

High Quality Whole Transcriptome RNA-Sequencing from Challenging Clinical Samples

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INTRODUCTION

In clinical studies, RNA sequencing (RNA-seq) of tumor biopsies and whole blood samples is widely used to elucidate drug mechanisms, distinguish between responders and non-responders, and investigate potential adverse effects. However, RNA-seq from clinical specimens presents several challenges, including limited input material, fragmented RNA, and high levels of ribosomal (rRNA) or globin RNA. These issues often result in high PCR duplication rates and reduced library complexity, which can compromise data quality and downstream analyses.

We evaluated three RNA-seq workflows developed by WatchMaker Genomics:

1. RNA library preparation with Polaris Depletion
2. RNA library preparation with exome capture
3. RNA library preparation with Polaris Depletion combined with exome capture

These workflows were applied to formalin-fixed, paraffin-embedded (FFPE) and whole blood samples to assess performance in challenging clinical contexts and compared to a reference method.

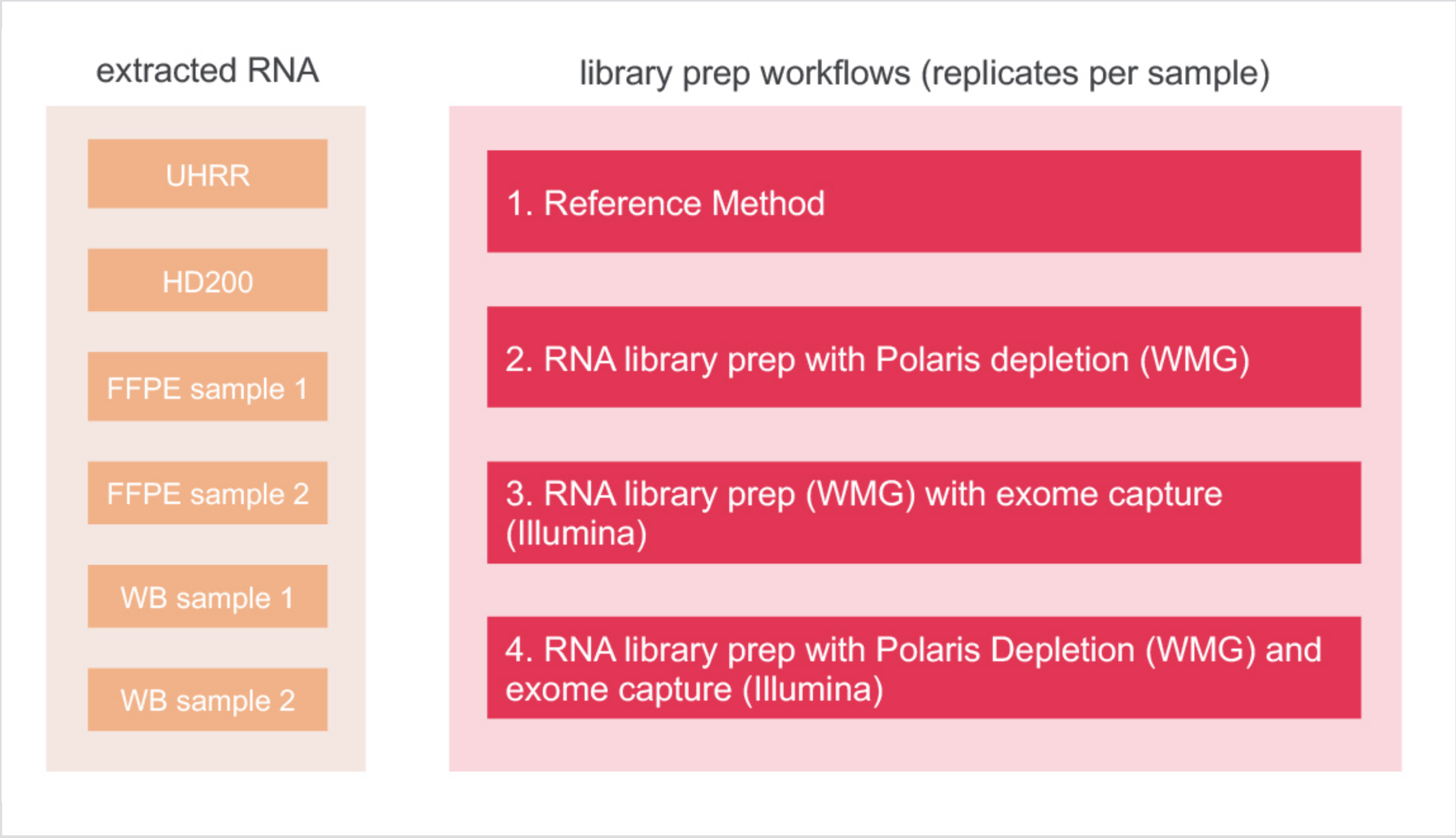


Figure 1. Overview of methods. This figure depicts the experimental design including sample types used and workflows executed.

RESULTS

QC metrics

Workflows incorporating the Polaris Depletion step generated high-quality libraries characterized by:

- Low PCR duplication rates
- High Unique mapping rates

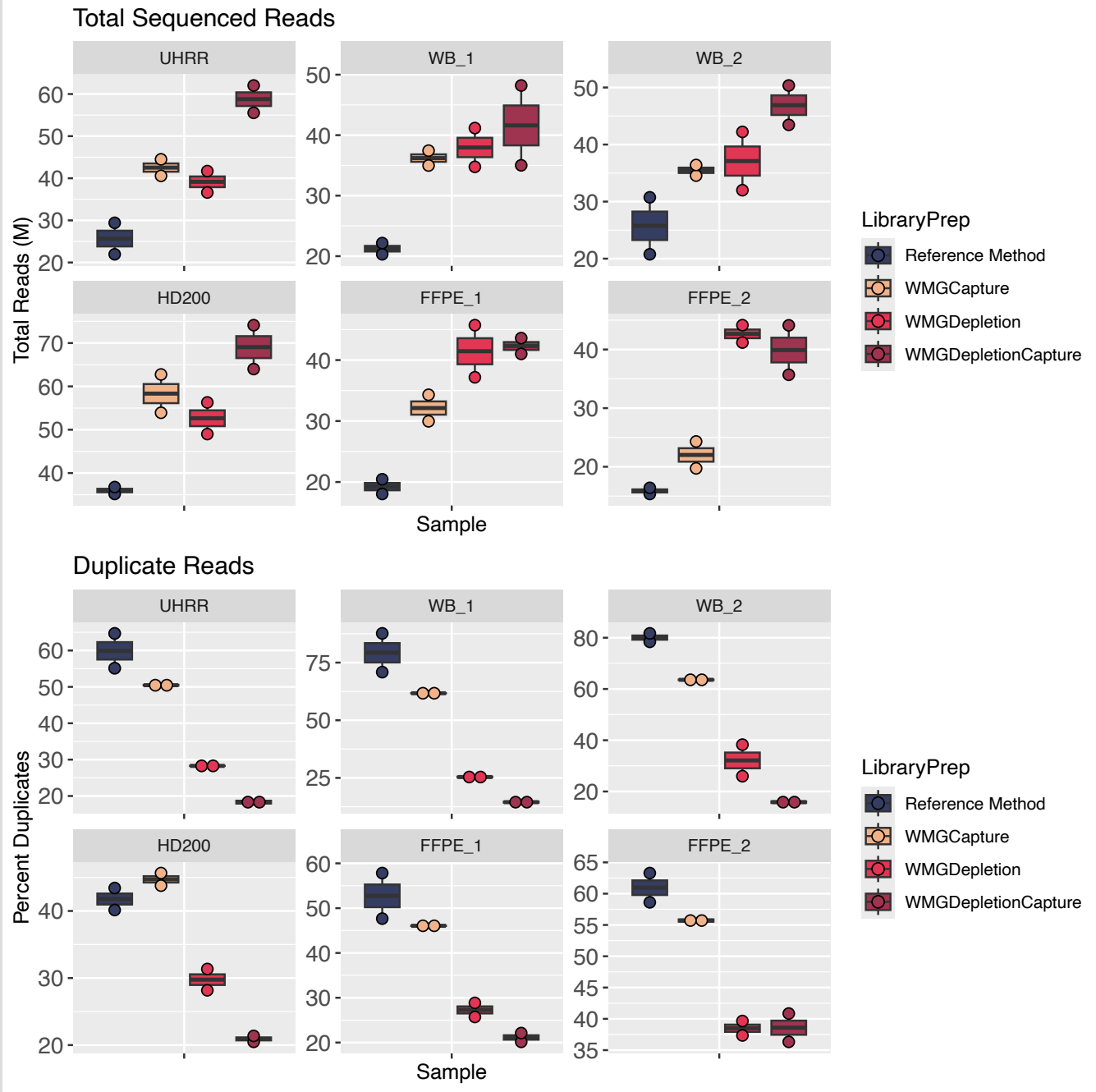


Figure 2a. Total Reads and Duplicate Levels. This figure shows low duplication rates for experimental workflows incorporating the Polaris Depletion step.

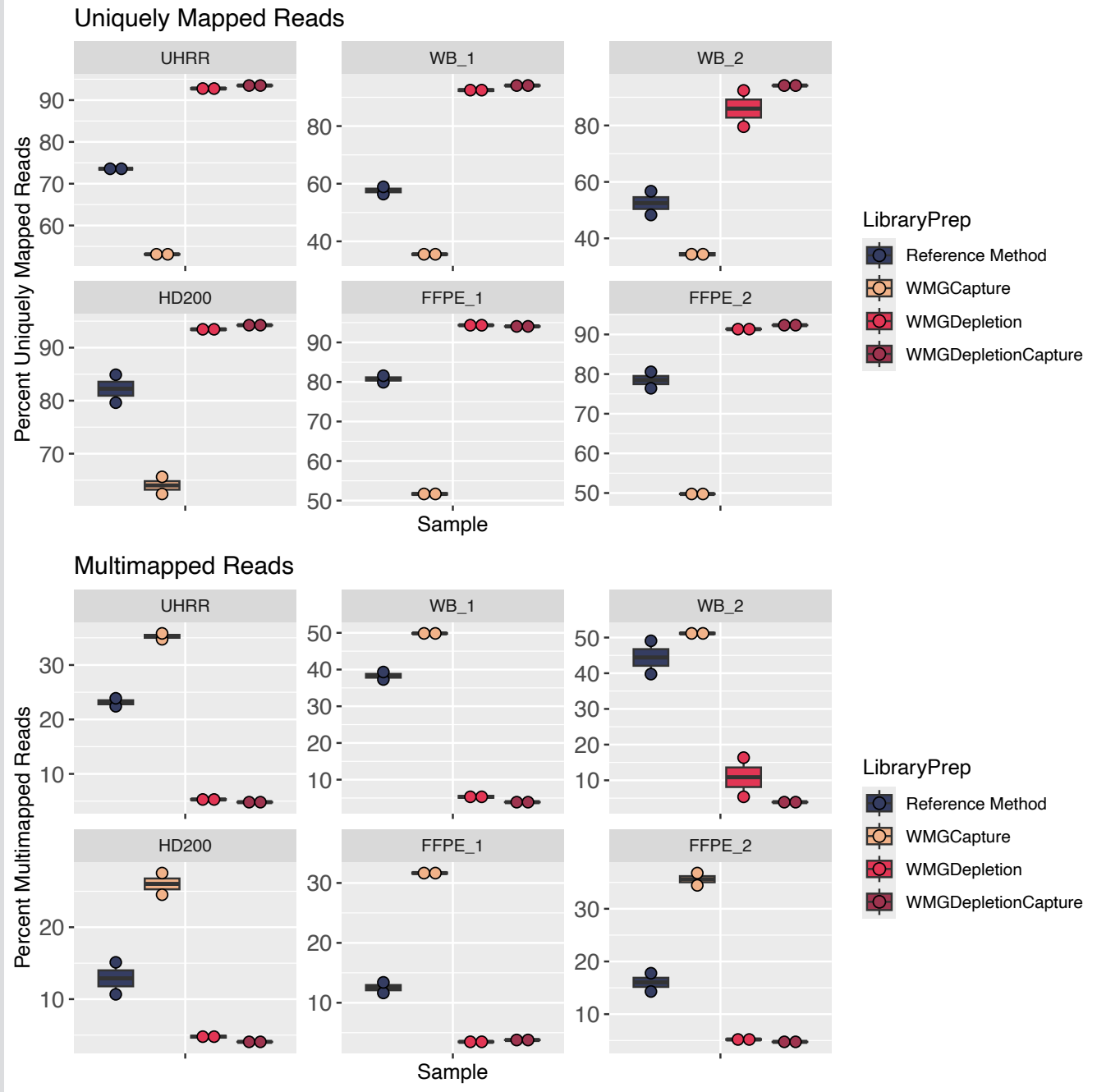


Figure 2b. Mapping Rates. This figure shows high unique mapping rates for experimental workflows incorporating the Polaris Depletion step.

- Efficient depletion of rRNA and globin RNA for whole blood samples

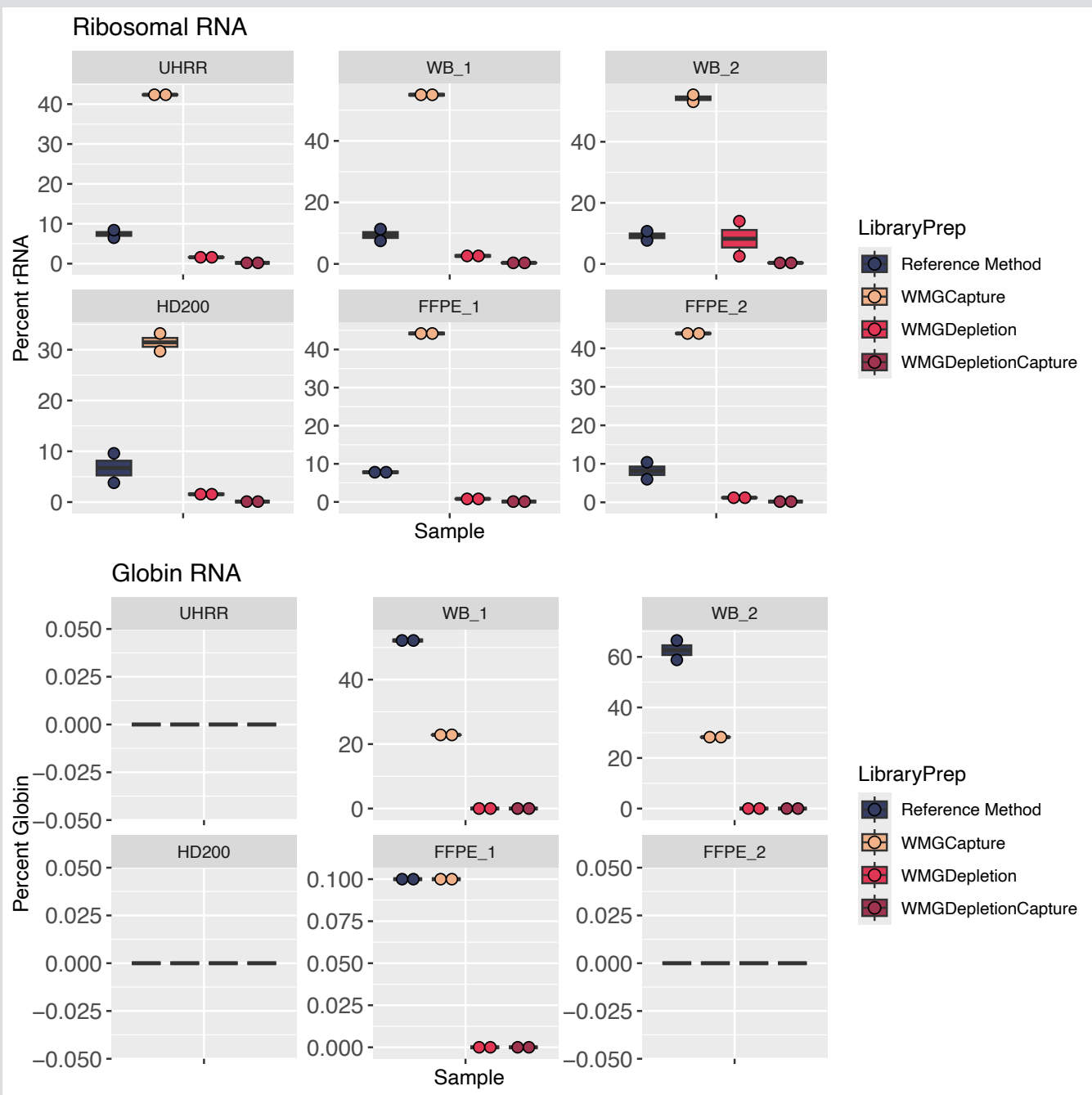


Figure 3. rRNA and Globin detection. This figure shows the efficient depletion of rRNA for all sample types and globin RNA for whole blood samples for experimental workflows incorporating the Polaris Depletion step.

- Higher Gene detection with a small increase in protein coding genes and more significant increase in lncRNA's using subsampled reads and a TPM cutoff of 1.

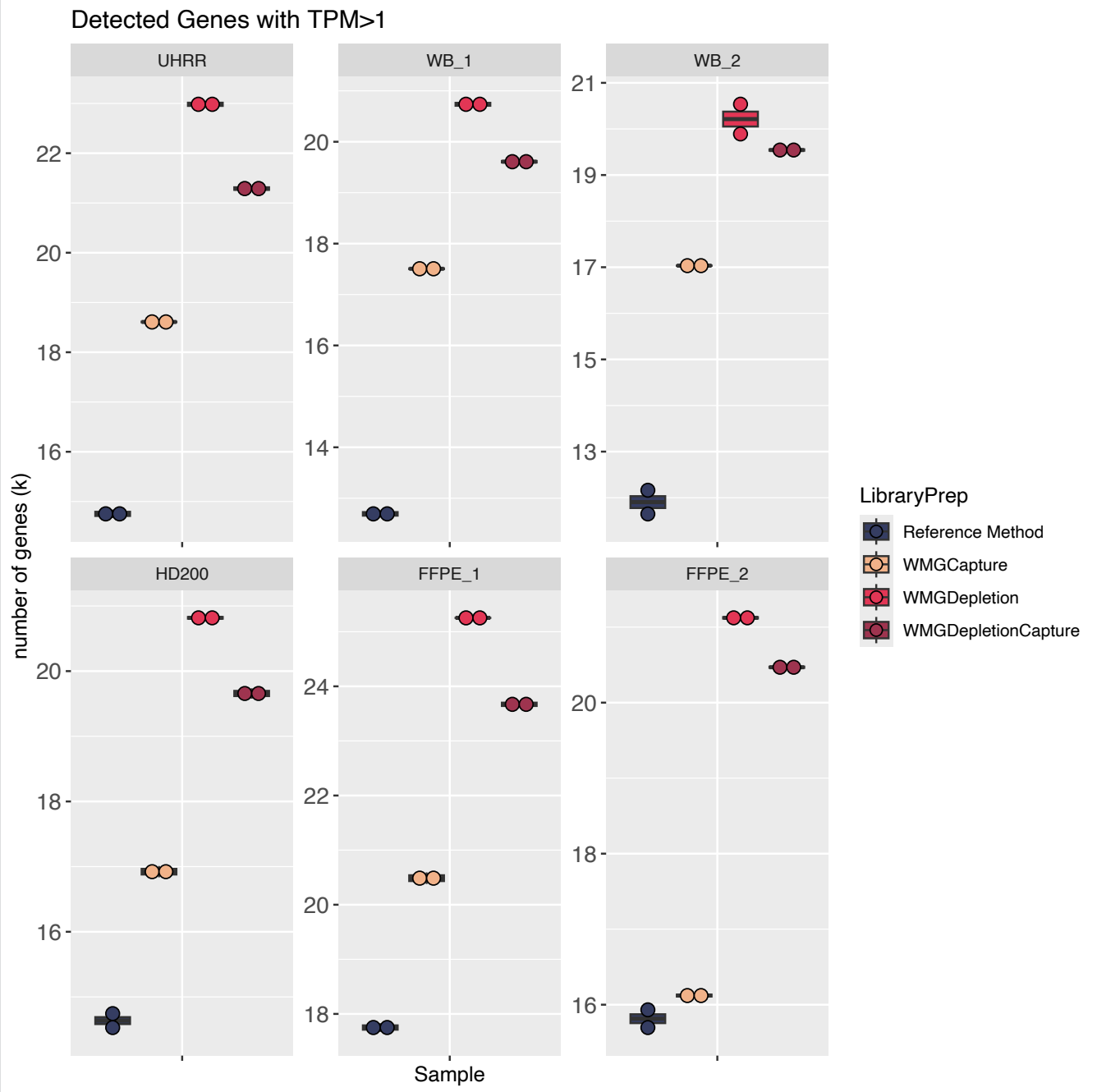


Figure 4a. Gene Detection. This figure shows higher gene detection for experimental workflows incorporating the Polaris Depletion step.

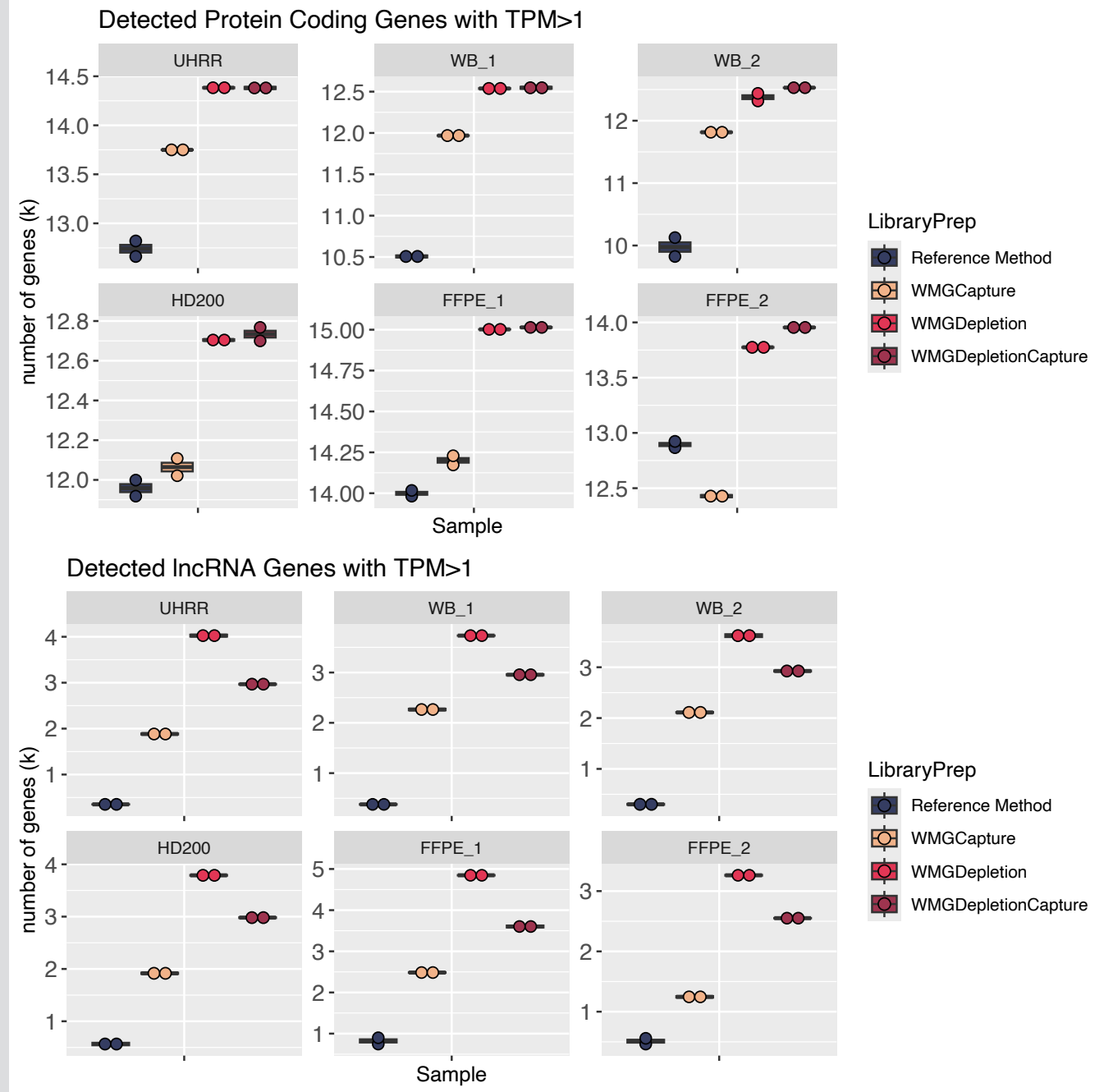


Figure 4b. Gene Detection Protein Coding Genes VS lncRNA's. This figure shows higher gene detection is primarily caused by a higher detection of lncRNA's.

Reproducibility

- All sample replicates show good correlation in expression levels amongst each other.

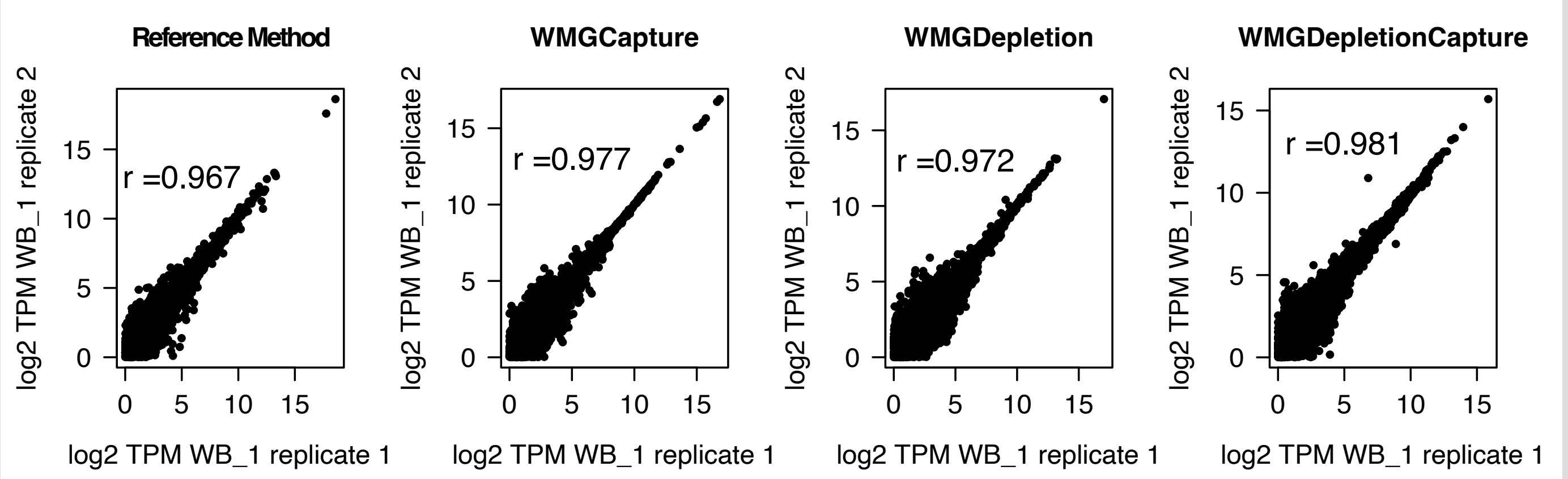


Figure 5a. Reproducibility Whole Blood and UHRR. This figure shows correlation in expression levels between replicates for a Whole Blood sample.

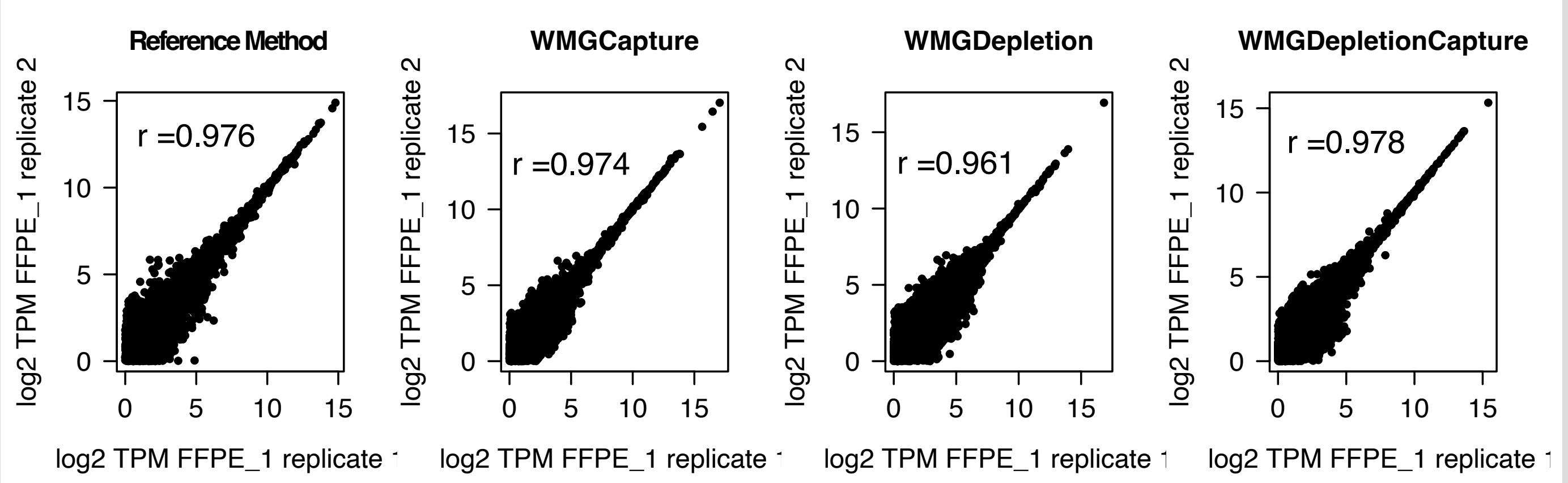


Figure 5b. Reproducibility FFPE. This figure shows correlation in expression levels between replicates for an FFPE sample.

- All three tested methodologies show good correlation regarding the reference method.

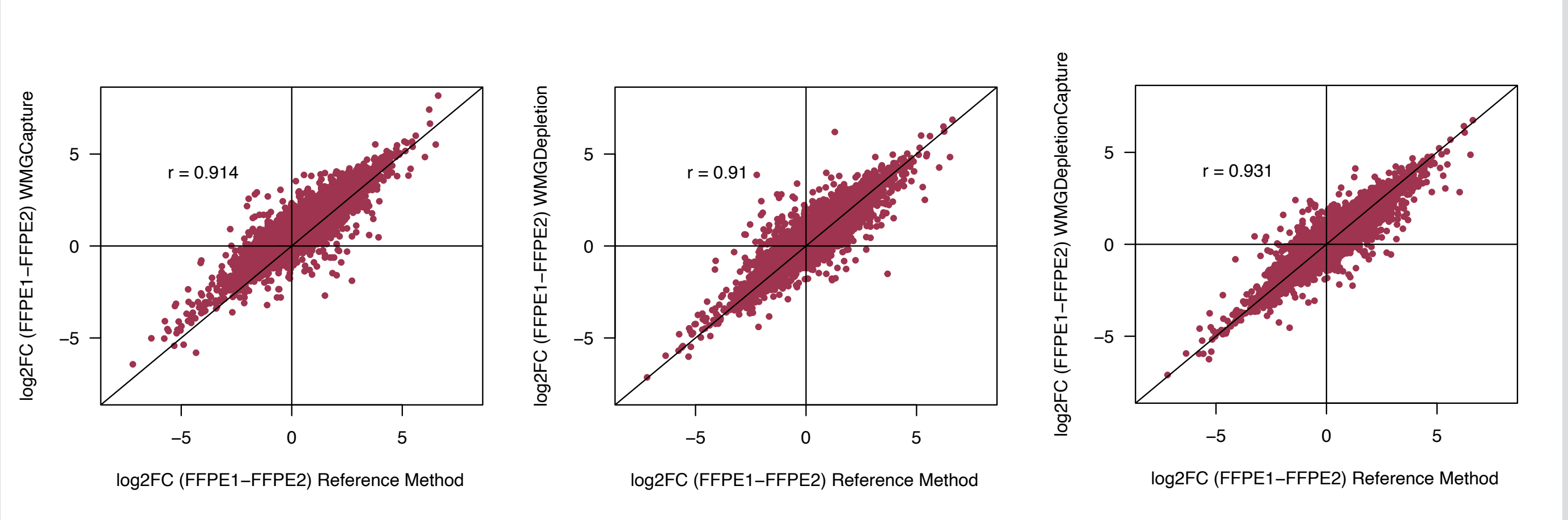


Figure 6. Fold Change Correlation to Reference method. This figure shows correlation in expression levels between experimental workflows and the reference method.

This performance was consistent across both FFPE and whole blood sample types. These workflows enabled sensitive and robust quantification of gene expression for both coding and non-coding RNAs.

DISCUSSION

The data demonstrate that Polaris Depletion in combination with the Watchmaker Genomics RNA-seq library prep is highly effective at overcoming common obstacles associated with clinical RNA samples. Importantly, the workflow using Polaris Depletion alone matched the performance of the more complex, capture based workflow. Therefore, exome capture is not necessary to achieve high-quality transcriptome data from challenging samples.

CONCLUSION

High-quality whole transcriptome RNA-sequencing from clinically relevant, degraded, or low-input samples is achievable using whole transcriptome workflows that only include Polaris Depletion without capture. This approach simplifies the workflow, reduces costs, and maintains high data quality—making it a practical and efficient solution for clinical RNA-seq studies.

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