Improved Small RNA Library Preparation Workflow for Next-Generation Sequencing

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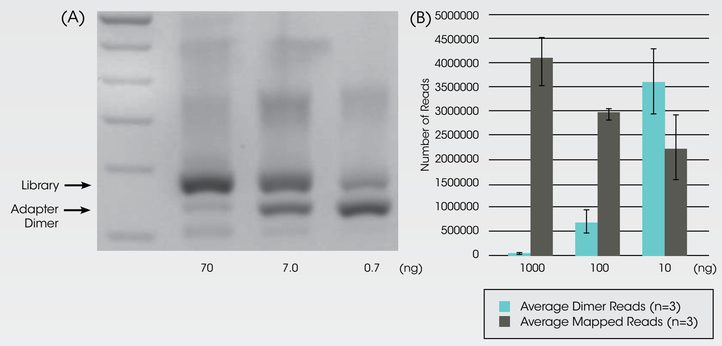
Abstract

Next-generation sequencing (NGS) is used to analyze microRNA (miRNA), a class of small non-coding RNAs that are important therapeutic targets and diagnostic markers. Commercially available small RNA sequencing library preparation kits require large inputs (>100 ng) and a laborious gel purification step. Additionally, commercial kits are hindered by adapter dimer formation, where 5' and 3' adapters ligate without an intervening RNA insert. Adapter dimers preferentially amplify during PCR. This is exacerbated at low RNA inputs where adapter dimer formation can greatly diminish usable sequencing reads.

We describe an optimized workflow which suppresses adapter dimers, works with low RNA input and eliminates the need for a gel purification step. Our workflow introduces chemically modified adapters that efficiently form libraries while reducing adapter dimer formation. TriLink's modified adapter workflow allows RNA inputs as low as 1 ng with less than 1% adapter dimer reads when gel purified. Furthermore, when our workflow is combined with bead-based size selection purification, automation becomes possible.

Figure 1: Small RNA 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 Adapters Block Adapter-Adapter Ligation

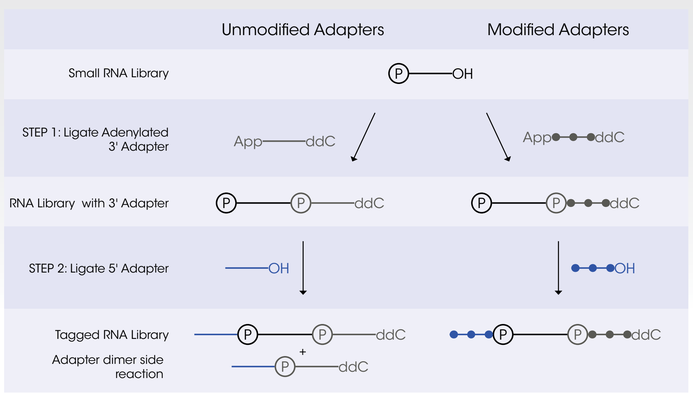
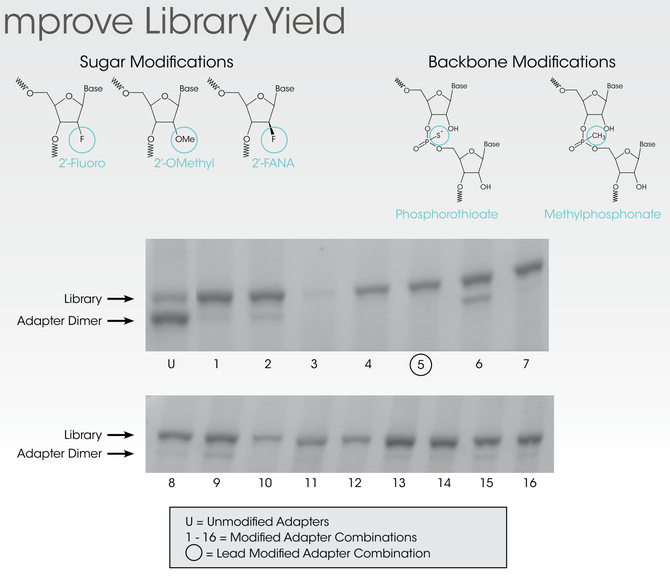
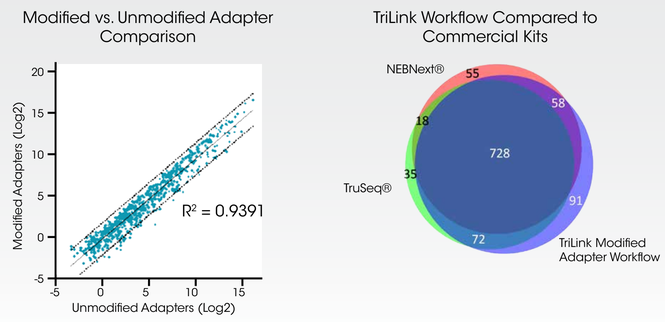


Figure 3: Identifying Modified Adapters that Reduce Adapter Dimer and Improve Library Yield



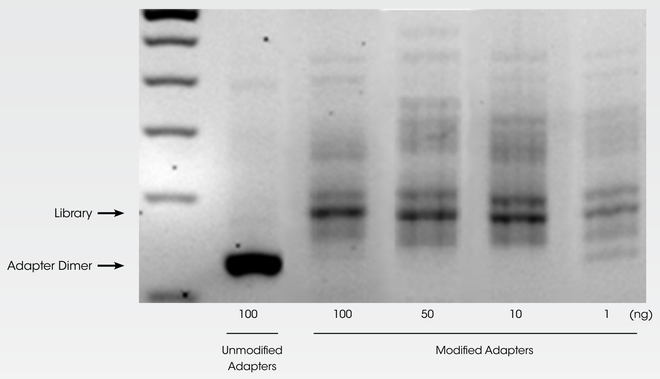
Agarose gel analysis of different 5' and 3' modified adapter combinations after PCR of tagged library. Synthetic randomer miRNA input (0.7 ng). AMPure® XP purified.

Figure 4: Unmodified and Modified Adapters Tag a Similar Population of miRNA



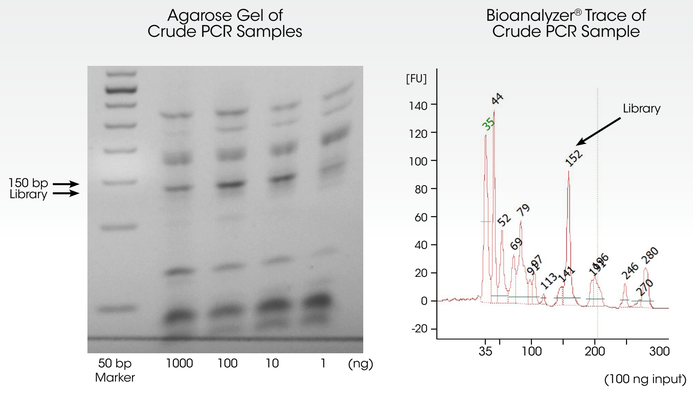
Human total brain RNA input. Gel purified.
Data analysis of brain miRNA identified in 3 replicates by The Scripps Research Institute.

Figure 5: Modified Adapters Allow Lower RNA Input Levels



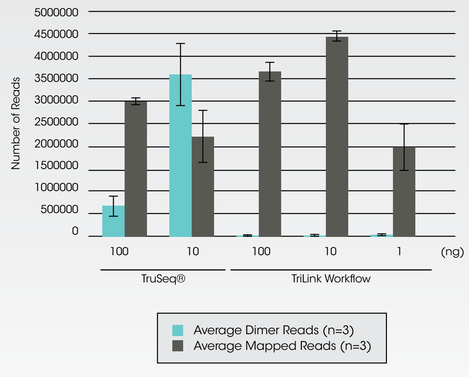
Human total brain RNA input. Ampure XP purified.

Figure 6: Modified Adapters Allow Library Preparation of Samples with Low miRNA Levels



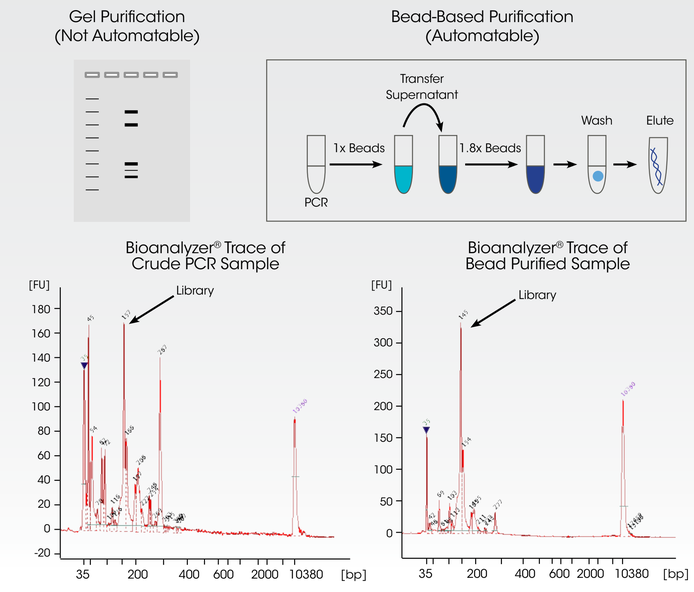
MCF7 total RNA input.

Figure 7: Even at Low Input, Modified Adapters Reduce Adapter Dimers While Maintaining Mapped Reads



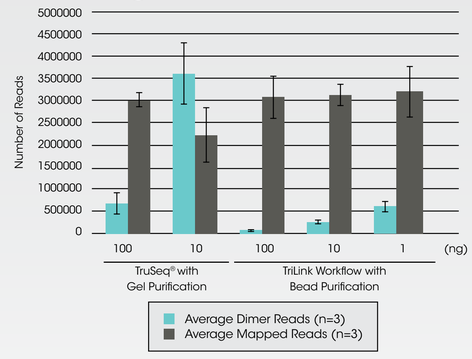
Human total brain RNA input. Gel purified.

Figure 8: TriLink Workflow Eliminates Need for Gel Purification



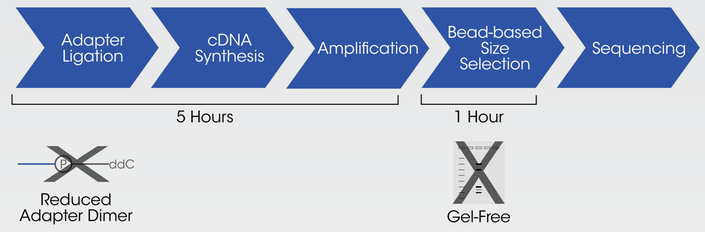
1000 ng Human total brain RNA input. AMPure® XP purified.

Figure 9: TriLink Workflow Results in Quality Sequencing Data Demonstrating Potential for Automation



1000 ng Human total brain RNA input. Gel or AMPure® XP purified.

Figure 10: TriLink Modified Adapter Workflow, 1-1000 ng RNA Input



Conclusion

- Modified adapters reduce adapter dimer.
- Modified adapters outperform commercially available kits.
- Modified adapters allow input as low as 1 ng total RNA.
- Modified adapters allow robust library prep even in samples with low miRNA abundance.
- Adapter dimer elimination makes bead-based purification feasible, and therefore automation is possible.

Reaction Conditions

Ligation Conditions: [Step 1] Buffer, Modified Adenylated 3' Adapter, RNA input, Truncated T4 RNA Ligase 2 KQ, 28°C for 1 hr, 65°C for 20 min; [Step 2] Buffer, Modified 5' Adapter, Step 1 product, T4 RNA Ligase 1, 28°C for 1 hr 65°C for 20 min.
RT: Superscript® II manufacturer recommended conditions. 50°C for 1 hr.
PCR: Q5® Master Mix manufacturer recommended conditions. 12-21 PCR cycles.
Analysis: 4% agarose gel or Bioanalyzer® with High Sensitivity DNA Kit. Buffer components may cause higher base pair shift in crude Bioanalyzer® traces.
NEBNext® Small RNA Library Prep Set: Per manufacturer recommended conditions unless noted.
TruSeq® Small RNA Sample Prep Kit: Per manufacturer recommended conditions unless noted.
Purification: Gel extraction or Agencourt® AMPure® XP.

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