DNA quality control of formalin-fixed paraffin-embedded and fresh-frozen tissues prior to target-enrichment and next generation sequencing

Application Note

Nucleic Acid Analysis

Authors

Melissa Huang Liu
Agilent Technologies, Inc.
La Jolla, CA USA

Maria Celeste Ramirez
Agilent Technologies, Inc.
Santa Clara, CA USA

Abstract

This Application Note describes the use of on-chip electrophoresis with the Agilent 2100 Bioanalyzer system for quality control of DNA samples from formalin-fixed paraffin-embedded and fresh-frozen tissues prior to and during SureSelect target enrichment in next generation sequencing workflows. Reliable DNA electrophoresis with the 2100 Bioanalyzer system provided smear profiles and details for library statistics, such as peak heights, average smear size, size distribution, and DNA concentration. FFPE DNA samples gave comparable results to DNA from fresh-frozen tissue and control DNA.

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Introduction

One of the most widely practiced methods of clinical sample preservation and archiving is the preparation of formalin-fixed paraffin-embedded (FFPE) tissue. There are over 400 million FFPE tissue samples archived in tissue banks, hospitals and laboratories worldwide. The tissues are collected by dissection or biopsy and then formalin-fixed and paraffin-embedded to maintain integrity for histopathology and microscopic investigations. Potentially, these collections of diseased and normal tissue may be valuable resources for molecular genetic studies. However, DNA extraction from FFPE samples has proved to be challenging[1]. Common issues with formaldehyde cross-linking, degradation, and mixtures of single-stranded and double-stranded DNA result in low amounts of suitable DNA material for downstream applications. Therefore, assessing the quality of samples to be processed for highly sensitive and costly applications, such as next generation sequencing (NGS), becomes a critical consideration.

The 2100 Bioanalyzer system has proven to be a valuable tool for sizing and quantification of fragmented DNA and DNA libraries in the NGS workflow[2]. This Application Note describes the use of on-chip electrophoresis with the 2100 Bioanalyzer system for the characterization of FFPE and fresh-frozen DNA samples in a NGS target enrichment workflow.

Experimental

Material

FFPE and DNA from fresh-frozen tissue were donated from Mount Sinai School of Medicine (New York, NY, USA), and NA10831-1 control DNA was acquired from the Coriell Institute for Medical Research (Camden, NJ, USA). A QIAmp DNA FFPE Tissue kit was purchased from QIAGEN GmbH (Hilden, Germany). An Ambion RecoverAll Total Nucleic Acid Isolation kit for FFPE was obtained from Life Technologies Corp. (Carlsbad, CA, USA.) An E220 Sample Preparation system was purchased from Covaris, Inc. (Woburn, MA, USA). 2100 Bioanalyzer System, an Agilent DNA 1000 kit, and an Agilent High Sensitivity DNA kit were obtained from Agilent Technologies (Waldbronn, Germany).

Sample preparation

Genomic DNA was extracted from patient FFPE tumor tissue using the QIAGEN FFPE Tissue kit and the Ambion Nucleic Acid Isolation kit. All samples were further processed through the SureSelect target enrichment protocol[3].

DNA quality control with on-chip electrophoresis

DNA electrophoresis was performed on the 2100 Bioanalyzer system in combination with the DNA 1000 kit or the High Sensitivity DNA Kit, according to the manufacturer's protocol[4,5]. Smear analysis of concentration and average sizes were performed in the Region table. Peak heights were determined in the Peak table, as described in the SureSelect protocol[3].

Results and discussion

For successful target-enrichment and NGS, it is critical that the quality of samples is evaluated at specific points during the sample preparation workflow. For this Application Note, genomic DNA (gDNA) derived from patient FFPE, matched gDNA from fresh-frozen tumor tissue, as well as control cell-line derived DNA were processed through the SureSelect protocol. DNA sample quality control (QC) was performed on the 2100 Bioanalyzer system at key steps: post-shearing fragmentation, precapture amplification, and post-capture amplification (Figure 1).

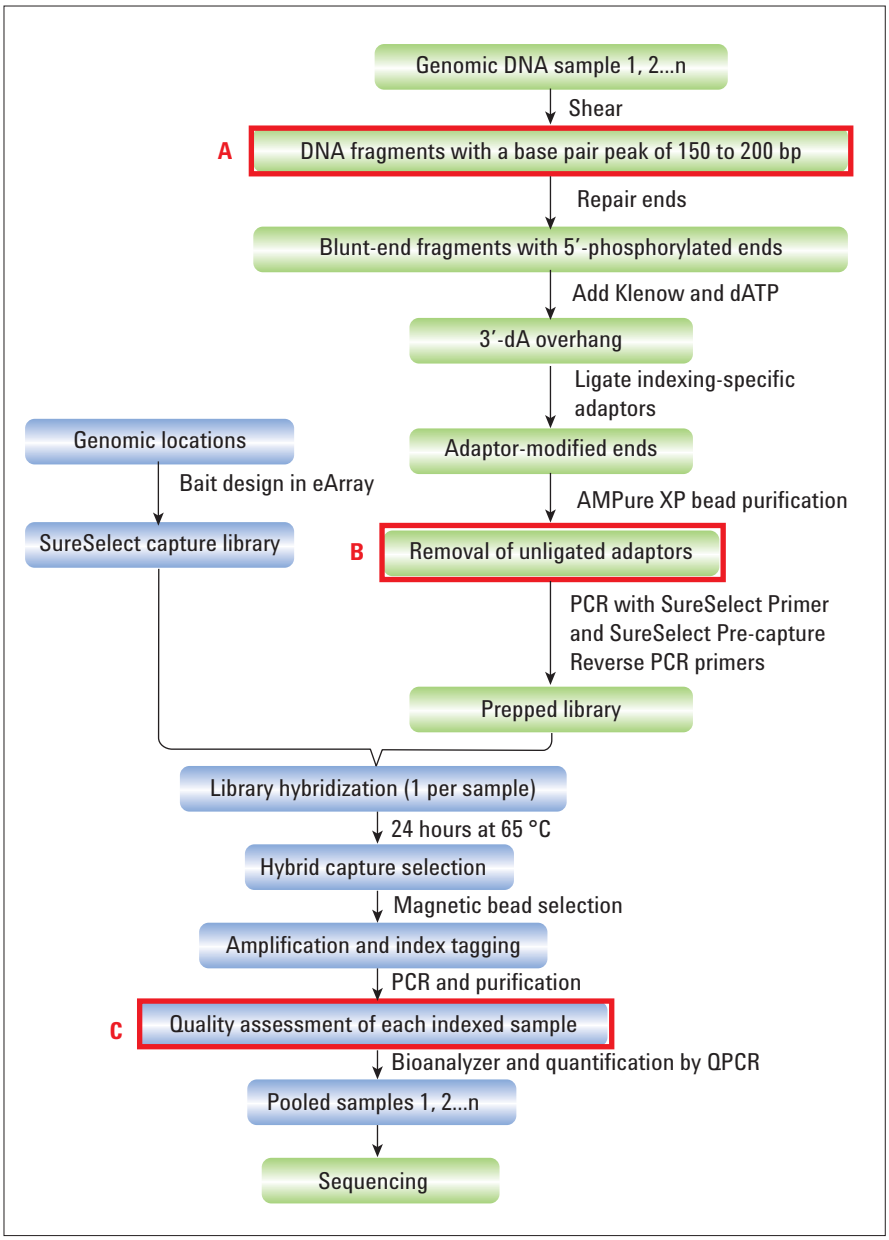

Figure 1
Sample preparation workflow for SureSelect target-enrichment. Steps requiring DNA QC are highlighted in red boxes (A: post-shearing, B: precapture amplification, C: post-capture amplification).

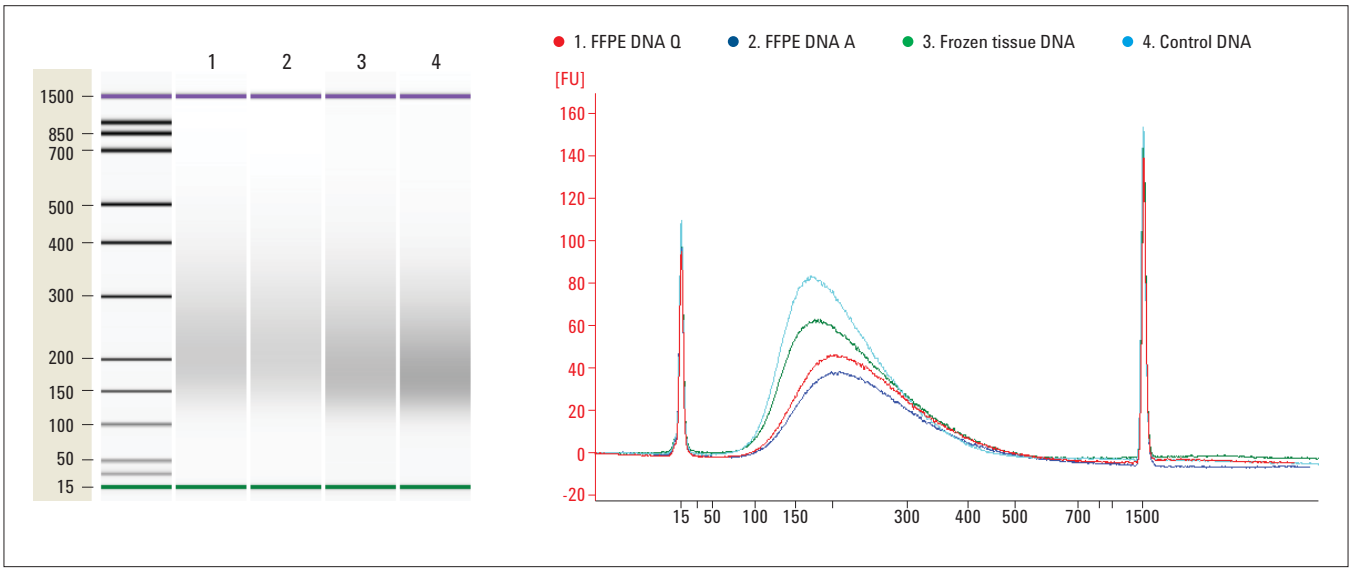

Figure 2
Post-shearing fragmentation profiles. Gel image and electropherogram overlay of samples run on the Agilent 2100 Bioanalyzer system with the Agilent DNA 1000 kit. Samples: FFPE DNA Q (red), FFPE DNA A (blue), DNA from fresh-frozen tissue (green), Control DNA (aqua).

Post-shearing fragmentation profile

DNA from FFPE samples was extracted using two different methods – the QIAGEN FFPE Tissue kit (FFPE DNA Q) and the Ambion Nucleic Acid Isolation kit (FFPE DNA A) – and compared to DNA from matched fresh-frozen tissue, and control DNA. All samples were fragmented using the Covaris system. Sheared samples were run on the DNA 1000 kit (Figure 2) showing comparable smears from 100 to 450 bases. In addition to sizing and purity information, the analysis with the 2100 Bioanalyzer system also provides the total DNA concentration and smear quantification within a specified region for each sample.

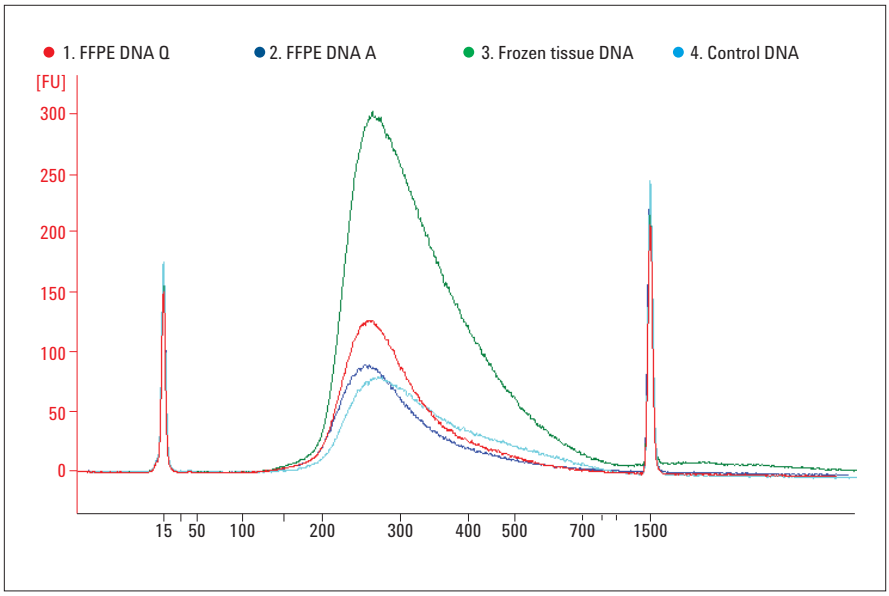

Figure 3
Electropherogram overlay of pre-capture amplified samples run on the Agilent 2100 Bioanalyzer system with the Agilent DNA 1000 kit. Samples: FFPE DNA Q (red), FFPE DNA A (blue), DNA from fresh-frozen tissue (green), Control DNA (aqua).

Sample	Average size [bp]	Peak height [bp]	Concentration [ng/µL]
FFPE DNA Q	307	264	34.8
FFPE DNA A	309	254	22.6
Frozen tissue DNA	331	264	89.5
Control DNA	348	271	23.2

Table 1
Average size, peak height, and quantification of precaptured amplified libraries.

Precapture amplification

The four DNA samples were further processed according to the sample preparation workflow (Figure 1). Five PCR cycles were used for the precapture amplification samples and then analyzed with the DNA 1000 kit on the 2100 Bioanalyzer system (Figure 3).

Similar profiles were observed for all DNA samples from approximately 100 to 900 bases. The average size ranged from 307 to 348 bases with maximum peak heights at 254 to 271 bases (Table 1). No amplification artifacts or primer dimers were seen. In addition, the 2100 Bioanalyzer system automatically provides the DNA concentration of the smears which varied from 23 to 90 ng/µL.

Post-capture amplification

The last DNA QC step in the SureSelect protocol required a 10-cycle amplification of post-capture samples, followed by analysis on the 2100 Bioanalyzer system with the High Sensitivity DNA kit (Figure 4). Comparable electropherogram profiles were observed for all four samples with smears from 225 to 600 bases with peak heights of 314 to 336 bases (Table 2). The size distribution and the yield of the DNA libraries were within the recommendations for Illumina paired-end multiplexed sequencing (2 × 76 bp reads).

Conclusion

The 2100 Bioanalyzer system was used for NGS sample QC of FFPE samples during the sample preparation workflow for SureSelect target enrichment. Reliable on-chip DNA electrophoresis provided smear profiles and details for library statistics, such as peak heights, average smear size, size distribution, and DNA concentration. FFPE, matched fresh-frozen tissue, and control cell line DNA gave suitable results appropriate for downstream sequencing on the Illumina platform.

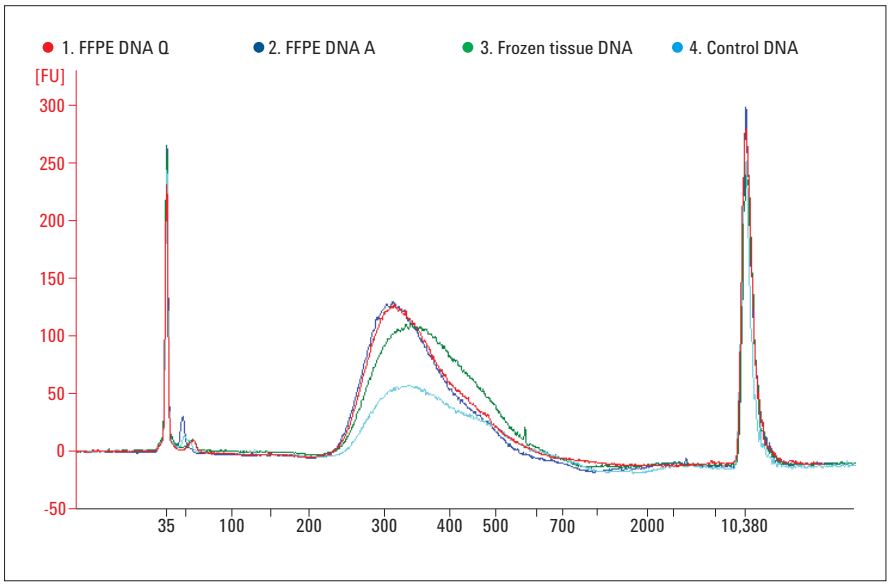

Figure 4
Electropherogram overlay of post-capture amplified libraries run on the Agilent 2100 Bioanalyzer system with the High Sensitivity DNA kit. Samples: FFPE DNA Q (red), FFPE DNA A (blue), DNA from fresh frozen tissue (green), Control DNA (aqua).

Sample	Average size [bp]	Peak height [bp]	Concentration [pg/µL]
FFPE DNA Q	357	316	572.0
FFPE DNA A	348	314	609.4
Frozen tissue DNA	376	340	676.3
Control DNA	382	336	415.2

Table 2
Average size, peak height, and quantification of post-captured amplified libraries.

References

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