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Higher Throughput with the TruSight™ Tumor Sequencing Panel

GE Healthcare pushes the boundaries of sample throughput and DNA input with the TruSight Tumor Sequencing Panel.

Introduction

Clarient, a GE Healthcare company, is a leading provider of cancer diagnostic testing and biomarker discovery and development. Its goals include establishing "collaborative relationships with the healthcare community to translate cancer discovery and research into better patient care." To become the leader in cancer diagnostics, Clarient and its genomic sequencing division, Seqwright, develop innovative technologies that can lead to faster and more accurate cancer biomarker discovery for their biopharmaceutical customers. In an effort to obtain a higher throughput, lower sample input solid tumor profiling method, Clarient recently evaluated the TruSight Tumor Sequencing Panel and the MiSeq® desktop sequencer from Illumina.

Methods

Library preparation

DNA was extracted from formalin-fixed paraffin- embedded (FFPE) tissue samples (supplied by Clarient) previously identified as having variants that can be confirmed using the TruSight Tumor panel, according to the recommendations in the TruSight Tumor User Guide. After extraction, DNA was qualified by PCR. Samples were then used to prepare libraries using the TruSight Tumor panel.

DNA input amount was also evaluated, with samples of various ΔCq values serially diluted so that total input amounts ranged from 10 to 100 ng.

Sequencing

Sequencing was performed on the MiSeq instrument, following the instructions in the MiSeq System User Guide. A confirmation run with four libraries was assayed to ensure concordance with product specifications in a standard run. To evaluate higher throughput capabilities, the four libraries used in the confirmation run, plus eight additional libraries, for a total sample index of 12, were used in a single run on the MiSeq system. A second evaluation of the 4 original libraries plus 12 additional libraries, for a total of 16 indexed runs, was performed in a single run on the MiSeq system (Table 1).

Table 1: Quality and Quantity of DNA Used to Generate Libraries with the TruSight Tumor Sequencing Panel

Sample No.	DNA qPCR QC (Δ Cq)*	Starting DNA Amount (ng)	MiSeq Run 1	MiSeq Run 2	MiSeq Run 3
1	-1.43	226	X	X	X
2	1.96	332	X	X	X
3	5.79	604	X	X	X
4	5.62	483	X	X	X
5	4.02	574		X	X
6	0.72	281		X	X
7	0.51	813		X	X
8	0.33	227		X	X
9	1.14	173		X	X
10	-0.72	190		X	X
11	0.77	176		X	X
12	2.41	211		X	X
13	0.31	167			X
14	0.65	364			X
15	-1.56	190			X
16	0.01	146			X

* Samples with a ΔCq > 4 are considered to be outside the suitable range of DNA quality for library preparation following the TruSight Tumor User Guide. These low-quality samples were included to test the boundaries of the assay with both high-quality and low-quality samples.

Results

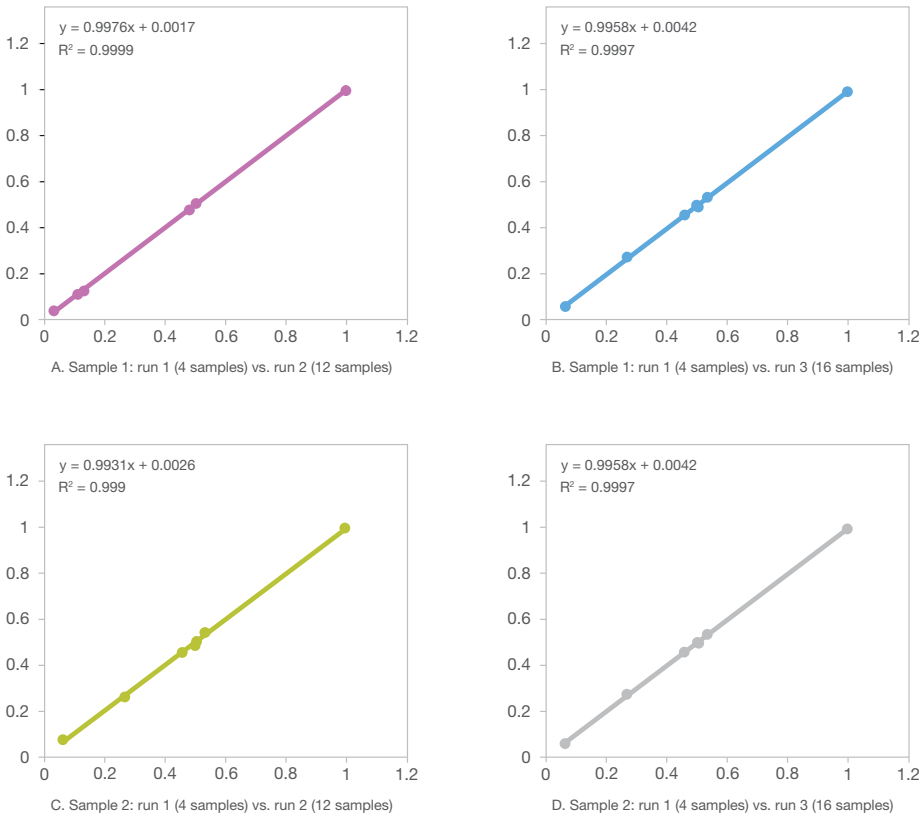
Sample indexing and DNA quality

Samples with a ΔCq < 4, those within the quality range recommended by the TruSight Tumor assay, showed excellent concordance between higher indexed runs. This was true for Samples 1 and 2 (ΔCq = -1.43 and 1.96, respectively, across the 4-, 12-, and 16-plex runs (Figure 1). Samples 3 and 4, with ΔCq = 5.79 and 5.62, respectively (both above the threshold recommended by the assay), showed lower concordance across the higher throughput runs, indicating that poor quality samples result in lower coverage and accuracy with higher multiplexed runs (Figure 2). Amplicon coverage can be reduced when indexing additional samples in a single run. The TruSight Tumor assay recommends minimum amplicon coverage of 1,000×, which is enforced by the analysis software. Figure 3 shows the relative number of reads per sample and the number, and nature, of amplicons that fall below the 1,000× threshold when samples are indexed above 4.

DNA quantity

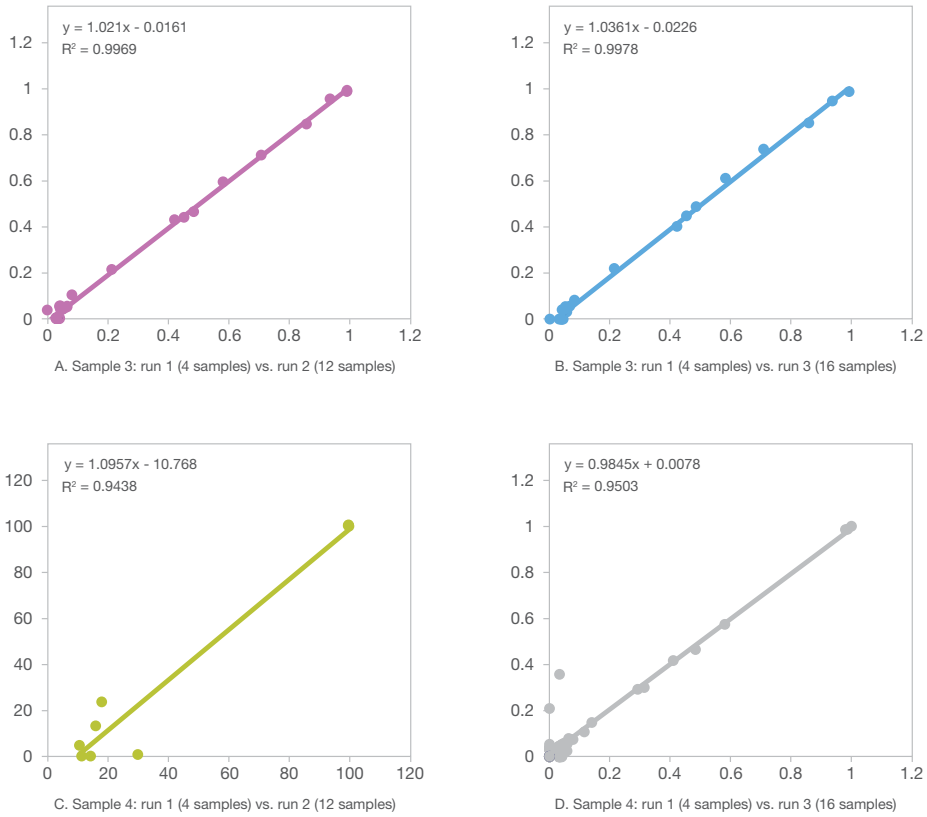
With TruSight Tumor, DNA input is determined based on the sample QC ΔCq values. To test the lower limits of DNA input, samples 1 and 3 were diluted and used to generate libraries. Libraries generated from Sample 1 show high concordance down to 20 ng (Figure 4). At 20 ng, sample 1 still showed high concordance with the standard DNA input used in library generation, but was divergent when lowered to 10 ng (Figure 5). Sample 3, which showed a ΔCq value beyond the recommended threshold for the assay, demonstrated poor performance when DNA input levels were diluted to 100 ng or less (data not shown). High-quality DNA samples, those passing sample QC, can be successful in the assay at lower than recommended DNA inputs. Poorly amplifying DNA samples, those with a ΔCq > 4, may not perform at any DNA input and alternate techniques, or an alternate sample, may be needed.

Figure 1: High-Quality Samples Show High Concordance Across Higher Sample Indexed Runs



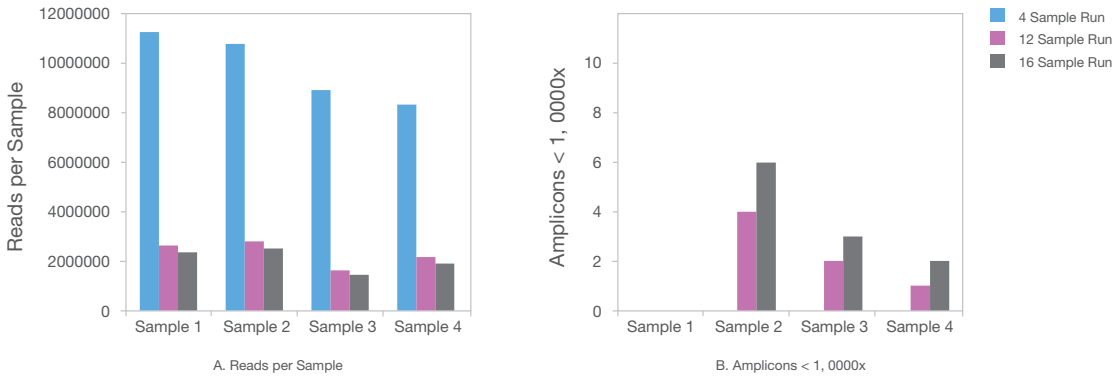
Two high-quality samples passing **Δ**Cq quality control were used in the TruSight Tumor assay across 4-, 12-, and 16-plex runs. Variant calls and frequencies were highly concordant between higher order indexed runs and the recommended 4 samples per run.

Figure 2: Samples With Higher ΔCq Values Show Reduced Concordance Across Higher Sample Indexed Runs



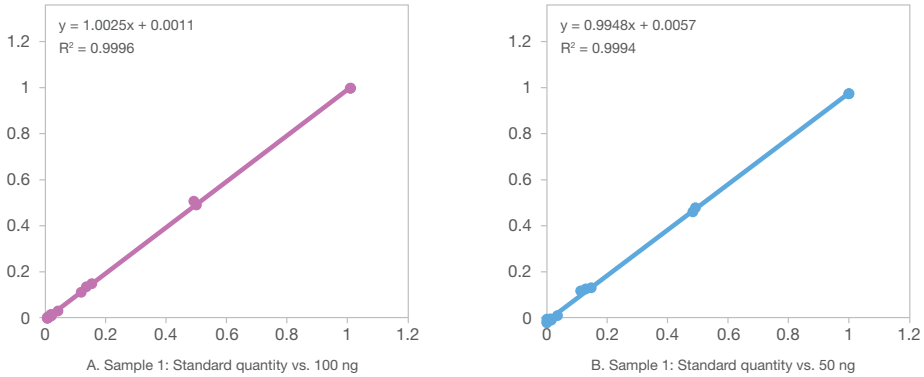
Two low-quality DNA samples (not passing **Δ**Cq quality control) were used in TruSight Tumor assays across 4-, 12-, and 16-plex runs. Variant calls and frequencies were less concordant among various levels of sample indexing compared to higher quality samples (ΔCq values < 4).

Figure 3: Reads and Amplicon Coverage with Higher Sample Indexing



The number of reads per sample did not decrease dramatically between 12 and 16 samples per run. Further, limited amplicons dropped below the 1,000× threshold set in the assay software at 12 and 16 samples per run. At 12 and 16 samples per run, up to 6 amplicons dropped below the threshold among the *FOLX2*, *SRC*, *STK11*, and *PTEN* genes. Sample 1, which passed QC, saw no amplicon drop out up to 12 samples per run. Sample 2, which also passed QC, saw amplicon drop out (< 1,000× coverage) in *FOXL2*, *STK11*, and *SRC*.

Figure 4: High Concordance of High-Quality Samples Down to 50 ng

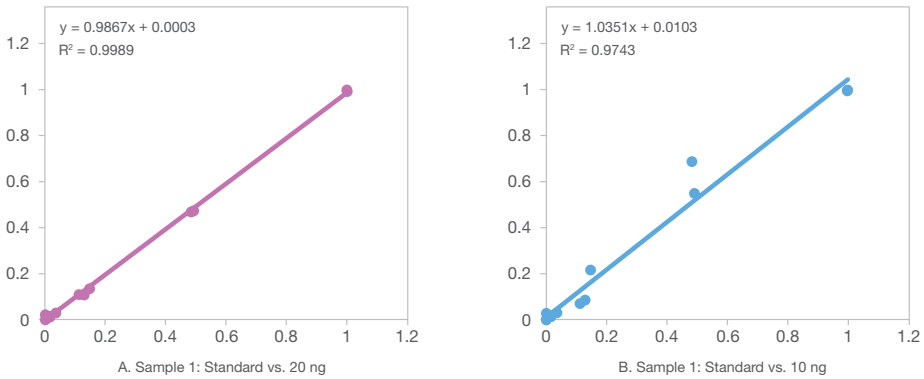


A. Sample 1: Standard quantity vs. 100 ng

B. Sample 1: Standard quantity vs. 50 ng

Samples were diluted to lower DNA inputs and used to generate libraries for the TruSight Tumor assay. High-quality samples showed high variant call and frequency concordance with the standard input amount, even when diluted by half.

Figure 5: Lower Limits of Input Amount with High-Quality Samples



A. Sample 1: Standard vs. 20 ng

B. Sample 1: Standard vs. 10 ng

Samples were diluted to lower DNA inputs and used to generate libraries for the TruSight Tumor assay. High-quality samples showed high variant call and frequency concordance with the standard input amount, even when diluted by half.

Conclusions

Using the TruSight Tumor Sequencing Panel on the MiSeq system, it is possible to multiplex up to 16 libraries and retain high-quality, accurate results, if the starting DNA is of high quality, with a ΔCq ≤ 4. Similarly, the amount of input DNA used to generate libraries can be decreased as long as the quality of the input material remains within the recommended limits.

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