# Arraystar Epitranscriptomic Microarrays

## Quantifying the epitranscriptomic modifications

## 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.

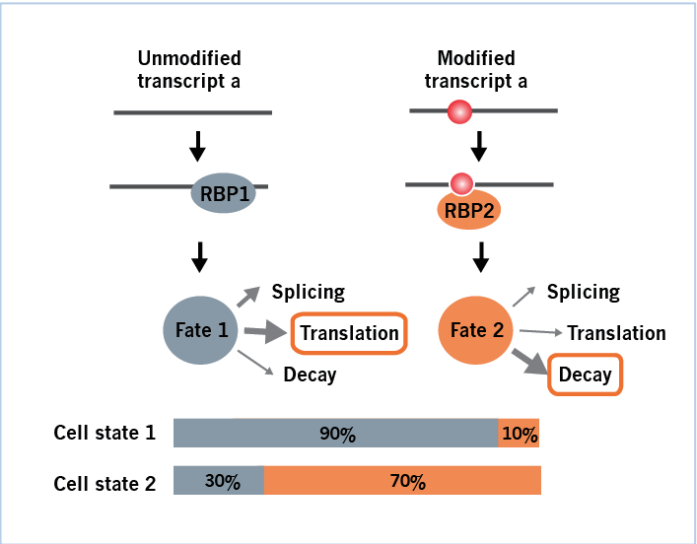


**Fig. 1.** The changing modification stoichiometry generates functional diversity from the same RNA transcript. The percentage of modified RNA "transcript a" can be very low under one cellular condition (e.g. Cell state 1), but change to high (e.g. Cell state 2) under another cellular condition. By 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.

## Introduction

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 two-color channel microarrays work with RNA modification immunoprecipitation (MeRIP) 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.

| 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 |
| **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) |

## 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.

## 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 RNAs.

## 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 ( ≥120 µg). Arraystar Epitranscriptomic Microarrays use as little as is 1 µg total RNA, opening up broad opportunities for research projects.

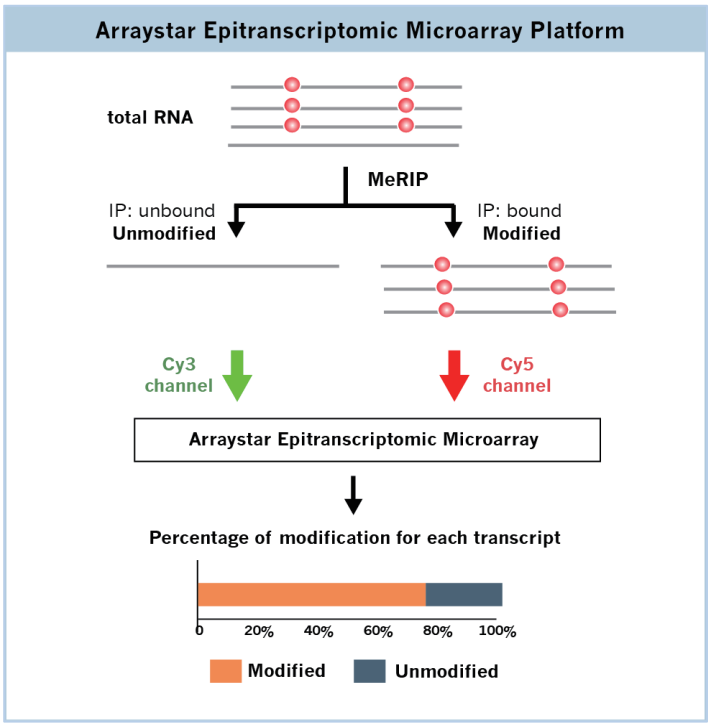


**Fig. 2.** Arraystar Epitranscriptomic Microarray measures Cy5 labeled modified RNA and Cy3 labeled unmodified RNA in two-color channels on the same array, such that the percentage of modification for each transcript can be measured.

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

## References


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