Application Note

Unlocking Superior Microbial Profiling with **iconPCR™**

Overview

16S rRNA gene sequencing is essential for exploring microbial diversity in various ecosystems, including soil, water, and human-associated microbiomes. However, conventional PCR methods used in 16S library preparation often introduce significant biases due to over-amplification, under-amplification, and the generation of chimeric sequences. These artifacts compromise the accuracy of microbial community profiling by distorting relative abundances and obscuring low-abundance taxa.

iconPCR, developed by n6, represents a transformative shift in PCR technology. Its core innovation lies in the concept of AutoNorm™, wherein each individual PCR reaction is monitored and terminated in real-time based on fluorescence thresholds. This eliminates the guesswork of fixed-cycle PCR and ensures every library is amplified optimally.

Figure 1. iconPCR, the world's first real-time thermocycler with 96 individually controlled wells.

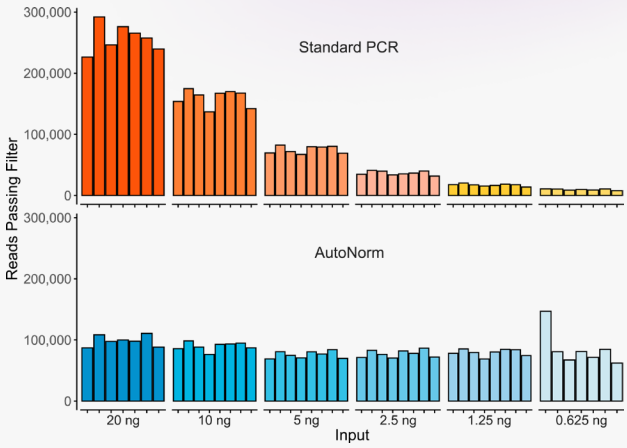


Figure 2. Here we show the significant yield variance when using a single PCR instrument with a fixed number of PCR cycles compared to iconPCR with AutoNorm where each sample is amplified to similar levels.

To evaluate performance, we prepared full-length 16S rRNA libraries from soil microbiome samples and sequenced them using the PacBio® Sequel® II platform. Known for its ability to deliver accurate, long-read sequencing data, PacBio technology was essential in capturing the full-length 16S gene (V1–V9), enabling high-resolution taxonomic analysis. The combination of iconPCR and PacBio sequencing yielded highly reproducible and comprehensive profiles of complex microbial communities.

This application note summarizes a comparative study using iconPCR and conventional PCR workflows. The results clearly demonstrate the superiority of iconPCR in data quality, taxonomic resolution, and workflow consistency—especially when paired with long-read platforms like PacBio for full-length amplicon sequencing.

Application Note

Advantages of iconPCR

- Automated normalization
- Single-tube workflow
- No chimeras, bias-free amplification
- Streamlined process, fewer hands-on steps
- Consistent diversity representation

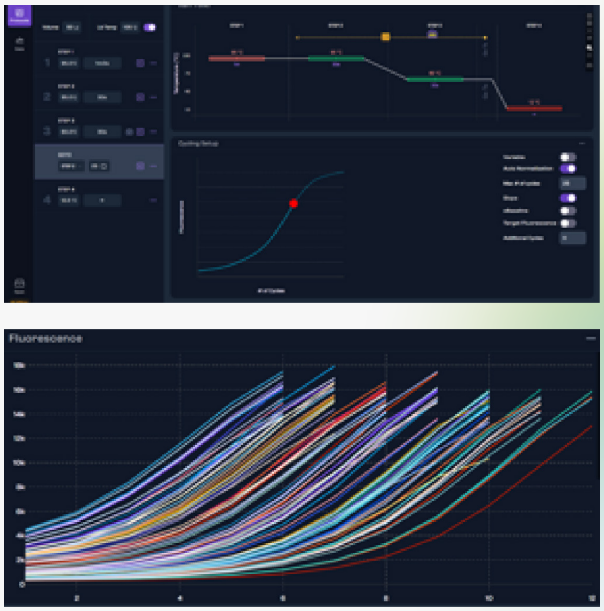


Figure 3a. Graphical view of setting AutoNorm on iconPCR and **3b.** resulting amplification of each sample to its set threshold.

Conventional PCR	iconPCR (with AutoNorm)
Fixed cycle count (e.g., 30 cycles)	Real-time fluorescence monitoring
One-size-fits-all amplification	Per-well cycle control based on signal, not guesswork
Under/over-amplification common	Optimal amplification per sample
High chimera rates	Reduced chimera formation
Variable library quality	Uniform library quality across all wells
Manual quant and normalization required	Automated normalization (no post-PCR quant)
More hands-on time	40–60% reduction in hands-on time
Increased reagent waste	Lower reagent waste, fewer failed libraries
Extra QC and rerun costs	Faster turnaround, minimal QC/rescue steps

Experimental Design

A total of 60 soil samples and 4 ZymoBIOMICS positive controls were split between two workflows:

- iconPCR using AutoNorm mode (AN)
- Conventional PCR using 30 fixed cycles (FC)

Libraries were sequenced using PacBio Sequel II, targeting full-length 16S (V1-V9). Fluorescence thresholds in iconPCR were set to 2.5x baseline, with each sample independently terminating at its ideal cycle.

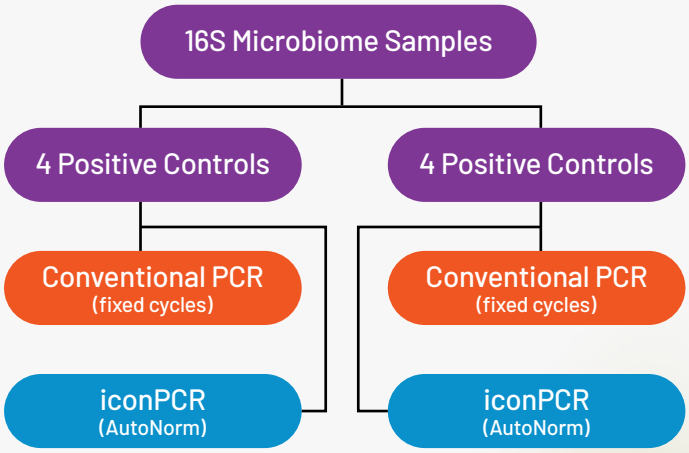


Figure 4. Experimental design showing sample distribution across workflows

Enhanced Data Quality

Data were processed using the DADA2 pipeline. iconPCR libraries consistently outperformed conventional PCR across multiple metrics:

- Reduced chimeras in all samples
- Increased trimmed and inferred reads
- Up to 10x more unique ASVs identified

The comparative analysis between iconPCR and conventional fixed-cycle PCR across 60 soil samples and positive controls demonstrates a consistent and substantial improvement with iconPCR. Both workflows began with identical raw reads (5,400 per sample), but iconPCR retained more trimmed reads, generated up to 10x more high-confidence DADA2-inferred reads, and produced zero chimeras in all samples—unlike the fixed-cycle protocol, which often showed significant chimera counts. Moreover, iconPCR resulted in higher chimera-free reads and a significantly greater number of unique amplicon sequence variants (ASVs), reflecting improved richness and sequencing efficiency. These results validate iconPCR's superiority in data quality, artifact reduction, and microbial diversity resolution.

Expanded Microbial Detection

iconPCR significantly enhanced species richness and taxonomic depth. Several microbial classes such as Chloroflexia, Opitutae, Thermomicrobia, and Caldilinea were detected exclusively in iconPCR-prepared samples.

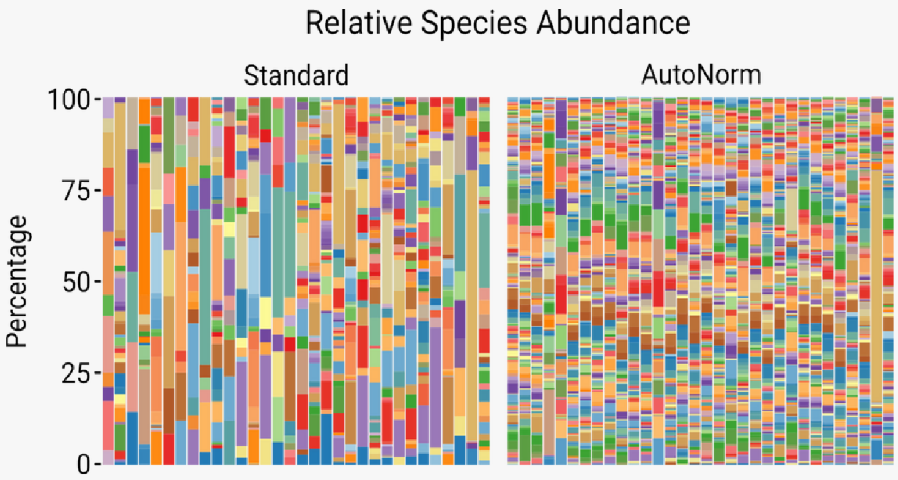


Figure 5. Species-level abundance profiles.

Figure 6. Core and unique taxa detected per workflow at the class level.

Diversity Metrics

Alpha and beta diversity analyses further underscored the enhanced performance of iconPCR:

- Alpha Diversity (Chao1, Shannon, Simpson): iconPCR-treated samples displayed significantly higher diversity ($p < 0.001$).
- Beta Diversity: PCoA plots using Bray-Curtis distances showed tighter clustering of iconPCR samples, indicating greater consistency and reproducibility.

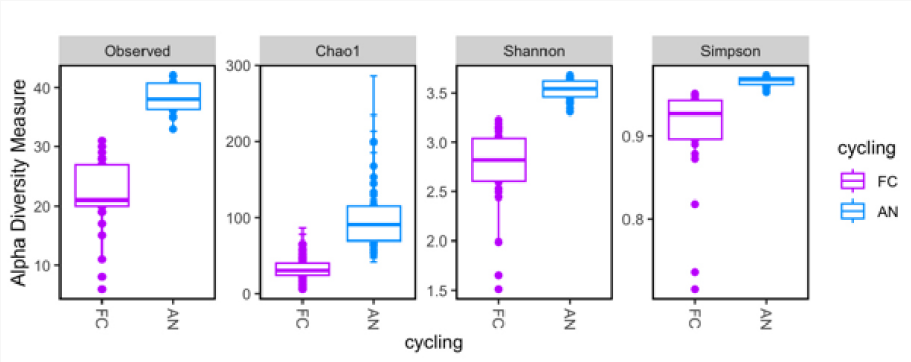


Figure 7. Alpha diversity analysis comparing observed richness and indices.

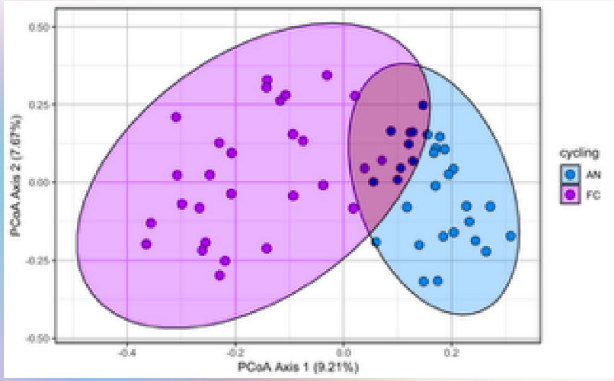


Figure 8. Beta diversity plot (PCoA) showing distinct clustering patterns.

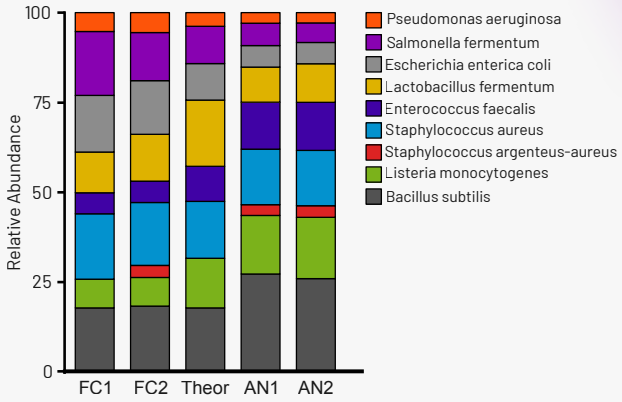


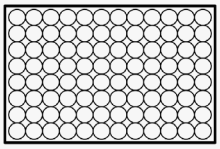
Figure 9. Relative species abundance of positive controls from fixed cycle PCR and iconPCR compared to the theoretical composition.

Application Note

Incorporating iconPCR into your 16S workflow

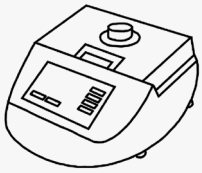
gDNA extraction

Standard protocol



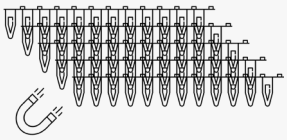
Prepare standard PCR reaction

- Kapa Hifi Supermix
- Barcoded F and R primers
- DNA



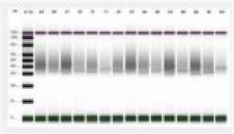
Run Conventional PCR

Amplification
Set 25 cycles



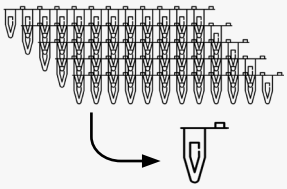
SPRI cleanup

Individual (1-96) cleanups



Quantification

Individual libraries

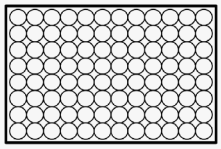


Normalizing & Pooling

1-96 rxns
Variable volumes

Sequencing

iconPCR protocol

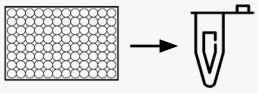


Prepare iconPCR reaction

- Same reagents as standard protocol *plus*
- SYBR Green (0.5X)

Run iconPCR

AutoNorm™
cycles vary per well



Pooling

1-96 rxns
(1-5 ul per library)

SPRI cleanup

Single (1) cleanup

Sequencing

Reduce cost by $1,000

Quant and cleanup, per 96 samples

Save 2.5 hours

hands-on time per 96 samples

Conclusion

iconPCR redefines 16S library preparation by eliminating the limitations of traditional PCR workflows. Through its unique per-well AutoNorm mechanism, iconPCR delivers higher data quality, reduces artifacts, and expands taxonomic discovery. When paired with long-read sequencing platforms such as PacBio, the result is a powerful, end-to-end workflow for generating high-resolution, chimera-free, full-length 16S profiles. These advantages make it an ideal solution for microbiome research in environmental, clinical, and industrial applications—empowering researchers with clearer, more reproducible insights into microbial communities.

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