Isobaric Labeling Optimization for Limited Samples and Large Experimental Designs

Michele Rodrigues Martins[1], Guillaume Nugue[1], Fábio César Sousa Nogueira[1], Magno Rodrigues Junqueira[1]

[1.] UFRJ, Universidade Federal do Rio de Janeiro, Departamento de Bioquímica, Instituto de Química, Cidade Universitária, Rio de Janeiro, RJ, Brasil.;

Isobaric labeling with Tandem Mass Tags (TMT) is extensively utilized in proteomics to facilitate sample multiplexing and enhance protein quantification reliability by mass spectrometry (MS). This multiplexing reduces equipment time by analyzing multiple samples in a single run and decreases the required sample size. modern MS machines require only a few micrograms of proteins to perform an in-depth analysis, whereas commercial kits are still designed for traditional samples of 100 µg. Nevertheless, the high costs of reagents, the number of samples, and the limitation of available biological material present significant challenges. This study aims to optimize isobaric labeling to improve cost-effectiveness and reduce the amount of biological material required without compromising data quality.

Samples were processed for proteomics using an extraction buffer containing urea, thiourea, and HEPES. Proteins were quantified by qubit and trypsin digestion, followed by desalting peptides using POROS R2 and C-18 columns. For isobaric labeling, peptides were dissolved in TEAB and labeled with TMT 10-plex. Reactions were performed with 8-15 µg of peptides and quenching with hydroxylamine. The samples were analyzed on an EASY-spray c-18 column integrated with an Easy 1000 nano-chromatograph coupled to an MS Q-Exactive Plus. The data was processed and analyzed using proteome discoverer, max quant software, and R programming language tools.

We performed 35 TMT analyses on complex samples, optimizing conditions to minimize reagent consumption. We observed inefficiencies in labeling following established protocols. Therefore, we developed a new protocol that minimizes reaction concentrations, ensuring labeling efficiency and overcoming competition between aminolysis and hydrolysis of NHS esters. We achieved labeling efficiencies between 98% and 99.5%, reducing reagent costs by up to 18 times.

Furthermore, additional experiments were conducted using multiple sets of TMTs simultaneously in a single experimental design. We implemented corrections for TMT effects, which allowed us to expand the number of conditions tested without the bias introduced by labeling. Currently, we are developing strategies to correct the compression effect of TMT ion signals, a widely discussed problem in the literature, to improve the precision of quantifications and confidence in the obtained data.

Optimizing the isobaric labeling protocol with TMT resulted in significant cost reductions and decreased biological material usage without compromising result quality. Applying this protocol demonstrated the feasibility of this technique in large experimental projects and limited samples. Data analysis and processing continue to validate the efficacy and robustness of the new method.

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