FFPE DNA Quality: Impact on Downstream Performance in Molecular Diagnostic Assays

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Introduction

Personalized medicine for oncology patients is driven by mutation profiling of tumors. Improved robust DNA extraction methods that produce high quality FFPE DNA template suitable for complex molecular diagnostic testing are needed. In addition to DNA fragmentation and protein-DNA cross linking, some FFPE tissues contain inhibitors and/or quenching substances (e.g. melanin) that adversely affect downstream performance in PCR and sequencing assays. In this study, we provide a side-by-side evaluation of two DNA isolation methods from FFPE tissue of various tumor types: a manual, column-based method (MO BIO) and the Maxwell CSC automated platform (Maxwell).

Materials & Methods

FFPE tissue from melanoma, GIST, endometrial, and NSCLC tumor types was obtained from equivalent numbers of slides for each extraction method. Manual extractions were performed using the MO BIO BiOstic FFPE Tissue DNA Isolation Kit followed in some instances by an additional purification step using the Zymo Research OneStep PCR Inhibitor Removal Kit to rid DNA isolates of visible melanin. Automated extractions were carried out using the Maxwell CSC DNA FFPE Custom Kit on the Maxwell CSC instrument with no additional PCR inhibitor removal steps. DNA eluate yields and optical densities were determined via spectrophotometry. Equivalent input DNA amounts from melanoma samples were assayed using both NRAS allele-specific qPCR (25 ng) and NRAS Sanger sequencing (200 ng) on the BIORAD CFX platform and Applied Biosystems 3730 DNA Analyzer, respectively. NRAS qPCR data were reported as delta-Ct values to determine qPCR differences. Sanger sequencing Phred scores were analyzed for the 51-base pair window surrounding the NRAS codon 61 hotspot to quantitatively determine sequence-trace quality. Quality metrics were further evaluated using the CodonCode Aligner and Sequence Scanner software packages.

Results

Both extraction methods require the same upfront steps of scraping FFPE tissue, reagent addition, and lysis/incubation, however, the Maxwell method requires fewer hands-on, liquid-handling steps (Fig. 1). Additional Zymo cleanup steps were required for 2 manually-extracted samples due to visible melanin in the FFPE tissue/eluted DNA (Fig. 2; Samples 02 and 07). DNA yields were greater for samples processed on the Maxwell CSC (30 of 34 total samples, 88%; 1.9-fold increase overall; Figs. 3a and 3b). Optical densities for both methods were largely congruent for the 260/280 ratio (Fig. 4a) but more variable for the 260/230 ratio (Fig. 4b). In qPCR, 11 of 12 (92%) of the Maxwell-extracted melanoma samples crossed the threshold 1.3 Ct sooner on average than the corresponding manually-extracted templates (Fig. 5a), and one manually-extracted, Zymo-purified sample failed to amplify (Sample 07). Two Maxwell-extracted samples (Samples 10 and 11) crossed the threshold markedly sooner (~2.5 Ct). Similarly, all Maxwell-extracted melanoma DNA isolates produced robust bidirectional sequence, whereas two manually-extracted isolates failed to yield NRAS sequence despite the inhibitor-removal step (Fig. 5b). Aside from the sequence failures of these templates and one additional template which exhibited elevated baseline noise, NRAS codon 61 mutation-detection results for the remaining nine manually-extracted eluates were identical to the Maxwell-extracted samples (data not shown). Templates from both extraction methods exhibited higher median Phred scores and narrower interquartile ranges (IQR) for reverse traces than for forward traces (Figs. 6a and 6b); this characteristic is attributed to the proximal location of the forward primer relative to the NRAS codon 61 hotspot. Sequences from Maxwell-extracted FFPE DNA exhibit higher mean Phred scores and less variability (insets; Figs. 6a and 6b). Overall, metrics generated using other software packages (i.e., quality values, trace scores, quality value 20+, continuous-read length, and signal-to-noise ratio) illustrate equivalent or better results for the Maxwell-extracted templates especially in regards to variability (see IQRs for boxplots; Figs. 7a –7e).

Figure 1. Comparison of extraction method procedural steps and hands-on time

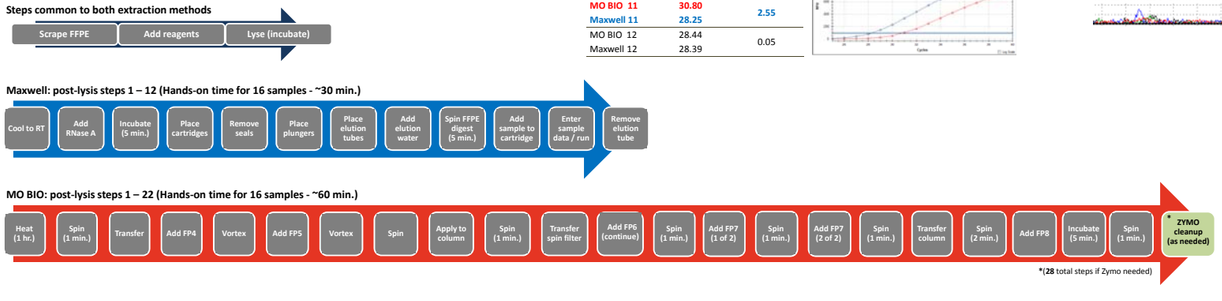


Figure 3a. Maxwell vs. MO BIO DNA extraction from different FFPE sample types (total DNA Yield). Overall, DNA yields were greater for samples processed on the Maxwell CSC (30 of 34 samples, 88%).

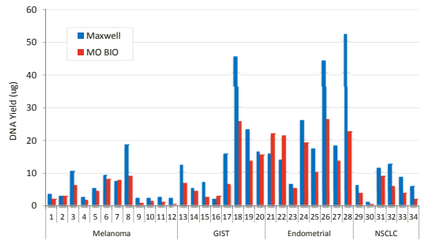


Figure 4a. Optical densities (260/280) Maxwell vs. MO BIO DNA extraction from different FFPE sample types.

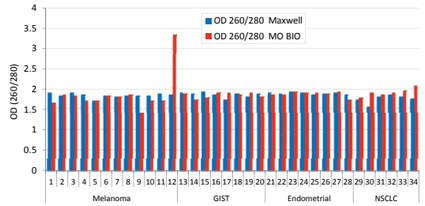


Figure 5a. NRAS allele-specific PCR for DNA extracted by both methods from melanoma FFPE; traces shown at right for samples with ΔC_t Values > 2.0 (Samples 02, 10, 11) and no amplification/amplification (Sample 07).

Sample	C_t Value	Δ C_t Value (MO BIO – Maxwell)
MO BIO 01	33.04	1.11
Maxwell 01	31.93	
MO BIO 02	35.42	2.17
Maxwell 02	33.25	
MO BIO 03	29.93	0.37
Maxwell 03	29.56	
MO BIO 04	32.09	0.66
Maxwell 04	31.43	
MO BIO 05	32.50	0.73
Maxwell 05	31.77	
MO BIO 06	34.64	1.94
Maxwell 06	32.70	
MO BIO 07	No amplification	N/A
Maxwell 07	35.07	
MO BIO 08	30.10	-0.02
Maxwell 08	30.12	
MO BIO 09	30.11	1.72
Maxwell 09	28.39	
MO BIO 10	30.93	2.60
Maxwell 10	28.33	
MO BIO 11	30.80	2.55
Maxwell 11	28.25	
MO BIO 12	28.44	0.05
Maxwell 12	28.39	

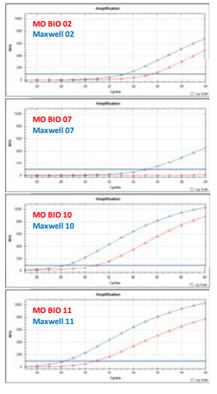


Figure 3b. Percent increase in DNA yield by Maxwell vs. MO BIO in different FFPE sample types (1.9-fold or 190% increase overall).

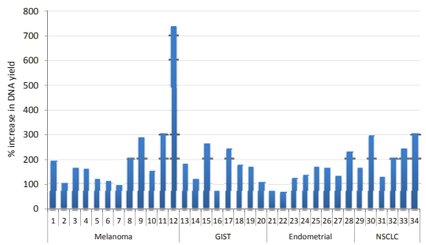


Figure 4b. Optical densities (260/230) Maxwell vs. MO BIO DNA extraction from different FFPE sample types.

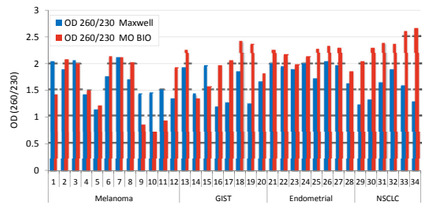


Figure 5b. Comparison of *NRAS* Sanger sequencing results for DNA extracted by both methods from two melanoma samples, both of which required the Zymo cleanup step for the MO BIO extraction.

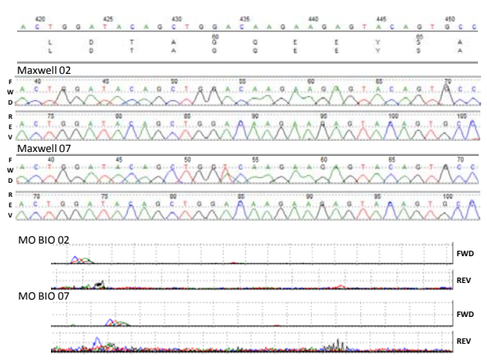


Figure 2. Representative specimen (Sample 02) requiring additional Zymo cleanup steps due to high melanin content (note red boxed area); H&E staining, 4X magnification.

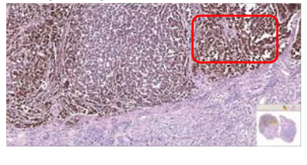


Figure 6a. Box plot graphs of Phred scores for Sanger sequences (see Figure 5; forward traces; 51-bp window; melanoma samples).

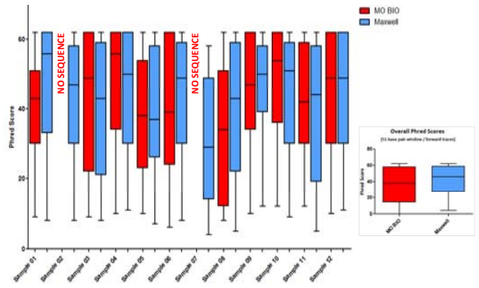
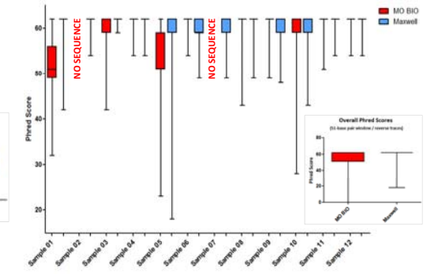
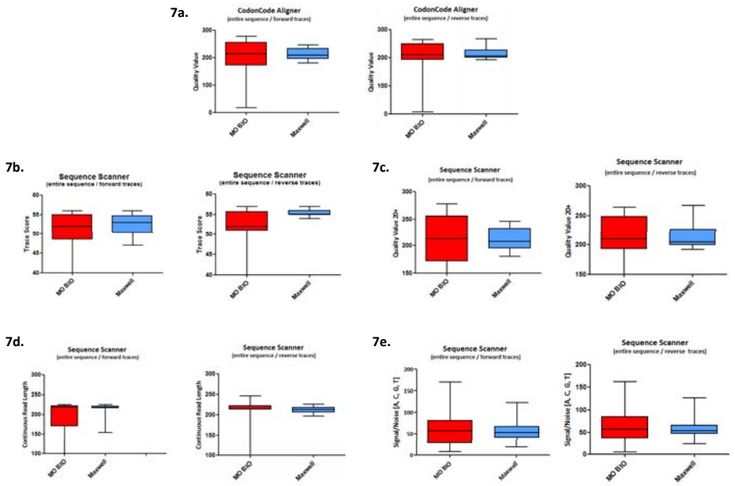


Figure 6b. Box plot graph of Phred scores for Sanger sequences (see Figure 5; reverse traces; 51-bp window; melanoma samples).



Figures 7a – 7e. Box plots representing sequence quality metrics using CodeCodon Aligner and Sequence Scanner software.



Conclusions

This comparison highlights the potential advantages of using an automated nucleic acid extraction platform for onco-diagnostic testing:

- Standardized workflow with fewer hands-on steps minimizing the likelihood of cross-contamination
- Higher throughput for faster turn-around-time of results
- Higher DNA yields, maximizing the potential data output from limited amounts of FFPE tumor tissue
- Higher quality DNA without extra purification steps, minimizing the potential need for repeat analysis

Acknowledgements

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