Ultra Low-cost DNA Miniprep Spin-Columns for Rapid Filter-Trapped Proteomics Sample Preparation

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Abstract:

Complete proteolytic digestion in the preparation of proteins for bottom-up proteomic analysis is substantially improved by the use of detergents for complete denaturation. This however is incompatible with many proteases, and highly detrimental to LC-MS/MS data collection. Recently, filter-based methods such as FASP (Filter-Aided Sample Prep) have seen wide use due to their ability to remove detergents and other harmful reagents prior to digestion and mass spectrometric analysis. Unfortunately, these techniques can be variable and time consuming. Suspension trapping (S-Trapping) is a newer method that utilizes a depth-filter to trap flocculated proteins, and has proven to be a faster approach for proteomic analysis. Sample preparation by these methods requires careful control of protein concentrations in order to flocculate the sample for collection, and the cost of commercial solutions can be high. We hypothesized that protein suspensions also retain on silica-based filters due to ionic interactions mediated by the presence of sodium (Na\(^+\)), SO\(_4\)\(^{2-}\) and PO\(_4\)\(^{3-}\). As such, we sought to investigate if very low-cost DNA purification spin-filters, so called ‘minipreps’ could efficiently and reproducibly trap proteins for digest and LC-MS/MS analysis. Using model proteins and whole-cell lysates we compared digestion efficiencies, capacities, recovery and identification rates from samples prepared using DNA-minipreps and FASP-based protocols. Samples were analyzed using nano uHPLC MS-MS/MS and Label-Free-Quantitative (LFQ) proteomics. DNA-filters show low variability, excellent recovery, sensitivity, and proteome depth from a commercially obtainable device which costs < $0.25 (US) per sample.

Introduction:

Effective sample preparation is central to all LC-MS based proteomics (1,2). Successful removal of harmful reagents or contaminants and having a complete protein digestion are essential components for analysis (3,4). Detergents are important for the solubilization of hydrophobic membrane proteins and complete denaturation of polypeptides (5,6). Sodium dodecyl sulfate
(SDS) is the most commonly used detergents for this task. However, it must be removed prior to analysis of signal suppression, adduct formation, and column poisoning among other instrumentation issues (7–9). Because of this, protein preparation for MS analysis often requires the extraction or purification of proteins prior to digestion into peptides (10,11). Two common techniques for prior to MS analysis are in-solution digests and in-gel digestions. In-gel digestion from one- or two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is still common today. This technique remains popular due to its ability to reliably generate peptides from bands and universality of the approach (12,13). However, in-gel digests are difficult to automate, lead to lower peptide yields, and the “gel-shifting” problems associated with reproducible membrane protein recovery and digestion (8,14). In-solution digestions are easier to automate and often employed if additional separation steps are undesirable (15,16). However, detergents must be entirely absent or removed via acidification or ion-exchange which leads to reduced peptide recovery (17,18).

Filter-based methods like filter-aided sample preparation (FASP) are widely-used for their gel-free handling of biological samples with detergent compatibility for MS analysis. The FASP method utilizes an ultrafiltration device and repetitive steps to disrupt protein-SDS micelles for the depletion of SDS and retention of protein samples (19,20). The filter retains comparatively large protein molecules that were fully denatured in an SDS solution. Washing with urea-containing buffers after loading protein disrupts SDS and protein micelles leading to the detergent being filtered out as flow-through. The digestion step occurs on-filter and will proteolyze the protein into smaller peptides, which can elute off the filter membrane to be captured for analysis (21,22). These filter-based methods are considered ‘universal’ and are compatible with a wide range of culture and digestion conditions and have shown considerably higher sensitivity and depth of protein identification than 2DGE/in-solution (22–25). Despite
these improvements, the procedure is tedious and time consuming, and has a high failure-rate (18,23,26).

Other filter-based methods like suspension trapping (S-Trap) also solubilize proteins in SDS buffers prior to filter loading. The filtering material is a quartz or borosilicate depth filter with reverse-phase membrane frits. The SDS containing protein sample is converted into a protein suspension through flocculation via acidification with phosphoric acid in a methanolic buffer. The protein suspension becomes trapped in the filtration material while SDS and other contaminants are removed during wash-steps (27). This method has enabled the preparation of SDS-containing protein lysates in a fraction of the time and effort as compared with FASP methods and post-preparation processing. The Banks group and others demonstrated that yields within these experiments are on-par or better than representative FASP proteomes (26–29).

Filter-based approaches for sample preparation are routinely targeted for improvements. This is done in order to make them more robust, sensitive, or tailored to a specific study (11,30,31). Construction of ‘in-house’ filter traps are widely employed to generate more affordable and/or reusable tools for ‘economical proteomics’ (32,33). This is in part due to the comparatively high cost of commercial filtration devices (~$10 US) and moderate-throughput needs of most proteomics preparations. Core facilities in particular are sensitive to the dual-need of robustness which commercial solutions promote and reduced costs from ‘in-house’ alternatives (ABRF personal communication). Here, we utilized a spin filter that has the cost-benefits of an in-house device, but the reliability and economy of scale from a commercialized product. Spin filters are mass produced in the genomics field for DNA miniprep. DNA has a high affinity for silica, it is the basis of spin-filter minipreps; so we hypothesized that commercially packed DNA miniprep spin filters are effective as filter-based tools for bottom-up proteomic analysis (34,35).
In this study, we examine the protein loading capacity, digestive efficiency, and reproducibility of DNA miniprep spin filters from a commercial vendor to proteomic comparisons against 3000 MWCO ultrafiltration for FASP. In order to measure protein loading capacity, and capacity for proteolysis, Bovine serum albumin (BSA) was used and analyzed using MALDI-TOF MS and LC-MS/MS. Subsequently we subjected whole-cell lysates of *E. coli* to mini-prep proteomics and FASP in parallel and analyzed the resulting peptide fragments by nano-UHPLC-MS/MS. Label Free Quantification (LFQ) [Flash-LFQ] in MetaMorpheus (MM-LFQ) was used to quantify protein yield and reproducibility across technical triplicate injections from biological triplicate samples (36,37). Each sample preparation method produced comparable protein and peptide yields as determined by MM-LFQ. Reproducibility was determined by the average coefficient of variation (CV) calculated from protein intensities between triplicate injections of samples for each method. The two methods had nearly identical CV's and comparable sample yield. This is the first work to provide a direct quantitative comparison of inexpensive DNA miniprep spin filters to other filter-based digestion methods, and the results illustrate miniprep filters compare favorably to existing filter-based tools in terms of yield and reproducibility.

**Experimental:**

**Reagents:** LB and LB-Agar were from Amresco (Solon, OH) and Teknova (Hollister, CA) respectively. All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless specified. LC-MS pure reagents (Water, Ethanol, Acetonitrile, and Methanol) were purchased from JT Baker (Radnor, PA). Urea was also from Amresco, the materials for staining and running gels (Coomassie brilliant blue, bromophenol blue, SDS, 1M Tris-HCl) were primarily from BioRad (Berkley, CA). Iodoacetamide (IAA) was purchased from MP Biomedicals (Solon, OH). The gels used for electrophoresis from Genscript Biotech (Piscataway, NJ) were run on a Mighty Small II from Hoefer Inc. (Holliston, MA) and imaged with a BioRad Gel-Doc EZ Imager. DNA miniprep spin filters were purchased from BioNeer (Oakland, CA) and the 3K vivaspin 500
ultrafiltration devices were from Sartorius AG (Wood Dale, IL). Hydrophilic–lipophilic balance (HLB) solid phase extraction (SPE) cartridges (10 mg) from Waters (Milford, MA) were used to desalt peptide samples prior to analysis on a Q-Exactive HF from Thermo Fisher (Waltham, MA) or an Ultraflex MALDI-TOF from Bruker Daltonics (Billerica, MA).

Miniprep Sample Preparation – BSA:
20 mg of BSA was dissolved in 50 mM Ammonium bicarbonate (ABC) and 6% SDS. Proteins were reduced with 10 mM TCEP and denatured at 95°C for 3-5 mins. After cooling to room temperature, they were alkylated with 10 mM IAA in the dark for 30 mins. The alkylation reaction was quenched with a 12% phosphoric acid solution, (to 1.2%) and the BSA was diluted in 50 mM ABC and 6% SDS to 10 mg/mL. 0-500 µg of BSA was removed from solution and diluted to 50 µL in 50 mM ABC and 435 µL in 95:5 MeOH:50 mM ABC. Samples were then loaded on to a Bioneer miniprep spin column and centrifuged at 1200 rcf for 30 secs. The flow-through was collected for 2DGE analysis. Samples were washed twice with the 95:5 methanol-buffer above and spun as before. Flow-through was discarded as waste. Trypsin was then loaded onto the filter at a 1:50 (enzyme:protein) ratio and left to digest for 8 hours at 37°C. After the digestion, all flow-through was collected and peptides were further eluted with two washes of 100 µL 0.1 % formic acid and one additional wash of 150 µL 0.2% formic acid in 50% ACN. Peptide solutions were dried briefly in a vacuum centrifuge (Genvac, MA) to remove acetonitrile and then resuspended in 0.1% formic acid prior to desalting with SPE. Solid Phase Extraction was performed as described below. Samples were dried completely prior to MALDI-TOF and LC-MS/MS analysis.

Gel-Electrophoresis of Flow-Through /capacity testing:
Flow-through from the first wash step was dried down in a speedvac and resuspended in a SDS-PAGE sample loading buffer (5X) made from 250 mM Tris-HCl, 10% SDS, 30% (v/v) Glycerol, 10 mM DTT, and 0.05% (w/v) bromophenol blue. TRIS-MOPS-SDS was used as the running buffer. 20 µL of sample was transferred into lanes, Precision Plus Protein Dual-Color Standard (BioRad) was loaded into as a molecular weight ladder, and 20 µL of unprocessed BSA control (1 mg/mL) was loaded into as a control. Electrophoresis occurred at 150 V for 90 mins. Gel staining solution was 60% DI water, 30% ethanol, 10% acetic acid, and 2g coomassie blue – G/R 250 dye. Gels were left in the staining solution at shaking conditions for 60 mins. Next, gels were transferred to a destaining solution that was 45% methanol, 45% DI water, and 10% acetic acid. Gels were left to destain overnight, and imaged on the Gel-Doc EZ Imager the next day.

**Bacteria Culture and Protein Harvest:**

*Escherichia coli (E. coli)* MG165 was struck to single colonies on LB Agar incubated overnight at 37°C. A single colony was then into 5 mL of LB Broth incubated shaking at 37°C overnight (250 RPM). Cells were diluted 1:200 in fresh LB media in an Erlenmeyer flask and grown for 4 hours or until an OD<sub>600</sub> 0.5. Media were centrifuged at 5,000xg for 8 mins at 4°C to pellet bacteria. LB broth was decanted and the cell pellet was resuspended in cold PBS before being centrifuged again at 5,000xg for 8 mins at 4°C. This was repeated again, and the cells were combined and pelleted in one tube which was stored at -80°C. until lysis.

**Cell Lysis:**

The cell pellet was resuspended in 2 mL of a buffer solution consisting of 100mM Tris HCl (pH 7.5), 150mM sodium chloride, 1mM phenylmethanesulfonylfluoride (PMF), 1% SDS, and 0.1% Triton X100. Once the pellet was dissolved, mixed with 0.5 mL 0.1mm diameter zirconia beads
(BioSpec) and kept on ice. Lysis was then performed with a bead beater (BioSpec) in three thirty second intervals. Between intervals, sample was left on ice. After lysis, cells and debris were clarified by centrifugation at 12,000xg for 12 minutes. Protein concentration was determined via BCA assay (Thermo).

**Miniprep Sample Preparation – E. coli Lysates:**

1 mg of whole-cell lysate lysate was dried down and resuspended in 50 mM ABC and 6% SDS and heated gently to resuspend. Proteins were then reduced with 10 mM TCEP and denatured at 95°C for 3-5 min. Protein was alkylated with 10 mM IAA in the dark for 30 mins. The alkylation reaction was quenched with a 12% phosphoric acid solution (To 1.2%). 50 µg of protein was then removed from the sample and diluted to 50 µL in 50 mM ABC and 435 µL in 95:5 MeOH:50 mM ABC. Samples were then loaded on to miniprep spin columns and centrifuged at 1200 rcf for 30 secs. Samples were washed twice with the methanolic:ABC as above and spun as before. Flow-through was discarded as waste. Trypsin was then loaded onto the filter at a 1:50 (enzyme:protein) ratio and left to digest for 8 hours at 37°C. After digestion, flow-through was collected and peptides were eluted with two washes of 100 µL 0.1 % formic acid and an additional wash of 150 µL 0.2% formic acid in 50% ACN. The peptide solution was dried in a vacuum centrifuge to remove acetonitrile and then resuspended in 0.1% formic acid prior to desalting.

**Filter-Aided Sample Preparation (FASP):**

50 µg of whole-cell lysate was mixed with a solution containing 1% SDS, 100 mM TCEP, and 100 mM ABC at a 1:5 ratio (sample:buffer) before mixing and heating at 95°C for 3-5 min. Sample was loaded onto the membrane and centrifuged at 9000xg for 15-20 min. Flow-through was discarded as waste. The membrane was washed three times with 8M Urea in 100 mM ABC.
at 9000xg for 15 min. 50mM IAA in 8M Urea was added to the filter and left in the dark for 10 min. before spinning at 9000xg for 15 min. The filter was washed again with urea and then twice with 50 mM ABC before trypsin was loaded added to the membrane at a 1:50 (enzyme:protein) ratio and left to digest for 8 hours at 37°C. After the digestion, all flow-through was collected and peptides were eluted with 300 µL 0.1 % formic acid and 300 µL 0.1% formic acid in 3-5% ACN. The peptide solution was dried in a vacuum centrifuge to remove acetonitrile and then resuspended in 0.1% formic acid prior to desalting

Solid Phase Extraction (SPE):
Oasis 10 mg SPE cartridges were brought under vacuum using a manifold. Each cartridge was wetted three times with 300 µL acetonitrile and then equilibrated with three additions of 300 µL 0.1% formic acid in water. Sample solution was pulled through the SPE cartridge and then washed twice with 200 µL 0.1% formic acid in water. The cartridges were removed from the manifold and peptides were eluted and collected in an epitube using a P1000 pipet providing positive pressure and three 200 µL elutions of 1:1 acetonitrile and water with 0.1% formic acid. The eluted peptides were evaporated to dryness in a speedvac and resuspended in 0.1% formic acid prior to LC-MS analysis.

MALDI-TOF MS:
0.25 µL of sample was pipetted onto the surface of a stainless steel MALDI target and then covered in 0.25 µL of 30 mg/mL 2,5-Dihydroxybenzoic acid (DHB) in a 1:1 mixture of acetonitrile and water with 0.1% trifluoroacetic acid. Sample was dried before introduction to the instrument. Data were acquired in reflector mode with a mass range of 800-2500 m/z. 3000 spectra were summed per sample at a pulse rate of 500Hz. Peptide Mass Fingerprinting was performed using Mascot (Matrix Sciences) from peak lists generated in Flex-Analysis (Bruker). Briefly, smoothed and deisotoped data were subjected to PMF with the following parameters: Bovine database,
Trypsin was the enzyme selected, and our search was sensitive to two missed cleavage sites. We searched with a mass tolerance of 100 ppm. Carbamidomethyl Cys was selected as a fixed modification, and Oxidation of Met (M) as variable.

*Analysis via LC-MS/MS:*

Dried digests were resuspended in 0.1% formic acid and water to a concentration of 250 ng/µL. 2 µL of solution was injected onto a 100mx100µm C18 BEH reverse phase chromatography column with an autosampler (Waters) as in (2). Peptides were separated on the column for a total of 90 minutes, at 900nl/min. MS-MS/MS was performed on a Q-Exactive HF mass spectrometer via electrospray. Samples were collected in biological triplicate, and each sample was analyzed in technical triplicate using a TOP17 Data-dependent method (11).

*Peptide-Spectral Matching/Database searching:*

RAW files from triplicate biological and technical replication were processed for peptide sequencing, protein inference and false-discovery rate estimation using the Meta-Morpheus engine (36,37). An *E. coli* FASTA from Uniprot UP000000625 was used with a common contaminant file. Data were calibrated prior to search (traditional) using default parameters. For main search, parameters included a maximum of two missed cleavages, two modifications per peptide, and a minimum peptide length of seven base pairs. The precursor mass tolerance was set to 5 ppm and the product mass tolerance was set to 20 ppm. The common fixed and common variable modifications were selected again, and results were normalized and filtered for a q-value of 0.01 (37). RAW data files are available through MassIVE (Massive Link).

**Results & Discussion:**

An efficient protocol for bottom-up proteomic analysis requires solubilization of proteins, digestion of proteins into peptides, and identification of peptides using mass spectrometry.
Detergents are very efficient at getting proteins into solution, but must be removed prior to analysis (5,38). Filter-based tools have become popular for their ability to isolate proteins and remove detergents from solution, but are time-consuming and tedious in their protocols, inconsistent digest performance, and comparatively expensive (18,23,26,32). In this study, we hypothesized that the silica substrates used in inexpensive DNA spin columns would efficiently trap proteins for a protease digest for proteomics analysis. We first compared the performance of DNA miniprep filters against FASP ultrafiltration membranes as described in Figure 1. DNA miniprep filters substantially less expensive than commercial protein filters, (A factor of 10-100) and their potential trapping capabilities allow for a much faster protocol with less wash steps. For analysis, *E. Coli* cell lysates were solubilized, denatured, and alkylated at high concentrations of SDS (~5-6%) prior to loading/washing and subsequent digestion on a DNA miniprep filter. In parallel, identical *E. Coli* cell lysates were prepared and digested on 3K vivaspin ultrafiltration devices following a FASP protocol (19). Both FASP and miniprep samples were analyzed using a Q-Exactive HF mass spectrometer. Protein and peptide quantification was performed using MetaMorpheus label-free quantification software FLASH LFQ (36,37).

In order to test the binding efficiency and capacity of the miniprep-columns, we prepared a bulk solution of 20mg BSA and loaded 0-500 µg increments on miniprep columns in triplicate after flocculation. Columns were spun at 1200 xg for 30 seconds. The resulting flow-through contains unbound protein. This was dried down and analyzed via SDS-PAGE (Supplemental Figure 1). We did not observe any breakthrough of BSA at any of the amounts collected. From this we conclude that the columns effectively trap protein in amounts from 20 to at least 500 µg and likely higher. The capacity appears only to be limited by clogging of the filter-substrate which would prevent flow-through of solvents.
The BSA stored on the columns were digested and spotted for MALDI-TOF MS analysis. The resulting peptide spectra (Supplemental Figure 2) were searched with MASCOT software to determine the efficacy of the digest (39,40). MASCOT score and sequence coverage were taken into account compared against a FASP digest control. For the MASCOT search, the protein score is \(-10 \times \log(P)\), where P is the probability that the observed match is a random event and thus protein scores greater than 50 are significant (p<0.05). For all digest methods, MASCOT scores nearly tripled what is considered significant and the sequence coverage was 68% or higher (Supplemental Figure 2). The positive identification paired with high sequence coverage and MASCOT scores for the DNA miniprep filters served as evidence of an efficient digest. In order to test sensitivity we repeated the above experiment; but loaded 500 ng of BSA onto a DNA miniprep. This protein was digested and subjected to nano-uHPLC-MS/MS as described above. A representative BPC is shown as Supplemental Figure 3. From these data we conclude that the DNA filter preparation is sensitive enough for low-volume / quantity proteomics samples.

Protein capacity was next tested with a complex sample; whole cell lysates of *E. Coli* lysates. 1.0 mg of cell lysate was prepared as described before, and 0-100 µg volume-normalized aliquots were loaded onto miniprep spin filters. Flow-through was collected and analyzed via SDS-PAGE. There were no protein bands present outside of the control (Supplemental Figure 1). Based on these results we conclude that DNA miniprep spin-columns effectively trap biochemically and proteomics-useful amounts of protein sample. Next we performed digests on miniprep isolated samples in parallel with FASP, a widely used filter-based proteomics tool. 50 µg of cell lysates were loaded onto three DNA miniprep and three 3K vivaspin 500 ultrafiltration devices. The cell lysates were loaded and digested as above and desalting via SPE. 500 ng of protein was loaded onto a nano UHPLC column and separated via chromatography prior for
MS/MS analysis on an orbitrap mass spectrometer. For each biological sample, three technical replicates were injected and analyzed. The RAW files were processed and peptide-spectral mass matching was performed using MetaMorpheus, which generated a list of all proteins and peptides identified in each sample. Proteins and peptides were quantified using LFQ and results were filtered at a q-value of 0.01 (FDR).

Proteins were organized from highest to least average intensity, and were counted as a positive ID if they appeared in two out of three technical runs. Using these parameters, 1945 proteins were identified from all samples. 1700 proteins were identified in the DNA miniprep samples and 1863 proteins were identified from the FASP samples. Of the 1945 total protein ID’s, 1646 were found in both the DNA miniprep samples and the FASP samples. 54 proteins were unique to the DNA miniprep samples and 245 proteins were unique to the FASP samples (Figure 2a). To better visualize this, a heat map was generated of the proteins organized from highest to lowest average intensity. If a protein is present in at least two of the three technical injections for a sample, it will appear dark blue, but if a protein is only present for one or none of the triplicate injections, it will appear yellow (Figure 2c). Of the 1700 proteins identified with DNA miniprep spin filters, 1536 (90%) were identified in two of the three biological samples, and 1400 (82%) proteins were identified in all three. Of the 1863 proteins identified with FASP membranes, 1747 (94%) were identified in two of the biological samples, and 1630 (87%) were identified in all three. The same analysis was applied to peptides and it was determined that 29834 peptides were identified through both methods. 22126 peptides were identified with DNA miniprep filters and 18525 were identified with FASP membranes. 11168 peptides were identified in with both filter methods. 10958 peptides were unique to the BioNeer miniprep filters and 8212 peptides were unique to FASP membranes (Figure 2b).
The coefficient of variation (CV) was measured for each set of replicate measurements and biological replicates in order to determine run-to-run reproducibility (41–43). The CV was determined from the replicate intensity (LFQ Abundance data). CV’s were then averaged for each biological sample both miniprep and filter method to determine an overall CV for both approaches. The combined CV for FASP was 18% and for the DNA miniprep filters it was comparable at 19%. For both methods, the majority of proteins had CV’s less than 10%, and over 65% of the proteins identified had CV’s less than 20% (Figure 3).

Conclusions:
The term “economical proteomics” suggests there is a desire for more affordable yet reliable tools for bottom-up proteomics sample preparation. Past research in this field has focused on self-assembling filter tools to create inexpensive and reusable “in-house” devices. In this work, we have shown that a more cost-effective alternative already exists, and that miniprep spin filters, typically used for DNA purification, can be utilized instead to purify protein for bottom-up proteomics sample prep to significantly reduce the cost and time associated with these experiments. Protein yield as determined by normalized LFQ data and reproducibility between runs as determined by the CV prove that DNA miniprep spin filters can be reliable tools for these studies when compared to universally accepted protocols like FASP. This is significant because DNA miniprep spin filters used in these experiments can be purchased in bulk for cents as opposed to dollars, and the protocol is less time-consuming. Future work can be dedicated to optimizing a procedure for these filters in bottom-up studies…
REFERENCES:

E. coli MG1655 cell pellets were harvested in a lysis buffer containing 100 mM Tris-HCl (pH 7.5), 150 mM Sodium Chloride, 1 mM PMF, 0.1% SDS, and 0.025% Triton X. Protein lysates were then dissolved in an SDS containing solution before reduction using TCEP and alkylation with IAA. Protein samples were filtered using BioNeer DNA miniprep spin columns or Sartorius Vivaspin 3K ultrafiltration membranes. Trapped protein was digested with Trypsin, and collected in epitubes. Peptide solution was desalted using HLB SPE prior to analysis via HPLC-MS/MS and searched using MetaMorpheus software.
Figure 2:
Figure 3:
Supplementary Information for:

Ultra Low-cost DNA Miniprep Spin-Columns for Rapid Filter-Trapped Proteomics Sample Preparation

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SI Figure 1: The resulting gels from protein loading capacity experiments with BioNeer DNA miniprep filters. These filters are shown to have a capacity for up to $500 \mu g$ of BSA (left) and $100 \mu g$ of *E. coli* lysate (right).
**SI Figure 2.** The resulting MALDI spectra from digests using the BioNeer DNA Miniprep filters for 10-50 µg BSA. The table below includes peptide matches, sequence coverage, and MASCOT scores derived from the MALDI spectra above.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peptides</th>
<th>Sequence Coverage (%)</th>
<th>MASCOT Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µg</td>
<td>48</td>
<td>62</td>
<td>194</td>
</tr>
<tr>
<td>20 µg</td>
<td>53</td>
<td>68</td>
<td>234</td>
</tr>
<tr>
<td>50 µg</td>
<td>49</td>
<td>61</td>
<td>197</td>
</tr>
</tbody>
</table>
SI Figure 3: A chromatogram generated from a 0.5 μg BSA digest on a BioNeer DNA miniprep spin filter. The resulting PSM’s, peptide ID’s, and sequence coverage from the run are listed below.

PSM’s - 315
Peptides - 39
Sequence Coverage - 52.1%