Protein(s) Identification and Characterization by tryptic digestion and Mass Spectrometry

**Principles:** Masses of peptides produced by the tryptic digestion of proteins are measured by mass spectrometry with high accuracy (known as a peptide mass fingerprint). Selected peptides are fragmented inside the mass spectrometer (tandem MS) and the masses of the resulted fragments are related to the peptide’s sequence. The combined data are matched against protein sequence databases to determine the proteins’ identity.

**Sample:** Protein from 1D or 2D gels (In-gel) or otherwise purified (In-solution)
Blue silver stain is strongly recommended although silver stain MS compatible may be used. Protein(s) identification could be obtained with a very weak band isolated from gel stained with Blue Silver.

**Experiment:** Reduced (with DTT) and S-carbamidomethylated (with IAA) proteins are digested in–gel or in-solution with trypsin. Peptides are extracted with combinations of acid and organic solvents, concentrated by evaporation, and subjected to mass spectrometry.

**Instrumentation:** Peptides are separated by reverse phase nano Liquid Chromatography (Easy nLC - PROXEON) and entered into the Mass Spectrometer (LTQ Orbitrap XL with ETD - Thermo Scientific) via nanoelectrospray ion source. Peptide ions are fragmented by Collision Induced Dissociation (CID), Higher energy Collision Dissociation (HCD) and Electron Transfer Dissociation (ETD).

**Data Analysis:** Data are processed using Proteome Discoverer 1.1 (Thermo Scientific) and subsequently searched with Mascot (Matrix Science) and Sequest (Thermo Scientific) against databases of Uniprot, IPI etc.

**Results:** A written report is provided describing the analysis undertaken and outlining the evidence for protein’s identification. If no hit is obtained, an indication of the possible reasons may be included. The report is sent by e-mail. Raw data are not included and they are archived by ProFI. If the data are used as a part of a scientific publication, reference to ProFI Lab should be made in the acknowledgements.