Reducing Sample Amounts for Isobaric Tagging Quantitative Proteomics Experiments

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Introduction

Several isobaric tagging methods have been developed for quantitative proteomics projects and are useful across many specimen types. Isobaric tagging allows for multiplexing across multiple conditions or replicates based on the TMT reagent area of reporter ions. The reporter ions are bound to peptides with a mass normalised linear region and then fragmented during the tandem MS (MS/MS) ejection of base. Because the individually labeled samples are analyzed in the same run, the spectral assignments for the different reporter ions/pseudomolecular ions are common observations. Two of the most commonly used isobaric tagging ions are the TMT reagents from AB Sciex and the iTRAQ reagents from Thermo Scientific. The TMT reagents in 4-plex and 6-plex kits, and TMT™/® are available in 2-plex and 6-plex kits. Kit instructions for both reagents recommend using 20-150 µg of digested protein per aliquot to ensure complete labeling. One technical difference is that TMT™ and iTRAQ™ utilizes the reporter ions flipped in sequence to increase peptide separation and decrease sample complexity in the mass spectrometer. This led to the requirements of large amounts of sample for labeling and subsequent fractionation step. However, with the advancement in MS cycle time and sensitivity, the required amount of sample per fractionation for the same number of identified peptides has progressively shrunk. These improvements have also reduced the amount of isobaric tagging needed per sample, allowing the use of smaller samples for multiplexing. The goal of this project is to test the possibilities of using smaller aliquot amounts to small sample sizes.

Workflow

Figure 1

Multiplex Protein Quantitation using Isobaric Reagents

Materials and Methods

HEK cell cultures were prepared by RIPA lysis and extraction, and proteins were methylated-proteolipid. The precipitate was re-suspended in 0.1% TFA reduced, alkylated, diluted to 25 µl, and run with LyoPac followed by Tronac. The HEK proteolytic peptide mixture was then digested by trypsin digestion. The TMT™ reagents were labeled from AB Sciex (Chagrin Falls, OH, USA) and the iTRAQ® kit from Thermo Scientific (Waltham, MA, USA). All additional buffers, solvents, and acids were purchased from Sigma Aldrich (St. Louis, MO, USA).

The C16- matrix (New England, South Boston, MA) dissolved H2O digests, previously quenched by trypsin and trypsin at 4 µl µg, were then treated and 1µl µg was aliquoted. The sample was dried in a speedVac, then diluted from 50 µl of water to remove residual acid from the C16 mass step. The sample was then dissolved in methanol/acetonitrile/buffer/trifluoroacetic acid (TFA) for 10 µg, TMT™ and iTRAQ® reagents were dissolved in anhydrous acetonitrile, so

Results

Figure 2

Protein and peptide ID results are presented in Figure 2. The 5 and 10 µg runs are highly paired to each other within each aliquot size, while the 1 µg runs show fewer proteins and peptides identified. This is likely due to losses of the final C18 cleanup step and reduction in lower amount of material packed in the mass spectrometer. Figure 3A shows the overall trend in chromatograms (IC) of the TMT™ samples. Figure 3B shows the same for the TMT™ samples. In both cases, the lower µg amount has a lower signal response, consistent with less material loaded, while the 5 and 10 µg loads are closely matched. Since the 1 µg/seed experiment would have at most 3 µg of total protein with lower losses, it is unsurprising that lower amounts of protein are available to load into the mass spectrometer. The nearly identical signal intensities for the 5 and 10 µg indicates that the losses are at the final C18 step and that the 1 µg/seed taking over high through protein concentration after losses during C18 derivatization give rise to results.

Conclusions

Isobaric tagging reagents label complexity and efficiency at lower sample amounts than required by the literature. High numbers of proteins and peptides can be identified with smaller amounts of material, but take losses at the lowest level due to increased peptide amount, independent of sample type or efficiency. Isobaric tagging kits can be used for greater numbers of reactions.

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