FFPE Protein Recovery and Optimization for Proteomics Analysis

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Mayo Clinic has amassed a large archive of Formalin Fixed Paraffin Embedded (FFPE) tissue blocks from diverse diseases such as cancer, multiple sclerosis, diabetes, immune disorders, neuropathies, *etc.* Protein profiling of such FFPE tissues offers a valuable opportunity to obtain new information regarding the molecular mechanisms of disease. However, the analysis of proteins from FFPE samples is both complex and challenging. With this in mind, we have designed a study to systematically investigate multiple different sample preparation methods to optimize a workflow for the proteomic analysis of FFPE tissues. The scope of this work was divided into two sections. We first focused on tissue sections corresponding to microgram amounts of protein (10-50µg) as samples large enough to be collected by macrodissection will typically fall in this range. To optimize the preparation of these samples we evaluated and compared six different methods, two methods using commercially available kits, and four methods that have been either adapted from the literature or developed in-house. The efficiency of protein extraction within each method was evaluated by the analysis of matched fresh frozen and FFPE tonsil core sections. The effectiveness of each method was compared to each other method by the identification of the total number of protein ID's using nanoLC-MS/MS on a LTQ Orbitrap instrument (Thermo Scientific).

After completion of the first step we sought to optimize the preparation method for samples collected by Laser Microdissection (LMD). This technique is extremely useful when collecting subpopulations of cells within the tissue of interest and typically results in sub-microgram amounts of protein. To accomplish this, four different methods were optimized and evaluated. Two of these methods were developed in house and two were adapted from the literature. Two different amounts of tissue, approximately 200 and 750ηg of FFPE tonsil section were processed

with each of these methods and run by nanoLC-MS/MS on a Q-Exactive (Thermo Scientific). The total number of protein and peptide identifications from each method was then compared to determine relative effectiveness. Results from this comparison indicate that the highest number of protein and peptide identifications arise from one of our in house developed methods. The effectiveness of this method was then demonstrated on a range of human tissue samples, where sub-microgram amounts of protein resulted in up to more than 2800 protein identifications in a simple 2 hour gradient.

By optimizing this important workflow investigators interested in retrospectively studying the mechanism of disease will have the opportunity to probe the proteome of Mayo's vast FFPE tissue biobank.

References

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