Development of a Multiplexed Panel for the Identification and Quality Control of Formalin-Fixed, Paraffin-Embedded Samples using the MassARRAY® System

INTRODUCTION

Highly-annotated, well-sourced bio-specimens are essential in elucidating cancer initiation and progression and in validating panels for clinically actionable use. Formalin-fixed, paraffin-embedded (FFPE) tissue archives are most commonly used for oncology research and validation studies. However, accurate mutation detection is often problematic in FFPE tissue since it tends to have highly variable levels of fragmentation that limit the amount of usable DNA templates. In addition, formalin-introduced sequence artifacts and PCR inhibitors further obstruct mutation detection. The lack of consistency between the various protocols for sample handling and extraction of DNA is one of the major obstacles in translating analysis to clinical practice.

This technical note describes the development of a multiplexed panel to determine sample quality and identification (ID) using the MassARRAY® System. This panel performs DNA quality assessment and template copy number enumeration across a broad dynamic range of copies and monitors sample fragment size over a 100-500 bp range, while uniquely identifying the sample using exonic SNPs.

ASSAY DESIGN

The Assays by Agena (ABA) Exome QC Panel is a set of pre-verified assays to assess DNA quality and amplifiable template copy number across a range of 100-100,000 copies (0.3-300 ng) and monitor sample fragment size between 100-500 bp. The DNA quality is assessed using 25 competitive PCR assays targeting known housekeeping genes. These genes are selected from cancer studies where they exhibit low levels of germline and somatic copy number alterations, SNPs, and somatic mutations within the coding sequence. The intact, amplifiable template copy number across a size range of 100, 200, 300, 400, and 500 nucleotides is assessed using 5 assays at each amplicon size, with 300, 1000, 3000, 10,000, or 100,000 copies of competitive template. Sample identification and matching is done via 21 exonic SNPs, with high minor allele frequency reported across major HapMart populations, along with 3 XY paralogues for gender determination. Completely automated software supports localized historical identity matching and sample quality assessment.

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ABA EXOME QC PANEL ASSAYS FOR SAMPLE IDENTITY, QUALITY, AND TEMPLATE COPY NUMBER

SAMPLE QUALITY			SAMPLE IDENTITY
Target	**Amplicon size**	**Competitive template copy number**	**Target**
POLR2A	100bp	300	rs1045728
POLR2A	100bp	1000	rs10495563
TBP	100bp	3000	rs1065457
ALB	100bp	10000	rs1127379
IPO8	100bp	30000	rs11998387
POLR2A	200bp	300	rs1200349
POLR2A	200bp	1000	rs12594531
TBP	200bp	3000	rs1344
IPO8	200bp	10000	rs17548783
HBG2	300bp	300	rs2246209
GUSB	300bp	1000	rs2273171
AHSP	300bp	3000	rs2301771
RPL27A	300bp	3000	rs3743165
TPT1	300bp	10000	rs3884596
VIM	300bp	30000	rs4478844
POLR2A	400bp	300	rs586421
RPL27A	400bp	1000	rs6420424
HBS1L	400bp	3000	rs6977125
TPT1	400bp	10000	rs7653897
IPO8	400bp	30000	rs773901
PDCD2	500bp	300	rs9131
TBP	500bp	1000	AHSP
PPIA	500bp	3000	AMEL
HBS1L	500bp	10000	ARSD
PDCD2	500bp	30000	

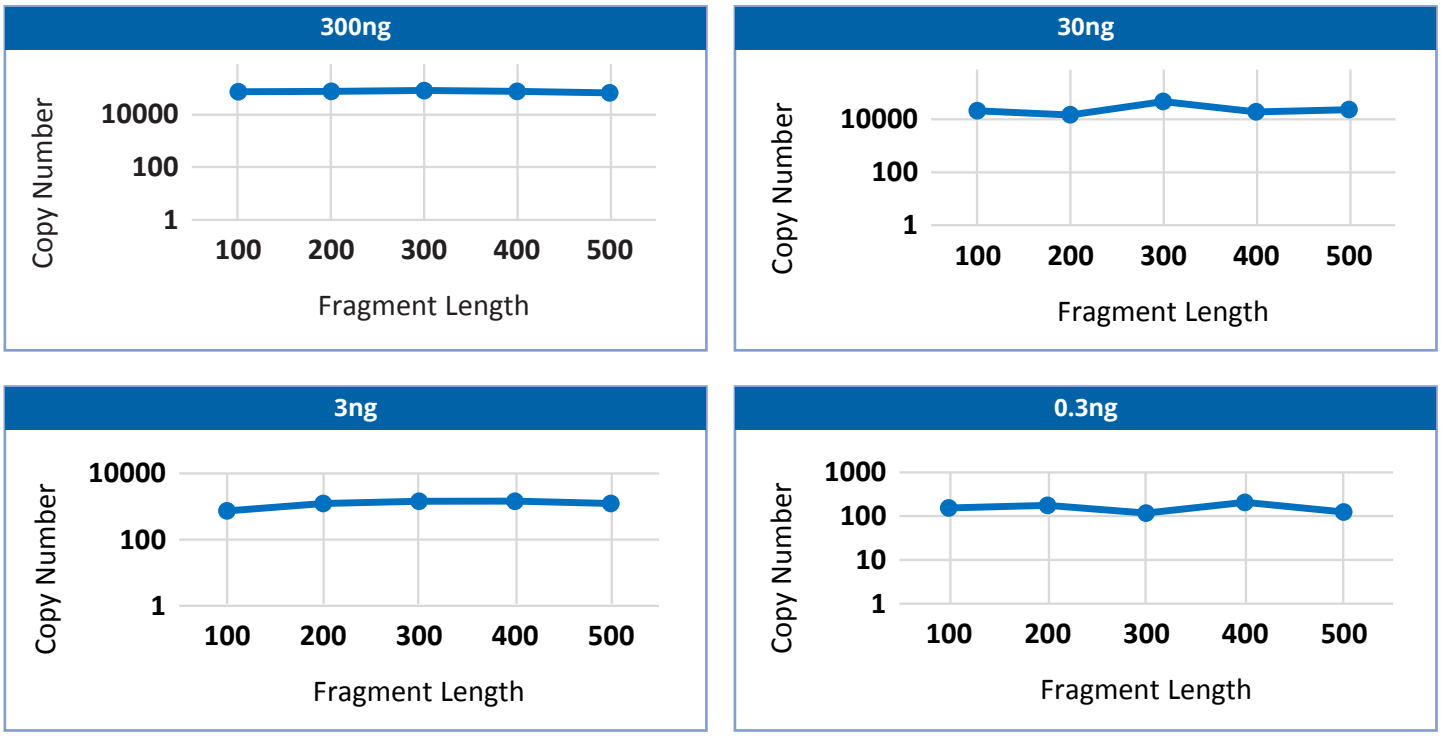
MATERIALS AND METHODS

DNA used to assess the Assays by Agena Exome QC Panel performance originated from two sources: 192 HapMap DNA samples and 48 archived FFPE tissue samples, which were extracted using the QIAamp® DNA FFPE Tissue Kit. The panel is composed of a single multiplexed reaction requiring only 2-10 ng input DNA. Twenty-one SNPs are sufficient to identify sample chain of custody disruptions in most clinical and NGS laboratory settings. Included in this panel are 3 X/Y paralogue assays for gender determination and 25 CNV and QC assays across 5X5 degradation curves (see Table 1). iPLEX® Pro biochemistry was utilized to amplify the specific regions, followed by single base extension into the known SNP (see Table 1). The extension products were then desalted, dispensed onto a SpectroCHIP® Array, and detected via MassARRAY MALDI-TOF mass spectrometry. Sample reports were generated and analysis performed using MassARRAY Typer 4 automated software. Microsoft Excel was used to generate the degradation curves.

RESULTS

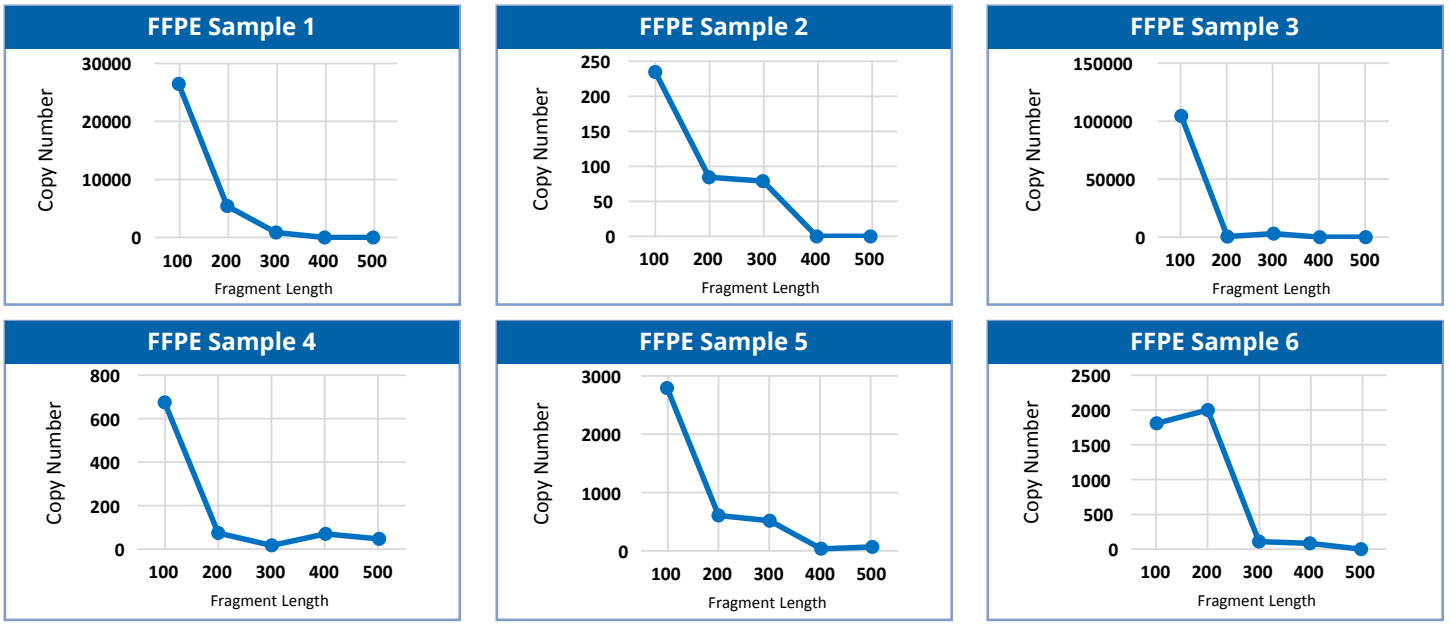
A dilution series with the 192 HapMap samples was performed, at varying starting concentrations. Sample NA11994 was used as a representative of these samples. With a starting concentration of approximately 300 ng, input DNA from NA11994 was titrated 10-fold to 30 ng, 3 ng, and 0.3 ng (Figure 1). Our data indicates that amplifiable intact template molecules (copy number) for each target amplicon size (fragment length) closely match input concentration throughout the dilution series. The Exome QC Panel was then applied to FFPE (n=48) samples that were archived for varying periods of time to assess amplifiable DNA copy number and template fragmentation. Varying levels of degradation/template fragmentation were observed (Figure 2) in each of the samples tested. As an example of this in Figure 2, Sample 3 shows extensive degradation with little to no fragments present above 100 bp. Although the samples had variability in copy number, all samples showed a trend towards lower template fragment size (100-200 bp), indicating high levels of degradation and fragmentation, with some samples having very low overall concentration.

Figure 1: Dilution Series



A dilution series of DNA from 192 HapMap samples were run using the Exome QC Panel. HapMap sample NA11994 is used as a representative of the 192. NA11994 had a starting concentration of approximately 300 ng (100,000 copies), was then titrated to 30 ng (9,000 copies), 3 ng (900 copies), and 0.3 ng (100 copies). The X axis represents the target assay amplicon size (fragment length in bp) and the Y axis represents the number of amplifiable intact template molecules or copy number for each target amplicon size. Template copy number closely matches input concentration throughout dilution series.

Figure 2: Degradation Curves



The X axis represents the target assay amplicon size (fragment length in bp) and the Y axis represents the number of amplifiable intact template molecules (copy number) for each target amplicon size. Six FFPE samples are shown with varying levels of degradation/template fragmentation. The Exome QC Panel was applied to archived FFPE (n=48) samples to assess amplifiable DNA copy number and template fragmentation.

SUMMARY

The Assays by Agena Exome QC Panel assesses DNA quality and amplifiable template copy number across a broad dynamic range while monitoring sample fragment size and identity. This improved QC is accomplished by the generation of a degradation curve using the absolute number of amplifiable template copies vs. amplicon length. From this study, it was found that FFPE samples exhibit a high degree of degradation. This degradation would severely impact the quality of downstream applications that require longer template such as Sanger sequencing, NGS, exome sequencing, and cytogenetics arrays.

By providing a single reaction solution for both sample ID and QC, the Exome QC Panel is a cost-effective and comprehensive resource for pre-analytical quality assessment and workflow identity authentication of potentially degraded clinical samples.

REFERENCE

1. American Association of Clinical Chemistry Annual Conference, Identification and Quality Control of Formalin-Fixed Paraffin-Embedded Samples using Agena Bioscience ExomeQC Panel, 2015 Poster (A-268).

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