNA for that transcript. The lack of such stoichiometric information has been a major concern for scientists [1,4]

Arraystar Epitranscriptomic Microarrays

Arraystar Epitranscriptomic two-color channel microarrays work with RNA modification immunoprecipitation to quantify the percentage of RNA that is modified for each transcript isoform. The microarrays cover the epitranscriptomes of mRNA, lncRNA, circRNA, pre-miRNA, pri-miRNA, snoRNA, and snRNA classes.

Quantifying the percentage of modification

The modified vs unmodified fractions of the same RNA transcript, which differ only in the structures or the bound readers, can assume different fates, functions and biological outcomes [4] (Fig. 1). While MeRIP-seq is used to map the modification sites, it does not quantify the relative fraction of modified RNA for a given transcript. Arraystar Epitranscriptomic Microarrays have the power to quantify the percentage by measuring the modified and unmodified transcripts in two color channels on the same array (Fig. 2), while simultaneously profile what gene transcripts are modified and the differential modification between conditions.

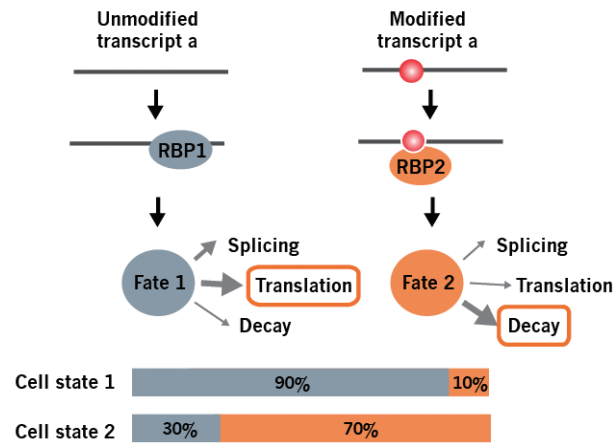


Figure 1. The changing modification stoichiometry generates functional diversity from the same RNA transcript. The percentage of modified RNA "transcript a" can be very low under one cellular condition (e.g. Cell state 1), but change to high (e.g. Cell state 2) under another cellular condition. By causing RNA structural changes and direct recruitment of modification reader proteins, the modified "transcript a" acquires a different fate, for example, from protein translation to increased RNA decay.

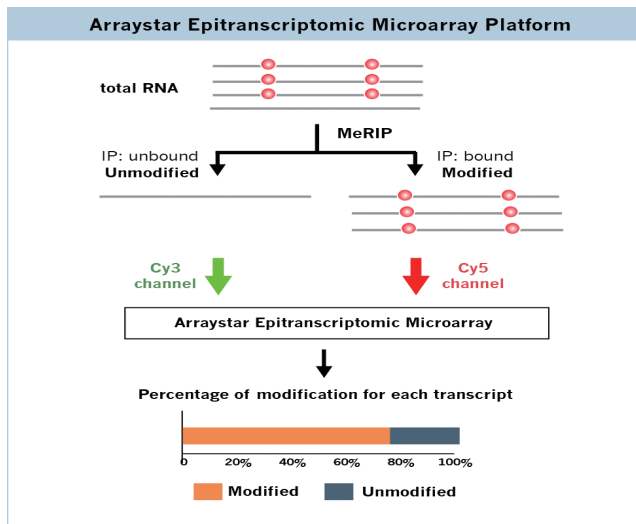


Figure 2. Arraystar Epitranscriptomic Microarray measures Cy5 labeled modified RNA and Cy3 labeled unmodified RNA in two-color channels on the same array, such that the percentage of modification for each transcript can be measured.

Covering coding and noncoding epitranscriptomes

The high sensitivity and accuracy of the microarrays are excellent even for RNA types (e.g. lncRNAs and circRNAs) otherwise technically difficult for MeRIP-seq.

• *Arraystar mRNA&lncRNA Epitranscriptomic Microarrays: For mRNA, lncRNA, and mid-sized noncoding RNA classes of pre-*

miRNA, pri-miRNA, snoRNA, and snRNA.

• *Arraystar circRNA Epitranscriptomic Microarrays: For circular RNA*

Transcript isoform specific profiling

Alternatively spliced transcript isoforms can have distinct tissue-specific expression and biological functions. For example, TRIM9 short isoform (NM_052978), but not the long isoform (NM_015163), promotes virus-induced interferon production [5]. The percentages of modified transcript isoforms have been associated with biological functions and diseases. Unfortunately, MeRIP-seq performs poorly at transcript-specific level due to required deep sequencing coverage, short read assembly, and quantification inaccuracy.

Arraystar Epitranscriptomic Microarrays use specific exon or splice junction probes to unambiguously, reliably and accurately profile the RNA modification in each individual transcript isoform, defining a new level of epitranscriptomic details.

Low sample amount requirements

Many biological samples are of limited supplies. Current MeRIP-seq requires a massive amount of total RNA ($\geq 120 \mu\text{g}$). Arraystar Epitranscriptomic Microarrays use as little as is $1 \mu\text{g}$ total RNA, opening up broad opportunities for research projects.

Arraystar Epitranscriptomic Microarray Specification

Epitranscriptomic Array Services	Array Contents
Human mRNA&lncRNA Epitranscriptomic Array (m6A/m5C)	44,122 mRNAs; 12,496 lncRNAs; 3,813 Mid-size ncRNAs
Mouse mRNA&lncRNA Epitranscriptomic Array (m6A/m5C)	48,161 mRNAs; 8,393 lncRNAs; 4,087 Mid-size ncRNAs
Rat mRNA&lncRNA Epitranscriptomic Array (m6A/m5C)	27,770 mRNAs; 10,582 lncRNAs; 2,505 Mid-size ncRNAs
Human circRNA Epitranscriptomic Array (m6A/m5C)	13,617 circular RNAs
Mouse circRNA Epitranscriptomic Array (m6A/m5C)	14,236 circular RNAs
Rat circRNA Epitranscriptomic Array (m6A/m5C)	14,145 circular RNAs

References

- Gilbert W.V. et al. (2016) Science [PMID: 27313037]
- Yang Y. et al. (2017) Cell Res. [PMID: 28281539]
- Alarcón C.R. et al. (2015) Cell [PMID: 26321680]
- Lewis C.J. et al. (2017) Nat. Rev. Mol. Cell Biol. [PMID: 28144031]
- Qin Y. et al. (2016) Cell Res. [PMID: 26915459]

Arraystar m6A Single Nucleotide Microarrays

Locate and quantify the exact m6A sites at single nucleotide resolution

Highlights

Arraystar m6A Single Nucleotide Microarrays add invaluable benefits which are lacking for conventional m6A-seq analysis:

- Systematic m6A profiling independent of m6A-antibody immunoprecipitation based approaches.
- Precise detection of m6ACA at single nucleotide resolution.
- Quantifying m6A modification stoichiometry and abundance.
- Low demand for sample amounts, as low as 1 ug total RNA.
- Specialized pipeline to collect and annotate the quantifiable Single-m6ACA sites.

Why Study Single-m6A sites?

The single-m6A modifications are often related to molecular functions in mRNA translation initiation or translation elongation dynamics. They also regulate noncoding RNA decay and activities.

Single-m6A site in 5' UTR for cap-independent translation initiation of Hsp70 mRNA

A single-base m6A modification at in the 5' UTR of Hsp70

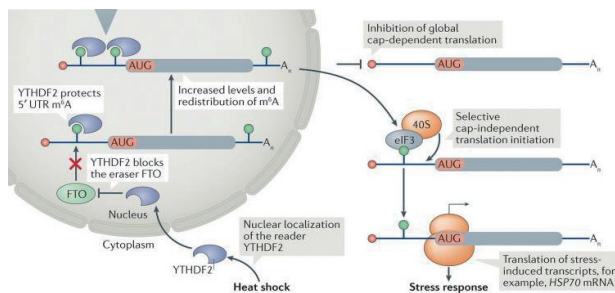


Figure 1. Molecular mechanism of cap-independent translation of HSP70 mRNA under heat shock stress by a single-base m6A modification [18, 19].

mRNA is necessary and sufficient to promote its noncanonical cap-independent translation (Fig. 1) [18]. Under stress conditions, nuclear localized YTHDF2 reader protein protects the m6A from demethylation by FTO. Translation initiation factor (eIF3) can directly bind to the m6A and initiate the internal translation, thereby allowing cap-independent translation of HSP70 when the global cap-dependent translation is shutting down in the stressed cells.

Single-m6A site in mRNA CDS affects mRNA translation dynamics

A single-base m6A modification in mRNA coding sequence (CDS) can affect the translation elongation dynamics [20]. An m6A in a codon can slow down the cognate-tRNA decoding and also act as a barrier/controller for tRNA accommodation and translation elongation. The m6A stalled translation may produce truncated proteins and have chain effects on co-translational protein folding, chaperoning, and localization signal recognition, all of which can potentially drive the protein for different fates or activities [21]. Conceivably, if m6A recodes a codon for another amino acid, it could lead to protein variants with an deviant function and substantial biological consequence even at low occupancy [20](Fig. 2).

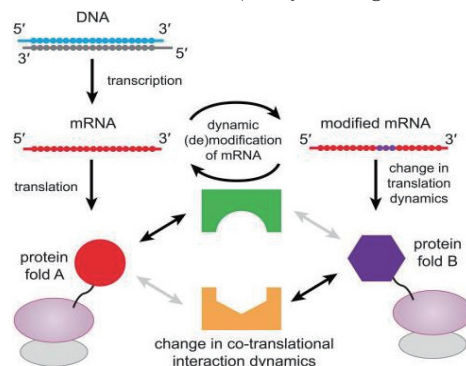


Figure 2. The changed translation elongation dynamics can influence co-translational nascent protein folding or interaction with other partnering factors [20].

Single-m6A site regulating lincRNA decay

Single-base m6A sites (A917, A1025 and A1056) in linc1281 are required to decoy let-7 family miRNAs (Fig. 3) [22]. By sequestering these pluripotency-related miRNAs, the m6A modified linc1281 ensures the cells of mESC identity.

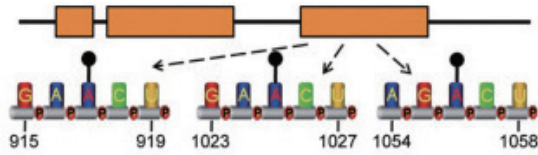


Figure 3. m6A methylation of single-m6A sites in the last exon of *linc1281* is required for the lncRNA to bind let-7 miRNAs and act as a competing endogenous RNA (ceRNA) to regulate mESC differentiation [22].

Arraystar m6A Single Nucleotide Microarrays

Arraystar has developed m6A single nucleotide resolution arrays that precisely locate the m6A modification at exact adenosine and quantify the stoichiometry of m6A modification fractions.

An orthogonal methodology for m6A detection

Due to the m6A-antibody cross-reactivity with other related modifications (e.g. m6Am)[1, 7-9], the assay specificity to m6A modification is limited. Also, in the absence of an orthogonal technique as an independent reference, the sensitivity of m6A-antibody based m6A profiling has not been systematically evaluated. For the first time, the microarrays based on methyl-sensitive MazF RNase now allow systematic m6A profiling independent of m6A-antibody immunoprecipitation based approaches such as MeRIP or miCLIP.

Single-nucleotide resolution for m6A site location

RNase MazF cleaves single stranded RNA 5' immediate to unmethylated (ACA) sequence, but not methylated (m6ACA) (Fig. 4) [1, 10]. The MazF digested RNA fragments with cleaved (ACA) and uncleaved m6ACA are labeled with Cy5 color, whereas the input RNA fragments without MazF digestion are separately labeled with Cy3 color. These RNAs are then combined and hybridized with Arraystar m6A Single Nucleotide Resolution Arrays, to quantify the m6A modification stoichiometry and abundance for each site (Fig. 5).

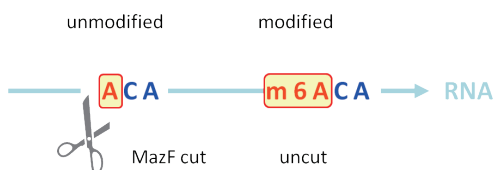


Figure 4. MazF enzyme cuts at unmethylated (ACA) sequence but not methylated (m6ACA).

m6A modification stoichiometry

The m6A stoichiometry, i.e. the fraction of m6A modification at that site, is critical for understanding its functional significance, answering questions about the m6A writing/reading/erasing, regulation, the dynamics in response to stimuli [3, 11, 12], and prioritizing the m6A sites for study. The fraction or percentage of m6A modification can be quantified by the two-color channel intensities at each site, addressing the long-standing unfulfilled needs in determining the dynamic m6A status.

Low RNA sample amount requirement

The microarrays use as low as 1 μ g total RNA. The highly sensitive and specific MazF enzymatically works well even on extremely low RNA amounts at nanogram or picogram level. m6A profiling can now be performed on rare samples, precious pathological specimens, particular histological sites, low yield sorted cells, or small animal models.

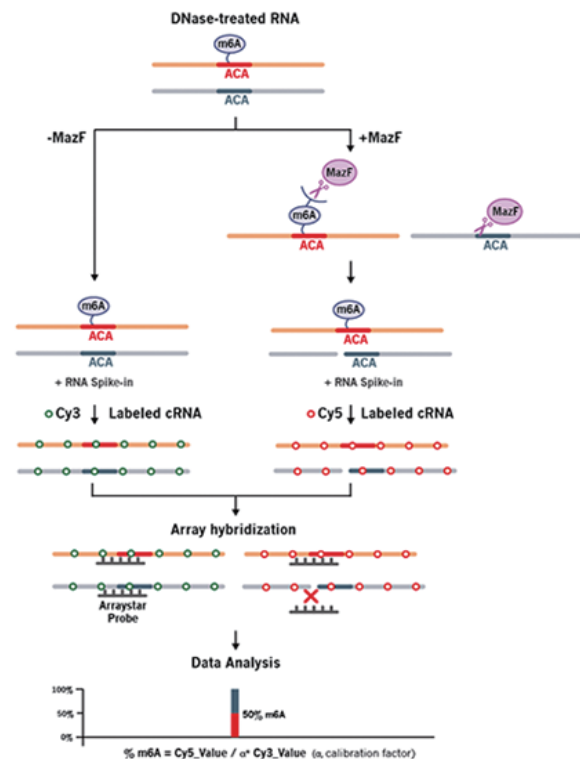


Figure 5. The workflow of Arraystar m6A Single Nucleotide Resolution Array.

Reliable collection and systematic annotations

Most m6A modifications occur in m6A motifs having a core (ACA) sequence, collectively referred to as m6ACA sites. For reliable collection of m6ACA sites, we have established a pipeline to discover all m6ACA sites that are quantifiable by

array probes. An ACA site without another ACA present within 40 nt can be uniquely detected by a probe and is defined as quantifiable Single-ACA site. Multiple ACA sites closely located within a 20-nt region can be collectively detected by a probe and is defined as quantifiable Poly-ACA site. Additionally, when multiple Single-(ACA) or Poly-(ACA) are contained within a 500 nt region and the distance between them is < 100nt, they are merged as one Clustered-ACA region (Fig 6), which can be analyzed by integrating the signals from the individual probes of constituent Single- and Poly-ACA sites. Not all ACA sequences in the RNAs are modified by m6A. All quantifiable ACA sites are further mapped to the cataloged m6A sites in the miCLIP dataset[13-16] and the m6A consensus near the m6A-seq peak summits[17], which defines a high and ultra-high confidence Single-, Poly-, and Clustered-m6ACA sets.

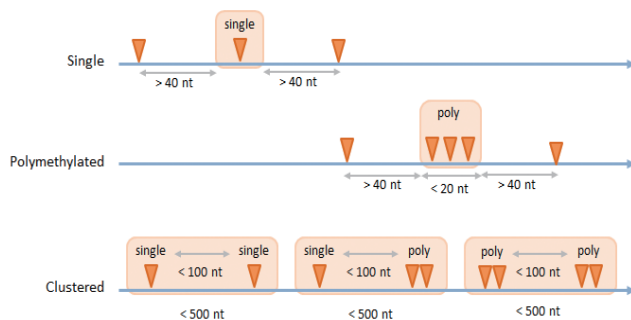


Figure 6. The collection pipeline of quantifiable Single-, Poly-, and Clustered-m6ACA sites.

Why use m6A Single Nucleotide Microarray over MeRIP-sequencing

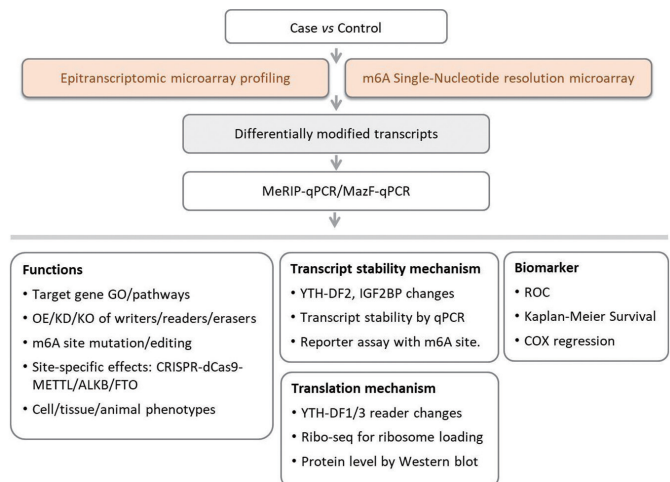
Profiling m6A at single nucleotide resolution has been challenging. Conventional methods such as m6A/MeRIP-seq have helped to make the analysis of m6A epitranscriptomics possible [1-5], but they cannot precisely identify which adenosines in a MeRIP-seq peak are actually modified, nor can they quantify the modification fraction for each site [6].

	m6A Arrays	MeRIP-Seq
Quantification	<ul style="list-style-type: none"> m6A stoichiometry as %Modified m6A RNA abundance Differential analysis of both %Modified and abundance 	<ul style="list-style-type: none"> Lack of modification stoichiometry Differential analysis of abundance only
m6A-site resolution	Single-nucleotide	~ 100 nt
Starting RNA amount	>= 1 µg total RNA	>= 120 µg total RNA
Poly(A) selection or rRNA removal	Not required	Required (scale-up needed)
RNA integrity demand	Tolerant	High

m6A Single Nucleotide Microarray Specifications

Array Name	Species	Size	Contents
m6A Single Nucleotide Array	Human Mouse Rat	8 x 15K	~14K probes targeting Single- or Poly-m6A sites.

m6A Epitranscriptomics Research Roadmap



References

- Garcia-Campos M A, Edelheit S, Toth U, et al. Cell, 2019,178(3):731-747.
- Knuckles P, Buhler M. FEBS Lett, 2018,592(17):2845-2859.
- Schwartz S. RNA, 2016,22(2):169-174.
- Meyer K D, Jaffrey S R. Annu Rev Cell Dev Biol, 2017,33:319-342.
- Yue Y, Liu J, He C. Genes Dev, 2015,29(13):1343-1355.
- Liu N, Parisien M, Dai Q, et al. RNA, 2013,19(12):1848-1856.
- Dominissini D, Moshitch-Moshkovitz S, Schwartz S, et al. Nature, 2012,485(7397):201-206.
- Schwartz S, Bernstein D A, Mumbach M R, et al. Cell, 2014,159(1):148-162.
- Linder B, Grozhik A V, Olarerin-George A O, et al. Nat Methods, 2015,12(8):767-772.
- Imanishi M, Tsuji S, Suda A, et al. Chem Commun (Camb), 2017,53(96):12930-12933.
- Grozhik A V, Jaffrey S R. Nat Chem Biol, 2018,14(3):215-225.
- Meyer K D, Jaffrey S R. Nat Rev Mol Cell Biol, 2014,15(5):313-326.
- Linder B. et al. (2015) [PMID: 26121403]
- Ke S. et al. (2015) [26404942]
- Chen K. et al. (2015) [25491922]
- Kai X. et al. (2017) [28809392]
- Schraga Schwartz, et al. (2013) [24269006]
- Zhou, J., et al. (2015) " Nature 526(7574):591-4 [PMID: 26458103]
- Kathrin Leppke, et al. (2018) [PMID: 29165424]
- Choi, J., et al. (2016) " Nat Struct Mol Biol 23(2):110-5 [PMID: 26751643]
- Kim, S. J., et al. (2015) Science 348(6233):444-8 [PMID: 25908822]
- Yang, D., et al. (2018) Nucleic Acids Res 46(8):3906-3920 [PMID: 29529255]

Selected Publications

Arraystar m6A Single Nucleotide Microarray

Specific RNA m6A modification sites in bone marrow mesenchymal stem cells from the jawbone marrow of type 2 diabetes patients with dental implant failure. Yan W, et al. *International Journal of Oral Science*, 2023

Arraystar Epitranscriptomic Microarray

mRNA&LncRNA

Sperm RhoA m6A modification mediates intergenerational transmission of paternally acquired hippocampal neuronal senescence and cognitive deficits after combined exposure to environmental cadmium and high-fat diet in mice. Zhang J, et al. *Journal of Hazardous Materials*, 2023

METTL3/IGF2BP3 axis inhibits tumor immune surveillance by upregulating N6-methyladenosine modification of PD-L1 mRNA in breast cancer. Wan W, et al. *Molecular Cancer*, 2022

Circular RNA

N6-methyladenosine hypomethylation of circGPATCH2L regulates DNA damage and apoptosis through TRIM28 in intervertebral disc degeneration. Chen Z, et al. *Cell Death & Differentiation*, 2023

METTL14-mediated m6A modification of circORC5 suppresses gastric cancer progression by regulating miR-30c-2-3p/AKT1S1 axis. Fan H N, et al. *Molecular Cancer*, 2022

Arraystar CircRNA Microarray

TGF- β signaling promotes cervical cancer metastasis via CDR1as. Zhong G, et al. *Molecular Cancer*, 2023

CircRNA DICAR as a novel endogenous regulator for diabetic cardiomyopathy and diabetic pyroptosis of cardiomyocytes. Yuan Q, et al. *Signal Transduction and Targeted Therapy*, 2023

Circular RNAs as biomarkers in liquid biopsy in colorectal cancer. Valladares-Ayerbes M, et al. *Journal of Clinical Oncology*, 2020

Arraystar LncRNA Microarray

The long noncoding RNA glycoLINC assembles a lower glycolytic metabolon to promote glycolysis. Zhu Y, et al. *Molecular Cancer*, 2022

A lncRNA signature associated with tumor immune heterogeneity predicts distant metastasis in locoregionally advanced nasopharyngeal carcinoma. Liang Y L, et al. *Nature Communications*, 2022

Arraystar Super-Enhancer LncRNA Microarray

Super-enhancer hijacking LINC01977 promotes malignancy of early-stage lung adenocarcinoma addicted to the canonical TGF- β /SMAD3 pathway. Zhang T, et al. *Journal of Hematology & Oncology*, 2022

tRNA Sequencing

Human SAMD9 is a poxvirus-activatable anticodon nuclease inhibiting codon-specific protein synthesis. Zhang F, et al. *Science Advances*, 2023

AAV-delivered suppressor tRNA overcomes a nonsense mutation in mice. Wang J, et al. *Nature*, 2022

nrStar™ tRNA PCR Array

N2-methylguanosine modifications on human tRNAs and snRNA U6 are important for cell proliferation, protein translation and pre-mRNA splicing. Wang C, et al. *Nucleic Acids Research*, 2023

A critical period of translational control during brain development at codon resolution. Harnett D, et al. *Nature Structural & Molecular Biology*, 2023

nrStar™ Functional LncRNA PCR Array

CAD increases the long noncoding RNA PUNISHER in small extracellular vesicles and regulates endothelial cell function via vesicular shuttling. Hosen M R, et al. *Molecular Therapy. Nucleic Acids*, 2021

One-stop Shop for All the Services You Need

Microarray Solutions

Epitranscriptomic Array Service
m6A Single Nucleotide Array Service
Small RNA Modification Array Service
Circular RNA Array Service
LncRNA Array Service
Super-enhancer LncRNA Array Service
T-UCR Array Service
Small RNA Array Service **NEW !**
piRNA Array Service

Next-Gen Sequencing Solutions

RNA Sequencing
miRNA Sequencing
tRNA Sequencing
tRF&tiRNA Sequencing
eccDNA Sequencing **NEW !**
DRIP-sequencing **NEW !**
MeRIP-sequencing
(h)MeDIP-sequencing with LncRNA Promoter Analysis
ChIP-sequencing

ncRNA PCR Arrays

nrStar™ Functional LncRNA PCR Arrays
miRStar™ Canonical Conserved microRNA PCR Array
nrStar™ tRNA PCR Arrays
nrStar™ tRF & tiRNA PCR Arrays
nrStar™ snoRNA PCR Arrays

mRNA PCR Arrays

NuRNA™ tRNA Modification Enzymes PCR Array
NuRNA™ Small RNA Biogenesis Proteins PCR Array
NuRNA™ Epitranscriptomics PCR Array
NuRNA™ Central Metabolism PCR Array

Contact us:



Tel.: 915 515 403

e-mail: info@bionova.es

www.bionova.es

