SCIENTIFIC WHITE PAPER

DNA amplifiability after xylene-free tissue processing: supporting precision medicine without molecular compromise

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Abstract

In modern diagnostic pathology, a single formalin-fixed, paraffin-embedded (FFPE) tissue block is increasingly required to support multiple analytical approaches, including morphological assessment, immunohistochemical biomarker profiling and molecular testing. The simultaneous preservation of tissue morphology, protein antigenicity and nucleic-acid integrity is therefore essential to ensure reliable diagnostic results and the accurate identification of prognostic and predictive biomarkers that guide personalized therapeutic decisions. Because pre-analytical tissue processing affects nucleic-acid integrity, it directly influences the reliability of downstream molecular pathology.

This white paper reviews the evidence on DNA integrity, amplifiability and antigen preservation in FFPE tissue processed with the Donatello tissue processor and Ottix reagents.

In an independent validation at the University of Siena, Donatello-processed tissue yielded all four PCR amplicons (100–400 bp), including those at 300 and 400 bp.

In the Diapath internal validation, all 34 DNA extracts tested by real-time PCR were classified as HIGH quality, with QC scores below the 0.04 optimal-range threshold. Genomic DNA ScreenTape showed size-resolved profiles extending beyond 300 bp (DIN 4.8–6.6), and DV200 values of 60.30–86.76% indicated preserved RNA integrity. In an independent matched comparison of three tissue processors at the University of Porto, Donatello produced the fewest processing artifacts on hematoxylin-eosin slides (17.8%, versus 38.4% and 50.7%) and best preserved PD-L1 staining intensity.

Together, these data demonstrate functional DNA amplifiability at and above 300 bp after Donatello processing, supported by orthogonal measures of DNA size distribution, RNA quality and tissue antigenicity.

1. Background and rationale

Tissue processing is one of the oldest and most essential stages of anatomic pathology. Its foundations were laid in the second half of the nineteenth century, with the introduction of paraffin-wax embedding in the late 1860s and of formaldehyde fixation by Blum in 1893, and the sequence established then is still in use today[1]. The FFPE workflow consists of four principal steps: fixation, dehydration, clearing and paraffin infiltration. From a physical standpoint, dehydration and clearing are diffusion-driven processes. Reagents move into and out of the tissue, which behaves as a porous medium, according to Fick's laws of diffusion, at a rate governed by an effective diffusion coefficient that depends on the reagent, the temperature and the tissue structure[2].

While these principles remain essentially unchanged, the requests placed on the processed tissue have not. Modern anatomic pathology integrates morphological, immunohistochemical and molecular analysis on the same FFPE block[3]. Because fixation and tissue processing precede DNA extraction, both steps may alter nucleic-acid quantity, purity and fragment length. The practical question is therefore not merely whether DNA can be extracted, but whether the recovered DNA remains sufficiently intact and amplifiable for downstream assays. Formalin fixation is known to fragment DNA and to introduce base lesions, so the quantity that governs assay performance is the amount of amplifiable template recovered rather than the total DNA yield[4]. Preserving morphology is equally important: in digital and computational pathology, whole-slide images are the substrate for visualization and quantitative image analysis, including artificial-intelligence algorithms, so processing artifacts translate directly into analytical error[5].

Tissue processing also has an occupational dimension. Xylene, the clearing agent in traditional protocols, is a volatile aromatic solvent with recognized health hazards and significant disposal costs. Replacing it is

acceptable, however, only if the substitute preserves the analytical quality of the block.
The workflow examined here combines the Donatello tissue processor with the Ottix reagent system, in which Ottix Shaper and Ottix Plus replace both the graded alcohols and xylene, so analytical equivalence is central rather than incidental.
The present paper synthesizes the available evidence into a publication-style technical review. It distinguishes direct evidence for the 300-bp threshold from supportive evidence on DNA quality, fragment distribution and downstream molecular compatibility.

2. Evidence bases

Two evidence classes were reviewed:

- Diapath internal matched-tissue validation records (2024), including FFPE DNA/RNA extraction, QIAGEN Real-Time PCR quality control, Agilent Genomic DNA ScreenTape and RNA ScreenTape analysis[6,7].
- Independent University of Siena validation of Donatello tissue processor and Ottix, including DNA extraction, PCR-based DNA fragmentation testing, gene-expression analysis, Sanger sequencing, FISH and immunohistochemistry (2015)[8].

3. Methods

The internal validation and the University of Siena study share the same processing approach: tissue processed on a Donatello instrument using the Ottix reagent system, which replaces the conventional sequence of graded alcohols and xylene (Table 1). Ottix Shaper is an alcohol-based mixture that initiates dehydration; Ottix Plus, a mixture of alcohols and linear hydrocarbons free of aromatic components, completes dehydration and clearing in a single reagent. The internal validation and the University of Siena study therefore interrogate the same reagent system with different analytical endpoints, although step durations and temperatures differ between the two protocols.
Two aspects of this workflow are particularly relevant to nucleic-acid integrity. Heat is a major driver of fragmentation, and in the protocol adopted here most Ottix steps run at room temperature, with heating limited to the last Ottix Plus step (37 °C) and to paraffin infiltration at around 60 °C. In addition, the Donatello tissue processor exchanges reagents through pressure–vacuum cycles with intermittent bubbling rather than passive immersion, which favors uniform penetration.

Table 1. *Protocol validated by Diapath in use on the Donatello tissue processors. This protocol is used for samples up to 4 mm. A, atmosphere; P/V, Alternating pressure/vacuum cycles.*

Reagent	Time	Temperature (°C)	Infiltration	Mix
Formalin	Fixation time should comply with ASCO, CAP, SIAPeC-IAP, RCPath, ESP and ESMO guidelines and recommendations[16–21]	20	A	30 min
Deionized Water	10 min	20	A	5 min
Ottix Shaper	30 min	20	P/V	10 min
Ottix Shaper	1h	20	P/V	20 min
Ottix Plus	1h	20	P/V	10 min
Ottix Plus	1h	20	P/V	20 min
Ottix Plus	1h30min	20	P/V	30 min
Ottix Plus	2h	37	P/V	30 min
Diawax 56-58°C	1h30min	60	P/V	15 min
Diawax 56-58°C	1h30min	60	P/V	30 min
Diawax 56-58°C	2h	60	P/V	30 min

3.1 Diapath internal molecular validation

Tissue specimens were processed on the Donatello tissue processor, using the protocol above. DNA and RNA were extracted from FFPE sections using the QIAGEN AllPrep DNA/RNA FFPE workflow.
Spectrophotometric measurements (Nanodrop) were recorded, followed by molecular quality assessment.
DNA amplifiability was assessed using StepOnePlus Real-Time PCR System with the QIAGEN Real-Time PCR quality-control assay (QIAseq DNA QuantiMIZE Kits) using 100-bp and 200-bp targets relative to a control of known concentration. DNA integrity was additionally evaluated by Agilent TapeStation with Genomic DNA ScreenTape, which provides size-resolved DNA profiles and a DNA Integrity Number (DIN). RNA integrity was evaluated using Agilent RNA ScreenTape; because RIN is not considered the most informative metric for FFPE-derived RNA, DV200 (the

percentage of RNA fragments at least 200 nucleotides long) was used as the principal RNA integrity descriptor. The use of DV200 in preference to RIN for FFPE-derived RNA is consistent with published comparisons of the two indices[9].

3.2 Independent University of Siena validation

Tissue samples. The study was carried out at the Section of Pathology in Azienda Ospedaliero-Universitaria Senese, where two Donatello tissue processors were installed in 2015 and process on average about 250 specimens each per day in routine diagnostics.

The University of Siena study compared an alternative tissue-processing workflow based on Ottix reagents and the Donatello tissue processor with a conventional alcohol/xylene protocol. Lung or lymphoma specimens were fixed in 10% neutral buffered formalin according to specimen size. For DNA quality control, DNA was extracted from 12 FFPE specimens and amplified using four PCR targets designed to generate products of different lengths. The standardized set of four targets is widely used to verify the integrity and amplifiability of DNA extracted from FFPE material before molecular testing[10] (Table 2). Amplification products were evaluated by 2% agarose gel electrophoresis.

Table 2. *DNA quality-control targets used in the University of Siena validation.*

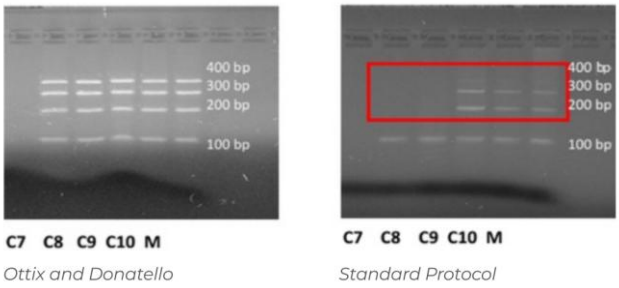
Locus	Target	Amplicon size
AF4 exon 11	AF4/X11	400 bp
PLZF exon 1	PLZF/X1	300 bp
RAG1 exon 2	RAG1/X2	200 bp
TBXAS1 exon 9	TBXAS1/X9	100 bp

4. Results

4.1 DNA amplifiability (PCR/qPCR)

The independent University of Siena validation directly evaluated DNA fragmentation using PCR products of 100, 200, 300 and 400 bp. The reported agarose gels show all four amplification products, including those at 300 bp and 400 bp, in every specimen processed with Ottix system and Donatello tissue processor. Because amplification requires an intact template across the complete target, these results provide functional evidence of amplifiable DNA at the 300-bp threshold and above (Fig. 1). The study further reported greater DNA fragmentation in several specimens processed with the standard protocol compared with the corresponding Ottix- and Donatello-processed specimens and interpreted the alternative workflow as favorable for DNA quality and downstream molecular testing.

Figure 1. University of Siena agarose-gel PCR results. *The Ottix and Donatello tissue processor column demonstrates all four amplification products, including the 300 bp and 400 bp targets, across the tested specimen series (C7–C10). The red box in the Standard Protocol panel marks lanes in which the higher-molecular-weight products were lost. Reproduced from Gazaneo et al.[8] with permission of the Department of Medical Biotechnologies, Section of Pathology, University of Siena.*



The internal Diapath validation (April 2024) provided direct quantitative evidence of molecular amplifiability after Donatello processing. In the first series (samples 1–5), all 10 resulting DNA extracts were classified as HIGH quality by the QIAGEN 100/200-bp Real-Time PCR assay.

In the subsequent series (samples 6–13), all 24 tested DNA extracts were again classified as HIGH quality. QC scores in this 24-extract series ranged from 0.003 to 0.027, remaining below the 0.04 threshold identified in the validation record as the optimal range.

These results demonstrate consistent PCR amplifiability across 34 tested FFPE DNA extracts generated after Donatello processing (Table 3).

Table 3. Summary of complementary molecular readouts: *PCR amplifiability.*

Dataset	Analytical readout	Observed result	Interpretation
Samples 1–5, n=10 DNA extracts	QIAGEN qPCR, 100/200 bp	10/10 classified HIGH quality	Consistent recovery of amplifiable FFPE DNA
Samples 6–13, n=24 DNA extracts	QIAGEN qPCR, 100/200 bp	24/24 classified HIGH; QC score 0.003–0.027	All tested extracts within the reported optimal QC-score range (<0.04)

4.2 DNA integrity (size-resolved electrophoresis)

As an orthogonal check on the PCR-based results, DNA integrity was assessed by size-resolved electrophoresis (Agilent TapeStation, Genomic DNA ScreenTape). The resulting electrophoretic profiles showed substantial DNA signal above the 300-bp region, frequently extending into the kilobase range. Among evaluable specimens in samples 1–13, reported DIN values were generally approximately 4.8–6.6, with several matched specimens around 6.0–6.6 (Table 4). The size-resolved profiles therefore provide orthogonal support to the PCR data by demonstrating recovery of DNA populations extending beyond the 300-bp threshold.

Table 4. Summary of complementary molecular readouts: *DNA integrity.*

Dataset	Analytical readout	Observed result	Interpretation
Samples 1–13, evaluable extracts	Agilent Genomic DNA ScreenTape	DIN values generally ~4.8–6.6; DNA signal extends beyond 300 bp	Size-resolved profiles confirm recovery of DNA populations extending beyond 300 bp; DIN describes overall integrity and is not by itself a 300-bp verification.

4.3 RNA integrity (DV200)

RNA quality was interpreted using DV200 rather than RINe[9]. In the sample subset 3–7, DV200 values ranged from 60.30% to 86.76% (Table 5). This indicates that a substantial fraction of recovered RNA consisted of fragments at least 200 nucleotides long and provides complementary evidence that the Donatello processing workflow can preserve nucleic acids suitable for downstream molecular applications.

Table 5. Summary of complementary molecular readouts: *RNA fragment preservation.*

Dataset	Analytical readout	Observed result	Interpretation
Samples 3–7	Agilent RNA ScreenTape, DV200	DV200 60.30%–86.76%	Majority of recovered RNA in this subset remained >=200 nt; supportive evidence of RNA quality

4.4 Morphology and biomarker preservation (immunohistochemistry)

In an independent study at the University of Porto, Caramelo and colleagues compared three tissue processors while keeping all other variables constant[11]. Each of 73 specimens (39 uterine leiomyomas, 17 placentas and 17 palatine tonsils) was divided into three fragments, one per processor, which were then re-embedded in a single paraffin block, so that every comparison was internally matched. Processor B was a Donatello tissue processor with Ottix Shaper and Ottix Plus; A was a conventional ethanol/xylene instrument and C a microwave-assisted instrument using isopropanol. Donatello produced the fewest processing artifacts on hematoxylin-eosin whole-slide images (17.8% of slides, against 38.4% for A, $p = 0.001$, and 50.7% for C, $p < 0.001$), and PD-L1 staining intensity in tonsil and placenta was significantly lower with A and C than with B (Fig. 2A).

In a murine model of cerebral cavernous malformations, Maderna et al. used paraffin-embedded spleen, liver, heart, kidney and bone marrow on Diapath automatic processor using a conventional ethanol/xylene protocol[12]. The resulting sections supported hematoxylin-eosin and Perls' staining, chromogenic immunohistochemistry for CD3, PAX5, Ter119, myeloperoxidase, F4/80 and PECAM1, and confocal immunofluorescence for VE-cadherin and endomucin, which the authors used for quantitative analysis of splenic architecture, hematopoiesis and vascular density (Fig. 2B).

The University of Siena validation[8] extended the assessment to a diagnostic panel. Twelve lymphomas and one reactive lymph node, processed both with Ottix on Donatello tissue processor and with a conventional alcohol/xylene protocol, were stained with eight antibodies (CD3, CD5, CD10, CD20, CD30, BCL2, BCL6 and Ki-67) covering membrane, cytoplasmic and nuclear antigens. The authors reported high-quality reactions with Ottix and Donatello tissue processors, overlapping those of the standard protocol.

At routine scale, a validation presented as a poster at the 31st European Congress of Pathology reported approximately 20,000 specimens processed with the Donatello tissue processor over five months at a single center, about 98% of them of high histological quality[13].

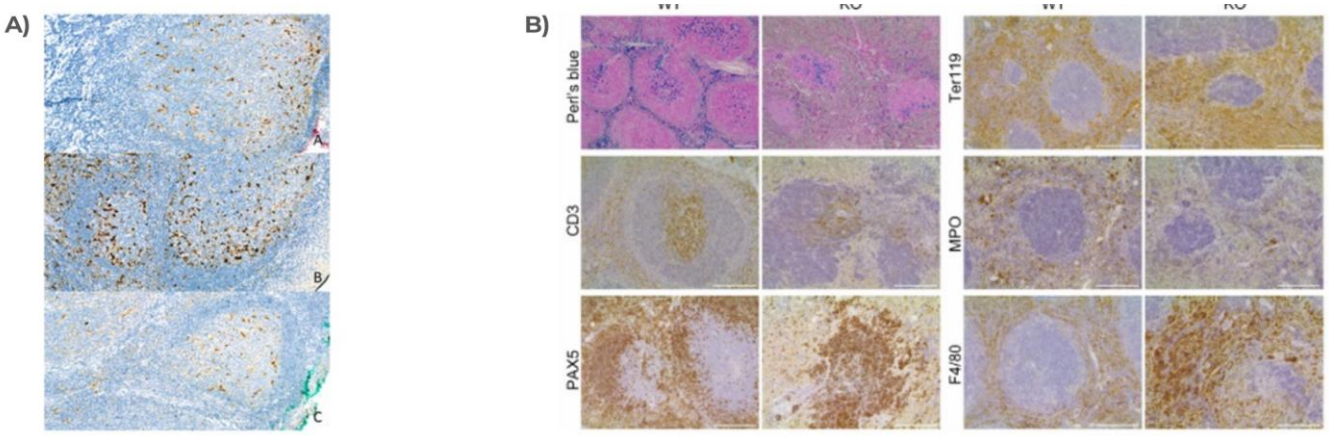
Taken together, these studies do not measure DNA fragment length and cannot substitute for the molecular evidence reported above; their contribution is convergent rather than direct. The matched, blinded comparison from Porto shows that processor choice materially affects downstream analytical quality, with

Donatello tissue processor performing favorably against both comparators, while the remaining reports, descriptive in design, document compatibility across five organs, a full diagnostic antibody panel and routine throughput. Since morphology, antigenicity and nucleic-acid integrity depend on the same processing variables (fixation, dehydration, clearing agent and heat), consistency across these endpoints reinforces the case for preserved template quality after Donatello processing.

Figure 2.

A) *PD-L1 immunohistochemistry on three matched fragments of the same palatine tonsil, each processed on (A) conventional processor using ethanol and xylene, (B) Donatello tissue processor with Ottix Shaper and Ottix Plus, (C) microwave-assisted processor using isopropanol. Illustrative case selected by the original authors. Reproduced from Caramelo et al.[11] under CC BY 4.0.*

B) *Histochemistry and immunohistochemistry on paraffin sections of spleen from wild-type (WT) and Ccm3-knockout (KO) mice, processed on Donatello: Perls' Prussian blue, CD3, PAX5, Ter119, myeloperoxidase (MPO) and F4/80. Reproduced from Maderna et al.[12] under CC BY 4.0.*



4.5 Downstream molecular applications

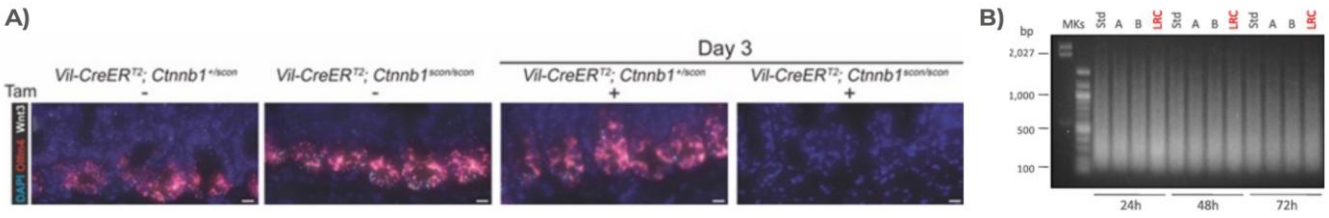
Tissues processed on Donatello instruments have been used as source material in peer-reviewed research in downstream molecular applications, including quantitative PCR and in situ hybridization. Wu et al. processed formalin-fixed mouse intestine on Donatello tissue processor and performed multiplex single-molecule RNA fluorescent in situ hybridization (RNAscope) on the resulting paraffin sections, resolving Olfm4 and Wnt3 transcripts in intestinal crypts[14] (Fig. 3A).

Furthermore, Amatori and colleagues processed normal human colon, fixed in neutral-buffered formalin for 24, 48 or 72 h, on Donatello tissue processor and extracted chromatin from 10-µm FFPE sections for chromatin immunoprecipitation[15]. The purified DNA was quantified, sized by agarose-gel electrophoresis and analyzed by real-time qPCR at active and silent gene promoters, with specific enrichment at all fixation times (Fig. 3B).

Figure 3.

A) *Single-molecule RNA fluorescent in situ hybridization (RNAscope) for Olfm4 (red) and Wnt3 (white) mRNAs on paraffin sections of mouse small intestine processed on Donatello. Scale bars, 10 µm. Adapted from Wu et al.[14] under CC BY 4.0.*

B) *Agarose-gel electrophoresis of input DNA purified from chromatin extracted from normal human colon, fixed for 24, 48 or 72 h and processed on Donatello. Fragment sizes reflect the controlled enzymatic and mechanical fragmentation required for chromatin immunoprecipitation, not the native integrity of the DNA; the DNA recovered at all three fixation times was subsequently analyzed by real-time qPCR. MKs, molecular-weight markers. Adapted from Amatori et al.[15] under CC BY 4.0.*



5. Conclusions

This review integrates evidence from an independent academic validation, two internal validation reports and four peer-reviewed studies involving tissue processed using the Donatello tissue processor. Collectively, these findings provide convergent evidence supporting the preservation of nucleic-acid quality and suitability for downstream molecular analyses in formalin-fixed, paraffin-embedded (FFPE) tissues processed using the validated workflow.

Importantly, the independent academic validation demonstrated successful amplification of 300-bp and 400-bp DNA targets, providing direct evidence of the preservation of amplifiable DNA fragments of these lengths.[8] These findings are further supported by internal validation data demonstrating consistent PCR performance, DNA fragment-size distributions extending beyond 300 bp, and RNA quality compatible with downstream molecular applications.[6,7]

Additional evidence from published studies demonstrates the successful application of Donatello-processed tissues in in situ hybridization[14] and chromatin-based analyses,[15] while comparative and descriptive investigations report preservation of tissue morphology and immunohistochemical biomarker detection.[11,12]

Taken together, the available evidence supports the conclusion that the validated tissue-processing workflow preserves DNA amplifiability for targets of at least 400 bp under the evaluated conditions, while maintaining tissue characteristics compatible with a range of histological and molecular applications. Although the evidence derives from studies employing different analytical endpoints and validation approaches, the consistency of the reported findings supports the suitability of this workflow for integrated histopathological and molecular investigations.

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Conflict of interest and funding

All authors are employees of Diapath S.p.A., which manufactures the Donatello tissue processor and the Ottix reagents evaluated in this review and which funded the preparation of this document.

The sources cited are of three kinds and should be weighed accordingly. The studies cited as references 11, 12, 14 and 15 were designed, conducted and published by independent groups with no Diapath involvement and no company funding. The University of Siena validation (reference 8) was carried out by an academic laboratory. The workflow validation presented at the 31st European Congress of Pathology (reference 13) and the diffusion study cited as reference 2 include Diapath employees among their authors, and the internal validation records (references 6 and 7) were generated in-house and have not been externally audited or peer-reviewed. These records are available from Diapath on request so that readers can assess them directly.