Quality Control of DNA from Formalin-Fixed Paraffin-Embedded and Fresh-Frozen Tissues Prior to Target-Enrichment and Next Generation Sequencing

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Introduction

There are over 400 million formalin-fixed paraffin-embedded (FFPE) tissue samples archived in biobanks worldwide. These diseased and normal tissue collections are valuable resources for molecular genetic studies. However, the challenges of DNA extraction from FFPE tissues, including formaldehyde cross-linking, degradation, and mixtures of single-stranded and double-stranded DNA, result in low amounts of usable high quality material for downstream assays. Given this, assessing quality of samples that are to be processed for highly sensitive and costly applications, such as next generation sequencing, becomes a critical consideration. On-chip and automated electrophoretic devices were evaluated for the characterization of FFPE and fresh-frozen DNA samples prior to and during target-enrichment and next generation sequencing workflows.

Sample Preparation Workflow

Analysis for sample size distribution, purity, and concentration throughout the sample preparation workflow is critical for successful next-generation sequencing (NGS).

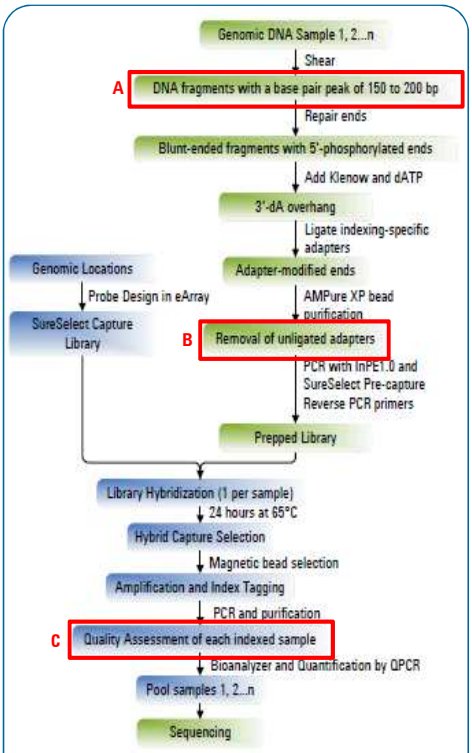


Figure 1. Sample preparation workflow for SureSelect target-enrichment. Steps requiring quality control (QC) are highlighted in red boxes (A: post-shearing, B: pre-capture amplification, C: post-capture amplification). The Agilent 2100 Bioanalyzer or 2200 TapeStation system is recommended for these steps.

Improvement in throughput capabilities of NGS instrumentation now requires an increase in automation and scalability of the library QC steps.

Here we compare the Agilent 2200 TapeStation, an instrument automating RNA, DNA and Protein QC, with the Agilent 2100 Bioanalyzer system, the industry standard for sizing and quantitation of fragmented DNA and DNA libraries in the NGS workflow.

On-chip Electrophoresis: Agilent 2100 Bioanalyzer System

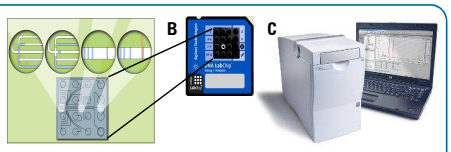


Figure 2. Components of the Agilent 2100 Bioanalyzer system: A) Electrophoretic analysis of samples within a microfabricated glass chip, B) Plastic caddy housing the glass chip and providing wells for sample loading, and C) Bioanalyzer system for control and analysis of data.

Automated Electrophoresis: Agilent 2200 TapeStation System

Figure 3. Components of the Agilent 2200 TapeStation system: A) ScreenTape with 16 individual separation lanes, and B) the Agilent 2200 TapeStation system which automates loading, separation, imaging, and analysis of samples in as little as 1 minute per sample.

Results

gDNA derived from patient FFPE tumor and matching fresh-frozen tumor, as well as control cell-line derived DNA were processed through the SureSelect protocol. QC analysis was performed on the Agilent 2100 Bioanalyzer and 2200 TapeStation systems.

Fragmentation profile

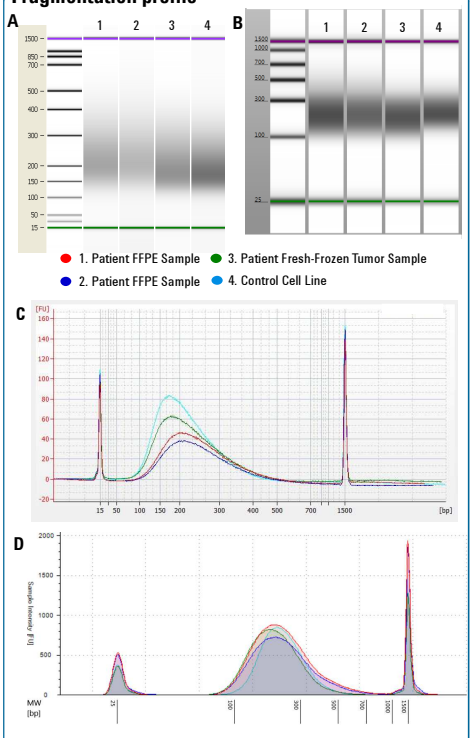
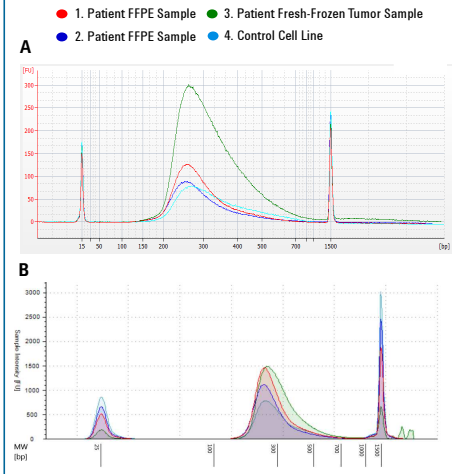


Figure 4. Post-shearing fragmentation profiles. Gel image of samples run on the Bioanalyzer system with the DNA 1000 kit (A) and on the TapeStation system with the D1K ScreenTape (B). Electropherograms of samples on the Bioanalyzer (C) and TapeStation (D).

Although peak shape differs, similar size distributions were detected with both systems.

Pre-Capture Amplification



C

Bioanalyzer Data	Avg size (bp)	Concentration (ng/µL)
1. Patient FFPE	309	34.8
2. Patient FFPE	313	22.6
3. Patient fresh frozen tumor	334	89.8
4. Control Cell Line	355	23.2

TapeStation Data	Avg size (bp)	Concentration (ng/µL)
1. Patient FFPE	305	31.7
2. Patient FFPE	301	20.0
3. Patient fresh frozen tumor	334	89.3
4. Control Cell Line	329	12.4

Figure 5. Quantitation and electropherogram profiles of pre-capture amplified samples. Electropherograms of libraries run on the Bioanalyzer system with the DNA 1000 kit (A) and on the TapeStation system with the D1K ScreenTape (B). Average size and quantitation of libraries from both systems (C).

Similar size distributions and concentrations were detected by both systems.

Post-Capture Amplification

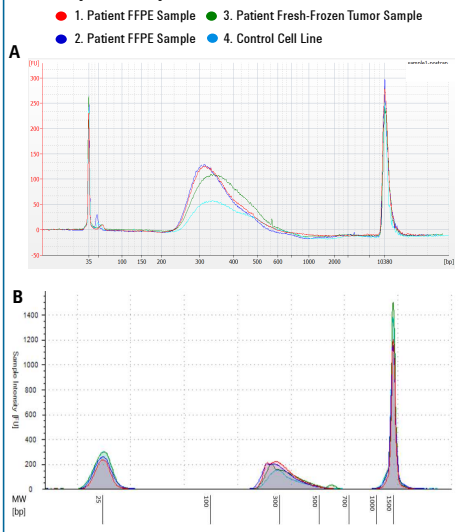


Figure 6. Electropherogram profiles of post-capture amplified libraries. Electropherograms of libraries run on the Bioanalyzer system with the High Sensitivity DNA kit (A) and on the TapeStation system with the High Sensitivity D1K ScreenTape (B).

Although profiles differ, assessment of all samples on both systems met yield and distribution recommendations for libraries going into sequencing (Illumina PE Multiplexed Sequencing, 2 x 76bp reads).

Conclusions

The Bioanalyzer and TapeStation systems yield comparable data for NGS sample quality control of FFPE samples. Although minor differences in the appearance of peak profiles exist, the calculation and determination of library statistics, specifically for peak size, distribution and concentration are comparable between the two systems. With its higher degree of automation the TapeStation system offers fast results, full scalability, and a simplified QC workflow.