Application Note

Enhancing Robustness of Microbial Sequencing Workflows Using seqWell ExpressPlex™ Library Prep with **AutoNorm™** Adaptive Amplification

Overview

Library preparation workflows for microbial genome sequencing require consistent amplification to achieve balanced sequencing output across multiplexed samples. ExpressPlex™ Library Prep from seqWell provides a streamlined, one-step workflow combining tagmentation and PCR into a single reaction. ExpressPlex includes built-in auto-normalization for up to a 10-fold range of DNA input depending on the application, when using traditional fixed-cycle PCR methods.

This normalization range can be significantly expanded when combined with the n6 icon96™ thermocycler, which performs real-time adaptive amplification using AutoNorm™ technology. This powerful combination enables the generation of full plates of sequencer-ready, auto-normalized libraries in a single reaction without the need for individual library QC. The one-step tagmentation and amplification workflow can be performed entirely on the icon96, delivering a highly streamlined library preparation process.

Figure 1. icon96 and icon16, the world's first real-time thermocyclers with individually controlled wells.

Traditional PCR workflows rely on predefined cycle numbers based on estimated DNA input, which can result in under- or over-amplification when sample inputs vary. In contrast, AutoNorm monitors amplification in real time and dynamically determines the optimal endpoint for each reaction. By adjusting cycle numbers based on actual amplification kinetics, AutoNorm enables consistent library amplification across samples with widely varying DNA inputs.

To demonstrate the utility of this approach, a study was conducted using ExpressPlex to evaluate normalization with *E. coli* genomic DNA across a 100-fold input range from 2–200 ng. An additional experiment was performed using microbial genomic DNA samples spanning high, moderate, and low GC content across a 24-fold input range. In both experiments, AutoNorm dynamically adjusted amplification cycles on a per-well basis, expanding the effective normalization range far beyond the standard 5-fold range achievable with fixed-cycle PCR.

Experimental Design

Two experiments were performed with varied microbial input using ExpressPlex Library Prep and n6 AutoNorm Technology run in Slope Mode. In each experiment, following one-step tagmentation and amplification on the icon96, wells were pooled by equal volume and purified together in a magnetic bead-based reaction clean up. The pooled library was then sequenced on an Illumina NextSeq 2000.

Experiment 1: 100-fold Range of *E. coli* Genomic DNA Input

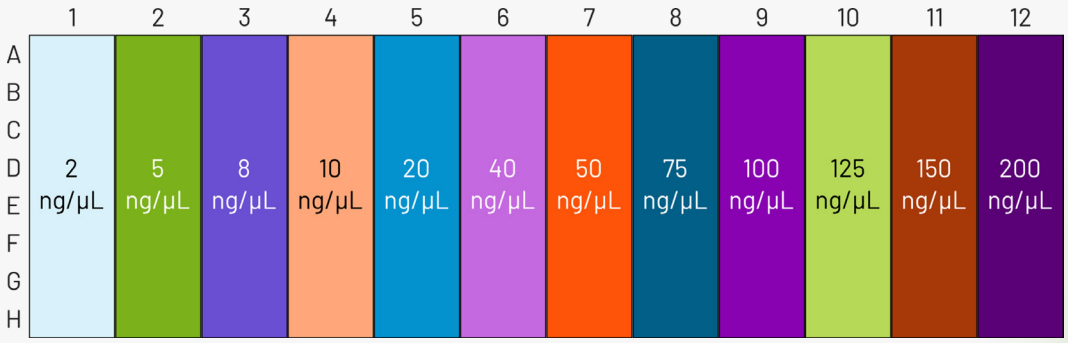

Figure 2. Plate layout for the *E. coli* input range experiment showing replicate wells spanning 2-200 ng DNA input.

Experiment 2: 24-fold Range using Microbes with Varied GC-content

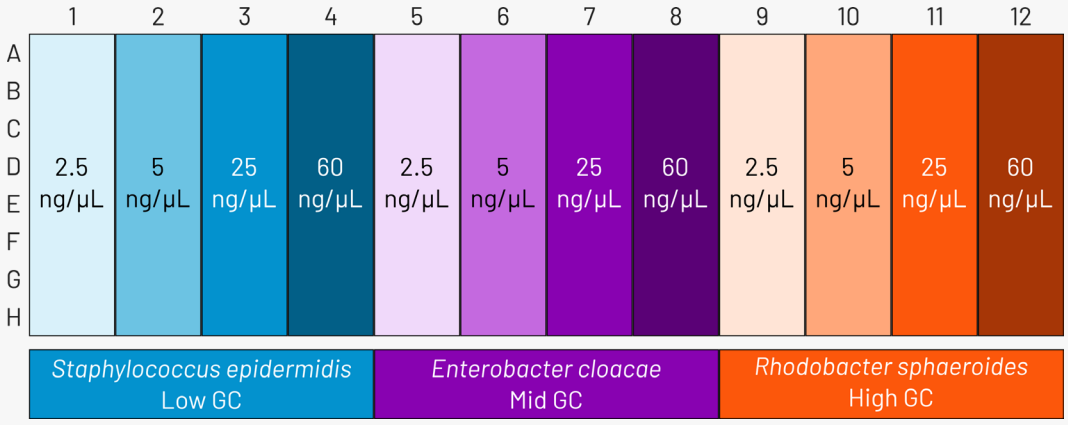

Figure 3. Plate layout for microbial genome experiment showing organisms with varying GC content.

Results

AutoNorm Expands Normalization Range of ExpressPlex

In both experiments, AutoNorm adaptive cycle termination using Slope mode resulted in amplification endpoints that varied across samples according to input DNA concentration (Figure 4). Lower input samples received additional amplification cycles while higher input samples terminated earlier in the PCR program ensuring consistent amplification outcomes across the plate. This behavior reflects per-sample control of amplification cycle number, where each reaction progresses to its optimal endpoint rather than using a fixed cycle number.

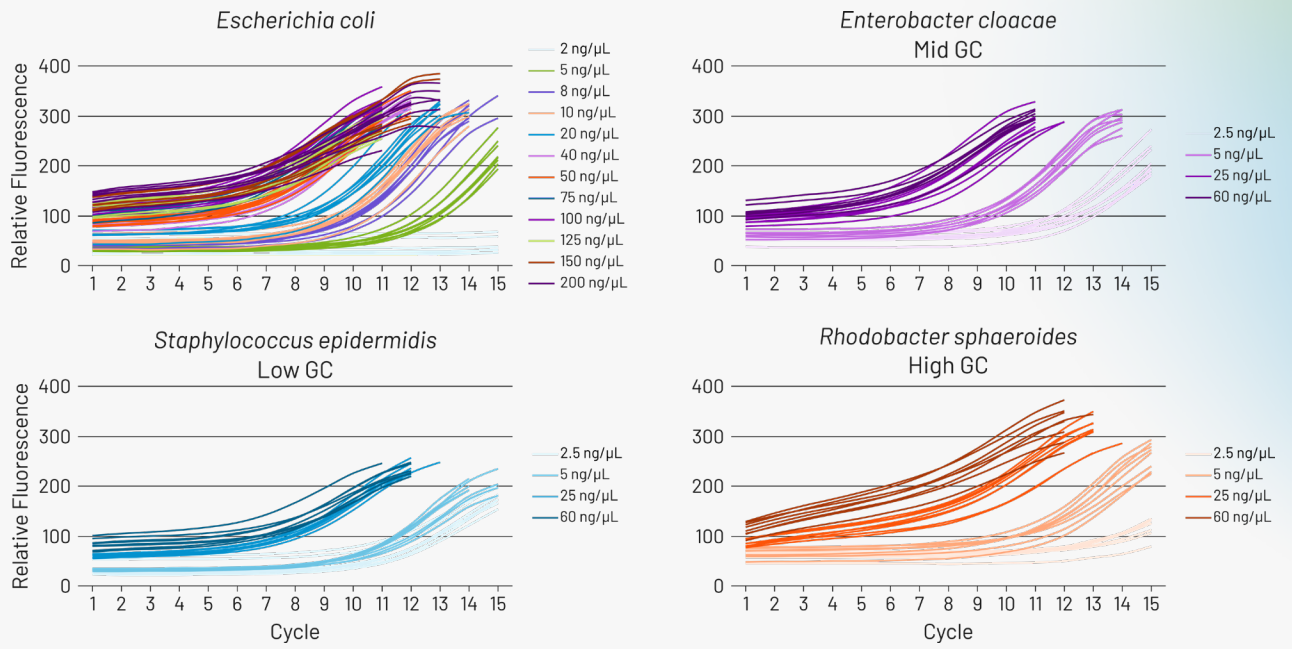

Figure 4. Amplification profiles from Experiment 1 (top left) and Experiment 2 (bottom left, top right, bottom right) show consistent amplification across a range of input concentrations and GC content.

AutoNorm Improves Sequencing Balance Across Variable DNA Inputs

Sequencing results demonstrated that adaptive amplification improved the balance of reads across multiplexed libraries. Libraries generated using AutoNorm produced more consistent sequencing representation across samples spanning a wide input range. Lower-input samples benefited from additional amplification cycles, resulting in increased library yield and improved representation within pooled sequencing runs.

Microbial sequencing workflows frequently include organisms with widely varying genome composition, including differences in GC content and genome complexity. These differences can influence amplification efficiency and may lead to uneven sequencing representation when fixed PCR cycling strategies are used. Sequencing results from libraries generated from organisms with varied GC content showed consistent library yield and balanced read representation across all organisms tested.

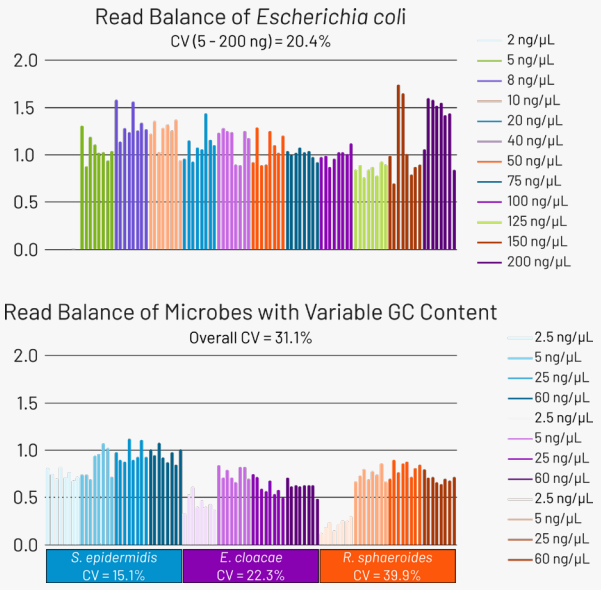

Figure 5. Read balance of *E. coli* libraries prepared across a 100-fold DNA input range (top) and multiplexed microbial genomes with varied GC content (bottom)

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Library Structure and Sequencing Quality Are Preserved

Sequencing analysis showed that adaptive amplification does not distort library structure. Insert size distributions remained consistent across libraries prepared using standard amplification and AutoNorm. GC bias profiles also remained comparable across genomic regions with varying GC content, indicating that adaptive amplification preserves expected library characteristics.

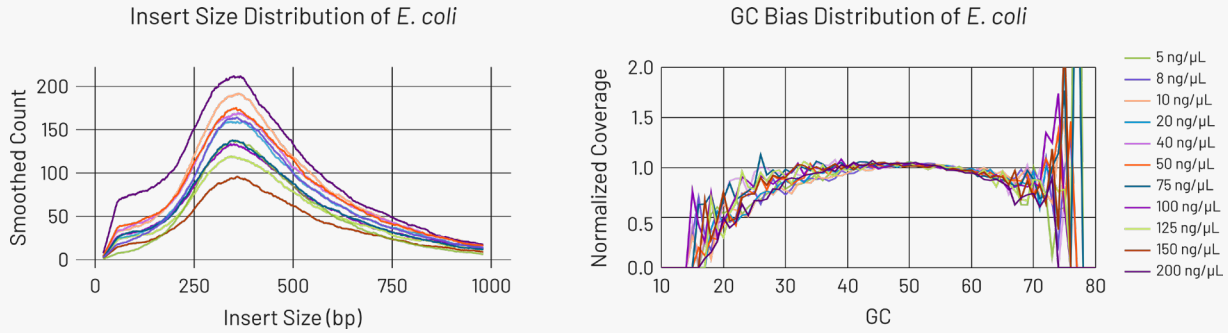

Figure 6. Insert size distributions (left) and GC bias profiles (right) demonstrate that adaptive amplification does not distort library structure nor introduce additional GC bias.

Conclusion

ExpressPlex Library Prep provides a streamlined and scalable workflow for microbial genome sequencing, with built-in normalization across a defined DNA input range using standard PCR methods. When combined with AutoNorm adaptive amplification on the n6 icon96 thermocycler, this workflow becomes significantly more robust to variation in DNA input.

By dynamically controlling amplification endpoints in real time, AutoNorm expands the effective normalization range from approximately 5-fold to up to 40-fold, reducing the need for precise input quantification and minimizing manual workflow optimization. This approach improves read balance across multiplexed libraries, increases yields for lower-input samples, and enables consistent library preparation across heterogeneous sample sets.

Importantly, these improvements are achieved while maintaining key library quality attributes, including consistent insert size distribution and minimal GC bias. Together, these results demonstrate that integrating AutoNorm with the ExpressPlex workflow simplifies microbial sequencing workflows and enables reliable, high-quality library preparation across a broad range of input conditions.

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