A rapid, automated whole transcriptome library preparation solution to support a variety of applications and sample types

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RNA sequencing is a highly sensitive and accurate method for transcriptome analysis that has broad utility across applications ranging from clinical and translational to agricultural. Though valuable for providing high-resolution data for gene expression profiling, alternative splicing analysis, and gene fusion detection, RNA-seq library preparation workflows are typically laborious, time consuming, and difficult to complete in a single day. There is a need for solutions that alleviate the library prep bottleneck while also meeting increasingly rigorous sensitivity requirements.

Here, we present an automated workflow on the Biomek i7 Hybrid NGS Workstation using the Watchmaker RNA Library Prep Kit with Polaris Depletion. The automated workflow is capable of processing up to 96 samples in 6 hours. An optional on-deck thermal cycler can be leveraged to automate the workflow in its entirety without user intervention, otherwise the number of user interventions are minimal (up to 7) for off-deck incubations. The high-throughput and fast processing of samples is partially enabled by the Watchmaker chemistry, which requires only three bead purifications and seven reaction steps, significantly reducing total and hands-on times as well as consumable use.

We evaluated the performance of the automated workflow by comparing automated and manual control libraries using commercially available, high-quality total RNA from a variety of vendors and human tissue sources. Library yield and sizing were consistent across manual and automated preps, as well as across the automated prep plate. Automated libraries were further characterized as having low residual ribosomal RNA rates, high uniquely mapped rates, and a high number of unique genes detected. These data, along with the highly streamlined and automated workflow, demonstrate the value of this solution for addressing the wide variety of applications and samples that core facilities support.