

CODE: 15128

2.- Information about methods used to analyze the samples.

A brief description of the experimental approach is summarized here (see Figure 1).

Lyophilized samples were depleted with ProteoPrep 20 Plasma Immunodepletion LC column (Sigma) according to the user guide. The non retained fraction (in 1x PBS) was collected and quantified using nanodrop system. Purification and quantitation was checked using a SDS gel stained with colloidal comassie blue.

Samples were digested and labeled using iTRAQ reagents-8 plex (Applied Biosystems) according to manufacturer protocol. Then, they were pooled, dried and resuspended in loading buffer for SCX. 40 fractions were collected and pooled up to 7 fractions. These fractions were chromatographically separated in reverse phase and collected directly into to a MALDI plate using a Probot (LC Packings). Samples were analysed using a 4800 MALDI-TOF/TOF instrument.

Since we were only interested in 4 proteins, an inclusion list was prepared for the MALDI. Theoretical peptides were calculated considering: trypsin enzyme, 0 miss cleavages, methylation of cysteins and 8 plex reagent for N terminal and K.

The MS/MS spectra were searched against UniProt Knowledgebase Release 14.6 using Paragon algorithm in Protein Pilot software.

3.- Experimental design used for relative quantitation

We used the 8-plex kit (Applied Biosystems). We labeled samples A-F with 114-119, respectively. We prepared a pool of the samples (ideally 1/6 corresponding to each sample) and labeled with 121. We had planned to label the same protein amount in all the cases, including the pool, but we had an experimental error.

When preparing the samples to be digested, the volume corresponding to sample D was added to the pool. So, this sample had less protein amount than the rest (4 ug vs 18 ug) since we did not have more volume. The total volume of the pool was divided by two in order to make similar protein amount and avoid a better ionization (if we had not done this, the protein in the pool would be 33 ug instead of 18 ug).

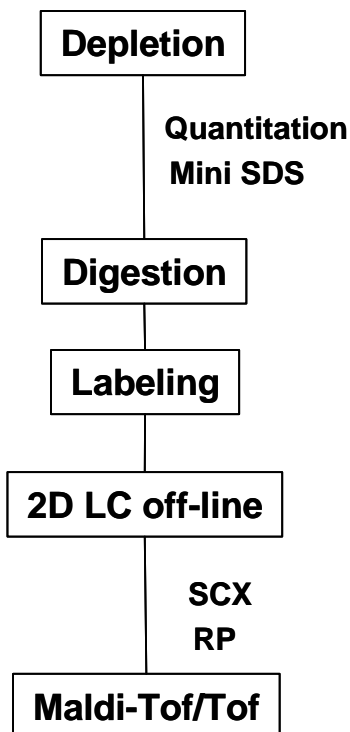


Figure 1: Experimental scheme