

Pushing the Limits of Small RNA-Seq with Chemically Modified Adapters

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Abstract

Next-generation sequencing (NGS) is an evolving tool for nucleic acid sequence identification. NGS is widely used for basic research and discovery as well as clinical diagnostics. It is critical to develop the most accurate and reproducible library preparation techniques, especially when dealing with patient samples. Both DNA and long RNA-Seq have advanced, low input, PCR-free, and automatable library preparation techniques. In the continuously growing field of small RNA (including microRNA, piwiRNA, tRNA, and snoRNA) sequencing methods still have limitations including high input requirements and difficulties in automation. Separation of tagged library from adapter-adapter ligation products (adapter dimer) has been a persistent challenge due to the minimal size difference between products, which hinders a gel-free workflow. In order to resolve this problem we have screened several chemical modifications of adapter oligos to suppress adapter dimer formation. We introduce a novel method of small RNA library preparation that applies these modified CleanTag™ Adapters and creates the potential for automation without the need for a gel separation to exclude adapter dimer. We replace the gel purification step with a two-step magnetic bead purification for size selection since adapter dimer no longer dominates the reaction. This could be carried out by a liquid handling robot for a fully automated library preparation procedure. Current commercial library preparation kits recommend inputs of 100-1000 ngs. Reduction in adapter dimer has allowed for library preparation from much less starting material (as low as 1 ng). Using 10 ngs of total brain RNA input in conjunction with CleanTag™ Adapters, adapter dimers were reduced by over 3,000,000 reads compared to a commercial kit. Additionally, libraries using modified adapters resulted in 1,000,000 more miRNA reads than a commercial kit at this low RNA input. With increased library coverage and decreased side products the data generated from modified adapters brings us closer to analysis on a single cell level. Our method of small RNA library preparation results in high quality libraries from various tissues, cells, and low RNA input biofluids (e.g. plasma and urine). The ability to use very low inputs and to automate library preparation will allow high throughput sequencing and improved clinical assays.

Figure 1: Small RNA Library Prep Is Prone to Adapter Dimer Formation at Low RNA Inputs

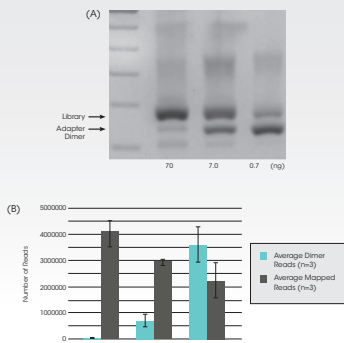


Figure 2: Chemically Modified CleanTag™ Adapters Block Adapter-Adapter Ligation

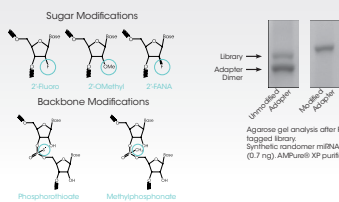


Figure 3: Unmodified and Modified CleanTag™ Adapters Tag a Similar Population of miRNA

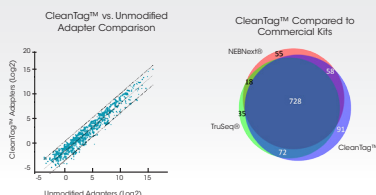


Figure 4: CleanTag™ Adapters Allow Library Preparation With Low Total RNA Input

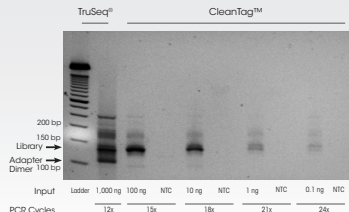


Figure 5: Evaluating CleanTag™ for Detection of Single Cell Quantities

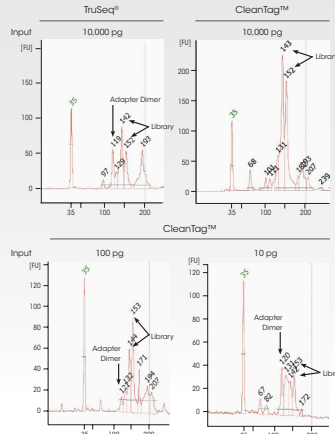


Figure 6: Improved Small RNA-Seq Data Using Plasma Samples

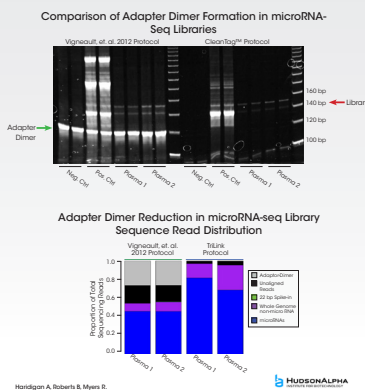


Figure 7: Robust Library Preparation from Samples of Low miRNA Abundance Using CleanTag™ Adapters

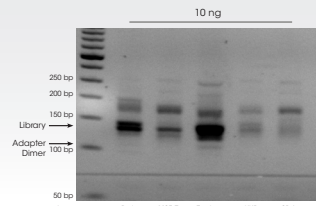


Figure 8: Even at Low Input, CleanTag™ Adapters Reduce Adapter Dimers While Maintaining Mapped Reads

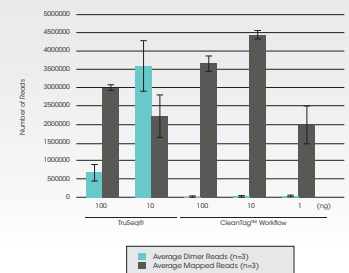


Figure 9: CleanTag™ Eliminates Need for Gel Purification

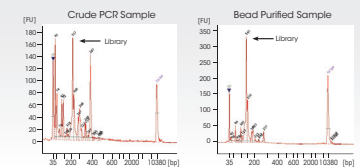


Figure 10: CleanTag™ Shows Potential for Automation with Quality Sequencing Data

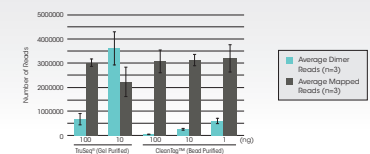
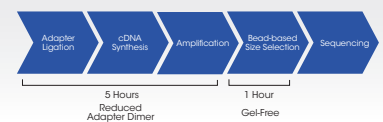


Figure 11: CleanTag™ Workflow, 1-1000 ng RNA Input



Conclusion

- Reduced adapter dimer with CleanTag™ Adapters
- Improved workflow compared to commercially available kits
- RNA inputs as low as 1 nanogram total RNA
- Robust system tags library even in samples with low miRNA abundance
- Suitable for diagnostic samples with limited RNA
- Bead-based size selection will allow automation by eliminating gel purification

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