Improved whole transcriptome sequencing for challenging clinical samples: A streamlined alternative to targeted RNA-seq

Watchmaker RNA Library Prep Kit with Polaris® Depletion

This study was performed in collaboration with CellCarta, a global Contract Research Organization (CRO) serving the biopharmaceutical industry. CellCarta evaluated whole transcriptome sequencing using Watchmaker RNA Library Prep with Polaris Depletion as an alternative to a standard RNA library prep into exome capture method across whole blood and FFPE samples. The results presented here summarize performance metrics including turnaround time, depletion efficiency, library complexity, and gene detection.

INTRODUCTION

RNA sequencing (RNA-seq) is a technology used in translational research and biomarker discovery, enabling insights into gene regulation, splicing, and transcript structure. In clinical studies, RNA-seq is routinely applied to both tissue- and blood-derived samples to characterize gene expression profiles associated with disease biology, drug mechanism of action, and immune response.

However, RNA-seq from clinical specimens presents several technical challenges. These include limited input material, severe RNA fragmentation and degradation in formalin-fixed paraffin-embedded (FFPE) tissue, and high levels of ribosomal (rRNA) or globin mRNA, particularly in whole blood. Together, these may limit sensitivity and compromise downstream analyses, especially in studies requiring robust detection of lowly expressed transcripts.

Hybrid capture-based sequencing workflows are commonly used to address some of these challenges, but they introduce longer turnaround times and increased workflow complexity and cost. Further, targeted approaches inherently restrict analysis to a predefined set of genes, limiting utility to uncover novel biomarkers. As translational studies scale, there is growing demand for RNA-seq workflows that are robust across challenging sample types, reproducible across replicates and conditions, and capable of higher throughput with reduced hands-on time.

CellCarta, a global leader in precision medicine services, evaluated whole transcriptome sequencing as an alternative to targeted RNA-seq using whole blood and FFPE samples spanning a range of RNA inputs and qualities. Key performance metrics included turnaround time, rRNA and globin depletion efficiency, library complexity, the number of genes detected, and expression profile reproducibility. This case study summarizes the results of that evaluation and highlights the performance of whole transcriptome sequencing using the Watchmaker workflow with clinically relevant sample types. Notably, the Watchmaker workflow reduced turnaround time from 16 hours down to just 5 hours, while enhancing data quality, increasing data yield, and demonstrating high reproducibility.

EXPERIMENTAL DESIGN, MATERIALS, AND METHODS

The study was conducted in two phases. Phase 1 focused on a direct comparison of the Watchmaker RNA Library Prep Kit with Polaris Depletion and a standard RNA library prep into exome capture workflow. Phase 2 extended this evaluation to assess the robustness of the Watchmaker workflow across FFPE samples spanning a range of RNA input amounts and quality levels.

Phase 1: Workflow comparison across sample types

Four sample types were used in Phase 1: Universal Human Reference RNA (UHRR), Horizon Discovery (HD200) reference FFPE material, FFPE tissue samples (FFPE), and whole blood samples (WB). UHRR and HD200 are commercially available reference materials. RNA from two unique FFPE and two unique whole blood samples were used. These sample types were selected to represent a range of transcriptome diversity and RNA qualities commonly encountered in clinical specimens. An RNA input amount of 100 ng was used to reflect moderate input amounts typical of clinical and translational studies.

RNA libraries were prepared in duplicate using either a targeted RNA-seq workflow or the Watchmaker workflow (Figure 1). Library QC was performed and sequencing pools were prepared using CellCarta's existing, validated protocols. Paired-end (2 x 100 bp) sequencing was performed using an Illumina® NovaSeq™ 6000 instrument. Sequencing performance metrics evaluated in Phase 1 included duplicate read rates, uniquely mapped read percentages, rRNA and globin depletion efficiency, number of genes detected (cutoff of TPM >1), and gene expression concordance.

Phase 2: Assessment of Watchmaker workflow with a range of FFPE samples

In Phase 2, a high-quality FFPE RNA sample from Phase 1 (DV200 approximately 74%) was heat treated to fragment the RNA and generate a contrived low-quality FFPE sample (DV200 approximately 36%).

Both the high- and contrived low-quality FFPE samples were evaluated using the Watchmaker workflow with 20 ng, 50 ng, and 100 ng inputs. Analyses focused on rRNA depletion efficiency, gene detection sensitivity, and reproducibility of gene expression profiles across RNA quality and input levels.

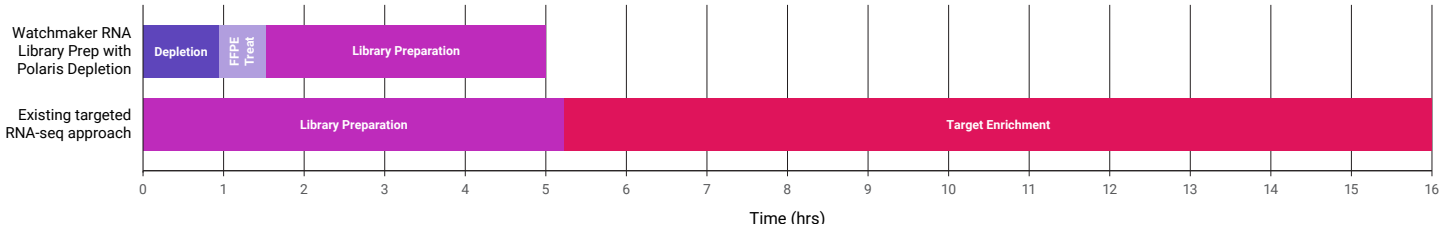

Figure 1. Comparison of RNA-seq library preparation processing times. The streamlined Watchmaker protocol (top) saves approximately 11 hours of processing time compared to the targeted RNA-seq workflow (bottom). Additionally, the Watchmaker workflow includes an optional FFPE treatment step to reverse residual crosslinks and improve RNA recovery.

RESULTS

Phase 1: Comparison of sequencing performance across workflows

Libraries prepared using the Watchmaker workflow showed substantially reduced duplication rates relative to the targeted RNA-seq method, with duplication levels below 30% across all sample types evaluated (Figure 2A). Further, the proportion of uniquely mapped reads was higher in Watchmaker libraries for all sample types (Figure 2B). Watchmaker's workflow also consistently reduced the percentage of reads aligning to rRNA and globin mRNA, with content below 3% across samples (Figure 2C). Together, these findings indicate the Watchmaker workflow delivers improved library complexity and more efficient utilization of sequencing reads, with a greater proportion of data contributing to biologically informative signal.

The combined effects of reduced duplication and improved depletion translated into broader transcriptome coverage. Across all sample types, libraries prepared using the Watchmaker workflow detected a greater number of unique genes (Figure 3A). This increase was driven by improved detection of both protein-coding genes and long non-coding RNAs (lncRNAs), reflecting transcriptome-wide coverage beyond exome-targeted regions. Gene expression profiles showed strong concordance across replicates, demonstrating high reproducibility using the Watchmaker workflow (Figure 3B).

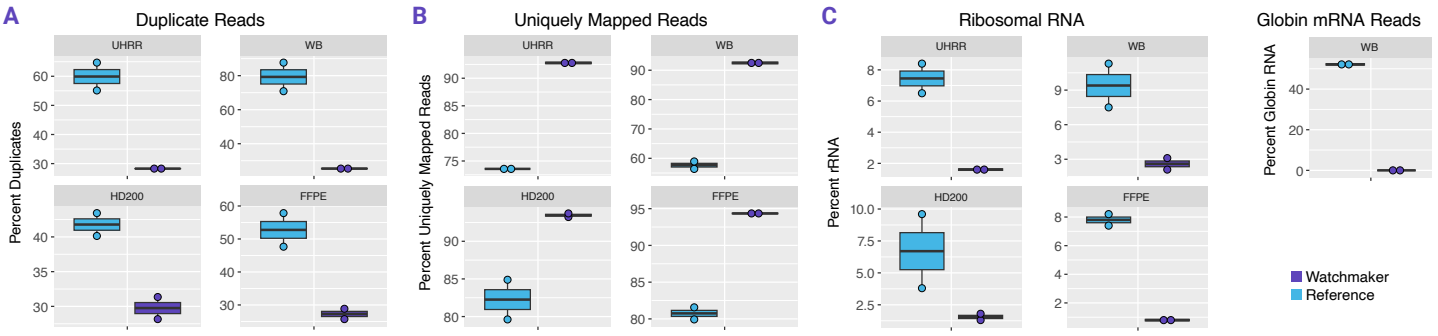

Figure 2. Key library construction metrics evaluated in Phase 1. Libraries prepared using the Watchmaker and targeted RNA-seq (reference) workflows were assessed for **(A)** percent duplication, **(B)** percent uniquely mapped reads, and **(C)** residual ribosomal RNA and globin mRNA reads. Across all sample types, including FFPE and whole blood (WB), Watchmaker libraries exhibited lower duplication, higher unique mapping, and more effective depletion of abundant non-informative transcripts (rRNA and globin mRNA).

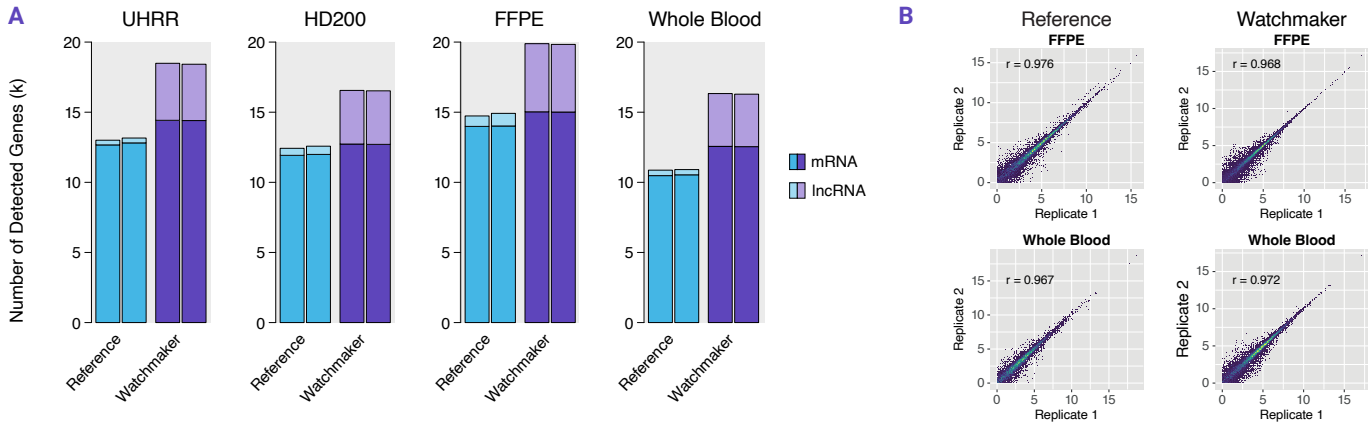

Figure 3. Gene detection and reproducibility in Phase 1. (A) Libraries prepared using the Watchmaker workflow detected a greater number of unique genes across sample types compared with targeted RNA-seq (reference), including increased recovery of protein-coding genes and detection of lncRNAs. **(B)** High correlation of gene expression profiles (log_2TPM, TPM >1) across FFPE and whole blood (WB) replicates prepared using the Watchmaker workflow, demonstrating high reproducibility for each sample type. The reference method is shown for comparison.

Phase 2: Performance with challenging FFPE samples

When the Watchmaker workflow was further evaluated using high-quality FFPE samples across a range of RNA input amounts, performance remained robust. rRNA depletion efficiency and gene detection were maintained across all input amounts evaluated (Figure 4A – B), with strong concordance observed in gene expression profiles between inputs (Figure 5A).

With contrived low-quality samples, increasing RNA input into library prep effectively overcame the challenges associated with RNA degradation. Libraries prepared from 50 ng and 100 ng inputs achieved depletion efficiency and gene detection comparable to high-quality FFPE samples (Figure 4A – B). Low-quality samples prepared with 20 ng input showed a modest reduction in rRNA depletion efficiency and the number of genes detected, but correlation remained strong across all input amounts for low-quality RNA, including 20 ng (r=0.900; Figure 5B). These results demonstrate that biologically meaningful transcriptomic information can still be recovered from low-quality, low-input FFPE samples.

When high- and low-quality samples were compared at matched input amounts, gene expression profiles remained highly concordant (Figure 5C). The high concordance maintained at 20 ng, even under the combined challenge of low RNA quality and reduced input, underscores the consistency of the Watchmaker workflow across clinically relevant conditions. Overall, these results demonstrate that the Watchmaker workflow performs robustly across a wide range of FFPE qualities and input amounts.

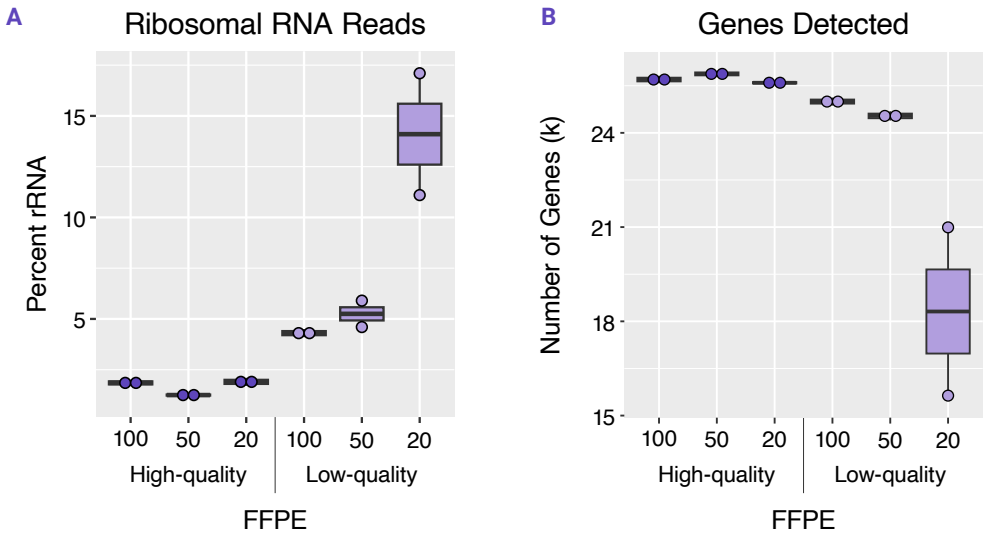

Figure 4. Key library construction metrics in Phase 2 study for libraries prepared from FFPE. Libraries prepared from FFPE samples spanning high and low RNA quality were assessed for **(A)** residual ribosomal reads and **(B)** number of unique genes detected. Results demonstrate robust depletion efficiency and a high number of unique genes detected across clinically relevant FFPE conditions.

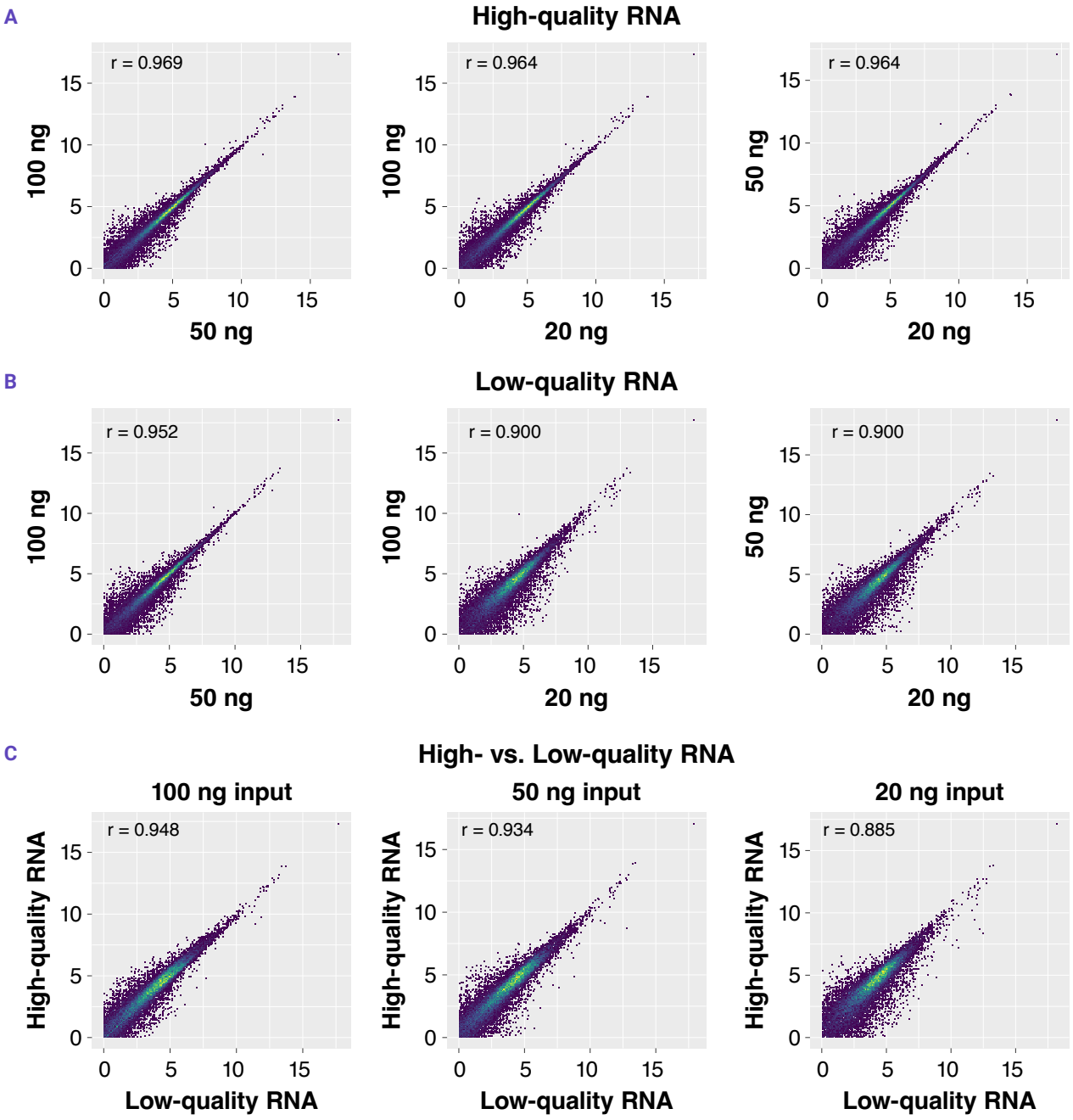

Figure 5. Gene expression reproducibility in Phase 2 across RNA input amounts and quality levels. Correlation of gene expression profiles (log_2TPM, TPM >1) for libraries prepared using the Watchmaker workflow from **(A)** high-quality and **(B)** low-quality FFPE RNA at three input amounts, and **(C)** high- versus low-quality FFPE RNA at matched input amounts. Results demonstrate high concordance of gene expression profiles across clinically relevant FFPE conditions.

CONCLUSION

This two-phase evaluation conducted by CellCarta demonstrates that whole transcriptome sequencing using Watchmaker RNA Library Prep with Polaris Depletion offers a streamlined and compelling alternative to exome capture-based RNA-seq workflows. By reducing turnaround time while improving library complexity and transcriptome coverage, the workflow addresses key performance limitations associated with targeted RNA-seq approaches.

The workflow is compatible with high-quality RNA (from tissues or whole blood) and degraded FFPE tissue, across a broad range of input amounts. Together, these results show that the Watchmaker RNA Library Prep Kit with Polaris Depletion provides a single-day, robust workflow capable of supporting diverse clinical and translational RNA-seq applications while delivering reproducible, biologically informative data.

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