Small RNA Library Preparation from Single Cell Quantities and Low Input Biological Samples

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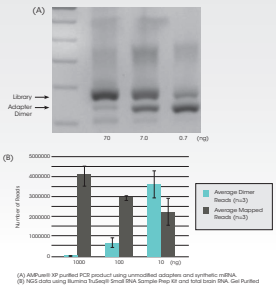
Abstract

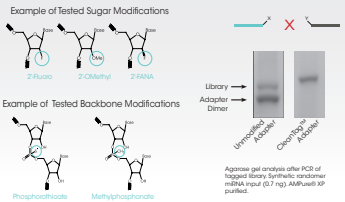
Next-Generation Sequencing (NGS) has greatly facilitated the exploration of new biomarkers for diagnostic applications. The discovery that biological fluids harbor cell free and exosomal genetic material has led researchers to prepare RNA and DNA libraries extracted from plasma and serum. However, library preparation from biological samples has been challenging due to inherently low amounts of genetic material. Small RNA biomarkers have recently received much attention in the diagnostics field however, small RNA library prep protocols require higher RNA inputs (> 100 ng). It is well known that the predominant obstacle in small RNA library preparation is adapter dimer formation. Adapter dimer out-competes library amplification and dominates valuable sequencing space, especially at low inputs. Overall, adapter dimer prevents lower limits of detection, mandates multiple purification steps including manual gel extractions, and prevents automation. Adapter dimer elimination is crucial to the small RNA library prep workflow. We have demonstrated this with the use of chemically modified adapters (CleanTag™ Adapters) which prevent adapter-adapter ligation. CleanTag™ adapters allow preparation of challenging sample types with ultra low levels of RNA such as Clip-Seq, CLASH, FFPE, FACS sorted cells, and biological fluids (plasma, urine, vesicles). These sample types have been successfully sequenced and resulted in reduced adapter dimer levels and quality NGS data. Until now, sRNA-Seq of single cell quantities was not possible with commercially available kits. Utilizing CleanTag™ adapters we have produced small RNA libraries and downstream NGS data using 10 picograms of total RNA (comparable to single cell levels). Preliminary results suggest that highly expressed miRNA are maintained and further investigation of single cell level inputs for small RNA will further advance the diagnostic field.

Figure 1: sRNA Library Prep is Prone to Adapter Dimer Formation at Low RNA Inputs



(A) AMPure® XP purified PCR product using unmodified adapters and synthetic miRNA.
(B) NGS data using Illumina TruSeq® Small RNA Sample Prep Kit and total brain RNA. Gel Purified

Figure 2: Chemically Modified CleanTag™ Adapters Block Adapter-Adapter Ligation



Agarose gel analysis after PCR of tagged library. Synthetic random miRNA input (0.7 ng). AMPure® XP purified.

Figure 3: CleanTag™ Workflow, 1-1000 ng RNA Input

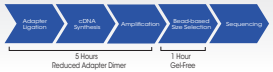
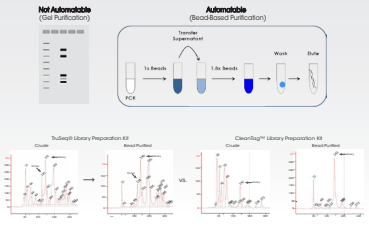
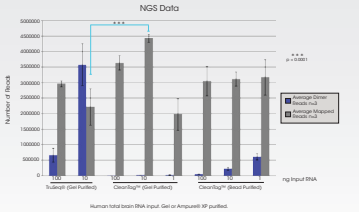


Figure 4: CleanTag™ Eliminates Need for Gel Purification



BioAnalyser® traces of 1000 ng human total brain RNA input

Figure 5: CleanTag™ Adapters Produce Quality NGS Data at Low Inputs Without Gel Purification



Human total brain RNA input. Gel or Ampure® XP purified.

Figure 6: Improved sRNA-Seq Data Using Plasma Samples

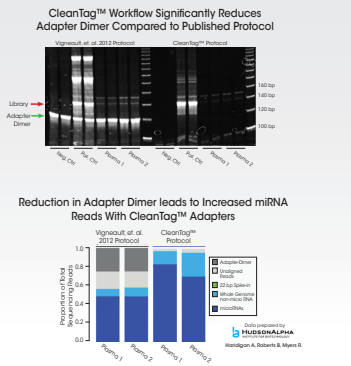


Figure 7: Detection of Parasitic Nematode-Derived miRNA From Plasma Samples

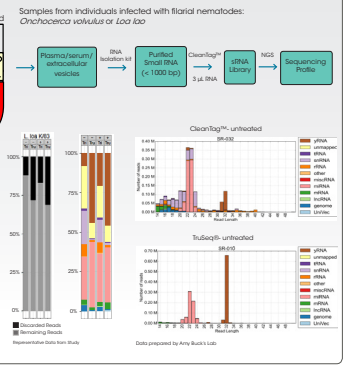


Figure 8: CleanTag™ Adapters Allow Robust Library Preparation From Extracellular Vesicles (EV) in MODE-K Cells.

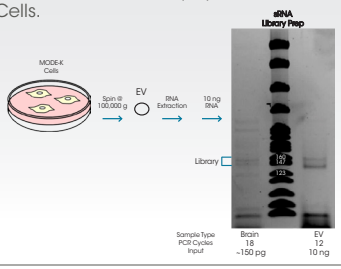


Figure 9: Significant Reduction of Adapter Dimer in Low Input Immunoprecipitation Assays

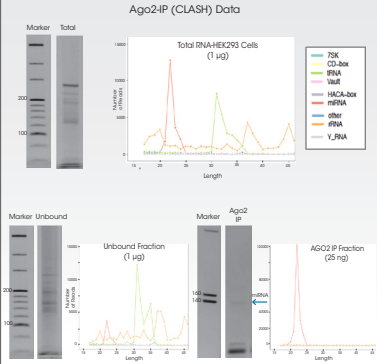
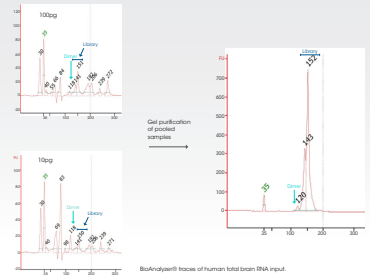
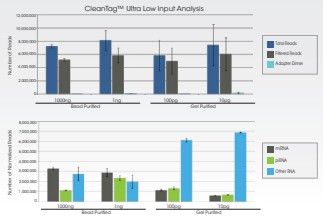


Figure 10: Small RNA Library Preparation From Single Cell Quantities of RNA Input Now Possible with CleanTag™ Adapters



BioAnalyser® traces of human total brain RNA input.

Figure 11: Next-Generation Sequencing Data from Single Cell Quantities of Total RNA



Human total brain RNA input

Conclusion

- Reduced adapter dimer with CleanTag™ adapters
- RNA inputs lower than 1 nanogram total RNA
- Potential to tag a more diverse population of small RNA
- Potential for automation by replacing gel purification with bead purification
- CleanTag ™ adapters provide robust library preparation for low input samples:
  - Biological samples (plasma and extracellular vesicles)
  - Immunoprecipitation assays- Ago-IP (CLASH protocol)
  - Single cell quantities of RNA
  - FFPE, urine, FACS sorted cells (Data not shown)

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