Enhanced MSI Status Determination from FFPE Tissue Sections Using an Integrated Workflow Combining QuantumCyte's QCPRECISE!™ with Promega's Maxwell® CSC DNA FFPE Kit

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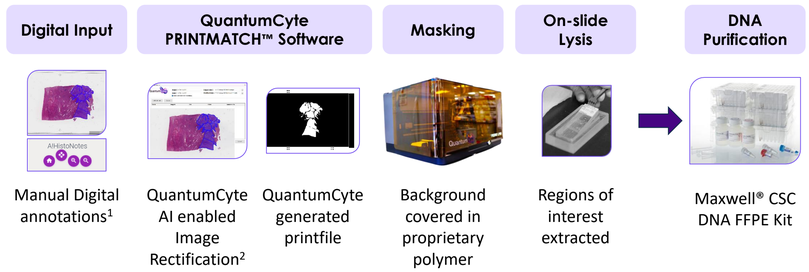
Background

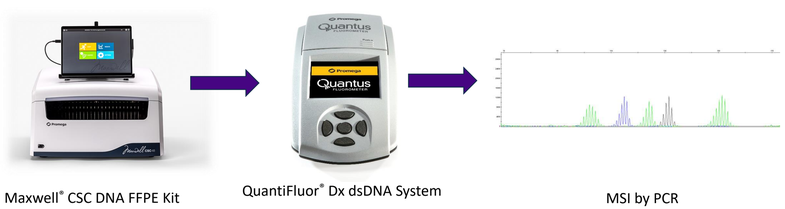
This research study utilized an integrated workflow combining QuantumCyte's QCPRECISE!™ tissue enrichment platform (Figure 1) and Promega's Maxwell® CSC DNA FFPE Kit to facilitate the extraction of nucleic acids from formalin fixed paraffin-embedded (FFPE) tissue sections. Extracted DNA was quantitated with the QuantiFluor® Dx dsDNA System and used as the template source for microsatellite instability (MSI) assessment by PCR (Figure 2). The QCPRECISE!™ platform employs semiconductor print mask technology integrated with digital pathology imaging to precisely target and extract nucleic acids from specific regions of interest (ROIs). QCPRECISE!™ can isolate nucleic acids from areas as small as 50 microns in diameter, with a targeting precision of +/- 5 microns. This comprehensive approach aims to improve the accuracy and reliability of determining MSI status in challenging samples.

Figure 1: The QCPRECISE!™ Tissue Enrichment Platform.



[1]For this study, manual annotations were done by a board-certified pathologist using the AIHistonotes manual annotation software. The QCPRECISE! integrates seamlessly digital annotations from most AI based digital pathology platforms.
[2] QuantumCyte proprietary AI enabled image rectification PRINTMATCH™ software.

Figure 2: Workflow Downstream of QCPRECISE!™ Platform.



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Materials and Methods

H&E-stained FFPE tissue sections from 7 samples (See Figure 4B) were sectioned at 5 microns thick on to QuantumCyte's pre-marked microscope slides, digitized and annotated by a board-certified pathologist. These annotated images were then incorporated into the QuantumCyte QCPRECISE!™ workflow (Figure 1). For the QCPRECISE!™ workflow, all slides were H&E stained, scanned using a KFBio scanner, and ROIs were identified using the AIHistonotes manual annotation system by a board-certified pathologist. For the QCPRECISE!™ process, Printfiles were generated using the QCPRECISE!™ software and the masks were printed onto the tissue mounted slides (Figure 1). The mask inhibits access of lysis buffer. Lysis buffer master mix, prepared as described in the Maxwell® CSC DNA FFPE Kit Technical Manual, was added directly to the slide using a Grace Bio-labs Hybriwell placed directly over the ROI. Lysis was then performed by placing the slide into a humidity chamber and incubated at 56°C for 60 minutes (Figure 3A). The crude lysate was recovered from the slide and gDNA was purified following the Maxwell® CSC DNA FFPE Kit using the Maxwell® CSC Instrument in IVD mode. The extracted samples were then quantitated using the QuantiFluor® Dx dsDNA System. Finally, MSI status was determined by PCR amplification followed by capillary electrophoresis separation. Data was analyzed at Promega using investigational software and results are reported in this poster (Figures 3B and 4).

Figure 3A: Pre- and post-lysis images from Sample S00149.

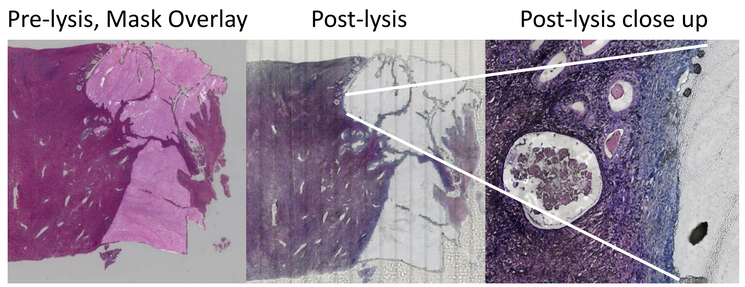
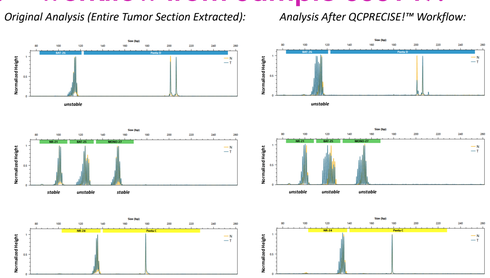


Figure 3B: Data comparison with and without the QCPRECISE!™ workflow from Sample S00149.



Results

The study successfully demonstrated that the Maxwell® CSC DNA FFPE Kit could extract gDNA from stained FFPE tissue masked by the QCPRECISE!™ system (Figure 2). Successful enrichment was achieved using the QCPRECISE!™ workflow as demonstrated in the pre- and post-lysis images shown in Figure 3A and the data in Figure 3B. This precise extraction enabled the determination of MSI status from samples that posed challenges for traditional MSI workflows, including macrodissection (Figure 4B).

Figure 4A: Electropherograms.

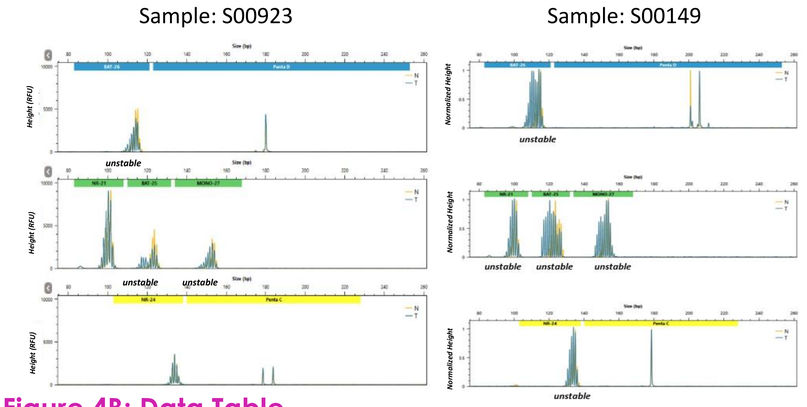


Figure 4B: Data Table.

Subject ID	Tumor Type	Initial Assessment	Workflow	Final Result
S00681	Salivary Gland	Insufficient normal tissue observed for macrodissection, macrodissection was not performed, not tested for MSI	QCPRECISE!™ → Maxwell® CSC DNA FFPE Kit → MSI by PCR	✓ Successful MSI determination (MSI-H)
S00694	Breast	Invalid MSI result due to insufficient Normal FFPE DNA	QCPRECISE!™ → Maxwell® CSC DNA FFPE Kit → MSI by PCR	✓ Successful MSI determination (MSS)
S00695	Melanoma	Insufficient normal tissue observed for macrodissection, macrodissection was not performed, not tested for MSI	QCPRECISE!™ → Maxwell® CSC DNA FFPE Kit → MSI by PCR	✓ Successful MSI determination (MSS)
S00923	Esophageal	Insufficient normal tissue observed for macrodissection, macrodissection was not performed, not tested for MSI	QCPRECISE!™ → Maxwell® CSC DNA FFPE Kit → MSI by PCR	✓ Successful MSI determination (MSI-H)
S01022	Ovarian	Insufficient normal tissue observed for macrodissection, macrodissection was not performed, not tested for MSI	QCPRECISE!™ → Maxwell® CSC DNA FFPE Kit → MSI by PCR	✓ Successful MSI determination (MSI-H)
S01024	Biliary-Cholangio	Insufficient normal tissue observed for macrodissection, macrodissection was not performed, not tested for MSI	QCPRECISE!™ → Maxwell® CSC DNA FFPE Kit (pooled extractions) → MSI by PCR	X Unable to determine MSI Status
S00149	Endometrial	Initial result generated by extracting entire curl, MSI-H with subtle shifts	QCPRECISE!™ → Maxwell® CSC DNA FFPE Kit → MSI by PCR	✓ Successful MSI determination (MSI-H, with more obvious shifts)

Conclusion

The integration of QuantumCyte's QCPRECISE!™ platform with Promega's Maxwell® and QuantiFluor® systems and chemistries offers a novel method for gDNA extraction upstream of MSI status determination from FFPE tissue sections. This workflow represents an alternative to traditional microdissection and macrodissection. We also demonstrate the utility of this workflow in samples representing various challenges, highlighting its potential utility in enhancing molecular diagnostics. This research study illustrates the potential of these combined technologies.

Discussion

The QCPRECISE!™ capability is being explored for clinical applications towards Precision Medicine and in pharma towards clinical studies.