MERCURIUS™ Full-Length BRB-seq Benchmarked Against NEBNext® Ultra™ II for mRNA-seq: Concordant Biology with a Quicker, More Cost-Effective Workflow

Concordant detection of 19,000+ genes and 85,000+ transcripts at 5M reads per sample, with 87–89% of DE genes shared between methods and high cross-method expression and fold-change correlations.

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Key Takeaways

- **Comparable gene and transcript detection sensitivity:** at 5M reads per sample, MERCURIUS™ Full-Length BRB-seq detected 19,478 genes and ~85k transcripts versus 19,307 genes and 90k transcripts for NEBNext®, with comparable detection at every depth tested.
- **Concordant expression levels:** within-method replicate correlations of R ≈ 0.99, and a cross-method correlation of R = 0.987.
- **Shared biology detection:** 6,445 differentially expressed genes were called by both methods (89% and 87% of each method's DE calls) with a fold change expression correlation of R = 0.931. Epithelial-to-mesenchymal transition was the most strongly enriched pathway in the shared set.
- **Faster and more cost-effective workflow at scale:** For preparation of 96 samples, MERCURIUS™ Full-Length BRB-seq protocol length is estimated at around 5.3 hours versus 8 hours for NEBNext® and has an approximately 3× lower library prep reagent cost per sample.

5.3 h
estimated protocol time for 96 samples

R = 0.987
cross-method gene expression correlation

6,445
DE genes identified by both methods

19,000+
genes detected at 5M reads per sample

Introduction

Standard mRNA-seq library preparation methods are widely used for transcriptome profiling because they enrich polyadenylated transcripts and generate reads across the full length of each transcript, supporting both gene- and isoform-level analysis. However, most commercial mRNA-seq kits are labor-intensive and relatively slow, limiting throughput and driving up per-sample cost, particularly for large cohort or screening studies.[1] They involve multiple single-tube reactions and cleanups per sample, with sample multiplexing occurring only at the final indexing step rather than early in the workflow. 3′ mRNA-seq methods such as BRB-seq address this throughput problem by barcoding samples early, but only sequence the 3′ end of each transcript, which limits transcript- and isoform-level information.[1]

MERCURIUS™ Full-Length BRB-seq (Bulk RNA Barcoding and Sequencing) retains the early multiplexing of up to 96 samples possible with BRB-seq, while providing full-length transcript information. Samples are barcoded during reverse transcription and pooled immediately, so all remaining steps are performed once per pool rather than once per sample, while the final library covers the whole gene body rather than only the 3′ end. This design reduces hands-on time and per-sample cost, but it is unclear whether early multiplexing affects sensitivity or biological conclusions compared with standard mRNA-seq methods.

To quantify assay performance and assess the trade-off between throughput, cost, and sequencing sensitivity, this tech note benchmarks MERCURIUS™ Full-Length BRB-seq against NEBNext® Ultra™ II RNA Library Prep Kit with Poly(A) mRNA Magnetic Isolation Module (NEBNext®), a standard non-multiplexed mRNA-seq approach.

Study Design

To generate a biologically relevant test case with a robust, reproducible transcriptional response, we induced epithelial-to-mesenchymal transition (EMT) in the Huh7 hepatocellular carcinoma cell line with TGFβ for 48 hours, alongside untreated (NT) controls.[2,3] We prepared libraries with MERCURIUS™ Full-Length BRB-seq and NEBNext® kits in parallel following the respective manufacturers' protocols. We used the same treated and untreated purified RNA samples for each technology to ensure our benchmarking reflected the technical capability of the library preparation methods, rather than biological variation between experiments. Experimental parameters are shown in Table 1.

Parameter	Value
Cell model	Huh7 hepatocellular carcinoma cell line
Conditions	Untreated (NT) vs. 10 ng/mL TGFβ-treated (48 h)
Replicates	4 technical replicates per condition
RNA input	100 ng purified total RNA
Kits compared (mRNA)	MERCURIUS™ Full-Length BRB-seq (Alithea Genomics, #10801), NEBNext® Ultra™ II RNA Library Prep Kit (NEB, #E7760L) with Poly(A) mRNA Magnetic Isolation Module (NEB, #E7490L), NEBNext® Multiplex Oligos for Illumina® (NEB, #E6609S)
Sequencing	Element Biosciences AVITI, paired-end
Depth used for analysis	Downsampled to 10M reads per sample (5M max for saturation plots)

Table 1. Experimental design summary.

Results

A Quicker, More Cost-Effective Workflow Than Standard mRNA-seq

Slow workflows compound with large sample numbers and can significantly delay project timelines. Therefore, to assess the feasibility of large-scale studies with each technology, we estimated protocol duration for users processing 96 RNA samples simultaneously.

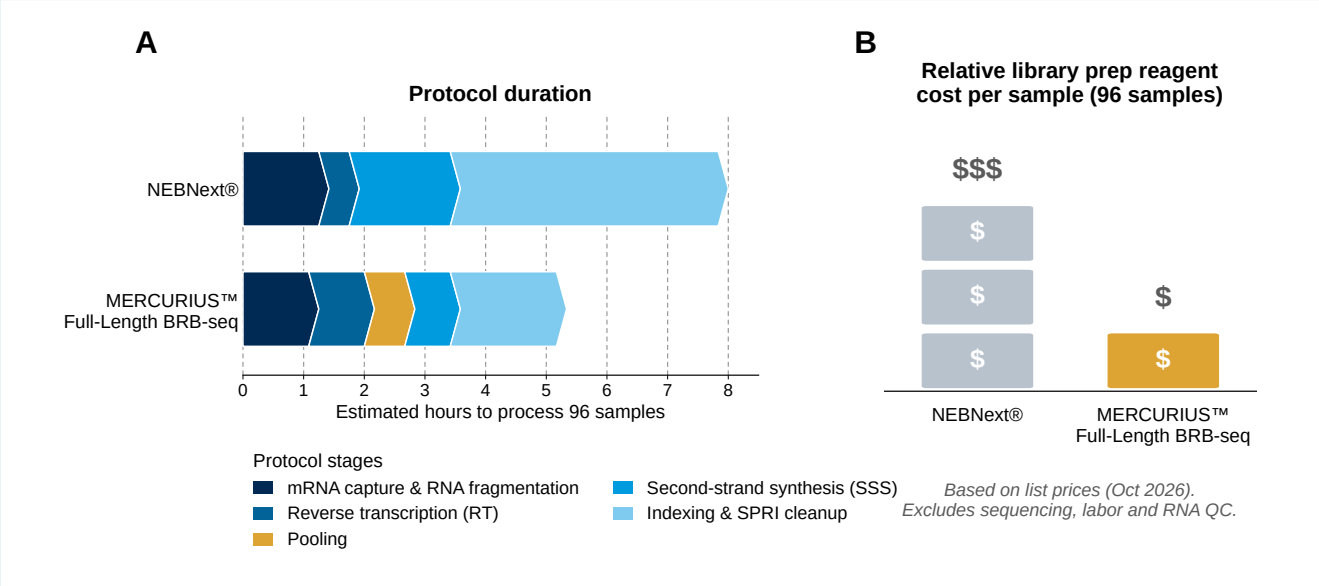


Figure 1. MERCURIUS™ Full-Length BRB-seq is faster and more cost-effective than NEBNext® for preparation of 96 samples. *(A) Comparison of estimated protocol duration to process 96 samples from purified RNA input to indexed library, including hands-on time plus reaction times. MERCURIUS™ Full-Length BRB-seq completes processing in approximately 5.3 hours per batch versus approximately 8 hours for NEBNext®. MERCURIUS™ Full-Length BRB-seq pools samples at the RT step early in the workflow for subsequent processing in a single tube. (B) Relative estimated library preparation costs to prepare 96 samples excluding sequencing, labor, and RNA QC.*

MERCURIUS™ Full-Length BRB-seq was the quicker protocol, estimated at 5.3 hours for 96 samples versus approximately 8 hours for NEBNext® (Fig. 1A). A major contributor to the efficiency of MERCURIUS™ Full-Length BRB-seq is the multiplexing of samples early in the workflow, with subsequent steps occurring on the pooled library instead of each sample individually (Fig. 1A).

In contrast, NEBNext® pools samples only after individual libraries are indexed, creating substantial user effort and per-sample consumable cost that grows sharply as batch sizes increase. In MERCURIUS™ Full-Length BRB-seq, the more samples multiplexed together, the larger the relative time and cost advantage versus standard approaches.

At October 2026 list prices for 96 samples, the library preparation reagents for MERCURIUS™ Full-Length BRB-seq cost an estimated three-fold less per sample than those for NEBNext® (Fig. 1B). Sequencing and labor costs depend on the facility and on the chosen read depth, so they are not included in this comparison. Therefore, from a workflow and cost perspective, MERCURIUS™ Full-Length BRB-seq is more suited to large-scale studies than standard mRNA-seq approaches.

Comparable Quality Metrics Between Technologies

Before assessing gene and isoform detection sensitivity, we first compared numerous quality control (QC) metrics for each technology. QC showed broadly similar outcomes across both technologies. MERCURIUS™ Full-Length BRB-seq and NEBNext® had comparable proportions of reads assigned to genes (82.2% vs. 84.1%) (Fig. 2A). For both technologies, reads mapped along entire gene bodies (Fig. 2B). NEBNext® coverage was slightly higher toward the 5′ region, while MERCURIUS™ Full-Length BRB-seq coverage was shifted modestly

toward the 3′ region, consistent with its barcoding chemistry (Fig. 2B).

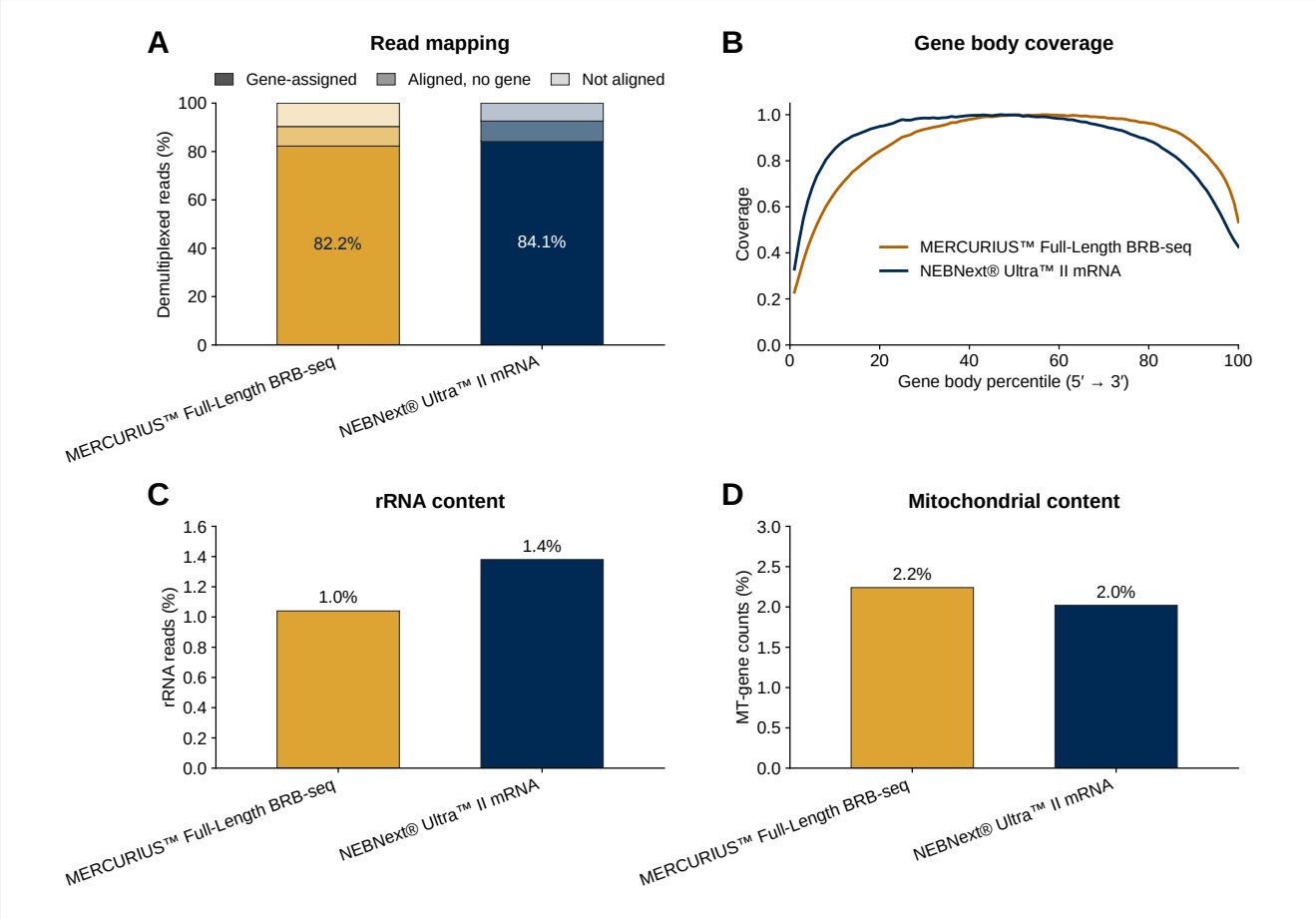


Figure 2. Both methods produce high-quality libraries with comparable read mapping, low rRNA and mitochondrial content, and coverage across the full gene body. *(A) Read mapping breakdown showing the percentage of demultiplexed reads that map to a gene, align without mapping to a gene, or remain unaligned, for each method. (B) Normalized gene-body coverage of mapped reads, 5′→3′. (C) Percentage of reads mapping to ribosomal RNA (rRNA) for each method, estimated on a 1M-read subsample per library. (D) Percentage of reads mapping to mitochondrial (MT) genes.*

Low ribosomal RNA carryover is necessary in mRNA-seq methods to ensure that sequencing budget is used to detect more biologically relevant transcripts. MERCURIUS™ Full-Length BRB-seq and NEBNext® kits both had low proportions of rRNA reads (1.0% vs. 1.4%) (Fig. 2C), and similar levels of reads mapped to mitochondrial genes (2.2% vs. 2.0%) (Fig. 2D). Together, these QC metrics indicate highly comparable technical performance between the two technologies, despite early sample multiplexing and a quicker workflow in MERCURIUS™ Full-Length BRB-seq.

Matched Gene Detection and Only Modest Reductions in Transcript Detection Despite Early Sample Multiplexing

After confirming each library's overall quality, we assessed potential trade-offs between method scalability and gene and transcript detection sensitivity. At maximum sequencing depths, both technologies detected close to 30k genes in total, with near-identical numbers at low, medium, and high expression levels (Fig. 3A).

Next, to assess the effect of sequencing depth on detection sensitivity, we downsampled each library to 100k–5M raw reads per sample and computed gene and transcript detection. Gene detection was essentially identical across all depths. At low read depths of 500k reads/sample, both kits detected approximately 13.6k genes, and at 5M reads/sample MERCURIUS™ Full-Length BRB-seq detected 19,478 genes versus 19,307 for NEBNext® (Fig. 3B).

For transcript detection, MERCURIUS™ Full-Length BRB-seq detected approximately 5% fewer transcripts than NEBNext® at all read depths, with approximately 85k versus 90k transcripts at 5M reads/sample (Fig. 3C).

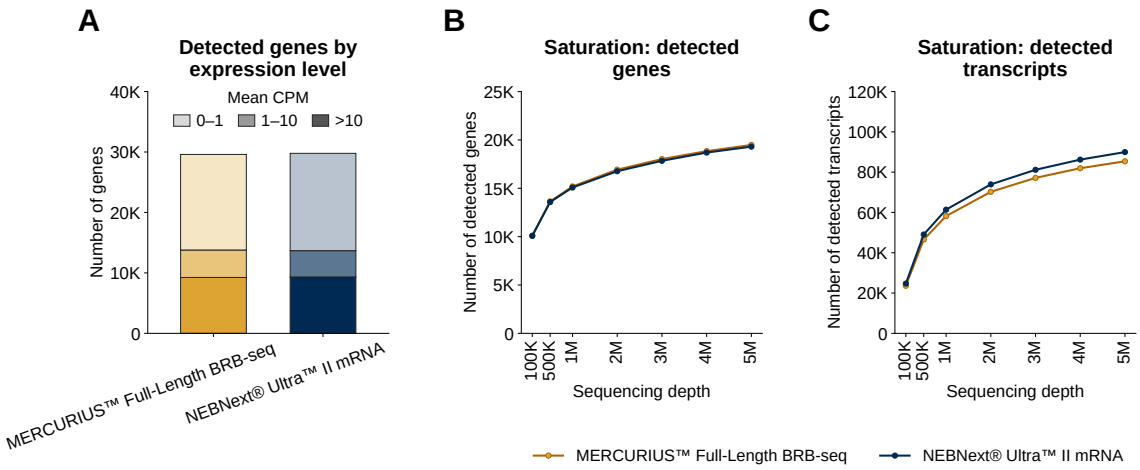


Figure 3. Gene detection is comparable at every sequencing depth, with around 5% fewer transcripts detected by MERCURIUS™ Full-Length BRB-seq at 5M reads per sample. *(A) Gene detection at maximum sequencing depths per technology, split by mean CPM. (B) Gene detection and (C) transcript detection after downsampling libraries to a common range of raw reads per sample (100k–5M).*

Together, for large-scale studies, MERCURIUS™ Full-Length BRB-seq matches the gene-level sensitivity of a standard non-multiplexed mRNA-seq kit, and its increased sample multiplexing capacity, lower cost, and quicker workflow likely offset its modest reduction in transcript-level detection.

High Reproducibility and Cross-Method Concordance in Gene Expression Levels

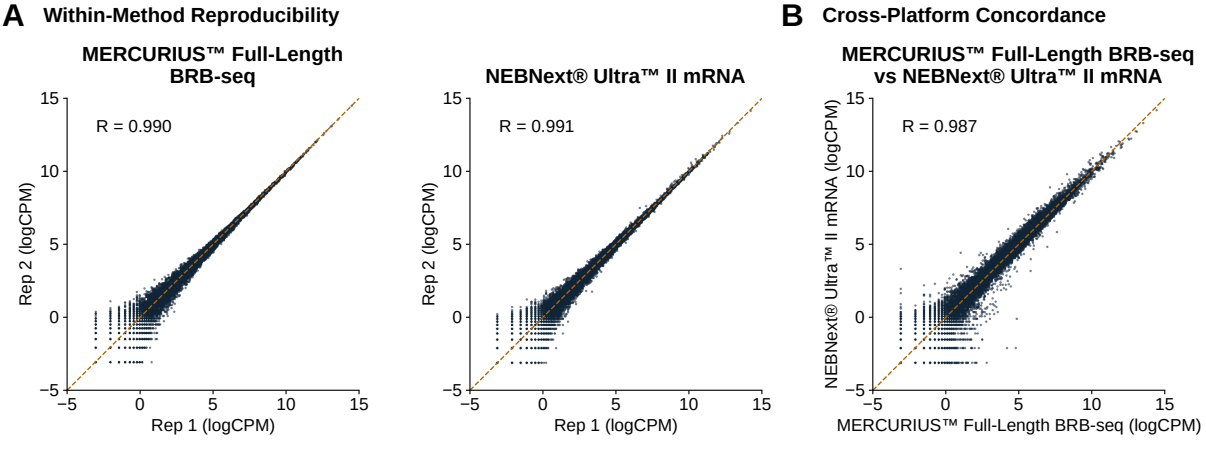


Figure 4. Both technologies are highly reproducible between replicates (R ≈ 0.99) and closely concordant with each other (R = 0.987). *(A) Pairwise scatterplots of gene expression levels comparing replicates within each library preparation method. (B) Pairwise scatterplot showing correlation of mean expression levels of MERCURIUS™ Full-Length BRB-seq versus NEBNext® libraries. Pearson correlation coefficients (R) are shown in the top-left corner of each plot.*

To evaluate reproducibility and cross-method concordance, we compared gene expression levels across replicates and between the two library preparation methods. Within-method correlations were R = 0.990 for MERCURIUS™ Full-Length BRB-seq and R = 0.991 for NEBNext®, indicating that

both approaches generate highly reproducible expression measurements between replicates (Fig. 4A). Similarly, the cross-method comparison showed a high gene expression level correlation of R = 0.987 (Fig. 4B).

Together, these results demonstrate that MERCURIUS™ Full-Length BRB-seq produces highly reproducible gene expression data that is closely concordant with an established mRNA-seq library preparation method, supporting its use as a reliable alternative for large-scale full-length transcriptome profiling.

Concordant Differential Expression and Pathway Detection Across Technologies

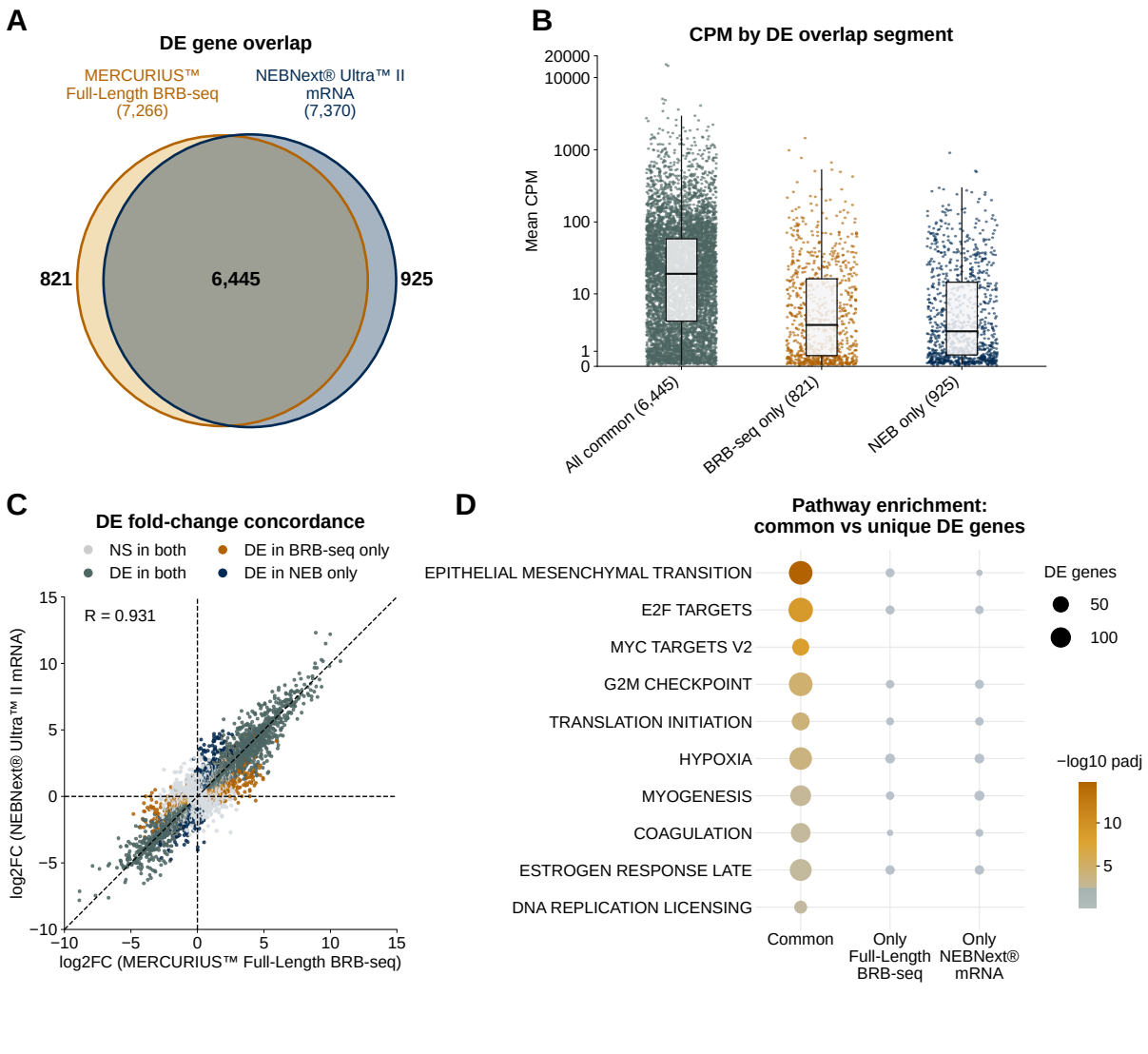


Figure 5. Most differentially expressed genes are shared between methods, with concordant fold changes, and the shared set is enriched for EMT pathways. *(A) Venn diagram showing the overlap of differentially expressed (DE) genes identified independently by MERCURIUS™ Full-Length BRB-seq (7,266 DE genes) and NEBNext® (7,370 DE genes). DE genes were determined by Benjamini–Hochberg-adjusted p < 0.05 and |log2FC| > 0.585 (|fold change| > 1.5x), from a DESeq2 Wald test of TGFβ vs. untreated run separately per protocol. (B) Distribution of mean CPM expression values for genes in each overlap segment from the Venn diagram, shown as scatter points overlaid with box plots (median, interquartile range, and whiskers). (C) Scatter plot indicating the high correlation in the fold changes of differentially expressed genes across technologies (Pearson R=0.931). (D) Dot plot showing pathway enrichment results for DE gene sets common to both library preparation methods and for DE genes unique to each individual method.*

To evaluate concordance in differential expression (DE) gene detection across library preparation methods, we compared the number and overlap of DE genes identified independently by MERCURIUS™ Full-Length BRB-seq and NEBNext® (Fig. 5A). Both technologies detected similar numbers of DE genes (7,266 vs. 7,370), with the majority (6,445 DE genes) identified as significant by both methods. This represents 89% and 87% of each method's DE calls, respectively, and substantial overlap in the underlying biologically relevant signal detected (Fig. 5A).

Each method also identified a smaller subset of DE genes not called by the other (821 unique to MERCURIUS™ Full-Length BRB-seq and 925 unique to NEBNext®). To determine whether these method-specific DE genes reflect genuine biological differences or simply detection sensitivity near the significance threshold, we examined mean CPM expression levels across each overlap segment. Genes common to both methods showed the highest and most variable expression levels (median mean CPM ~19), while genes unique to either method were predominantly low-expression transcripts (median mean CPM ~3), consistent with these genes falling near the limit of detection where small differences in sensitivity between platforms are most likely to influence significance calls (Fig. 5B). This supports that the observed discordance is driven primarily by expression-level-dependent detection sensitivity rather than systematic biological or technical discrepancies between the library preparation methods.

Fold changes of DE genes were also highly correlated between methods (Pearson R = 0.931), further confirming the ability of multiplexed MERCURIUS™ Full-Length BRB-seq to provide highly robust biological information in line with non-multiplexed methods (Fig. 5C).

Next, to determine the biological relevance of the common and method-specific DE gene sets, we performed a pathway enrichment analysis on each overlap segment (Fig. 5D). DE genes common to both methods showed strong enrichment across multiple cancer- and EMT-relevant pathways, as expected given the TGFβ-induced EMT model used for benchmarking (Fig. 5D). In contrast, DE genes unique to either method showed minimal to no significant pathway enrichment across the same gene sets (Fig. 5D). This indicates that both library preparation methods capture the same biologically meaningful information, and that the modest differences in transcript detection and DE counts do not translate into a materially different biological conclusion, despite MERCURIUS™ Full-Length BRB-seq's early multiplexing, quicker protocol, and scalability for large projects.

Conclusion

In a TGFβ-induced EMT model using identical Huh7-derived RNA, MERCURIUS™ Full-Length BRB-seq produced results closely concordant with NEBNext®. Library QC metrics were comparable, and gene and transcript detection was within ~5% at every sequencing depth tested. Within-technology replicate correlations in gene expression were R = 0.99 and cross-method correlation was R = 0.987.

The 6,445 DE genes shared by both methods represent 87–89% of each method's DE calls with a correlation in fold change levels of R = 0.931. These common DE genes were enriched in EMT-associated pathways as expected. Together, these data indicate that the two kits lead to the same biological conclusion and that early multiplexing of samples in MERCURIUS™ Full-Length BRB-seq has little impact on assay performance.

An additional advantage is that early barcoding with MERCURIUS™ Full-Length BRB-seq shortens the workflow to an estimated 5.3 hours for 96 samples, versus roughly 8 hours for NEBNext®, time-efficiency that would grow with batch size. The library preparation reagent cost per sample for 96 samples is also estimated to be approximately 3× lower with MERCURIUS™ Full-Length BRB-seq than NEBNext®. This cost efficiency can substantially increase sample throughput within existing budgets, without compromising data quality or biologically interpretable results.

For perturbation screens and large cohorts, where sample number, cost, and throughput matter most and gene- or transcript-level expression is the main readout, multiplexed MERCURIUS™ Full-Length BRB-seq provides comparable performance to standard mRNA-seq approaches and can be easily implemented into existing experimental workflows.

References

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3. Cook DP, Vanderhyden BC. Context specificity of the EMT transcriptional response. Nature Communications. 2020 May 1;11(1):2142.

Questions about running MERCURIUS™ Full-Length BRB-seq in your lab? **Reach out to speak to our experts.**