

PROTEORED MULTICENTRIC EXPERIMENT 2009 (PME5): QUANTITATIVE PROTEOMICS METHODS

Objectives:

- Test each laboratory abilities to perform quantitative proteomic analysis.
- Comparison of methodologies for relative quantitative analysis of proteomes. The study should provide data to assess and compare performance of different methodologies and intra- and inter-lab reproducibility of these.
- Evaluation of data reporting and data sharing tools (MIAPE documents, standard formats, public repositories).

Samples:

Each participant laboratory will receive two protein mixture samples, labeled A and B, containing each 100 µg of total protein.

Samples are in solution, in 20mM Tris.HCl, 50 mM NaCl, and are sent frozen.

Concentration of both samples is 6.6 µg/µL

Samples contain:

- A mixture of around 150 E. Coli proteins (identical in each sample)

This mixture has been prepared by fractionation of the cytoplasmatic proteome of E.Coli. It contains soluble proteins, of a wide range of pI and Mw.

- Four spiked mammalian proteins:

CYC_HORSE (Cytochrome C, Mw 12362), added at the ~ 1 pmol/ 1 µg total protein level.

MYG_HORSE (Apomyoglobin, Mw 16952), at ~ 200 fmol / 1 µg total protein

ALDOA_RABIT (Aldolase, Mw 39212), at ~ 25 fmol / 1 µg total protein

BSA_BOVIN (Serum albumin, Mw 66430), at ~ 1 fmol / 1 µg total protein

These four proteins have been spiked in different amounts in samples A and B, with ratios ranging from 1.5:1 to 5:1 between the two samples.

Purpose of the analysis:

The intended purpose of the analysis is to measure the ratios between samples A and B for the four spiked proteins. The “matrix” E. Coli proteins, which should be unchanged, will provide a measure of dispersion for the method used.

The samples can be also used to test methods for absolute quantitation, if desired.

In order to evaluate reproducibility in an homogenous dataset, we ask to perform a minimum of 4 replicate analysis of the samples. (Depending on the method of choice this would demand a maximum of 4 + 4 LC-MS runs).

Methods:

- Sample complexity has been chosen to allow for the analysis of the mixture on single LC-MS runs. In principle, there is no need for pre-fractionation. A long enough gradient (90-120 min) gradient is suggested, but this of course will strongly depend on the MS instrument available for analysis.
- 1-2 micrograms of total protein per run should be enough to cover the range of abundances of the spiked proteins in the samples. Again, this will depend a lot on the instrument used, and should be adjusted by each Lab. according to their expertise.
- The sample is primarily intended to test non-targeted relative quantitation methodologies. Both label-based methods (ICPL, iTRAQ, TMT, O18,...) and label-free methods (based on spectral counts, Hi3, "LCMS Image analysis"...) can be performed and tested to analyze the samples. Some of them will require 4 + 4 LC-MS runs, while others (i.e. 8-plex iTRAQ) could require a single run to provide comparable measurements of reproducibility. Try to choose the number of replicate analysis in a way that 4 independent measurements of each A:B ratio are obtained, so that comparable statistics can be calculated.
- The sample can be also used if desired to test targeted methods, such MRM methods for relative or absolute quantitation. The concentration of the spiked proteins is probably too high to provide a real challenge for those methods, but it can still be useful for test purposes (one can test accuracy, sensitivity on serial dilutions of the sample...)
- The amount of sample provided, as well as the concentration of the spiked proteins, should allow also a 2D-DIGE analysis of the samples, although this is not the main purpose of the experiment.

Data analysis and report:

Together with Salvador Martínez de Bartolomé, who will be coordinating this, we will send you soon a separate document on how to analyze data and report protocols and results. The general idea is performing an "In-lab" analysis, with the lab's own tools, and a second "centralized" analysis, by sharing the data in standard formats and using different software tools available for comparison.