

High quality whole transcriptome RNA-sequencing from challenging clinical samples

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INTRODUCTION

RNA sequencing (RNA-seq) of clinical specimens poses several challenges due to limited sample material, RNA fragmentation, and high fractions of ribosomal RNA (rRNA) and globin RNA. These factors often result in elevated PCR duplication rates and reduced library complexity, compromising transcriptome data quality and downstream analyses.

METHODS

We evaluated the Watchmaker Genomics RNA Library Prep with Polaris Depletion workflow and compared its performance to a standard RNA-seq and exome capture workflow, further referred to as the reference method. Performance was assessed across a range of sample types, including reference materials (universal human reference RNA – UHRR, Horizon Discovery HD200 FFPE reference standard), whole blood (WB), and tumor FFPE samples with varying RNA integrity.

RESULTS

Reproducible Reduction in PCR duplication rates and improved mapping rates

PCR duplication rates were approximately twofold lower with the Polaris Depletion workflow (30.2%) versus the reference method (62.5%).

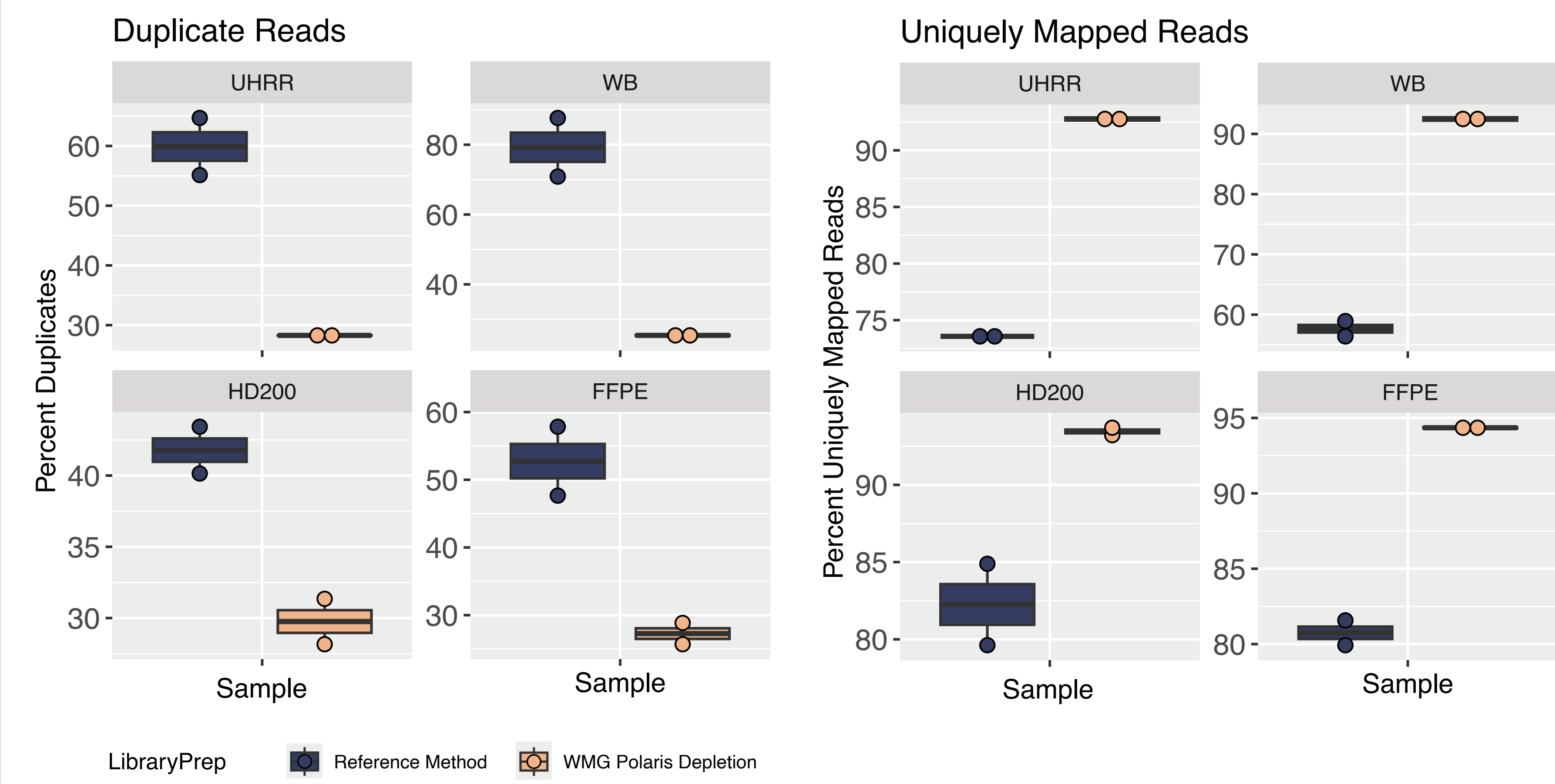


Figure 1: Percent duplicates and percent uniquely mapped reads for 4 different sample types (universal human reference RNA (UHRR), whole blood (WB), HD200 and tumor FFPE) processed in duplicate with the reference method and the Polaris Depletion workflow.

Efficient depletion of rRNA and globin RNA and higher gene detection

The Polaris Depletion workflow achieved efficient depletion of both rRNA (< 5%) and globin RNA (< 1% in whole blood), compared to > 5% rRNA and > 50% globin RNA observed with the reference method (Figure 2A). This was accompanied by a substantial increase in the number of detected genes (Figure 2B).

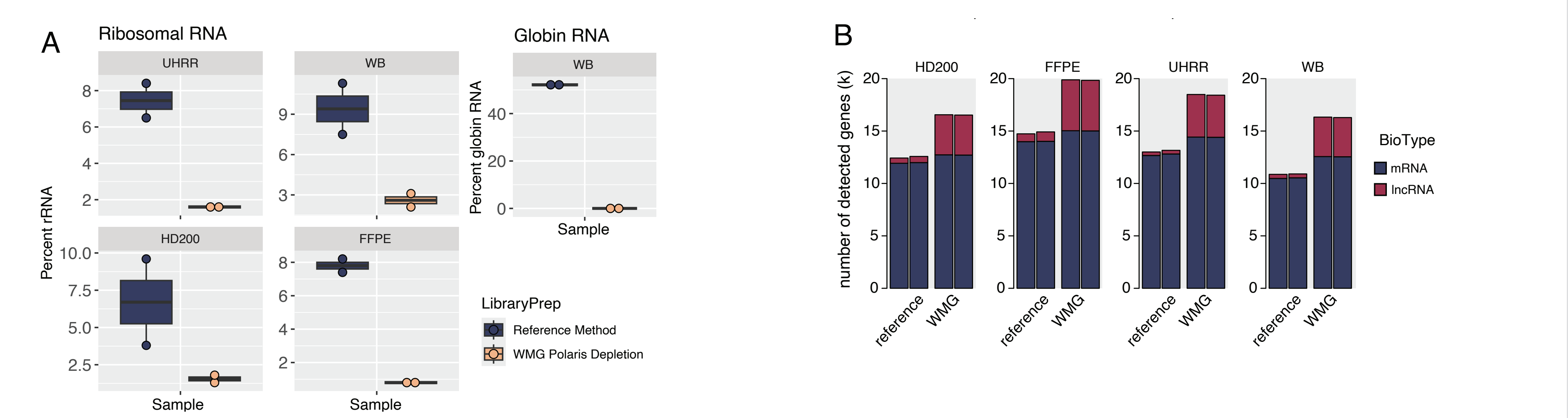


Figure 2: (A) Percent rRNA and globin RNA in samples processed with the reference method and the Polaris Depletion workflow. (B) Number of detected genes in samples processed with the reference method and the Polaris Depletion workflow.

Reproducible gene expression quantification

Both the Polaris Depletion workflow and the reference method generate highly reproducible gene expression data in both FFPE and whole blood samples.

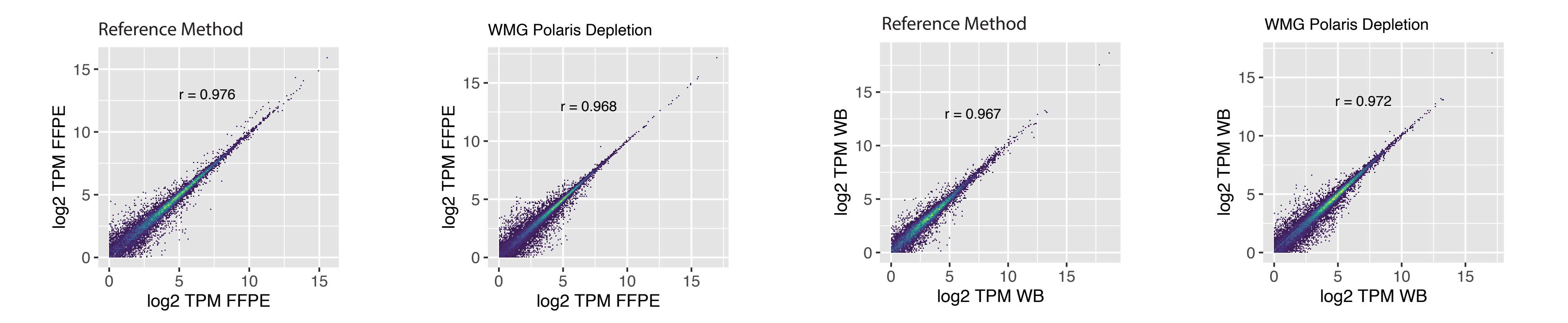


Figure 3: Gene expression correlation plots (log2 TPM) between replicate FFPE and WB samples processed with either the reference method or the Polaris Depletion workflow. Only genes with a TPM > 1 in both replicates are included.

Robust performance across samples with varying RNA quality

The Polaris Depletion workflow demonstrated robust performance across samples of differing RNA quality, provided sufficient RNA input was used for library preparation (Figure 4). For low quality FFPE RNA samples, at least 50 ng of input RNA was required to obtain reproducible gene expression data.

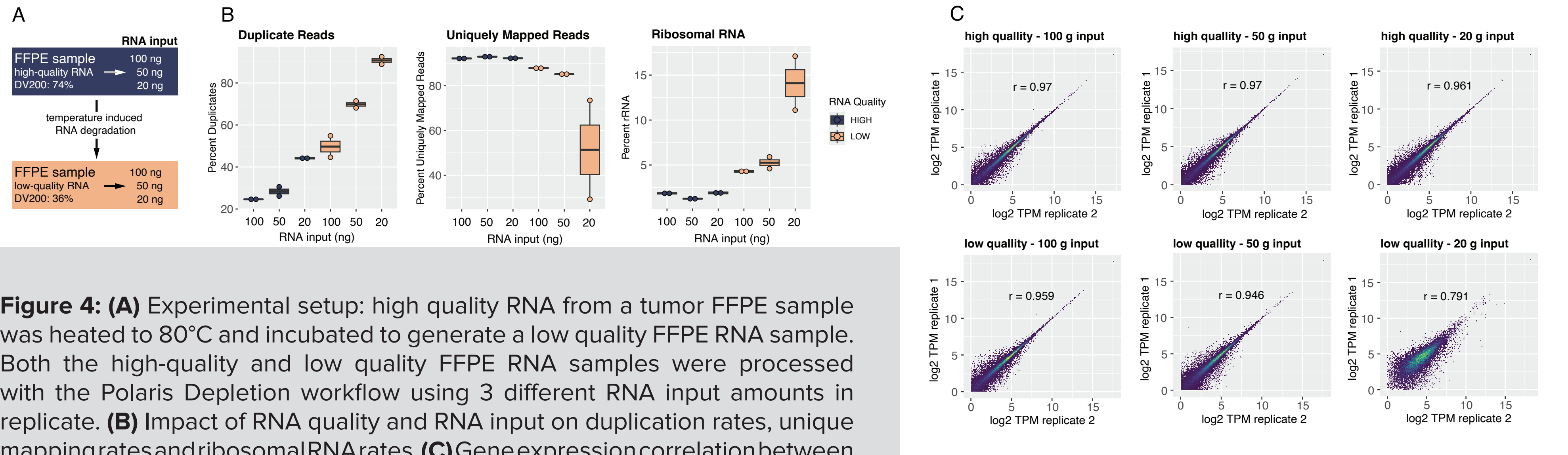


Figure 4: (A) Experimental setup: high quality RNA from a tumor FFPE sample was heated to 80°C and incubated to generate a low quality FFPE RNA sample. Both the high-quality and low quality FFPE RNA samples were processed with the Polaris Depletion workflow using 3 different RNA input amounts in replicate. (B) Impact of RNA quality and RNA input on duplication rates, unique mapping rates and ribosomal RNA rates. (C) Gene expression correlation between replicates for each RNA quality-RNA input combination

High gene expression reproducibility was observed between different input conditions and between high and low quality RNA from the same sample (Figure 5).

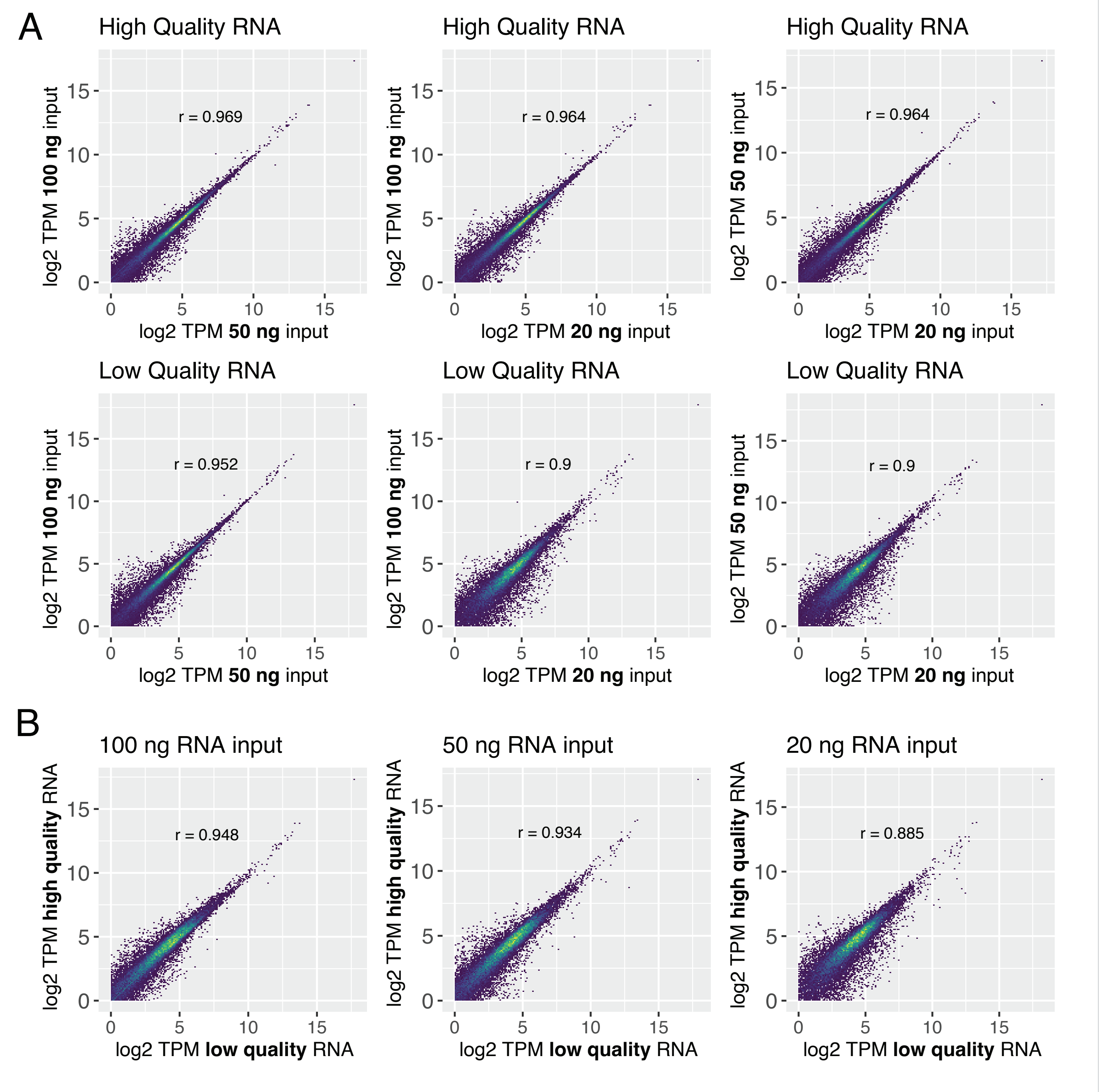


Figure 5: (A) Gene expression correlation between different RNA input amounts of the same high or low quality RNA sample. High quality RNA displays high correlation between all RNA input conditions whereas for low quality RNA, correlations with the 20 ng condition are lower. (B) Gene expression correlation between different RNA quality levels for the different input conditions. High and low quality RNA show higher correlation with higher RNA input amounts.

CONCLUSION

The Watchmaker Genomics RNA Library Prep with Polaris Depletion workflow enables the generation of high-quality RNA-seq libraries with efficient rRNA and globin depletion, reduced PCR duplication, and reliable quantification of both coding and non-coding transcripts. These results demonstrate that RNA sequencing of challenging clinical samples can be effectively performed without the need for costly and time-intensive exome capture, thereby improving both sensitivity and workflow efficiency.

Send inquiries to isales@cellcarta.com with AACR 2026 RNA-sequencing in email subject.