Proteomics Analysis FAQ

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1. What are the requirements for proteomic analysis?

   Proteomic analysis requirements are as follow:

   - Animal sample tissues and microorganism samples should weigh no less than 200mg in wet weight. Plant sample tissues and fungus samples should weigh no less than 2g in wet weight.
   - Samples with high concentrations of impurities or that are low in protein content should weigh no less than 3g in wet weight and the cell count should be ~5x107.
   - Solutions with volumes above 5mL should contain no hemolytic agents. Solutions with blood serum should be greater than 500uL.
   - For protein extracts, the concentration should be no less than 2mg/mL, and the protein content should be no less than 1mg.
   - If the samples needed to be enriched, such as modification analysis, the protein content should be no less than 2mg.
   - Protein solutions should contain no ionic detergent as SDS. Please notify BGI of buffer concentration as a measure for quality assurance.
   - Protein solutions should contain no nucleic acids, lipids, polysaccharides, or molecules of any type that alters electrophoretic results.
   - If samples are rich in nucleic acids, salts, pigments, polysaccharides, or molecules of any type that alters two-dimensional electrophoresis, this will require purification or concentration of the sample. Please notify us in advance of such conditions..

2. What mass spectrometry services are available at BGI?

   BGI employs the latest and most advanced mass spectrometers, including AB SCIEX QTRAP® 5500, LTQ Orbitrap Velos from Thermo Scientific, and maXis and ultrafleXtreme from Bruker Daltonics.

3. What proteomic analysis services do BGI platforms provide?

   Currently, BGI provide analysis services with platforms as shown below:

Analysis Type	Method of Analysis
Proteome profiling	SDS-PAGE-LC-MS/MS
	HPLC-LC-MS/MS
Quantitative proteomics	iTRAQ
	Label-free
Modification proteomics	Requires enrichment experiment
	Requires no enrichment experiment
Target proteomics	MRM

4. How many types of proteins does proteome profiling analysis typically provide?

   The number of protein types provided depends on the sample's complexity, database capability, protein content, and the level of proteomic separation. LC-MS/MS follows protein digestion and can identify about 500-1000 types of proteins. One-dimensional proteomic separation at the peptide and protein level can identify about 1000-3000 types of proteins.

   Note that under standard conditions, the protein count in serums is 1/10 of that which can be identified in cell lines and tissue samples.

5. What are the comparisons between iTRAQ and label-free quantitative techniques?

   Using iTRAQ quantitative technique, multiple (max. 8/group) protein samples from different sources are tagged, combined, and undergo mass spectrometry analysis simultaneously. Every secondary peptide's mass spectrum corresponds to its respective ionic strength and characterizes the individual protein's relative concentration in the sample. This method identifies and quantizes proteins simultaneously.

   Using a label-free technique, from large-scale protein identification mass spectrometry data, we can compare the corresponding peptide strength between different samples; and can conducts relative protein quantification on corresponding peptides.

   iTRAQ quantizes protein of low molecular weight, with highly accurate results. Label-free requires little sample operation and is not limited to the sample's condition which is superior to the tagging technique in many respects. Liquid chromatography and mass spectrometry, however, require higher stability and repeatability.

6. What protein modification analysis is used at BGI?

   BGI chiefly conducts protein phosphorylation modification processes. The four steps for phosphoproteome analysis are sample preparation, peptide phosphorylation/protein enrichment, LC-MS/MS detection, and data analysis. Other protein modification processes may be conducted depending on the requirements of the collaboration.

7. What is the target protein analysis approach?

   Multiple reaction monitoring, MRM, sets up mass spectrometer detection limits based on known data and hypothetical data, and records signals released by ions. It removes intervening signals from the large quantity of ions, which do not fit the norm. MRM is a highly specific and highly sensitive mass spectrometry data acquisition model. Compared to unbiased proteomics, MRM is ideal for proteomic studies that require high sensitivity and selectivity.

   MRM's target proteomic approach can be applied to low abundance protein detection, post-translational modified protein identification, high abundance homologous protein isoforms check, biomarker validation, and protein-RNA interaction studies.

8. Does BGI provide a summary report, and if so, what information is included?

   Yes, BGI does provide a summary report.

The summary report contains basic information (contract and sample information), research analysis, experiment protocol, lab, and analytic results (statistical data, protein identification, protein quantification, modification analysis, protein functions analysis, functions analysis of difference proteins, protein GO categories, protein metabolic pathway analysis,etc). Summary reports will include personalized laboratory results and data analysis based on client's request.

9. How long does it take to perform a typical proteomic profiling study?

   Proteome profiling studies typically take approximately 40 business days; quantitative, modification and target proteomics studies take around 60 business days.