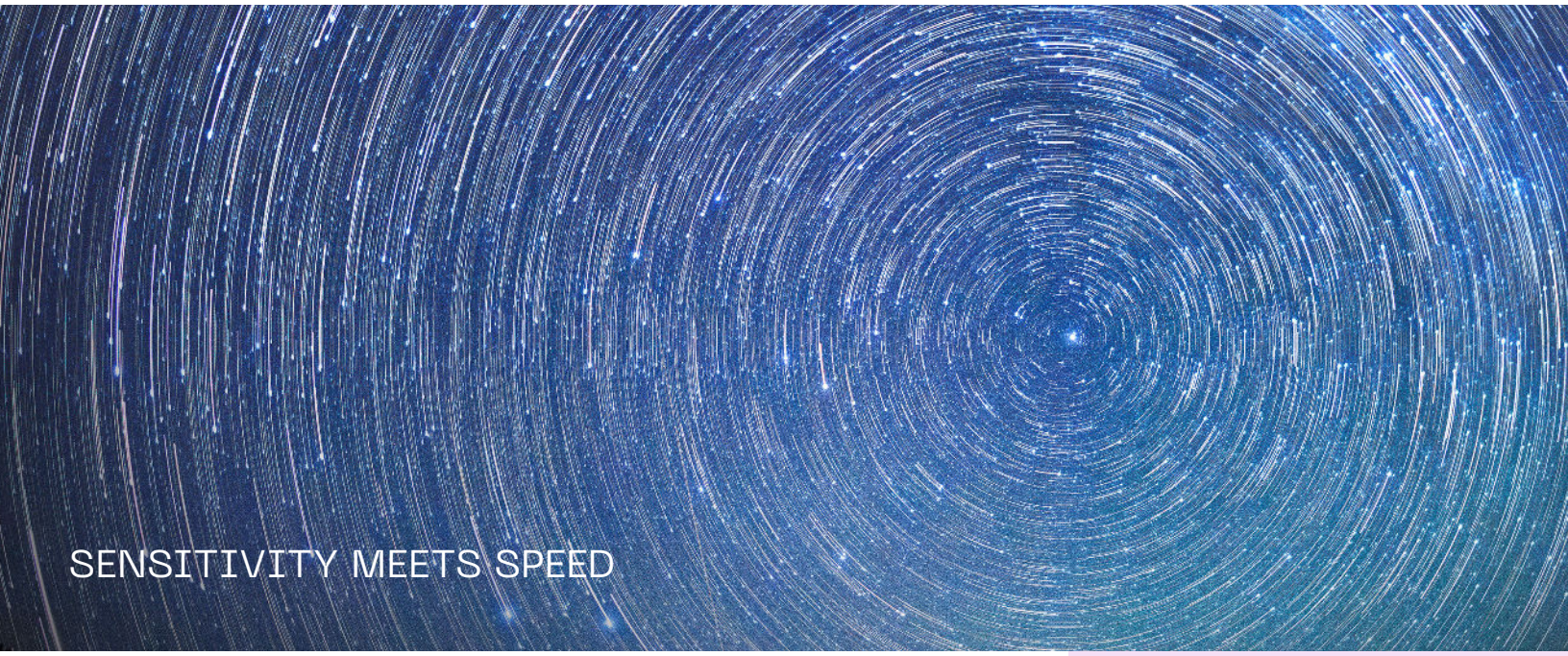
WATCHMAKER GENOMICS

WATCHMAKER RNA LIBRARY PREP KIT WITH POLARIS DEPLETION



Rapidly prepare stranded, whole transcriptome sequencing libraries using **Watchmaker RNA Library Prep Kits with Polaris™ Depletion**. The highly streamlined Polaris Depletion module depletes highly abundant rRNA and globin transcripts in human, mouse, and rat samples, providing improved coverage of biologically interesting transcripts, including long non-coding RNAs.

The automation-friendly workflow reduces total turnaround time, hands-on time, and consumable use through a reduction in bead purification and reaction steps. A novel, engineered reverse transcriptase improves the conversion of RNA to cDNA, enabling high-quality performance with 1 ng to 1000 ng of total RNA, as well as FFPE-derived RNA.

KEY FEATURES & BENEFITS

- Increase sensitivity and **retain quantitative gene expression information** with low-input samples, **down to 1 ng**
- Access more biologically relevant information from **FFPE samples**
- Generate **stranded** libraries in **under 4.5 hours**, including rRNA and globin depletion
- Improve automatability with **fewer purifications and reagent additions**
- Effectively sequence **blood samples** while covering **lncRNAs**

APPLICATIONS

- Gene fusion, isoform, and splice variant detection
- Gene expression analysis
- Single nucleotide variant detection
- Novel transcript discovery
- Hybridization capture
- Detection of long non-coding RNAs (lncRNAs)
- RNA sequencing with degraded samples, such as FFPE

PROCESS MORE SAMPLES EASILY WITH A SIMPLE, RAPID WORKFLOW

With fewer bead cleanups, shortened incubation times, and fewer handling steps, the Watchmaker RNA Library Prep Kit with Polaris Depletion delivers sequencing-ready libraries in under 4.5 hours. The numerous workflow improvements ensure ease of automation and reduce consumable use by up to 1,000 pipette tips per 96 libraries.

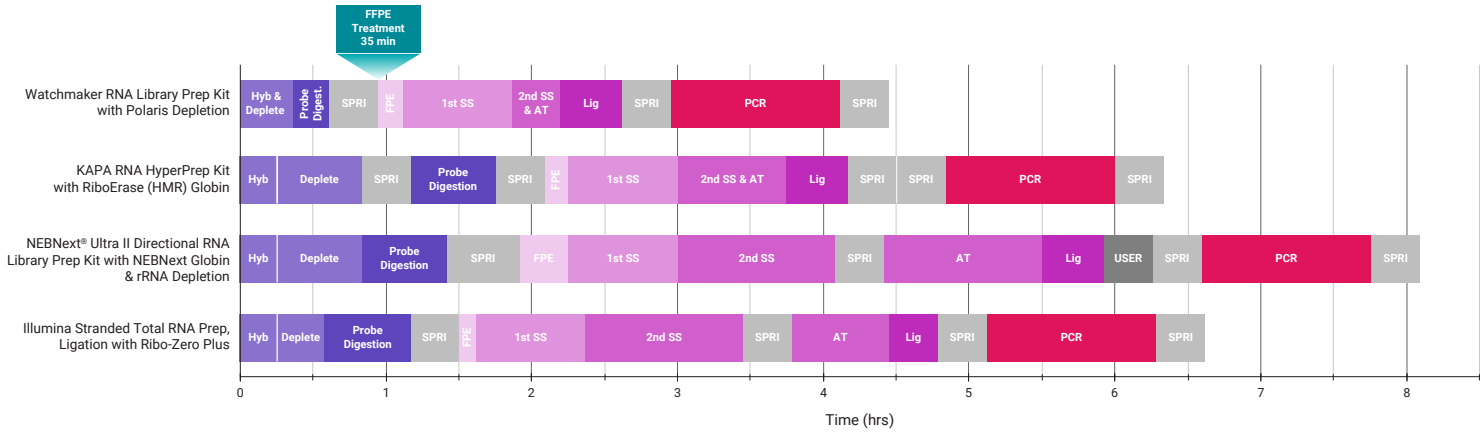


FIGURE 1. Improve automatability and reduce turnaround time. The Watchmaker solution combines and shortens enzymatic steps and has fewer bead purifications in comparison to commercially-available kits, resulting in a highly automatable workflow with significantly reduced hands-on time (up to one hour per plate).

LIBRARY PREPARATION TAILORED FOR FFPE SAMPLES

Processing FFPE samples is inherently challenging due to the template damage incurred during fixation and the presence of residual cross-links. Watchmaker's novel FFPE treatment step, paired with a reverse transcriptase specifically engineered for RNA-seq applications, delivers excellent sensitivity and makes more clinically relevant samples addressable to researchers.

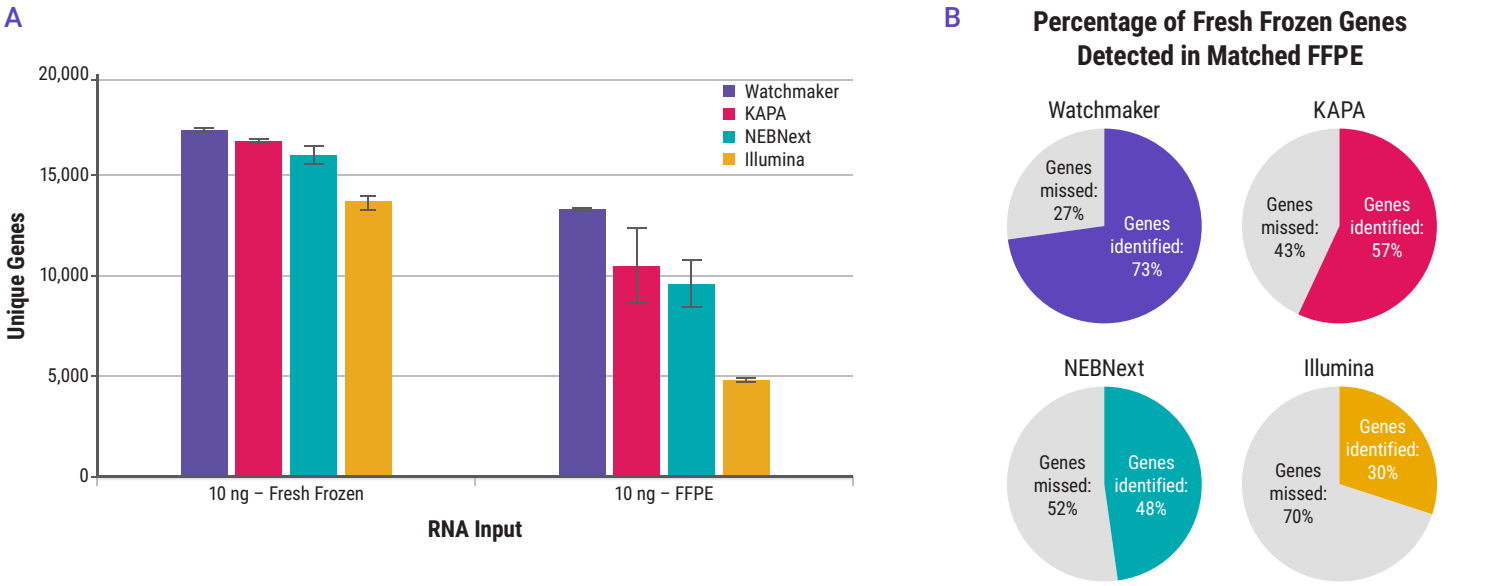


FIGURE 2. Access more information from FFPE. Libraries were prepared in duplicate from 10 ng of a matched fresh frozen and FFPE sample set using the workflows listed in Figure 1. **(A)** Watchmaker detects more unique genes for both the fresh frozen and FFPE samples. **(B)** In a gene detection overlap analysis between the fresh frozen and FFPE data sets for each chemistry, Watchmaker FFPE libraries detect a significantly higher percentage of the unique genes identified in the fresh frozen control. Data were downsampled to 11M paired reads per library. Unique genes were identified using featureCounts with a cutoff of 5 deduplicated raw reads. Only genes identified in both technical replicates were counted.

INCREASE SENSITIVITY WITH ULTRA-LOW-INPUT SAMPLES

Robust and reproducible sample processing can be difficult for applications and samples where RNA quantities are restrictive, such as fine needle biopsies, limiting the sensitivity of associated assays. Watchmaker RNA Library Prep Kits with Polaris Depletion enable the generation of high-quality libraries with as little as 1 ng of RNA.

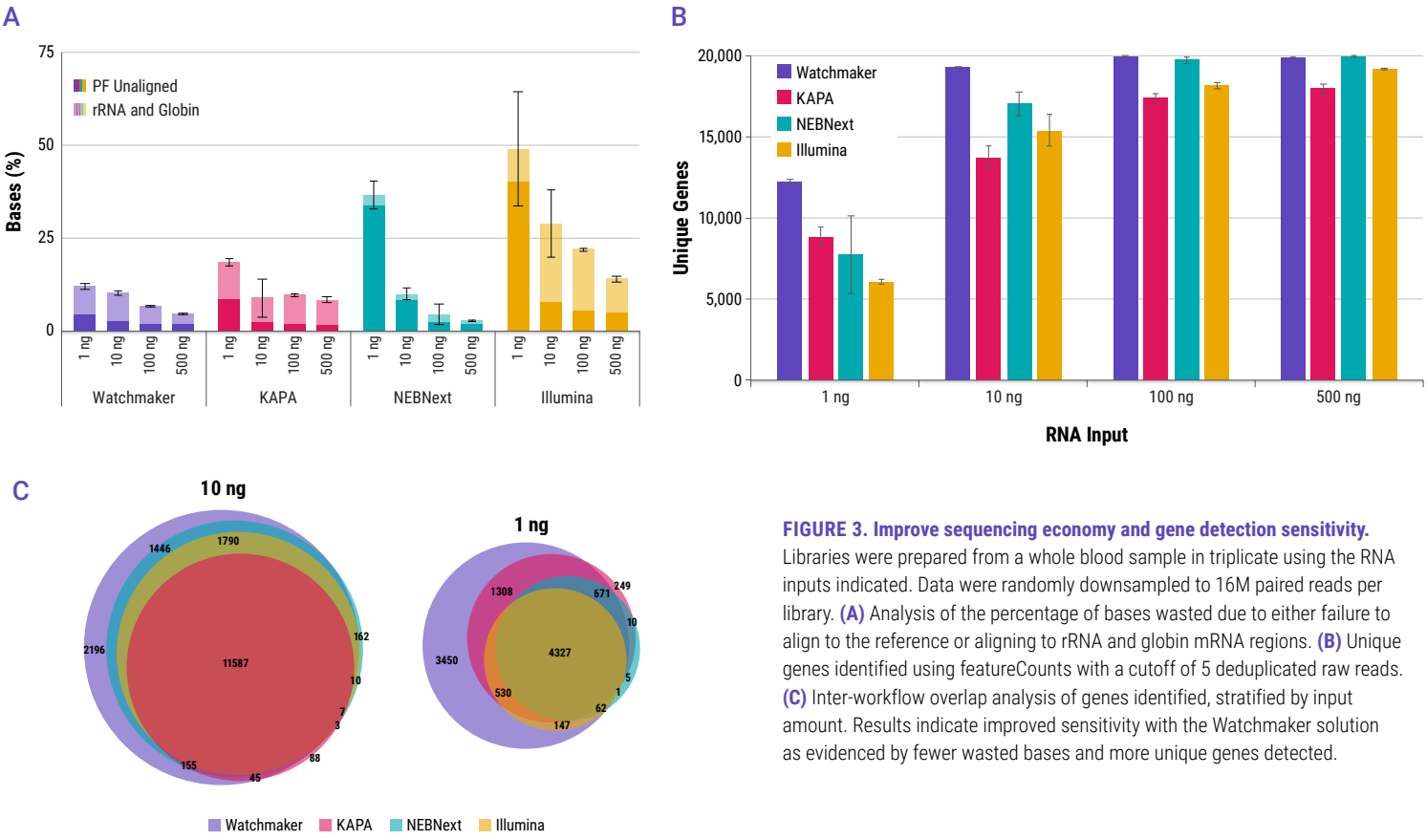


FIGURE 3. Improve sequencing economy and gene detection sensitivity. Libraries were prepared from a whole blood sample in triplicate using the RNA inputs indicated. Data were randomly downsampled to 16M paired reads per library. **(A)** Analysis of the percentage of bases wasted due to either failure to align to the reference or aligning to rRNA and globin mRNA regions. **(B)** Unique genes identified using featureCounts with a cutoff of 5 deduplicated raw reads. **(C)** Inter-workflow overlap analysis of genes identified, stratified by input amount. Results indicate improved sensitivity with the Watchmaker solution as evidenced by fewer wasted bases and more unique genes detected.

RETAIN QUANTITATIVE GENE EXPRESSION INFORMATION ACROSS INPUT AMOUNTS

Agreement between high- and low-input libraries with respect to genes identified and their abundance is indicative of how well sample complexity is maintained as RNA input decreases. Watchmaker better preserves the true gene expression profile as RNA input amount decreases from 500 ng to 10 ng, whereas other commercial solutions result in data distortion with low inputs.

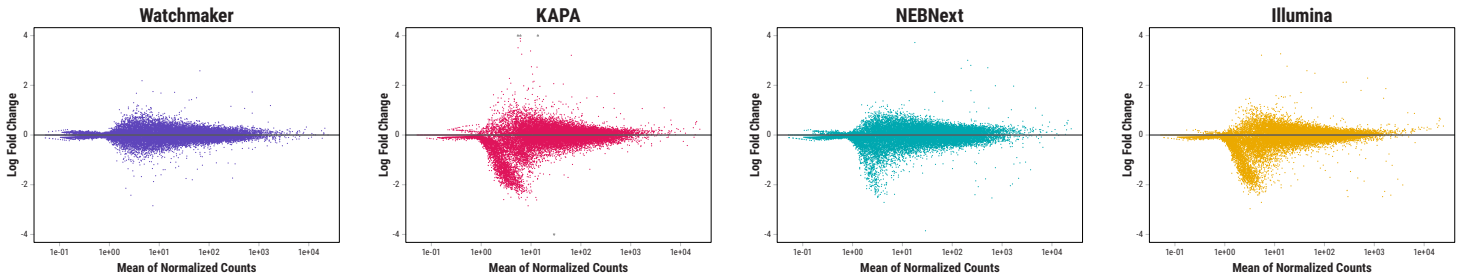


FIGURE 4. Prevent data distortion with low inputs. Differential expression analysis between averaged 500 ng (control) and 10 ng whole blood samples using DESeq2. Results indicate that other vendors lose representation of low abundance genes at 10 ng, while the Watchmaker solution does not.

EFFICIENTLY SEQUENCE BLOOD SAMPLES WHILE COVERING lncRNAs

Overabundant globin mRNAs in blood samples pose a challenge for efficient RNA sequencing, as ≥30% of bases map to these transcripts if traditional mRNA capture is used. Additionally, mRNA capture omits a significant subset of long non-coding RNAs (lncRNAs) that have demonstrated regulatory functions.[1] Polaris Depletion removes both rRNA and globin mRNA while also providing excellent coverage of lncRNAs, such as MALAT1, which has been implicated in at least 17 cancer types, including lung, breast, and pancreatic.[2]

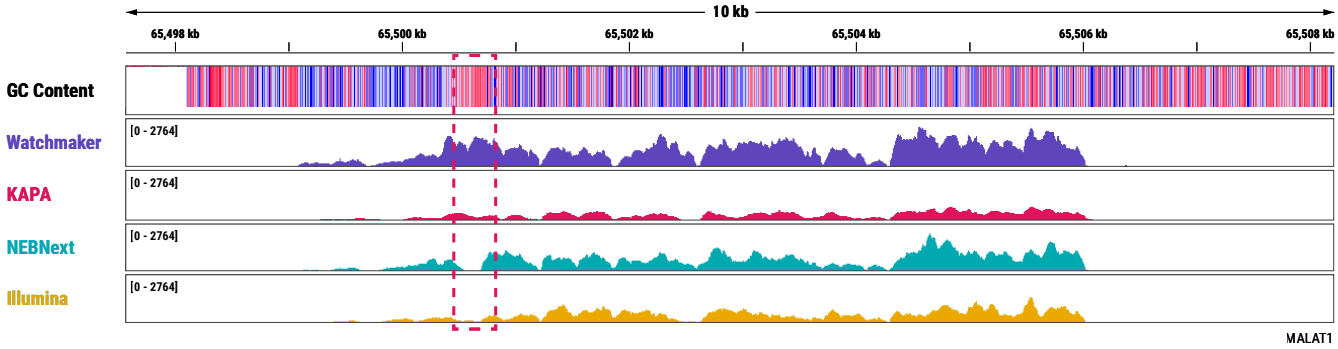


FIGURE 5. Improve efficiency with whole blood and characterize lncRNAs. Polaris Depletion improves both library prep and sequencing efficiencies with whole blood through the combined removal of rRNA and globin mRNA. This focuses sequencing reads on what matters: informative exonic and lncRNAs. The Watchmaker solution provides improved coverage across MALAT1, especially the GC-rich region outlined in red. Libraries were prepared from 10 ng of whole blood-derived RNA.

[1]Statello, L., Guo, CJ., Chen, LL., et al. Gene regulation by long non-coding RNAs and its biological functions. *Nat Rev Mol Cell Biol* 22, 96 – 118 (2021).
[2]Amodio, N., Raimondi, L., Juli, G., et al. MALAT1: a druggable long non-coding RNA for targeted anti-cancer approaches. *J Hematol Oncol* 11, 63 (2018).

Contact sales@watchmakergenomics.com
or visit watchmakergenomics.com/RNA-with-polaris to learn more.

PRODUCT	24 rxn	96 rxn
Watchmaker RNA Library Prep Kit with Polaris Depletion *Incl. reagents for rRNA and globin depletion, RNA library prep, and amplification*	7BK0002-024	7BK0002-096

M165 | WM109 | v3.0.0924