

Precision at any input: High-throughput automated workflow ensures uniform gene expression profiles across broad RNA inputs



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Abstract

This study demonstrates ElevateBio's seamless integration of the Watchmaker mRNA Library Preparation Kit with the Biomek i7 automated liquid handling system and shows that Next Generation Sequencing libraries generated from a broad range of starting RNA input yield highly concordant sequencing results in Universal Human Reference (UHR) samples.

The robust, automated integration system aims to reduce hands-on time and consumable use, supports relaxed input normalization, and minimizes batching constraints to enable efficient high-throughput library preparation.

Utilization of this workflow underscores ElevateBio's ability to support diverse experimental needs and evolving customer workflows, reinforces confidence in platform adoption, and drives sustained customer engagement.

Experimental Design & Methods

Sample	RNA Input (ng)	Replicates	Stubby Adaptor Conc (uM)	Post Lig. SPRI	PCR Cycles	Post PCR SPRI
UHR	10	14	1	0.7X	12	1X
UHR	50	14	1	0.7X	12	1X
UHR	200	14	1	0.7X	12	1X

Table 1. Experimental design table.

Libraries were constructed using the Watchmaker mRNA Library Preparation (Fig 1) kit with input groups of 10 ng, 50 ng, and 200 ng of UHR RNA (Table 1). IDT xGen Stubby Adaptors and UDI barcode primers were used to individually tag samples for multiplexing.

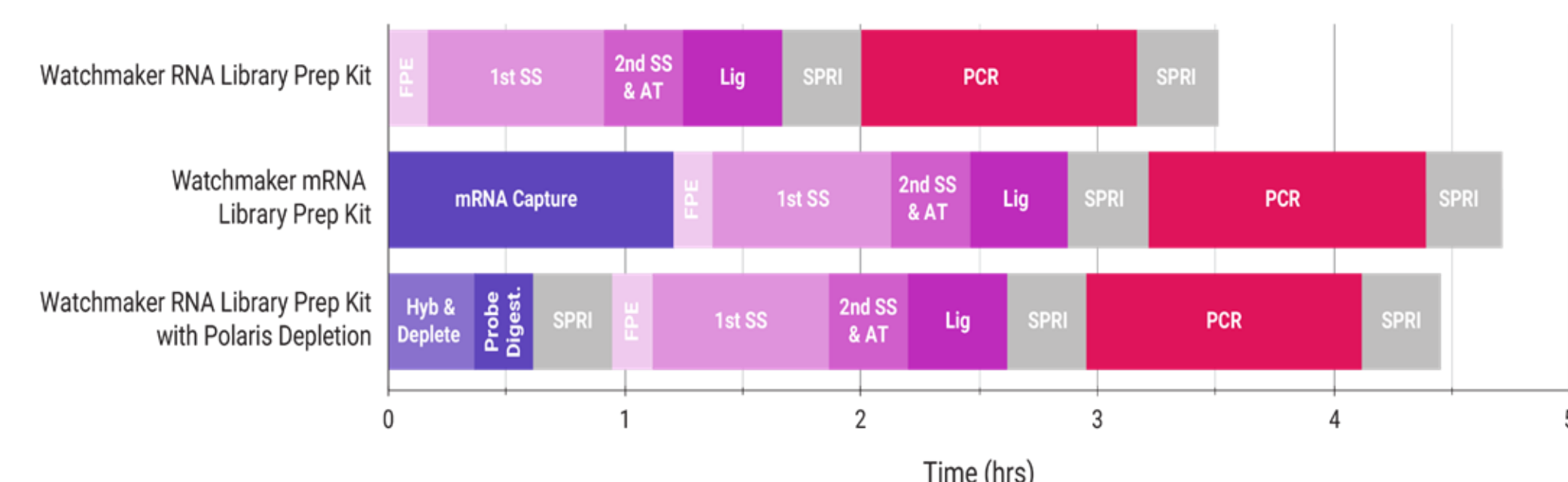


Figure 1. Watchmaker mRNA Library Preparation kit workflow.

Automated Library Preparation Process and Quality Control



Figure 2. Biomek i7 creates sequence-ready mRNA libraries from start to finish.

Plated RNA samples (50uL) underwent Watchmaker mRNA library preparation on the Biomek i7 liquid handler. Final libraries were then sequenced on an Illumina NextSeq 2000 (paired end 150bp, P4 flow cell) (Fig 2).

Overlaid TapeStation traces of three replicates from each input group illustrates reproducibility, final library size, and shows sample similarity in expected base pair range (Fig 3).

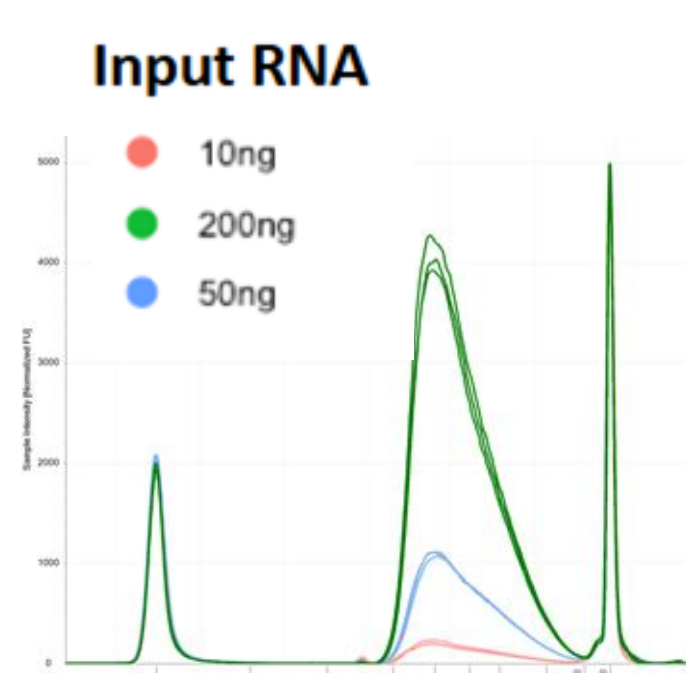


Figure 3. D1000 Assay TapeStation traces of input groups and replicates.

Post-Sequencing Metrics

Run	Q30 (%)	PF (%)	Avg Mapping (%)	Avg Exonic Reads (%)	Avg Ribosomal Reads (%)
UHR_exp	91.7	83.9	98.9±0.04	75.6±0.07	1.7±0.2

Results indicate overall high-quality sequencing (Q30, PF) and pipeline metrics (high mapping, high proportion of exonic reads, low ribosomal reads; Table 2). Expectedly, raw reads and unique gene counts increase as RNA input increases (Fig 4).

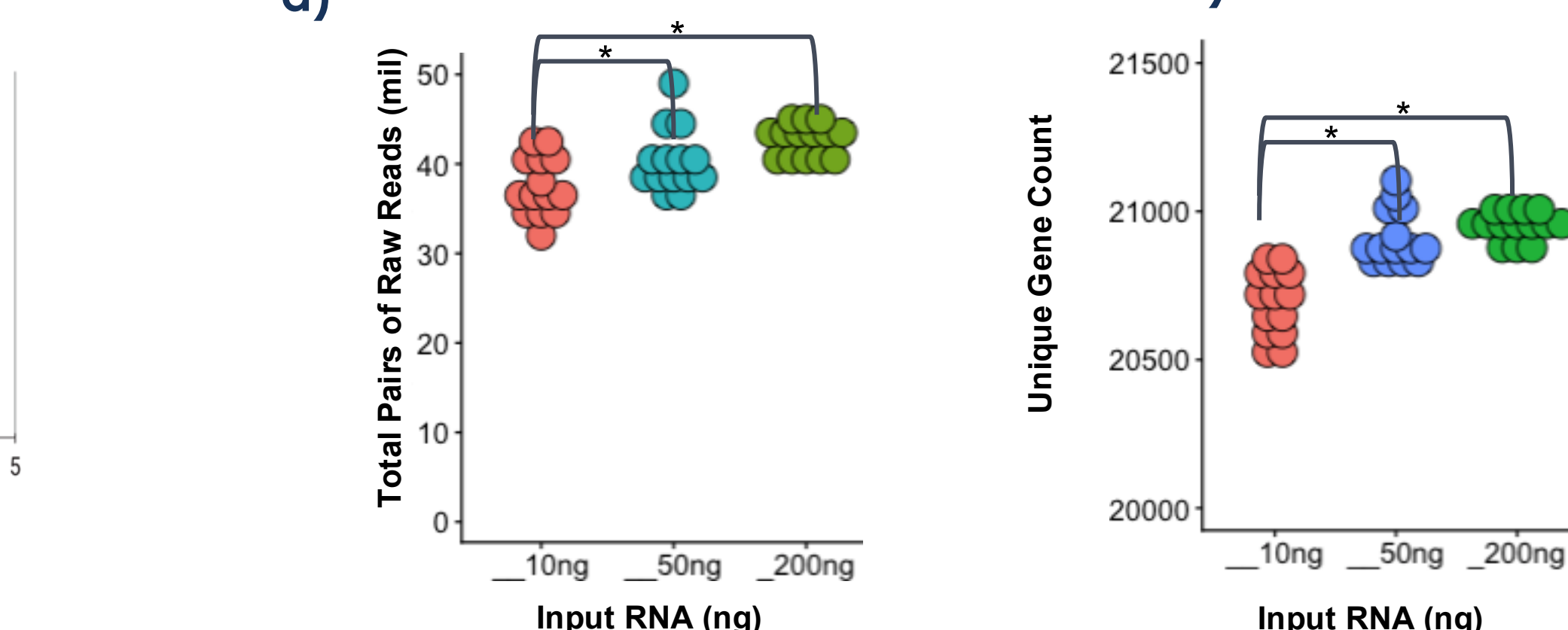


Figure 4. Dot plots of a) total raw reads and b) unique gene count per RNA input group. Significance differences in means determined by one-way ANOVA, Tukey with $p < 0.05$.

Sequencing Results Reveal Strong Sample-to-Sample Concordance

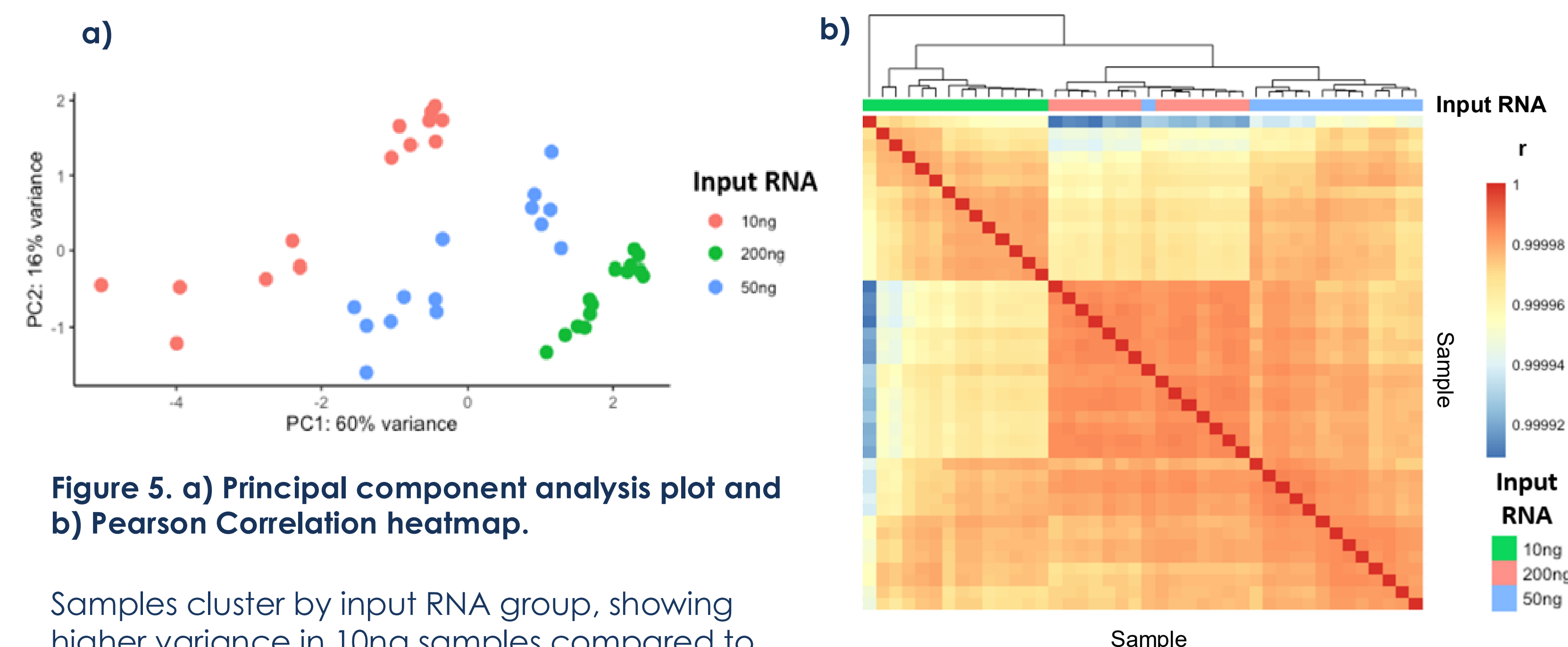


Figure 5. a) Principal component analysis plot and b) Pearson Correlation heatmap.

Samples cluster by input RNA group, showing higher variance in 10ng samples compared to 50ng and 200ng samples (Fig 5a). Despite clustering by input group, overall high sample-to-sample correlation ($r > 0.99$) is maintained across all samples (Fig 5b).

Nearly identical expression profiles are further observed among input groups with low numbers of differentially expressed genes (DEGs) identified (Fig 6). In Fig 6b, 10 DEGs were identified ($< 0.05\%$ of genes) when comparing the 200ng vs 10ng input groups, however 9 of 10 DEGs are non-coding RNA, which are not a common transcript of interest for mRNA capture. This further emphasizes the robustness of expression profiling even between the lowest and highest input levels.

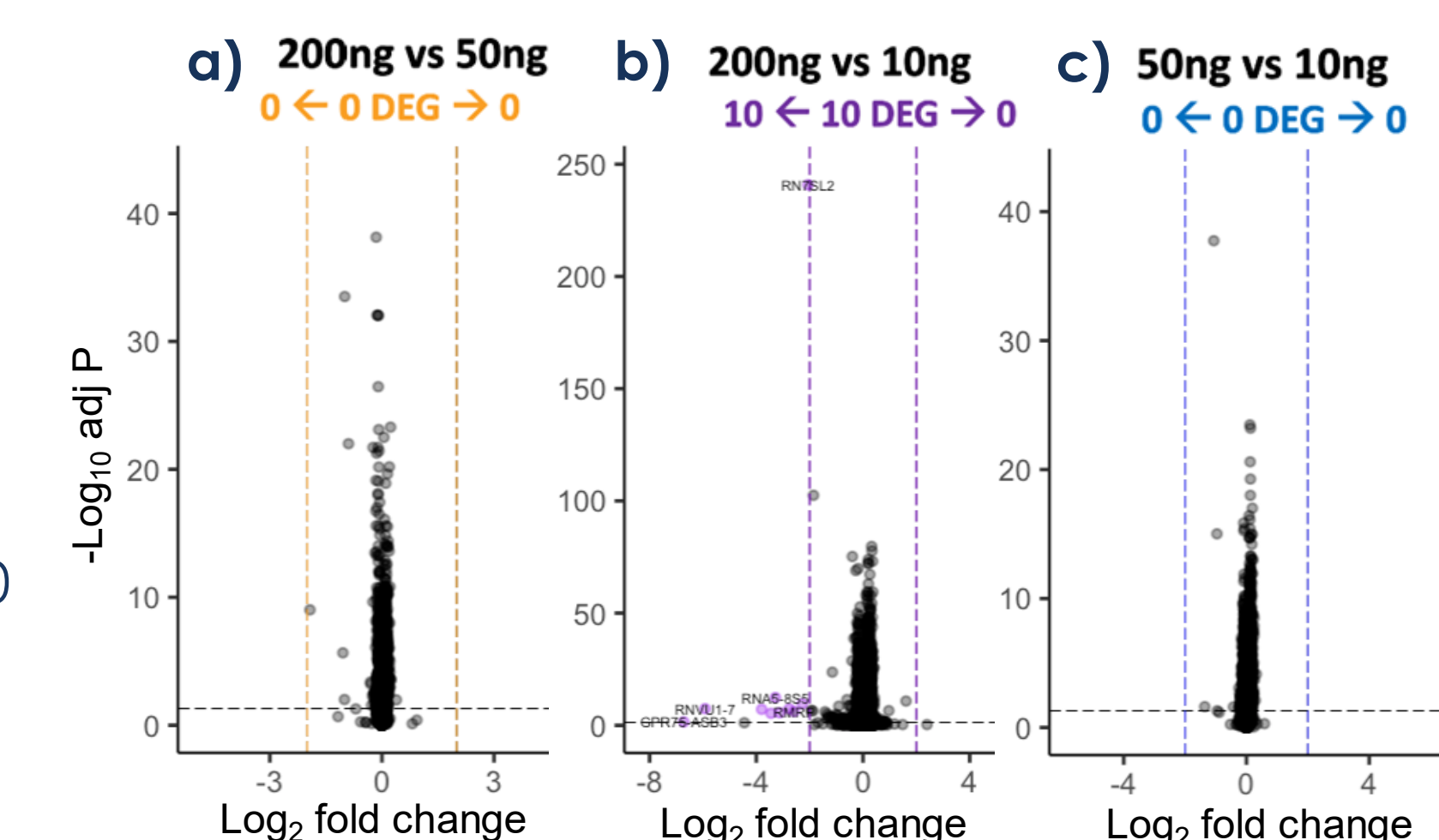


Figure 6. Differential expression volcano plots.



Conclusion

- ElevateBio's automated workflow **generated high-quality libraries** and **maintained highly consistent gene expression profiles** across input groups using the Watchmaker mRNA Library Preparation kit
- This work supports **the robustness of ElevateBio's system** and **suitability for high-throughput library preparation** with flexible input ranges
- ElevateBio's automated solution can provide customers with a validated method capable of **delivering highly reliable and concordant data** without constraints of sample variability or batching
- Further evaluation of inputs near 1 ng, below the recommended minimum of 2.5 ng, will help define lower performance limits

References

1. User Guide. (n.d.). Retrieved January 9, 2026, from https://www.watchmakergenomics.com/media/wg/asset/%2Fm%2F2Fm223_mrna_library_prep_kit__ug_wmug214_v2-2-1224.pdf

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