Using Isotachophoresis as a Novel Method to Improve the Yield and Quality of Nucleic Acid Purification from FFPE Samples

Lewis A. Marshall, Mercedes C. Morales, Cameron Pospisil, Austin P. So, Shripa Patel, Nathan P. Hoverter, Klint A. Rose
Purigen Biosystems | Pleasanton, CA

INTRODUCTION

The Purigen Ionic™ Purification System uses isotachophoresis (ITP) to extract, concentrate and purify nucleic acid from biological samples. ITP is a gentle, microfluidic, process that separates nucleic acid from impurities using electrophoretic mobility.

Ionic™ FFPE to Pure DNA Fluidics Chip

Ionic™ Purification System

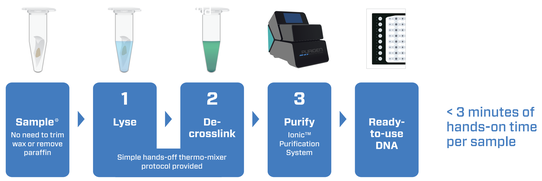
To compare the performance of the Ionic system, we purified DNA from 32 FFPE tissue blocks with both the Ionic system and a commercially available column-based purification kit for FFPE samples. We assessed the quantity and quality of the purified DNA with the Qiagen MRef multicopy reference assay and the Agilent Technologies QC Plex Assay. We also enriched libraries for next-generation sequencing using the SureMASTR (amplicon-based) and SureSelect (hybrid capture) technologies from Agilent Technologies.

WORKFLOW

The Ionic system protocol greatly simplifies the processing of FFPE samples. The workflow requires a hands-on time of less than three minutes per sample and enables working directly from scrolls. The workflow also eliminates the need for paraffin removal and any impact on data quality from this time consuming, manual process.

Column-based **WORKFLOW**

Purigen **WORKFLOW**



< 3 minutes of hands-on time per sample

RESULTS

The Ionic system delivers **more DNA** compared to columns.

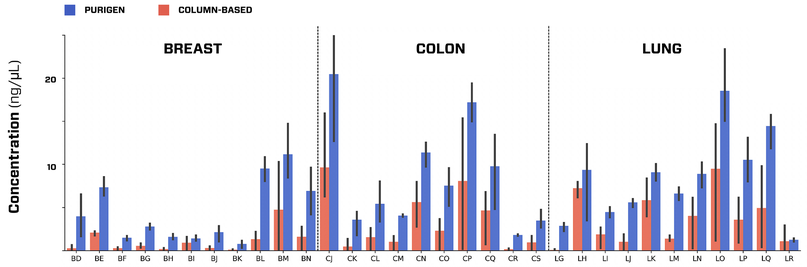


FIGURE 1: Samples were quantified using the 80-bp target from the MRef multicopy reference assay (Qiagen).

DNA purified by the Ionic system is **more compatible** with amplicon-based library preparation

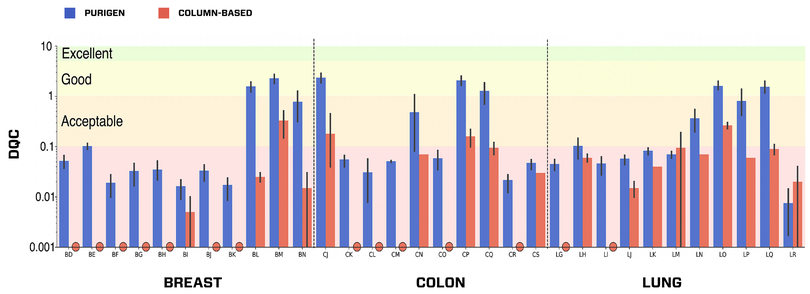


FIGURE 2: Samples were assessed with the QC Plex assay (Agilent) and the resulting amplicons were analyzed on the Agilent Technologies 4200 TapeStation. The resulting traces were scored using the DQC algorithm (Agilent). The results show better amplification from the samples purified with the Ionic system. In addition, 14 of the samples purified by column resulted in no amplification. Amplification was observed from every sample purified by the Ionic system.

SUMMARY

- Simplified workflow for purification from FFPE samples
- Higher yield of DNA when compared to columns
- Improved compatibility with amplicon-based library preparation
- Improved coverage uniformity for NGS
- Less bias toward length or GC content

Purification by the Ionic system **reduces bias against long targets** when compared to columns

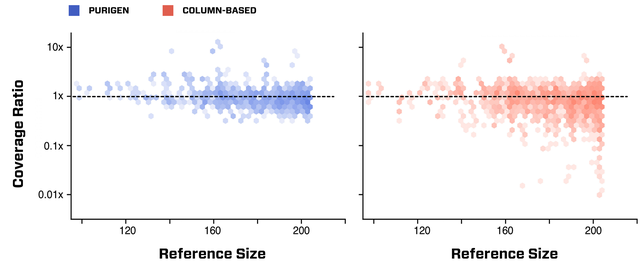


FIGURE 3: Libraries were prepared and enriched for the SureMASTR Tumor Hotspot Panel (Agilent) and sequenced on the Illumina MiSeq sequencer. Coverage was assessed against control libraries constructed from a high-quality reference sample, Coriell Institute GM24835 DNA. Data from the sequencing of the SureMASTR Tumor Hotspot libraries is plotted here as coverage ratio against the size of the reference for the target. The underrepresented targets in the libraries made from column-purified DNA occur most often in the longer targets. DNA purified via ITP does not show substantial bias toward length.

Purification by the Ionic system **reduces GC-bias** when compared to columns

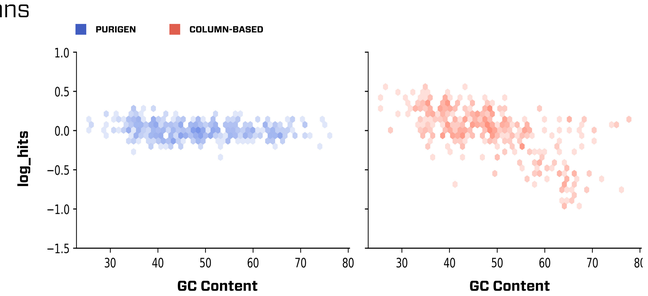


FIGURE 4: Data from the sequencing of the SureMASTR Tumor Hotspot libraries is plotted here as coverage ratio against the % GC-content of the target. The samples purified using a column-based kit show variation in coverage based on GC content, whereas the samples purified on the Ionic System are not substantially impacted by GC content.

Purification by the Ionic system **improves repeatability of variant calls** when compared to columns

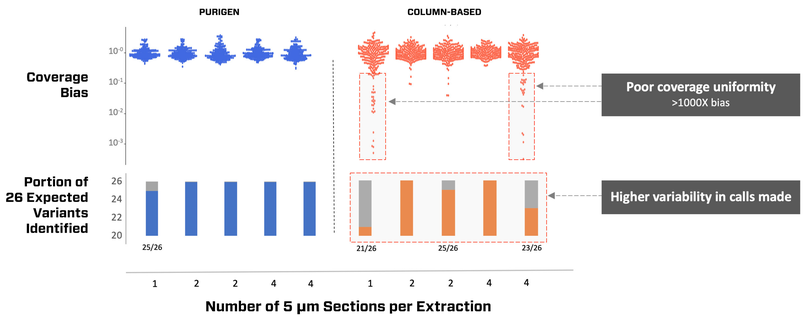


FIGURE 5: Libraries were prepared and enriched for the SureMASTR Tumor Hotspot Panel (Agilent) and sequenced on the Illumina MiSeq sequencer. Coverage was assessed against control libraries constructed from a high-quality reference sample, Coriell Institute GM24835 DNA and plotted as log ratio. Samples purified on the Ionic system have similar depth of coverage for target amplicons in comparison to the reference sample. 100% of the 26 variants identified by both technologies were detected in 4 out of 5 of the samples purified on the Ionic system.

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