MICRONBRANE MEDICAL

Comparative Evaluation of DNA Library Preparation Kits for Ultra-Low Input Metagenomic Sequencing

Library preparation from low-input DNA remains a significant barrier to effective sequencing in both research and clinical settings. Whether studying rare taxa, working with degraded material, or analyzing low-volume samples, investigators often face the challenge of preparing high-quality libraries from sub-nanogram quantities of DNA[1,2].

The issue is especially pronounced in clinical applications, where fluids like cerebrospinal fluid or plasma contain vanishingly small amounts of microbial DNA, and re-collection is often impossible. In these cases, even modest gains in library efficiency and fidelity can affect diagnostic sensitivity and downstream interpretation.

Microbiome research—both in and beyond clinical contexts—faces similar constraints. Although microbial communities can be rich and complex, sample types such as biopsy tissue, swabs, or post-depletion DNA extracts often yield limited input. As a result, the ability to generate representative and reproducible libraries from picogram to low-nanogram inputs has become a defining requirement for modern metagenomic and microbiome workflows.

Study Background

To assess kit performance at sub-nanogram input levels, AMILI PTE. LTD., a Singapore-based microbiome company combining research and commercial services, conducted a benchmarking study using serial dilutions of stool-derived microbial DNA. Although not a low-biomass matrix, stool provides a compositionally rich and consistent model for testing input sensitivity under controlled conditions. This approach isolates the effect of DNA quantity on library performance while minimizing variability from extraction method or host contamination.

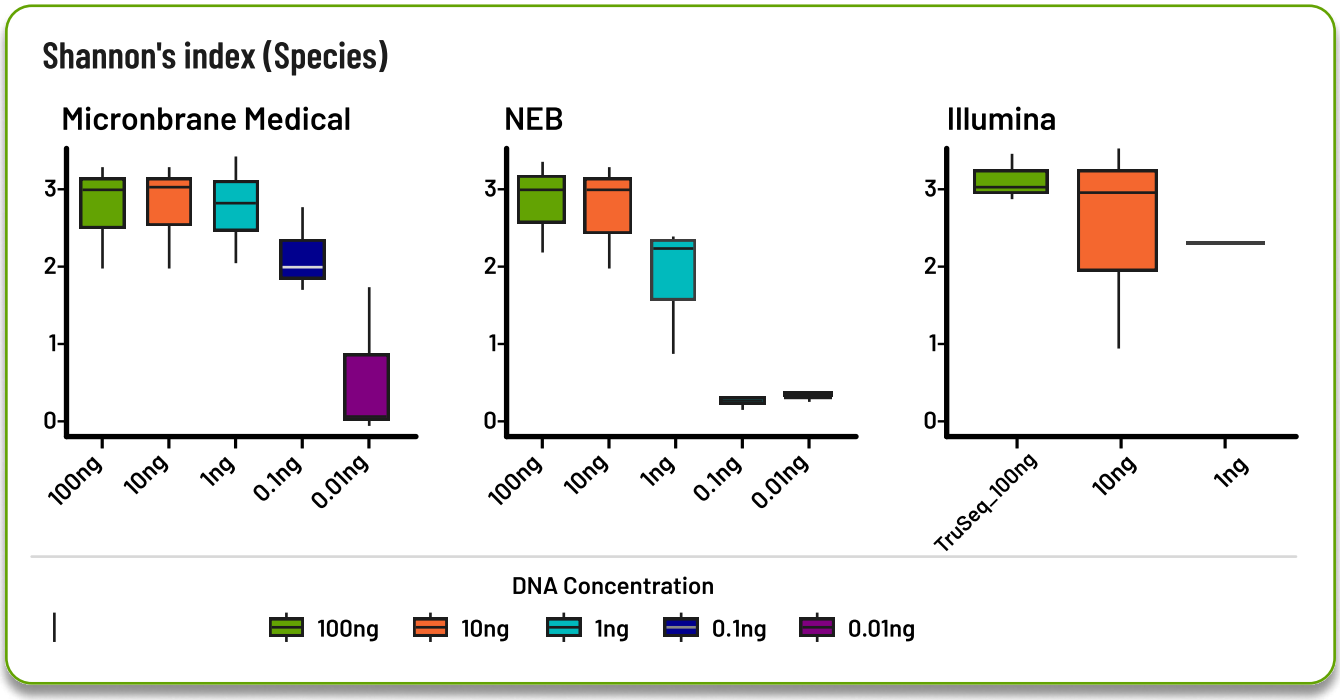
Materials and Methods

Stool microbiome DNA was extracted from a single healthy donor using the QIAamp PowerFecal Pro DNA Kit (Qiagen) and serially diluted to generate input amounts ranging from 100 ng to 10 pg. For each input level, triplicate libraries were prepared using four different library preparation kits —the **Micronbrane Medical** Unison Ultralow DNA NGS Library Preparation Kit, **Illumina** TruSeq DNA Nano, **Illumina** DNA Prep, and the **New England Biolabs** NEBNext Ultra II—according to their respective manufacturers' protocols. Due to input limitations and quality control outcomes, Illumina TruSeq DNA Nano was tested only at 100 ng, while Illumina DNA Prep was evaluated at 10 ng and 1 ng; lower input libraries (0.1 ng and 0.01 ng) failed to meet the quality thresholds for downstream sequencing.

All libraries were sequenced on the Illumina NovaSeq platform (~20 million paired-end reads per sample), followed by quality control with KneadData (v0.12.0) to remove host and low-quality reads. Taxonomic profiling was performed using MetaPhlAn4 (v4.1.1)[3] within the bioBakery[4] framework. Downstream analyses, including alpha and beta diversity metrics, and taxonomic composition comparisons, were conducted in R (v4.2).

Results

Unison produced consistent alpha diversity (Shannon and Simpson indices) down to 1 ng input. In contrast, NEB required ≥10 ng to maintain comparable diversity, and Illumina DNA Prep showed marked diversity loss below 10 ng.



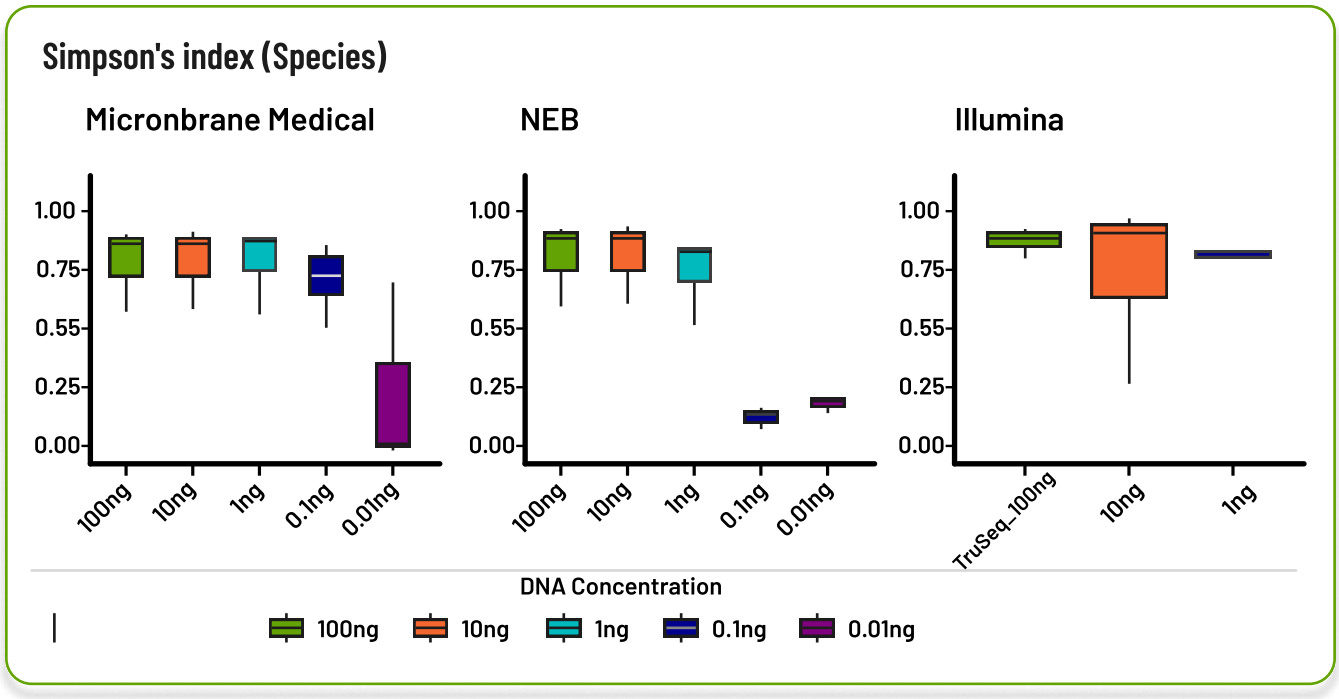


Figure 1. Unison maintains microbial diversity at inputs down to 1 ng, while NEB and Illumina show substantial declines below this threshold.

Beta diversity analysis showed tight replicate clustering for Unison at DNA inputs as low as 1 ng, indicating high reproducibility. NEB and Illumina kits showed greater inter-replicate dispersion at low input levels, suggesting amplification bias and reduced consistency.

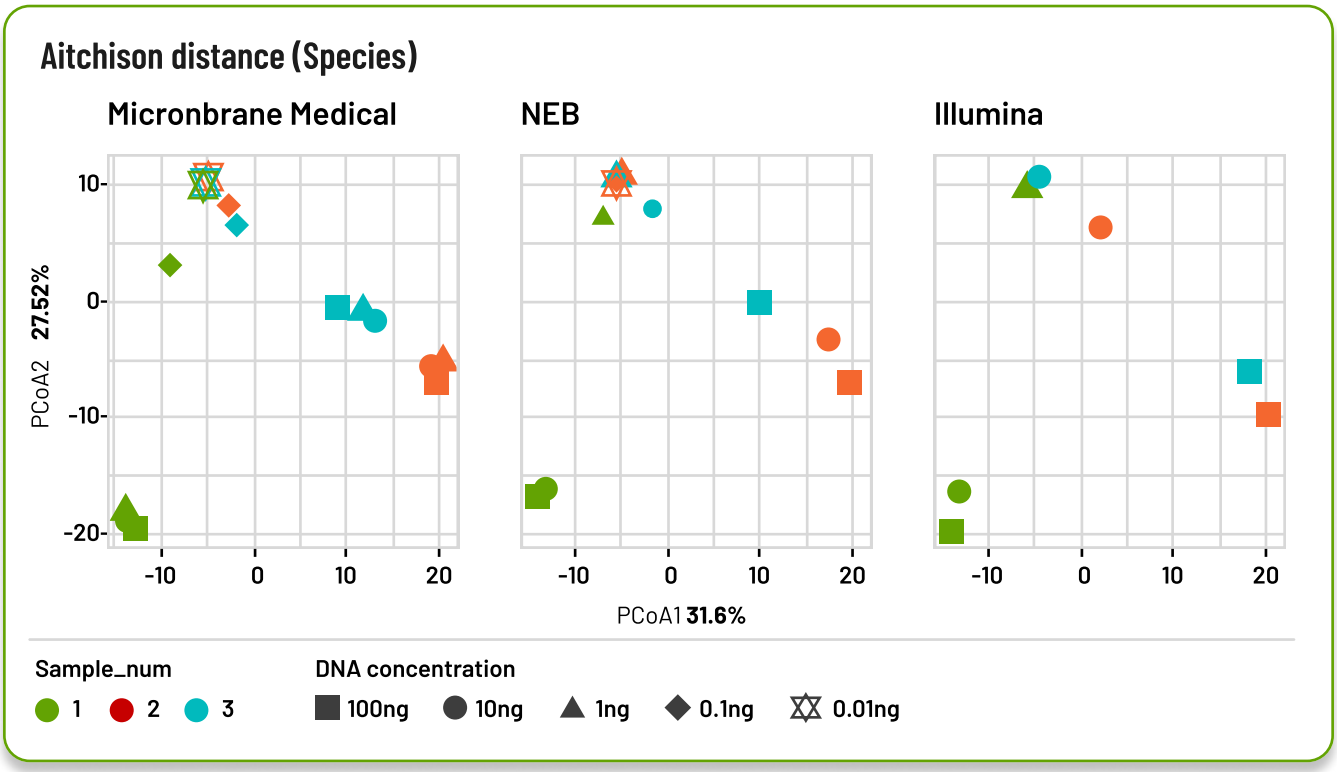


Figure 2. Unison demonstrates strong replicate consistency at 1 ng, unlike NEB and Illumina kits, which show increasing dispersion with lower input.

At the phylum level, Unison maintained stable community structure down to 0.1 ng. NEB and Illumina kits displayed compositional distortion, including overrepresentation of Actinobacteria likely a result of input-dependent PCR bias.

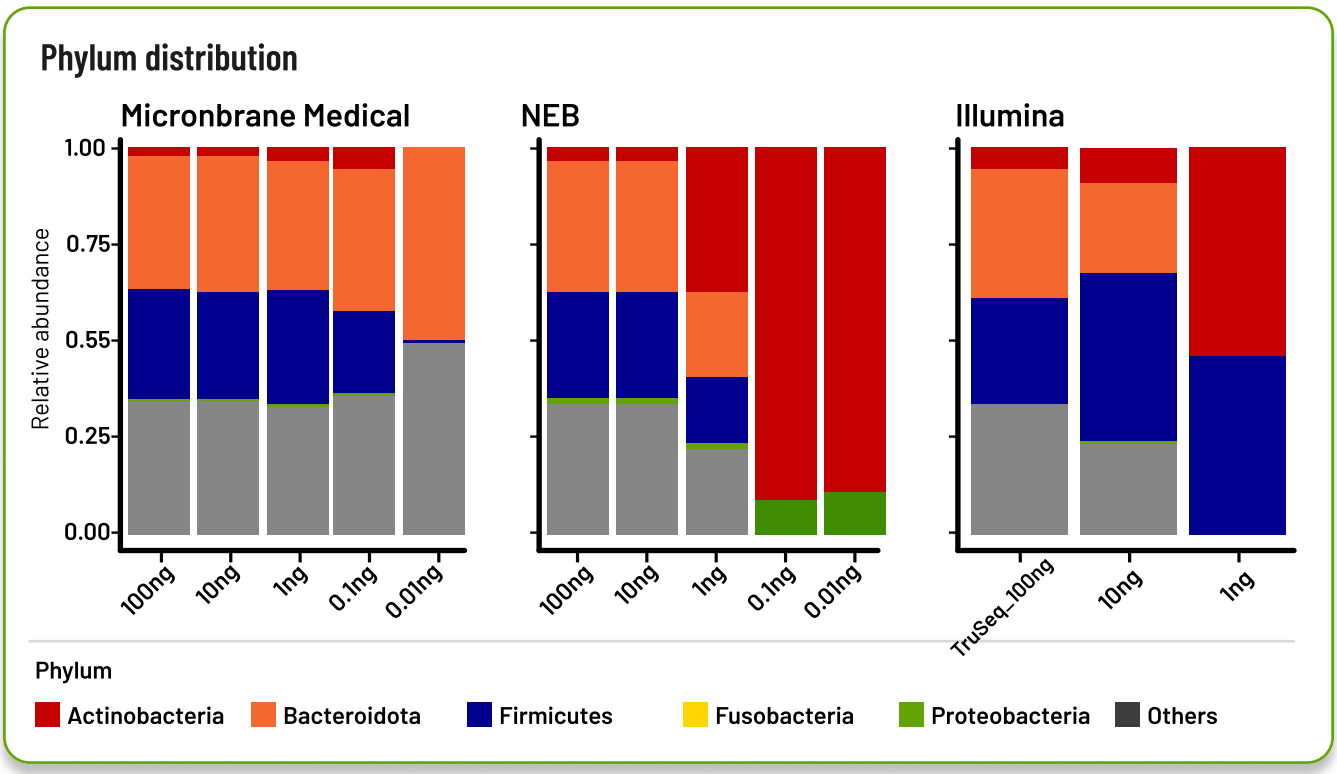


Figure 3. Unison preserves phylum-level structure at 0.1 ng input, whereas NEB and Illumina skew microbial representation.

Table 1. Library Kit Comparison

Library Prep Kit	Minimum Effective Input	Diversity Preservation	Reproducibility	Taxonomic Fidelity	Notable Limitations
Micronbrane Medical Unison Ultralow DNA NGS Library Prep Kit	0.1ng	Stable down to 1ng	Tight clustering even at 0.1ng	Accurate down to 0.1ng	None observed; performs optimally across ultra-low input levels
Illumina TruSeq DNA Nano	100ng only (tested)	High at 100ng	High at 100ng	Accurate at 100ng	Cannot handle low input; protocol not suitable below 100ng
Illumina DNA Prep	≥10ng	Significant drop <10ng	Poor below 10ng	Skewed below 10ng	Failed at 0.1ng and lower; diversity and consistency compromised
NEB Next® Ultra™ II	≥10ng	Declines <10ng	Moderate at low input	Distorted at sub-10ng	Requires ≥10ng for reliable results; shows PCR bias at low input

Conclusion

Unison consistently generated high-quality libraries across a broad input range, including sub-nanogram quantities. When applied to challenging metagenomic samples, it demonstrated strong preservation of sample complexity and taxonomic structure. Key performance outcomes included:

- **Reproducibility at ultra-low input:** Reliable library generation from as little as 0.1ng of DNA
- **Preservation of diversity:** High microbial diversity retained across replicates
- **Minimized taxonomic bias:** Accurate composition maintained at low input concentrations
- **Sample integrity conservation:** Streamlined workflow reduced input loss
- **Versatility across sample types:** Compatible with degraded, low-biomass, or trace input DNA

Used as part of the PaRTI-Seq™ workflow—including host depletion and integrated bioinformatics—Unison supports high-confidence microbial and pathogen detection, even from limited or complex research samples.

References

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2. Marquet, M., Zöllkau, J., Pastuschek, J., Viehweger, A., Schleußner, E., Makarewicz, O., et al. (2020). Sensitivity of shotgun metagenomics to host DNA: Abundance matters. Microbial Genomics, 6(2), e000377.
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4. McIver, L. J., Abu-Ali, G., Franzosa, E. A., Schwager, R., Morgan, X. C., Waldron, L., et al. (2018). bioBakery: A meta'omic analysis environment. Bioinformatics, 34(7), 1235–1237.