

Overview of Current Proteome Profiling Technologies^a:

| Technology | Labeling Required | Detect Post-translational Modifications? | Proteins that are Optimally Quantified | Approximate Dynamic Range | Max. Number of Proteins or Spots Quantified | Analytical Issues |
|---|--|--|--|---------------------------|---|---|
| SELDI or MALDI-MS Disease Biomarker Discovery | None | Yes | <i>Naturally</i> occurring forms of <10 kD proteins | 25 | Not applicable | Separate experiment required for protein identification |
| Traditional 2D Gel electrophoresis (2DGE) | None | Yes | <i>Naturally</i> occurring forms of 10 kD - 200 kD proteins | 1,000 | 3,000 | Quantitation and replication difficult |
| Amersham Differential 2D Fluorescence Gel Electrophoresis (DIGE) | <i>In vitro</i> with Cy-2,3 or 5 fluorophores at primary amines | Yes | <i>Naturally</i> occurring forms of 10 kD - 200 kD proteins | 10,000 ^b | 3,000 ^c | Only detects proteins expressed at high levels, that have long half-lives ^{b,d} and are amenable to 2D gel analysis |
| Proteome Lab PF 2D Automated 2D Chromatofocusing/Reverse Phase HPLC | None | Yes | <i>Naturally</i> occurring forms of >5 kD peptides <i>and</i> proteins | 100 ^e | 2,500 ^e | Limited to UV detection unless coupled to MS |
| Multi-dimensional LC/MS/MS Protein Identification (MudPit) | N ¹⁴ /N ¹⁵ <i>in vivo</i> at nitrogens in amino acids | Yes | Tryptic peptides from digests of protein extracts | 10,000 ^f | 872 ^g | Mixture highly complex, requires fractionation prior to MS |
| Acid-Labile Isotope Coded Affinity Tag (ICAT) - LC/MS | <i>In vitro</i> with C ¹² /C ¹³ cleavable ICAT reagent at cysteine | No | Cysteine-containing tryptic peptides from digests of protein extracts | 10,000 | 496 ^h | Only detects cysteine-containing proteins, cannot generally detect post-translational modifications |
| Targeted proteomics: protein browsing and MS/MS scanning | Some procedures use ICAT reagents | Yes | May be used to quantify any protein expressed at sufficiently high level | No data | 100 ⁱ | Need to validate each internal synthetic peptide calibrant prior to use |
| Protein Microarray | <i>In vitro</i> with Cy-3 or 5 fluorophores at primary amines | No | Proteins for which commercial antibodies are available | 100 ^j | 512 ^j | Limited to proteins with monoclonal antibodies, a range binding affinities between antibodies, cross-reactivity between antibodies, and limited to using specific binding and reaction buffers. |

Literature cited: ^a(This table is property of KeckBRL and should not be used or reproduced without consent from Christopher Colangelo or Kenneth Williams),^b(Tonge et al. *Proteomics* **2001** 1:377-396), ^c(Hoving et al. *Electrophoresis* **2000**, 21:2617-2621), ^d(Gygi et al. *Mol. Cell. Biol.* **1999b** 19:1720-1730), ^e(Betgovargez and Simonian Beckman Coulter Application Information Bulletin **2003**, A-1964A), ^f(Wolters et al. *Anal. Chem.* **2001**, 73:5683-5690), ^g(Washburn et al. *Anal. Chem.* **2002**, 74:1650-1657), ^h(Han et al. *Nat. Biotechnol.* **2001**, 19:946-951), ⁱ(Kalkum et al. *Proc. Natl Acad. Sci. USA* **2003**, 100: 2795-2800), ^j(http://www.bdbiosciences.com/clontech/archive/APR03UPD/Ab_microarray.shtml)