Improving data quality and reproducibility with FFPE-derived RNA using a novel, rapid whole transcriptome library prep workflow

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Introduction

Formalin-fixed, paraffin-embedded (FFPE) tissue provides access to an immense variety of archived disease specimens and their associated donor information—making them an invaluable oncology research tool. While the fixation process preserves the overall tissue structure, it is incredibly damaging to nucleic acids. FFPE-derived RNA is particularly challenging to process as a result of residual cross-linking and template damage. As a result, RNA sequencing remains a challenge with unpredictable and high failure rates. To address these needs, we developed a rapid whole transcriptome library preparation workflow specifically tailored for processing FFPE material.

Methods

We compared the **Watchmaker RNA Library Prep Kit with Polaris Depletion** to three other commercial products (KAPA RNA HyperPrep with RiboErase (HMR) Globin, NEBNext Ultra II Directional RNA Library Prep Kit with Globin & rRNA Depletion and Illumina Stranded Total RNA Prep with Ribo-Zero Plus). RNA was extracted from whole blood and five FFPE blocks, and libraries were prepared in triplicate with inputs ranging from 1 ng to 500 ng (blood) and in duplicate with 100 ng inputs (FFPE). Libraries were sequenced on a NovaSeq 6000 S2 flow cell with 2 x 75 bp read lengths. Data sets were randomly subsampled to 16M paired reads.

Table 1. FFPE RNA DV200 values

Block ID	DV200
A	32%
B	36%
C	47%
D	50%
E	55%

Workflow

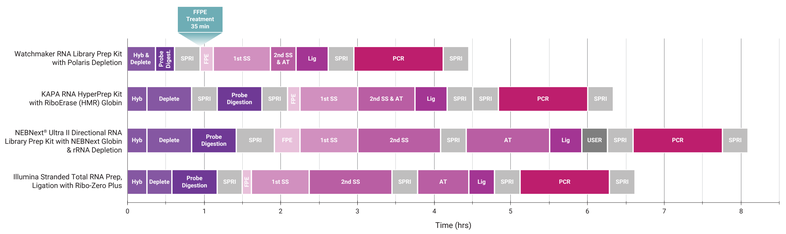

Figure 1. Improved automatability and reduced turnaround time. The Watchmaker solution combines and shortens enzymatic steps and has fewer bead purifications in comparison to other commercially available kits, resulting in a highly automatable workflow with significantly reduced hands-on time (up to one hour per plate) and consumable requirements (up to 1,000 tips per plate). A novel FFPE treatment step facilitates reversing residual cross-links that may persist after RNA extraction for certain extraction methods.

Increased Sensitivity with FFPE

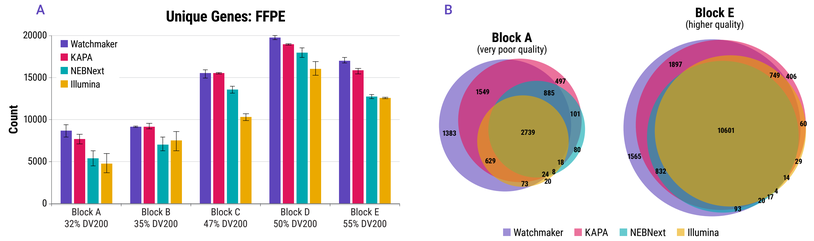

Figure 2. Detect more genes from degraded inputs. Analysis of **(A)** unique genes identified using featureCounts with a cutoff of 5 deduplicated raw reads and **(B)** inter-workflow overlap of unique genes identified, stratified by FFPE block. Only genes identified in both technical replicates were included in the overlap analysis. The Watchmaker solution detects more unique genes across the blocks assessed.

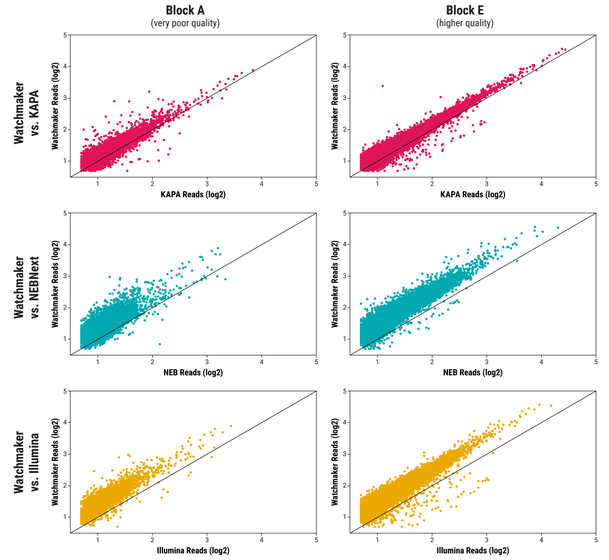

Figure 3. Identify genes with confidence. Per-gene comparison between Watchmaker and each alternative vendor for FFPE blocks A and E. An x = y line is included for visual reference. Data were averaged across technical replicates. Genes plotted above this line have more supporting reads in the Watchmaker libraries, indicating higher library complexity.

Improvements with Low Inputs

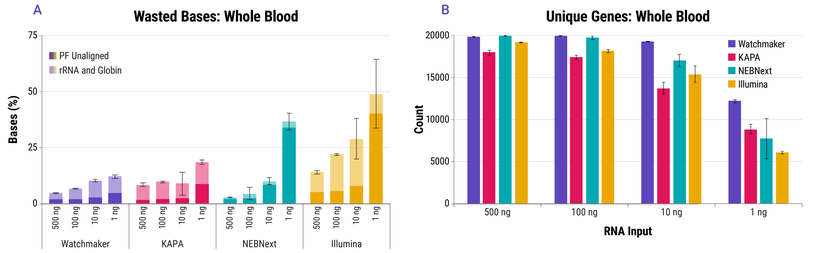

Figure 4. Improved sequencing economy and gene detection. Analysis of **(A)** the percentage of bases wasted due to either failure to align to the reference or aligning to rRNA and globin mRNA, and **(B)** unique genes identified from whole blood samples. A gene was called using featurecounts and a deduplicated raw read cutoff of 5. As a note, the rRNA reference included the 45S ETS and ITS regions. This inflates the measured amount of residual rRNA but also provides a more comprehensive and accurate analysis.

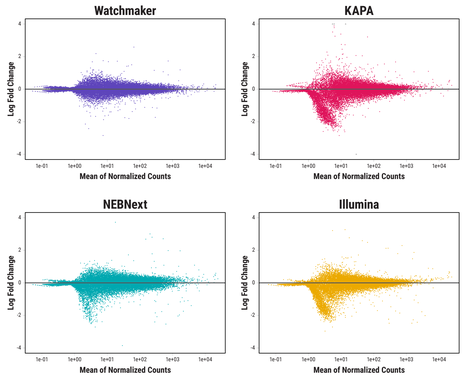

Figure 5. Retain quantitative expression information across input amounts. Differential expression analysis between averaged 500 ng and 10 ng whole blood samples. Results indicate that other vendors lose representation of low abundance genes at 10 ng, while the Watchmaker solution does not.

Conclusions

The **Watchmaker RNA Library Prep Kit with Polaris Depletion** delivers::

- Increased gene detection sensitivity with FFPE-derived RNA
- Accurate quantitative expression profiling across a wide range of input amounts
- A novel, simplified workflow that enables library construction in under 4.5 hours and reduces hands-on and consumable use by 25%