

# CHOICE OF FFPE DNA ISOLATION METHOD AFFECTS BOTH YIELD AND FUNCTIONAL QUALITY AND IMPACTS VARIANT CALLING BY TARGETED NGS

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## SUMMARY

- Advances in precision medicine in oncology mirror innovations in molecular profiling techniques and the utility of new biomarkers, yet these approaches can be limited by the quality and quantity of sample input.
- The choice of DNA isolation technology, particularly for low-quality or low-quantity FFPE samples, can impact downstream assay results.
- To quantify this impact, we compared and contrasted multiple isolation methods, including a novel isotachopheresis (ITP) technology, for pre-analytical functional DNA yield and quality and analytical results following targeted NGS.
- Here we show that functional DNA yield and quality varies across different FFPE sample preparation methods, and performance in downstream NGS is definitively linked to the number of input amplifiable copies. Such information is critical to the development of research and clinical molecular profiling assays.

## INTRODUCTION

A common denominator for molecular profiling methods for precision medicine is the up-front sample preparation of tumor biopsies. The choice of isolation method can affect the yield, quality, and purity of recovered nucleic acids. In the study we compare sample preparation technologies using low-quality/quantity samples to demonstrate differences in functional yield as well as differences in the removal of contaminating substances and show how these differences manifest in NGS data quality.

## METHODS

For each study phase, sections from residual clinical FFPE (Asuragen) or cell-line FFPE (Horizon Dx) blocks were procured, and nucleic acid (NA) extracted using three fundamental isolation methods (Fig. 1). The recovered NA was evaluated for total recovery by mass (NanoDrop), recovery of amplifiable copy number (QuantideX<sup>®</sup> qPCR DNA QC Assay), and relative sequencing error rates down to an input of 25 functional DNA copies using the QuantideX NGS Pan Cancer Panel\* paired with an intentionally generic variant caller (Figure 2). Results are presented in a side by side comparison of the methods within each phase.

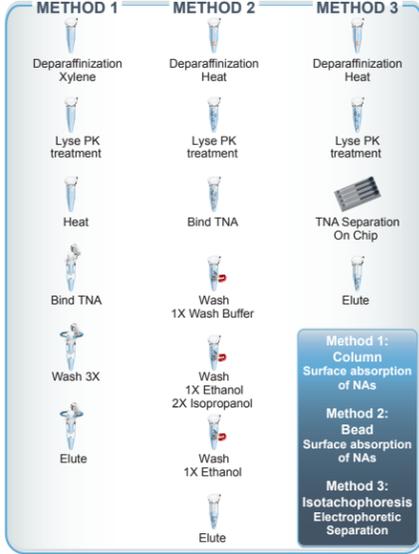


Figure 1. Isolation technologies: overview of isolation technologies used to recover total nucleic acid (TNA) from FFPE samples. Note that DNA isolation methods would have an additional RNase step included in the workflow. Isolations using kits from Qiagen, Norgen, and an additional supplier (β-kit) use method 1, FormaPure uses method 2, and a custom on-chip isotachopheresis (ITP) from Purigen uses method 3.

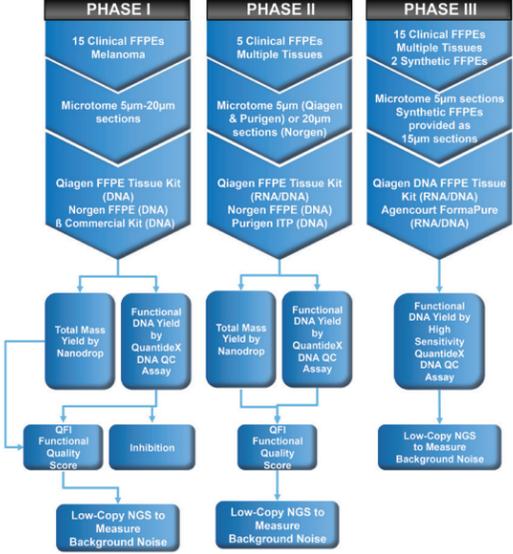


Figure 2. Overview of experimental workflow by phase. For each phase, curls from clinical FFPE samples were processed by the different recovery methods in parallel. Replicate isolations for each sample and method were conducted. Recovery (mass and amplifiable copies), inhibition, and NGS (subset) analyses were performed in various phases to assess the impact of each recovery method.

## RESULTS

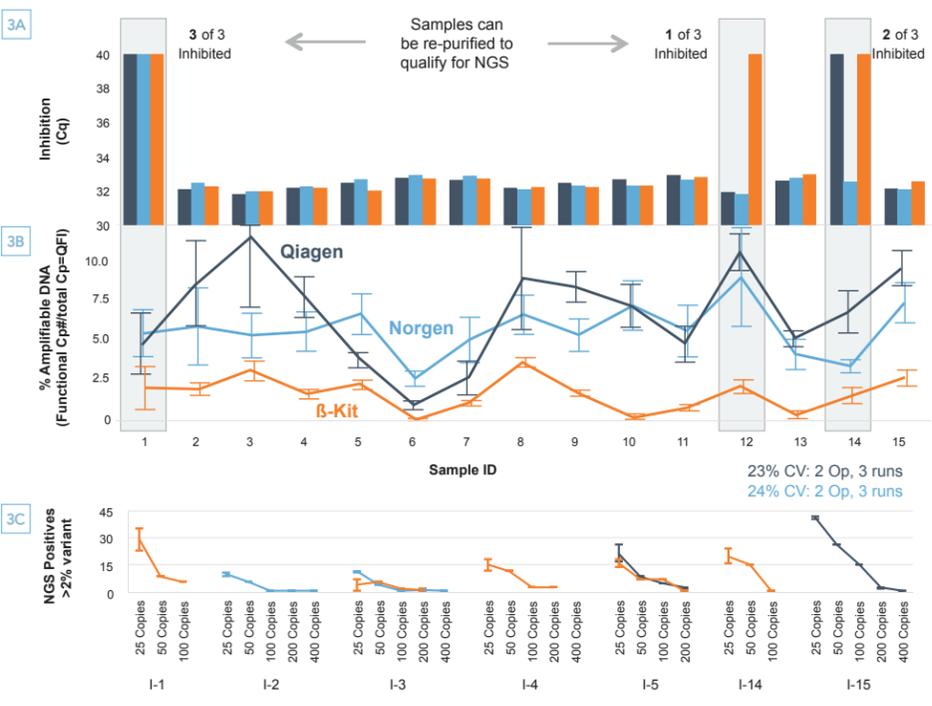


Figure 3. Phase I results for inhibition, copy number recovery and low-input NGS false-positive calls. A) Samples demonstrating inhibition yield high Cq values in response to qPCR of an exogenous template sequence (QuantideX qPCR DNA QC assay). Inhibition varied across methods. B) The number of amplifiable copies were obtained by evaluating the samples after dilution to remove any observed inhibition. The % amplifiable DNA is expressed as the number of functional copies divided by the total recovered DNA (QFI). A β-version of a commercial column-based kit yielded lower quality material than the Qiagen and Norgen isolations. C) NGS results from a subset of samples demonstrated that when using any of the methods false positives accumulated at less than 100 amplifiable copies using a generic NGS caller.

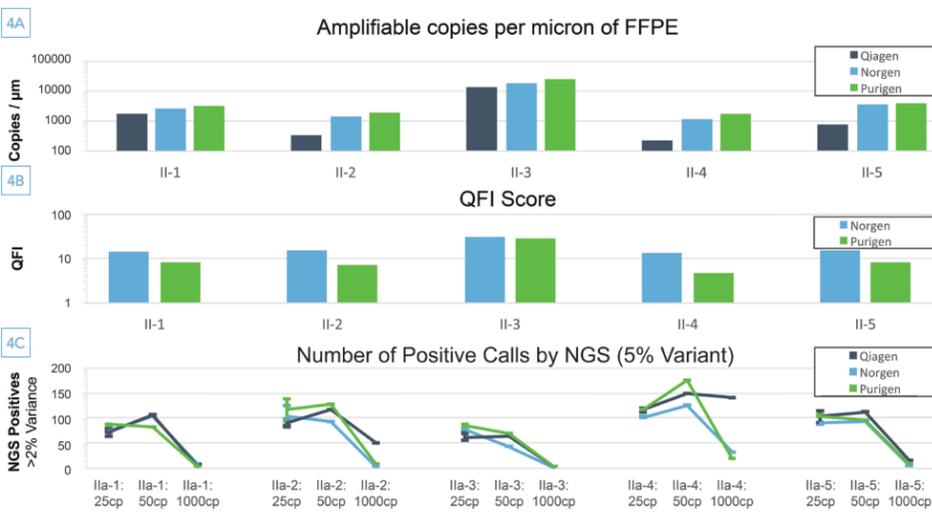


Figure 4. Phase II (DNA) results for inhibition, copy number recovery and low-input NGS false-positive calls. A) Amplifiable copies recovered per micron of tissue (Purigen > Norgen > Qiagen), and B) QFI score (Norgen > Purigen) varied consistently across methods. Norgen isolations were performed on 20µm sections, Qiagen and Purigen isolations were performed on 5µm sections. No QFI score could be assigned to the Qiagen isolations because both DNA and RNA were recovered and thus bulk DNA quantification by A260 was not possible. C) NGS variant data were consistent across methods except the Qiagen isolates of IIa-4 and IIa-2 input at 1000 copies, which correlate to the samples with lowest recovery of amplifiable copy numbers per micron of FFPE. No functional inhibition was observed in any of these samples.

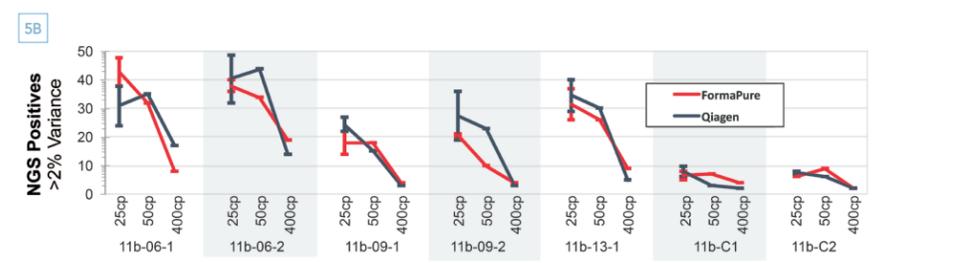
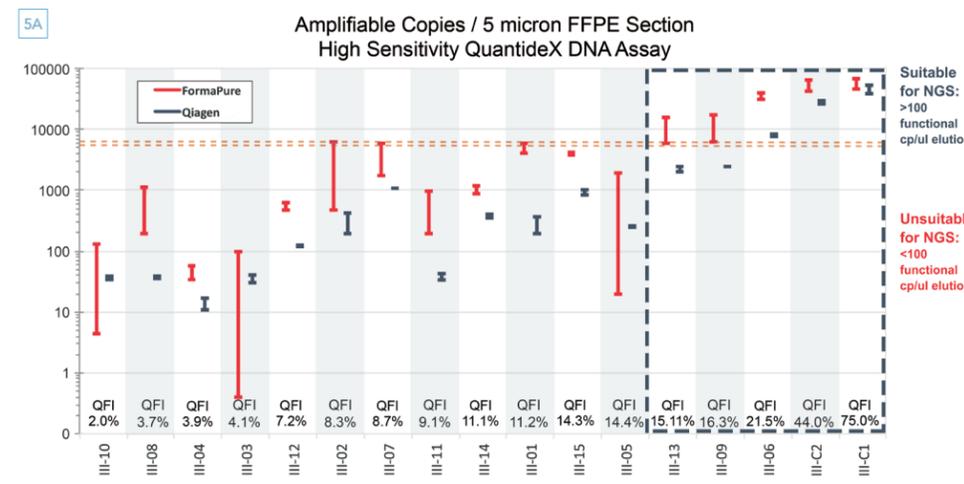


Figure 5. Phase III (TNA) results for copy number recovery and low-input NGS false-positive calls with reference QFI values for FFPE blocks. A) Variation in functional DNA copies per 5µm FFPE section (control samples were 15µm and data were normalized to copies per 5µm) measured across two methods when challenged with low-input amounts of poor-quality tumor biopsies. The functional quality of each FFPE sample is referenced to QFI scores obtained using a larger-scale isolation (Qiagen). The line (---) indicates the copy value threshold needed to ensure a minimum concentration of amplifiable copies (>5000 total copies eluted in 50µL → 100 cp/µl elution) to support adequate inputs and accurate variant calling by NGS. Of note, total functional yields for NGS-ready samples were directly correlated to those FFPE samples with the historically highest QFI. The FFPE cell-line controls (C1, C2; Horizon Dx) produced 4 to 10 times higher QFI scores than are typically observed in clinical FFPE samples. B) These controls also failed to replicate the low-input NGS noise profiles of clinical FFPE DNA.

## CONCLUSIONS

- Different isolation protocols have variable efficacy in removing PCR inhibitors from tissue biopsies, as shown in melanoma FFPE samples.
- Even when using the same core isolation methodology, the choice of commercial kit/protocol can result in differences in functional DNA yield and quality.
- In a pilot set of 5 residual clinical FFPE tumors, a prototype ITP method outperformed column-based isolation kits from Qiagen (mean 2.9-fold lower functional DNA yield; range 1.9- to 7.7-fold) and Norgen (mean 1.4-fold lower; range 1.1- to 1.5-fold).
- Bead-based DNA isolation (FormaPure) exhibited the lowest variances, but also delivered the lowest overall yield.
- Lower-quality sample recoveries carry the risk of a categorically elevated “noise” profile in NGS if the functional copies are not considered. This risk can be mitigated by inputting sufficient functional DNA copies.



\*Research Use Only – Not For Use In Diagnostic Procedures  
Preliminary research data. The performance characteristics of this assay have not yet been established.  
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