Ultra-High Throughput Library Prep for High Quality, Large-Scale using Ultima Genomics & iconPCR
Driving Down the Cost, Scaling Up the Sample Volume, Production-Scale Sequencing

YEARS OF AGBT™

Abstract and Study Goals:

Ultima Genomics has revolutionized high-throughput, production scale sequencing while driving down the sequencing cost per genome to under $100. This increase in throughput has shifted the total cost per sample to the library preparation process. Labor, reagent and consumables represent the key cost contributors and bottlenecks, even when using liquid handling robots. The individual processing of each sample through SPRI cleanup, quantification, QC assessment and normalized pooling is a major bottleneck, particularly when attempting to scale to current sequencing output.

Using iconPCR, this study shows the efficiencies gained by employing iconPCR's AutoNormalization to enable >95% reduction in the time, consumables including tips, tubes and 96-well plates.

A dilution series of input DNA spanning 10-120 ng input were processed and sequenced to demonstrate the workflow improvements, cost savings, while maintaining high degree of uniformity in representation of each sample in a multiplexed sequencing run.

96—plex Droplet-Emulsion PCR (dePCR) Preparation of WGS Libraries

Study Goals:
To assess workflow optimization and library preparation efficiency, a 96-reaction study was designed to evaluate drastic reductions in the hands-on-time and manipulation steps using liquid handling robotics. In this study, DNA from the Genome in a Bottle sample HG006 (NA24694) was prepared in a dilution series from 10 ng to 120 ng input quantities in a 96-well plate using 8 replicates per concentration. A Control plate was prepared and processed through the standard amplification workflow. In parallel, replicate plates were processed using the iconPCR system to identify optimal Auto-Normalization settings.

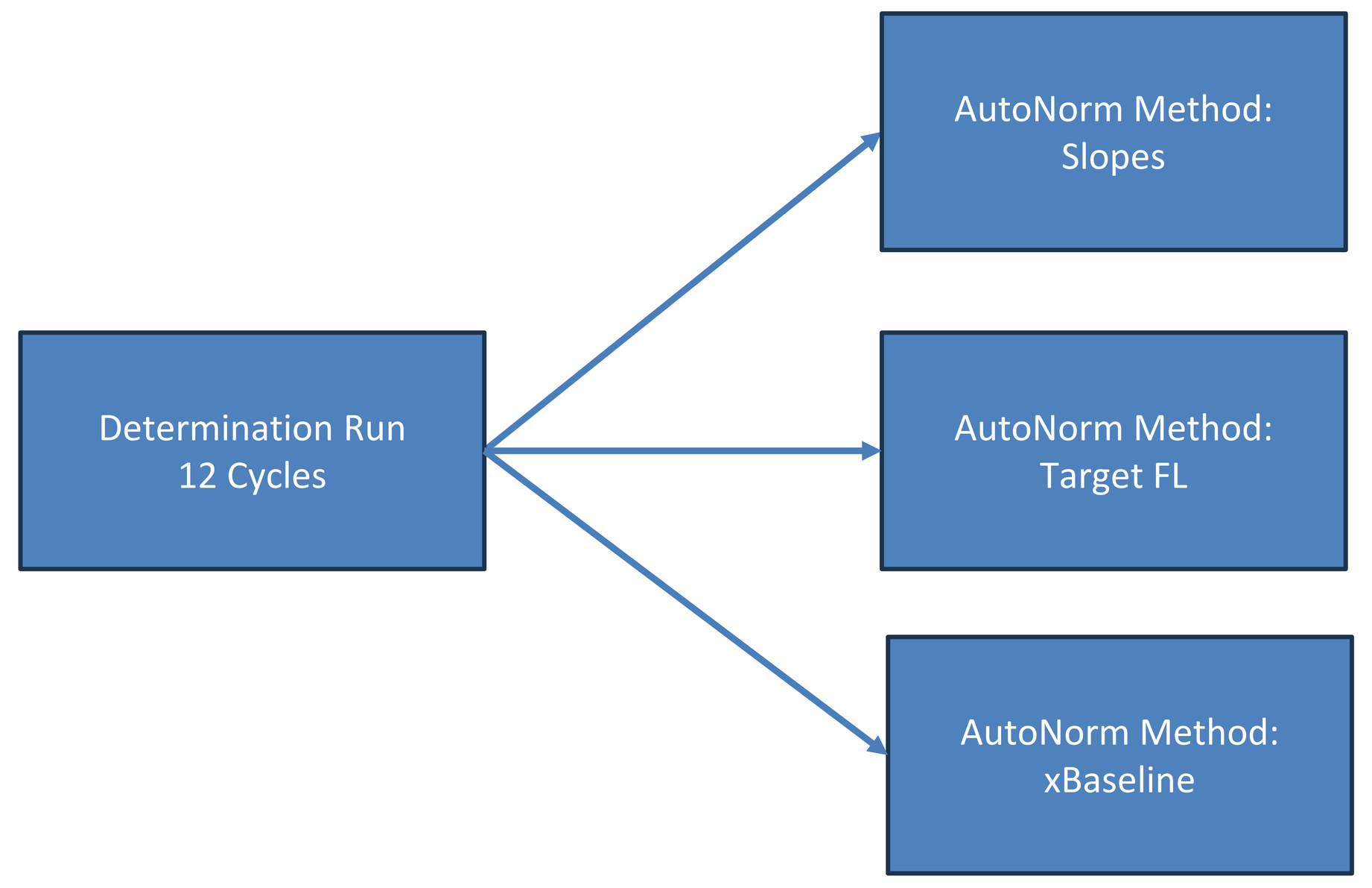
Control Plate Setup: The Control plate was split by input quantity and amplified in three separate thermocycler runs to enable different levels of amplification.

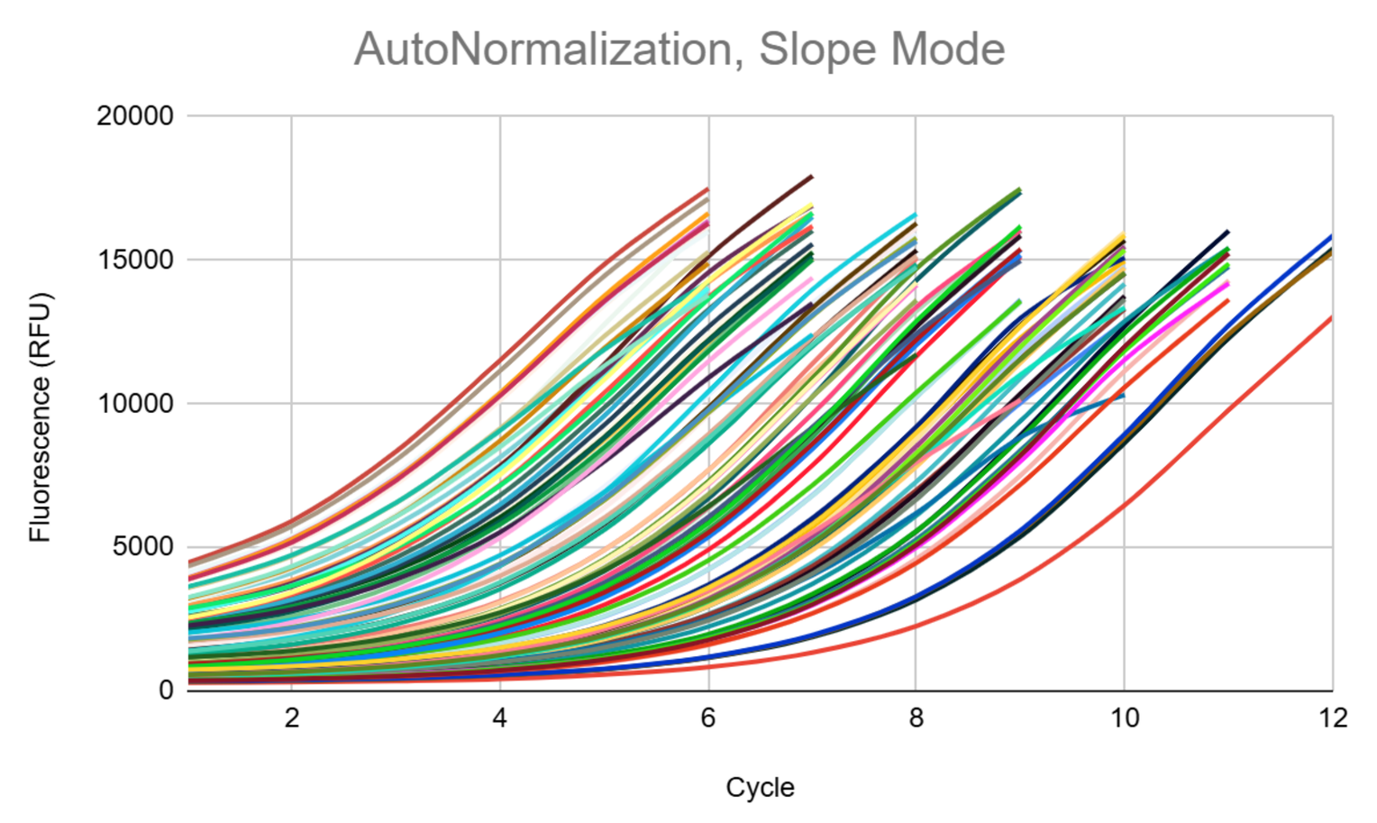
Post-amplification, samples were combined into a single 96-well plate for processing on a Hamilton liquid handling robot.

Control Samples: Input vs. # PCR Cycles

8 PCR Cycles		7 PCR Cycles					6 PCR Cycles				
10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng

IconPCR Plate Setup: Using a Determination run, the optimal thresholds were selected for all three AutoNorm methods: Slope, Target Fluorescence (FL) and xBaseline. Three plates were amplified with the different AutoNorm methods to demonstrate as options for variable yields and AN levels. The Target FL method was chosen for sequencing and comparison to the Control.

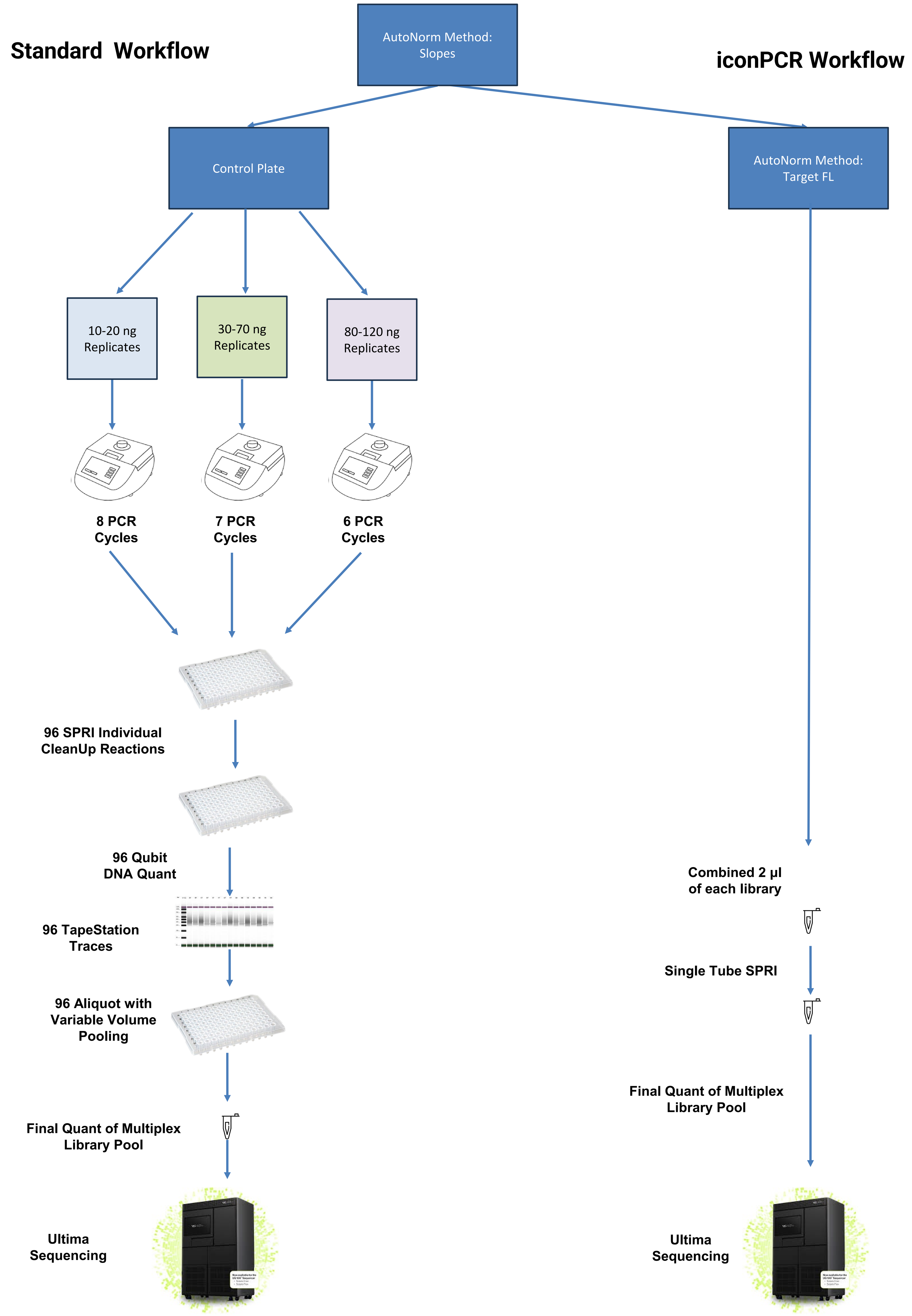




Slope Example Shown Above: Target FL AutoNorm selected for further processing and sequencing

The Target FL plate was selected for sequencing. A 2 µl aliquot from each sample wase pooled by combining an equal volume of each reaction into a single multiplexed pool.

Library Prep and Sequencing Summary Schematic



Assessing Library Recovery and Sample Dropouts

Sample Dropouts:
The Control workflow recorded seven sample dropouts and a high degree of variability in the 30 ng input amount. iconPCR had one dropout in the 30 ng input column

Control Workflow

	1	2	3	4	5	6	7	8	9	10	11	12
A	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
B	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
C	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
D	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
E	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
F	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
G	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
H	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng

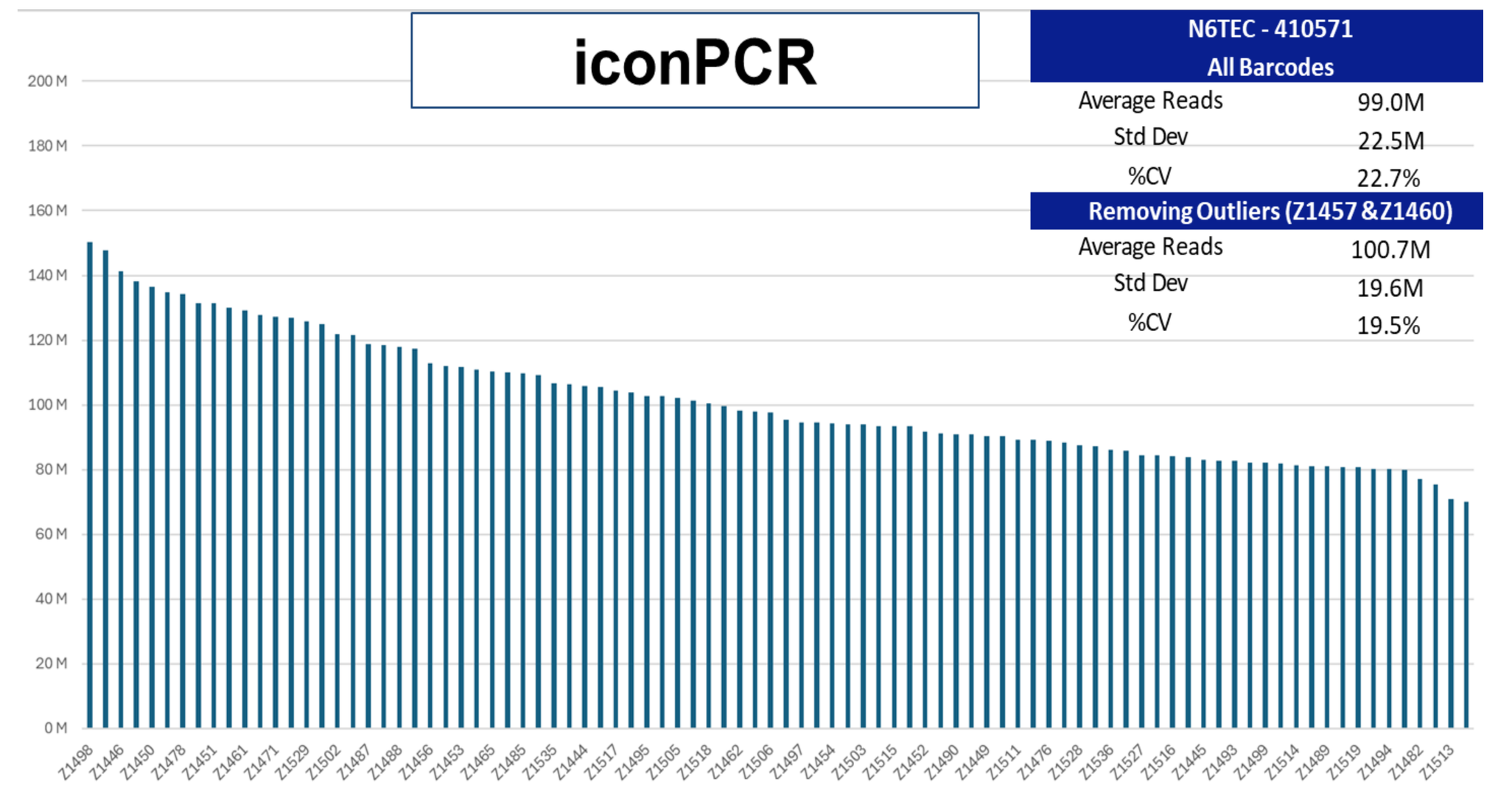
iconPCR

	1	2	3	4	5	6	7	8	9	10	11	12
A	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
B	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
C	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
D	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
E	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
F	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
G	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng
H	10ng	20 ng	30 ng	40 ng	50 ng	60 ng	70ng	80ng	90ng	100ng	110ng	120ng

The column containing the 30 ng input showed considerable variability compared to the rest of the plate in both the Control and iconPCR plates, most likely due to a technical error during plate setup. iconPCR was able to rescue 3 of 4 of the dropouts observed in the Control plate. Additionally, individual replicates in the 50, 80 and 110 ng inputs had dropout samples.

Library Balancing using Manual Normalization vs iconPCR AutoNormalization

Both the Control multiplex library and the AutoNormalized iconPCR pool were sequenced using a 2x150 bp sequencing run. The quantity of each library was measured and represented by each barcoded sample index. Average representation and variation (%CV) are shown for both workflows.

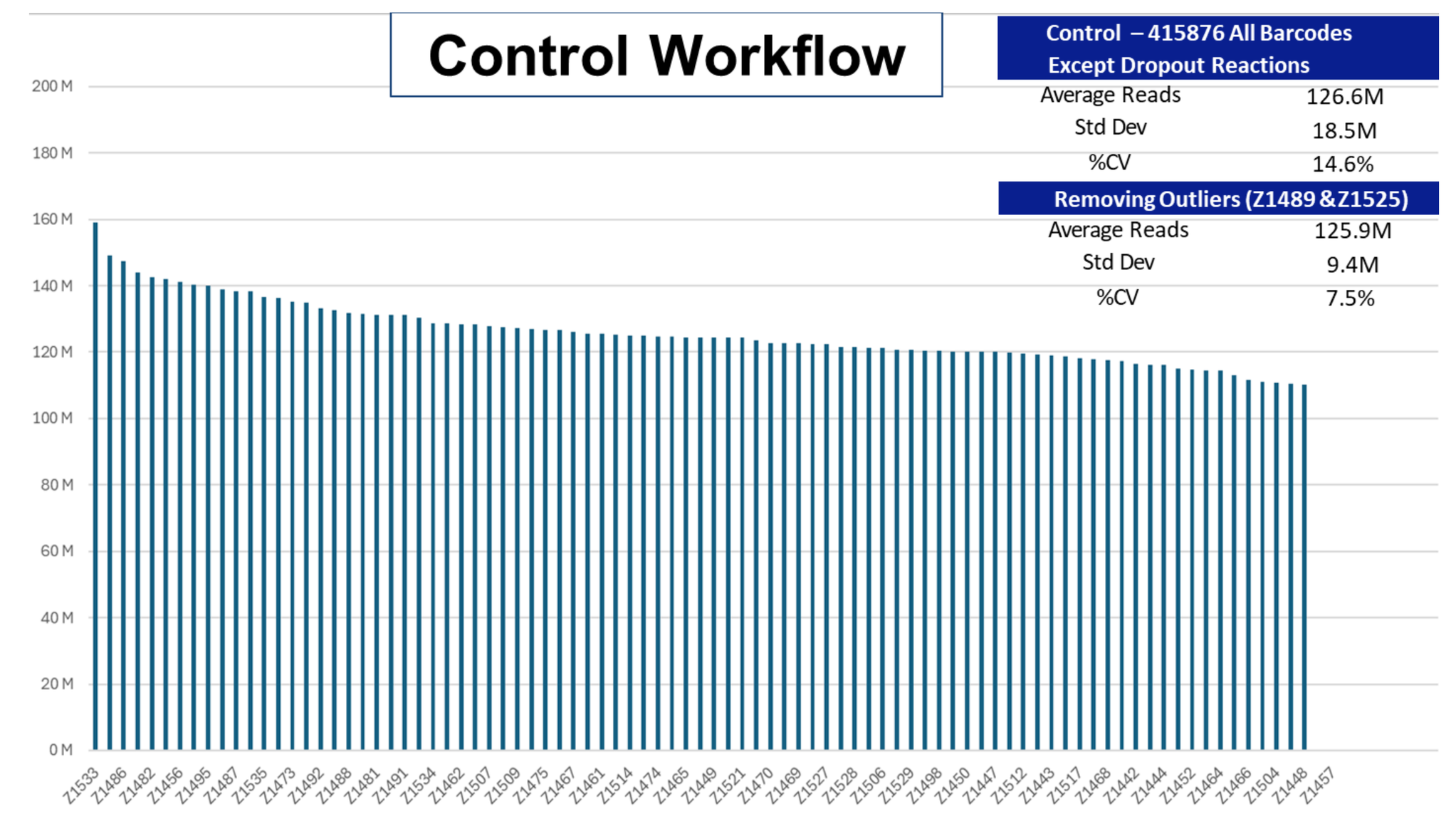


Pooling of the AutoNorm Run:

The multiplexed sequencing library was created by pooling 2 µl of each reaction. This method will reflect slight variations within the final concentration and is the most course method of pooling reflecting in up to 1 Ct difference between samples.

Refined balancing can be achieved by using the iconPCR Pooling by Fluorescence (FL) method. This uses the final RFU values from each well to adjust the volume of each sample.

Pooling by FL will result in barcode balancing on par with manual pooling (see below)



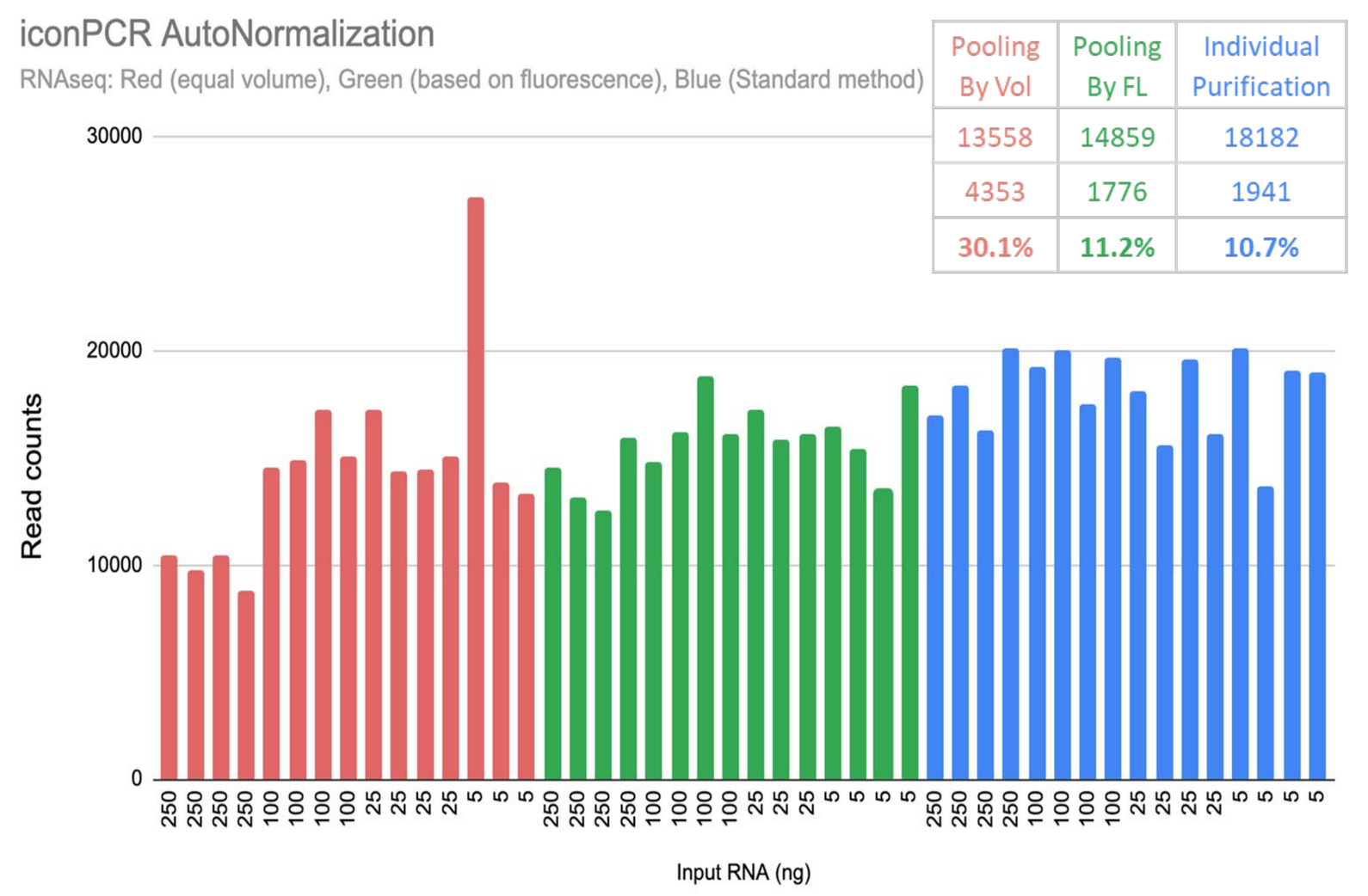
Pooling of the Control Run:

The percent coefficient of variation for the Control run generated a %CV of 7.5% which shows the high precision that reflects the additional time, effort and costs associated with legacy NGS workflows.

Pooling by Vol versus Pooling by FL (example):

From previous studies, using the final RFU value allows for higher precision for assay types requiring larger sequencing requirements per sample, such as WGS.

For sequencing-light assays, including RNA-seq, 16S and scRNA-seq, Pooling by Volume results provides a simple automation workflow as all samples are aliquoted in the same volume.



Example of Pooling Methods:

A 50X fold dilution series was generated for RNA-seq libraries ranging from 250 ng to 5 ng input. Libraries were amplified using iconPCR AutoNorm and the subsequent material was further processed using the standard purification, quant and pooling of each library (standard) as well as both of n6's recommended methods: Pooling by Vol and Pooling by FL.

Thus, researchers are able to choose between the different methods for the most economical and efficient sequencing option depending on assay type.

Pooling by FL Provides Equal Library Balancing as Manual Method:

Making adjustments to the pooling volume based on the End-point RFU values, the same level can be achieved as the laborious legacy method. N6 provides an analysis tool to directly make recommendations for all methods.