

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)