Protein Identification by Mass Spectrometry

GUIDELINES FOR SAMPLE SUBMISSION

Protein identification is performed both in-gel or in solution by proteolytic digestion of samples followed by LC-MS/MS or MALDI/MS analysis of the resulting tryptic digest. Database search is included in the Protein Identification Service and the results will be organized in a report identification table.

The complete identification procedure consists of 3 phases:
1. **Protein digestion:** protein sample either in solution or from 1D or 2D gel is reduced, alkylated and submitted to proteolytic hydrolysis with trypsin. For gel sample, peptides are extracted from the gel by a specific washing procedure.
2. **Peptides analysis:** the peptide mixtures are directly analyzed by LC-MS/MS (preferred procedure) or alternatively by MALDI/MS (mainly for well resolved spots from 2D gel sample).
3. **Database Search:** results from the mass spectra analysis are used to search for non redundant protein databases using an in house version of the Mascot software.

The LC-MS/MS analysis is performed using an LC/MSD XCT Ultra Ion Trap equipped with a 1100 HPLC system and a chip cube (Agilent Technologies) whereas MALDI mass spectra are recorded on a 4800 plus MALDI TOF-TOF (AB Sciex) mass spectrometer equipped with a reflectron analyzer and used in delayed extraction mode with 4000 Series Explorer v3.5 software.
Mass spectral data from either MALDI/MS or LC-MS/MS analysis are used to search for a non redundant protein database using an in house version of the Mascot software (Matrix Science, Boston, MA, USA).

Submission of sample for Protein identification

Protein identification by proteomic approaches can be performed on proteins either in solution or separated by mono- and/or bidimensional electrophoresis.

A) Samples from 1D or 2D gels:

<u>During sample preparation and electrophoresis analysis:</u>
Powder free gloves and lab coats should always be used. Work under a laminar flow hood is advisable to minimize the possibility of contamination by dust, hair, flakes of skin thus eliminating interfering keratins. When possible, 1 mm thickness gel should be used with the narrowest gel lane width possible to achieve a protein to gel volume ratio as high as possible.
<u>After electrophoresis experiment:</u>
Gel staining with colloidal Coomassie is necessary.
Please contact Primm before using different staining procedure.

After staining, please send a copy of the gel image indicating the bands or spots of interest. If possible, send the intact gel; alternatively, excise the bands or spots of interest (using a clean scalpel and tweezers) and send them in a sealed tubes with MilliQ water (to keep each gel plug wet).

B) Sample in solution or lyophilized:

Single purified protein is preferentially provided in aqueous solution or organic solvents (i.e. Acetonitrile, TFA). Lyophilized proteins are also acceptable.
Please, indicate the last step in sample preparation. Provide information on protein concentration, buffer composition and protein molecular mass, if known.

Requested amount

Indicative amount of proteins for identification analyses consists of 500 pmol of lyophilized or soluble protein or visible Coomassie stained spot or band.
It is also possible to analyze spot or band stained with different method or lower amounts of sample in solution, please, contact Primm to discuss the feasibility.

Important for sample submission

Please, avoid the presence of high concentration of detergents and glycerol. Indicate the organism or source of protein sample. The organism must have a sequenced genome. Please, contact Primm when the organism genome is unknown.
Identification might be difficult if a band or spot is not visible on colloidal Coomassie-stained gel.
Positive identification for spots or bands cannot be guaranteed if the gel is not stained with colloidal Coomassie.

Analysis Time

Usually 7-14 working days from the arrival of the samples.