Use of isotachophoresis for successful nucleic acid extraction from difficult specimens in the Biospecimen Processing Core Facility at the University of North Carolina at Chapel Hill.

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Introduction: As a core facility it is necessary to stay abreast of new technologies, particularly with those that provide superior results for research clients. Many institutional pathology departments contain thousands of disease-specific formalin-fixed paraffin-embedded (FFPE) samples, representing a vast resource for genetic analyses. Although FFPE samples can be stored indefinitely at room temperature, this method of preservation commonly results in damaged and fragmented DNA and RNA. Despite this, nucleic acids extracted from FFPE tissues are used in many downstream analyses such as PCR, microarray, targeted sequencing, and RNA expression analyses. Column-based extraction methods have been the standard for years, however these methods are time-consuming and do not perform well when FFPE samples contain little tissue. An isotachophoresis (ITP) system developed by Purigen Biosystems (now part of Bionano) promised higher yields and better-quality isolations when compared with existing column-based methods. As one of the first users of the ITP system, we have seen both claims borne out on particularly difficult sample types in our facility as demonstrated below.

1. Study 1 – Evaluation of the transcriptome from tumor biopsies from 3 patients. The BSP received FFPE slides of lymph nodes from 3 patients, each containing very little tissue. In our experience, column-based methods would not have been successful and thus the isolations were performed on the ITP system. The extractions worked well and >1 ug of RNA was obtained from the slides, with 3/5 samples expressing a DV200 of >30. All extractions had successful library preps made and sequencing performed.

2. Study 2 – Extraction of DNA from EndoCervical Curettage (EEC) FFPE slides for the analysis of HPV genotypes. As with Study 1, these samples contained very little starting tissue. Using ITP we were able to isolate DNA which was successfully typed in an HPV assay that amplified small DNA fragments using a TypeSeq v2 assay.

3. Study 3 - Methylation for small volume blood samples. DNA extractions from 150 ul of whole blood from young adult cancer patients were performed on the ITP for subsequent methylation analysis. Extractions yielded an average of 1.5 ug and successfully underwent bisulfite conversion for methylation analysis on an Illumina EPIC array.