Arraystar Small RNA Modification Arrays

o8G/m7G/m6A/Ψ/m5C in miRNAs, pre-miRNAs & tsRNAs

Quantifying Modifications in Small RNAs with High Sensitivity & Accuracy

Highlights

- Detecting and quantifying a variety of small RNA modifications
- Coverage of multiple small RNA classes
- Gold standard for accurate quantification of modified small RNAs
- High sensitivity for modified small RNAs at lower levels
- Low sample amount required, starting from as little as 1 ug total RNA

Why Study Small RNA Modifications?

Small RNAs, including microRNAs (miRNAs) and tRNA-derived small RNAs (tsRNAs, i.e. tRFs and tiRNAs), harbor a diversity of RNA modifications. RNA modifications such as 5-methylcytidine (m5C), 7-methylguanosine (m7G), 8-oxoguanine(O8G), pseudouridine (Ψ) and m6A-methylation (m6A) modulate the activities of small RNAs in diverse biological processes and play pivotal roles in pathological conditions.

- Small RNA modifications influence miRNA targeting

Modifications in the seed regions of miRNAs are known to alter the miRNA-mRNA base pairing and targeting specificity, having profound biological consequences [4-6]. For example, Reactive Oxygen Species (ROS) can convert guanine (G) to 8-oxoguanine (O8G) in miRNAs. O8G base pairs with adenine (A) instead of unmodified G pairing with C. Thus, O8G modification in the seed region of a miRNA alters the mRNA targeting through O8G•A base pairing (Fig. 1, Top). When modified with o8G, miR-184 binds its new mRNA targets BCL-XL and BCL-W and suppresses their translation, resulting in increased cardiomyocyte cell death (Fig. 1, Bottom)[1]. In another example, introducing o8G in the seed region of miR-1 alone is sufficient to cause cardiac hypertrophy in mice [2]. Therefore, modifications installed in response to pathophysiological conditions can coordinate the gene expression by influencing miRNA targeting[2].

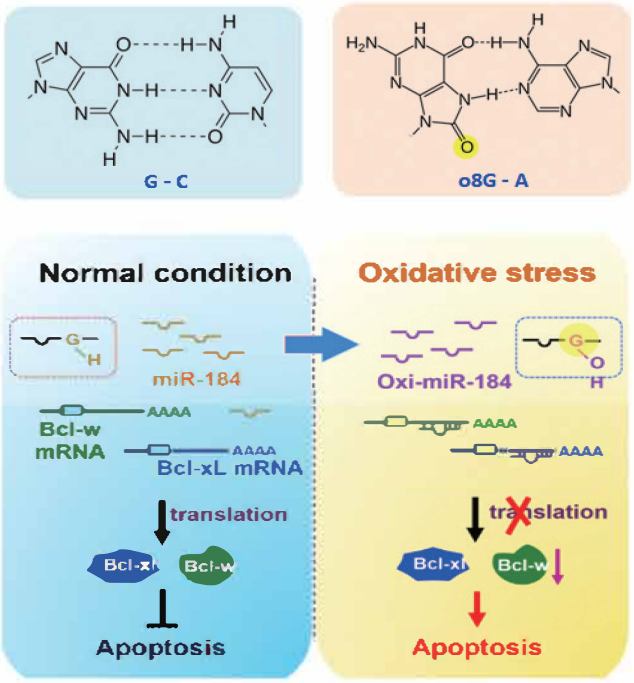


Fig.1. Top: o8G modification converts G-C pairing to altered G-A pairing. Bottom: Under oxidative stress, miR-178 is o8G modified and re-targets anti-apoptotic Bcl mRNAs, causing apoptosis [1].

- Small RNA modifications inhibit protein translation

Modified small RNAs can decoy RNA binding proteins (RBP), thereby displacing the RBP from mRNAs, inhibiting its binding to the target mRNAs, and suppressing the translation. For example, the 5' terminal oligoguanine (TOG) in tsRNAs can either have an unmodified uridine (U8) or pseudouridine (Ψ8) modified by PUS7 at the 8th position. The U8/Ψ8 status determines the different binding affinities with polyadenylate-binding protein 1 (PABPC1) as a translational initiation factor. Ψ8-TOG-5' tsRNAs bind more tightly to PABPC1, displace it from mRNAs, and inhibit the translation, whereas U8-TOG-5' tsRNAs do not (Fig. 2)[3].

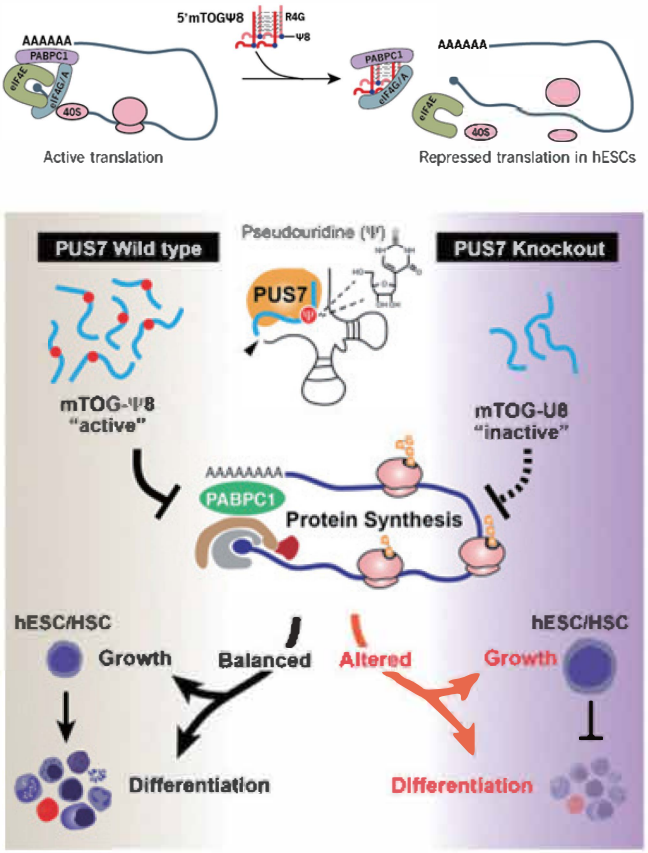


Fig. 2. 5' TOG-tsRNAs with Ψ modification at position 8 decoy and displace PABPC1 from the translation initiation complex, thereby repressing the translation of capped mRNAs [3]

- Small RNA modifications as biomarkers

The associations of small RNA modifications with diseases offer opportunities for a new class of epitranscriptional biomarkers for potentially superior diagnostic/prognostic performance [4]. For example, o8G oxidation of miRNAs coordinates redox-mediated gene expression and is correlated with pathophysiological conditions of cardiomyocytes [2]. Overall, m6A modification of miRNA is significantly increased in cancers compared with normal tissues. For example, m6A modified miR-17-5p level in serum indicates early pancreatic cancer with extremely high sensitivity and specificity (Fig.3) [4].

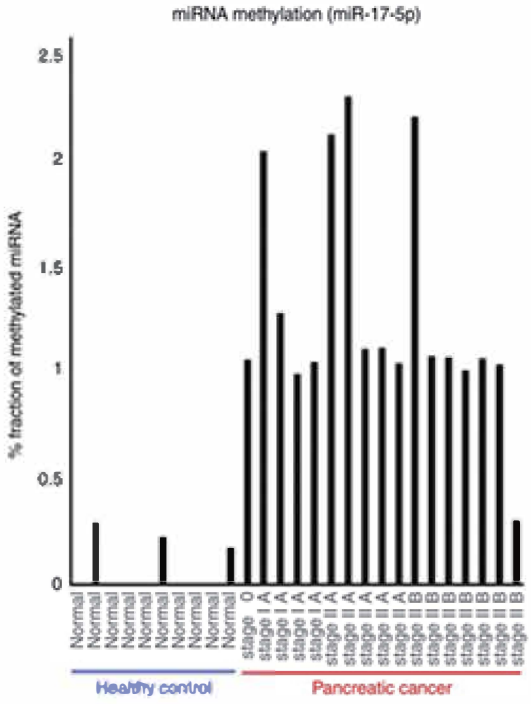


Fig. 3. m6A methylation of serum miR17-5p displays superior diagnostic value in pancreatic cancer [4].

The Challenges to Quantify Small RNA Modifications Accurately

Although sequencing has been used for small RNA profiling, the influence of RNA modifications on the sequencing quantification has largely been ignored. Various RNA modifications, such as m1A, m3C and m1G, do interfere with and block the reverse transcription reaction during sequencing library construction, thereby making accurate quantification of small RNAs and especially their modifications impossible (Fig.4). For example, the presence of m1A in the TUC loop blocks the proceeding of reverse transcriptase during cDNA synthesis. Most of the past small RNA sequencing data were obtained using the library construction methods above without regards to the modifications, which could mislead the interpretation.

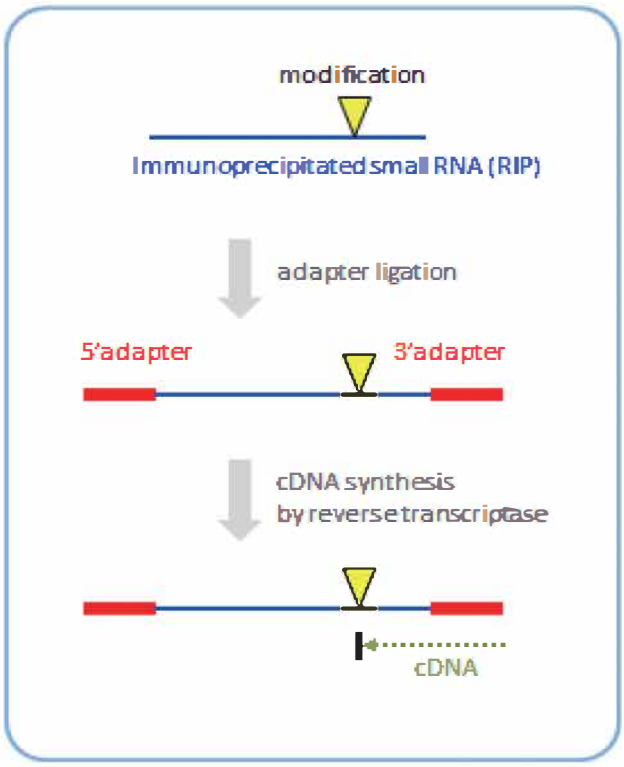


Fig.4. Small RNA MeRIP-seq limitation: cDNA synthesis during sequencing library construction is blocked by RNA modifications.

Also, small RNA profiling by small RNA-seq requires multiple PCR amplification steps, which incurs significant quantification bias/inaccuracies. The situation therefore necessitates the use of independent, orthogonal methodologies.

In practice, most sequencing based methods for modification profiling require large amount of input total RNAs (> 100 ug), precluding many studies with only limited sample amounts.

Furthermore, small RNA-seq commonly uses Reads Per Million RNA reads (RPM) for normalization and to represent the relative RNA abundances in the sample. However, RPM depends on the composition of the small RNA population in a sample. A change in one small RNA' s RPM will adjust all the other small RNAs' values even their actual absolute expression levels are not changed.

In order to identify and quantify the full spectrum of modified-small RNAs with high sensitivity and accurate stoichiometry, there is a need for overcoming the limitations of the sequencing-based approaches and developing non-sequencing based methods.

The Non-Sequencing Based Solution – Small RNA Modification Arrays

Arraystar Small RNA Modification Arrays are designed to quantify a choice of o8G, m7G, m6A, Ψ, or m5C modifications in miRNAs, pre-miRNAs, and tsRNAs on a single array. Combined with RNA immunoprecipitation (RIP), the arrays simultaneously measure the modified and unmodified small RNA levels in two-color channels on the same array, providing the key information to study regulatory impacts of the modification in small RNAs.

- Gold standard for accurate quantification of modified small RNAs

The arrays use direct RNA end labeling to ensures high fidelity of quantification, without the problems of RNA modifications blocking cDNA synthesis during RNA-seq library prep (Fig. 5). Here, the 3' -end of the RNAs are ligated with cytidine-5'-phosphate labeled with Cy5 (for modified IP-RNA) or Cy3 (for unmodified supernatant RNA) by T4

RNA ligase in one step ligation. No problematic reverse transcription or PCR amplification are used. Such directly labeled RNAs most faithfully represent the RNA abundance levels.

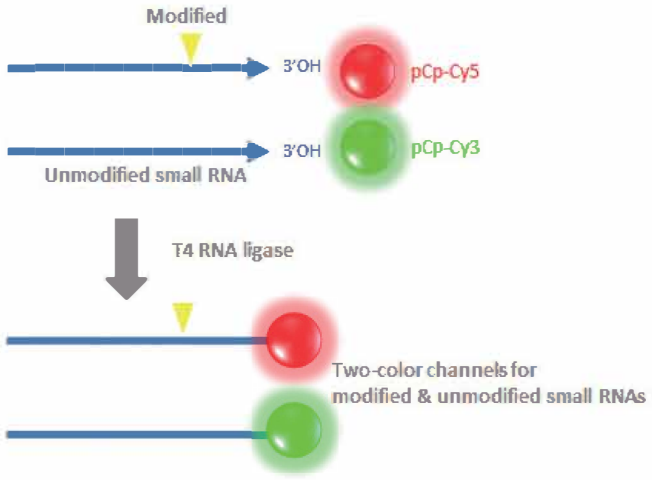


Fig. 5. One-step direct RNA end labeling of modified and unmodified RNAs by cytidine-5'-phosphate Cy5 or Cy3 labels in separate two-color channels, to achieve the most faithful quantification accuracy.

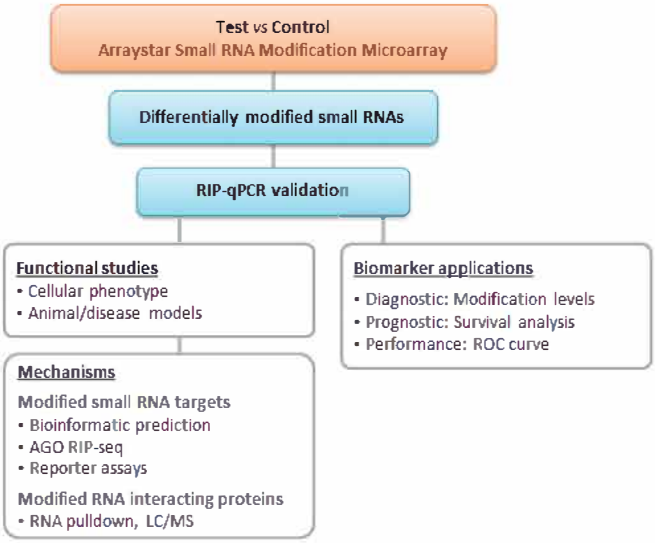
- *Low sample amount requirements*

Very often, many biological samples are of limited supplies. Arraystar Small RNA Modification Microarrays use as little as 1 ug total RNA, opening up opportunities for broad research projects.

- *Array coverage of multiple small RNA classes*

	Human	Mouse
miRNAs	2,628(1,319 **5-p-miRNAs** & 1,309 **3-p-miRNAs**)	1,949 (966 **5-p-miRNAs** & 983 **3-p-miRNAs**)
pre-miRNAs	1,745	1,122
tsRNAs	5,128	1,809
Small RNA sources	**miRNA:** miRBase (v22) **pre-miRNA:** miRBase (v22) **tsRNA:** tRFdb, GtRNADb (18.1 2019.08) **Literatures:** Scientific publications (up to 2019)	

Research Roadmap for Small RNA Modification Profiling



References

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[2] Seok, H., et al. (2020) "Position-specific oxidation of miR-1 encodes cardiac hypertrophy" Nature 584(7820):279-285 [PMID: 32760005]

[3] Guzzi, N., et al. (2018) "Pseudouridylation of tRNA-Derived Fragments Steers Translational Control in Stem Cells" Cell 173 (5):1204-1216 e26 [PMID: 29628141]

[4] Konno, M., et al. (2019) "Distinct methylation levels of mature microRNAs in gastrointestinal cancers" Nat Commun 10(1):3888 [PMID: 31467274]

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