Application Note

Streamlined FFPE DNA Library Preparation Using NEBNext® UltraShear® and Multiplex Oligos Kits with **iconPCR™**

Overview

Fixed-cycle PCR workflows force researchers to choose a single cycle number based on assay requirements and input quantity. This approach is prone to inefficiency, particularly when processing samples with widely varying inputs or quality, because it requires precise quantification, batch segregation, and often multiple thermocycler runs. The challenge is exacerbated for FFPE-derived samples, where nucleic acid degradation breaks the link between input mass and the number of amplifiable fragments. This can lead to inconsistent amplification, over- or under-cycling, and neccessitates additional pre- and post-PCR quality control steps on a per-sample basis.

icon96™ overcomes these limitations with iconPCR technology and AutoNorm™, a real-time monitoring system that automatically ends each reaction when a predefined fluorescence threshold is reached. By tailoring amplification to the actual kinetics of each library, AutoNorm removes the guesswork of fixed-cycle PCR, eliminates the need for input-matched batching, and ensures that every sample reaches its optimal endpoint.

Figure 1. icon96, the world's first real-time thermocycler with 96 individually controlled wells.

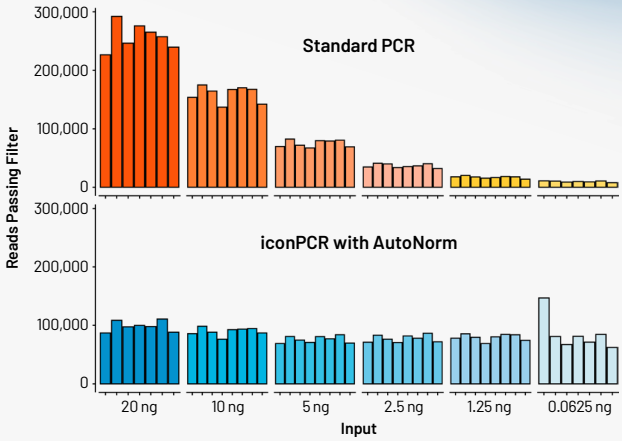

Figure 2. Here we show the significant yield variance when using a single PCR instrument with a fixed number of PCR cycles compared to iconPCR with AutoNorm where each sample is amplified to similar levels.

Experimental Design

Libraries were prepared from 1, 10, and 100 ng of DNA obtained from FFPE (colon and liver) and control (NA12878) samples. Each DNA input was processed using the NEBNext UltraShear® FFPE DNA Library Prep Kit (E6655L) and indexed with NEBNext® Multiplex Oligos for Illumina® (E6442S), followed by amplification using either standard PCR or iconPCR with AutoNorm.

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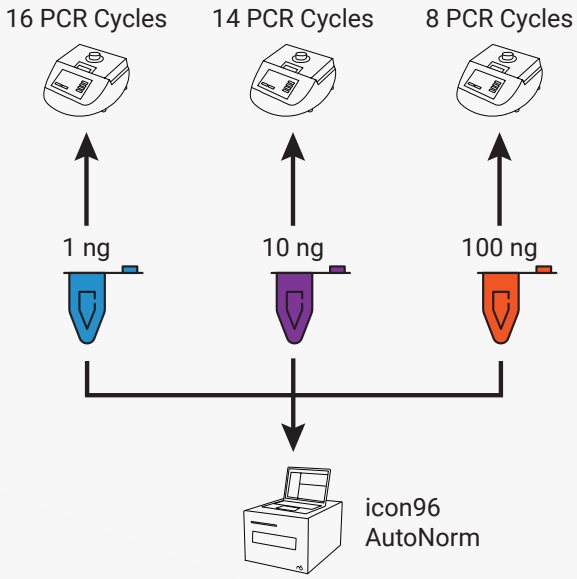

Figure 3. Experimental design comparing sample distribution across workflows. In the standard PCR workflow (top), samples were split into separate runs by input amount, increasing thermocycler time and handling steps. In the iconPCR workflow (bottom), all samples, regardless of input, were processed in a single run using AutoNorm, reducing run time and simplifying sample handling.

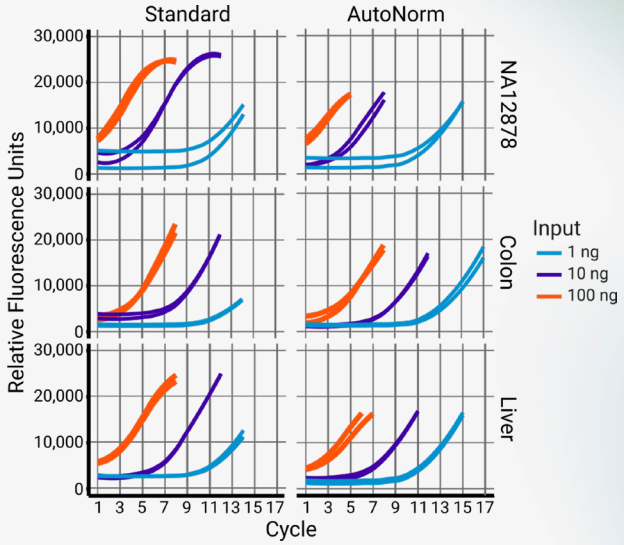

Figure 4. Consistent amplification across variable inputs achieved in a single icon96 run using AutoNorm. Standard PCR exhibited significant variability in endpoint fluorescence both between different input amounts within the same tissue and across tissues at identical inputs. In contrast, iconPCR with AutoNorm produced uniform endpoint RFU values, demonstrating improved amplification consistency.

Optimized Workflow

Standard PCR required three separate thermocycler runs of 14, 12, and 8 cycles, corresponding to inputs of 1 ng, 10 ng, and 100 ng, respectively. In contrast, all samples, regardless of input amount, were processed simultaneously on the icon96. With AutoNorm, cycle numbers were determined dynamically, and each well terminated amplification when fluorescence reached the point of maximal slope during the linear phase.

	Standard	AutoNorm		
Input	All Samples	FFPE Colon	FFPE Liver	NA12878
1 ng	14	17	15	15
10 ng	12	12	11	8
100 ng	8	8	7	5

Table 1. AutoNorm dynamically controls cycle numbers based on sample-specific amplification. The table presents the average stop cycle for each sample in the study. AutoNorm enables individual reactions to terminate at different cycles within a single PCR run, ensuring optimal amplification tailored to each sample. Notably, samples with the same input amount may require different cycle numbers to reach comparable amplification levels.

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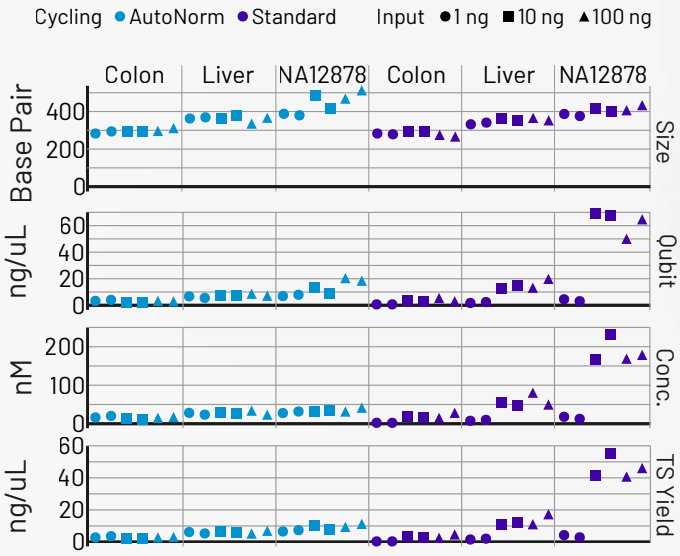

Figure 5. iconPCR enables effective normalization across input amounts. After library preparation, samples were individually SPRI purified and quantified. Libraries generated with iconPCR displayed markedly reduced concentration variability compared to those prepared with standard PCR, highlighting enhanced reproducibility.

Data Quality

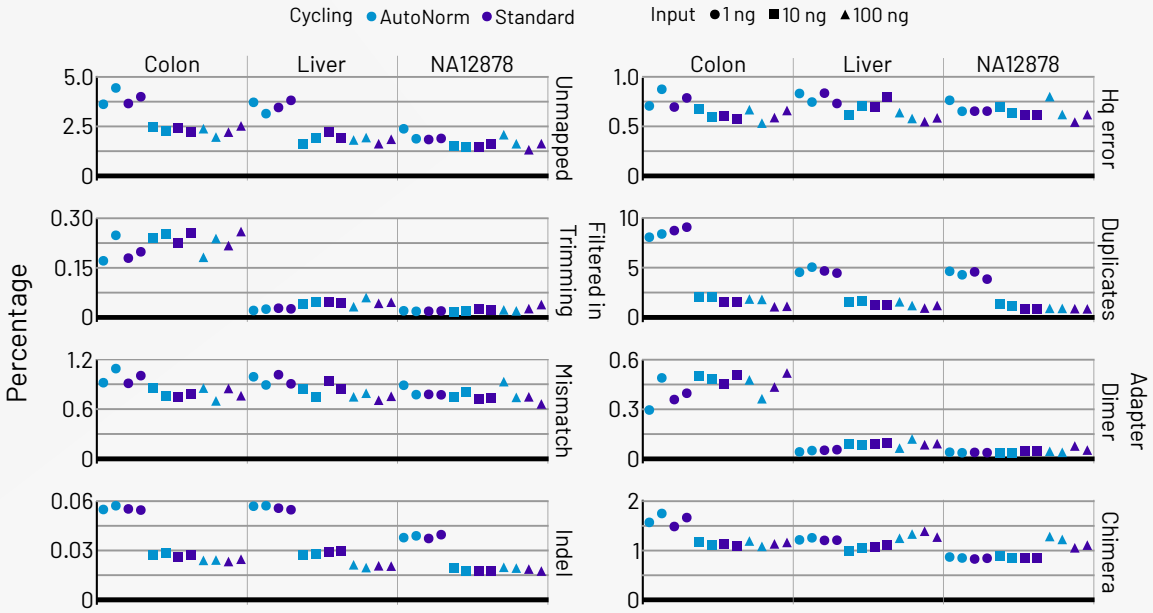

Figure 6. Variation in cycle numbers does not affect data quality. Across all input amounts and sample types, no significant differences in quality metrics were observed between fixed-cycle and AutoNorm conditions.

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Workflow Advantages of iconPCR

Conventional PCR	iconPCR (with AutoNorm)
Fixed cycle count (e.g., 16 cycles)	✓ Real-time fluorescence monitoring
One-size-fits-all amplification	✓ Per-well cycle control based on signal, not guesswork
Under/over-amplification common	✓ True-to-biology amplification: no overcycling, reduced dropouts
Variable library quality	✓ Uniform, balanced libraries across every well
Manual quant and normalization required	✓ Fully automated normalization
More hands-on time	✓ 40-60% reduction in hands-on time
Increased reagent waste	✓ Lower reagent waste and fewer failed libraries
Extra QC and rerun costs	✓ Faster turnaround with fewer QC/rescue steps

Conclusion

iconPCR redefines DNA library preparation by overcoming the constraints of traditional PCR workflows, particularly in the context of challenging FFPE samples. The unique per-well AutoNorm mechanism simplifies amplification and enables simultaneous processing of samples with varied input quantities and qualities, ensuring optimal endpoints for every sample. By integrating NEBNext UltraShear library preparation and NEBNext Multiplex Oligos for Illumina indexing with iconPCR automation, this combined approach establishes a reproducible and scalable workflow designed for consistency across diverse sample types. The innovation streamlines processing while maintaining high data quality, minimizes overamplification and batch variability, and delivers increased efficiency and reliability for both research and clinical sequencing applications. This synergy leverages the strengths of NEBNext kits and iconPCR to drive more robust and dependable genomic analyses.

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