

An Alternative Approach to Quantitative Protein Profiling: Differential 2D Fluorescence Gel Electrophoresis (DIGE)

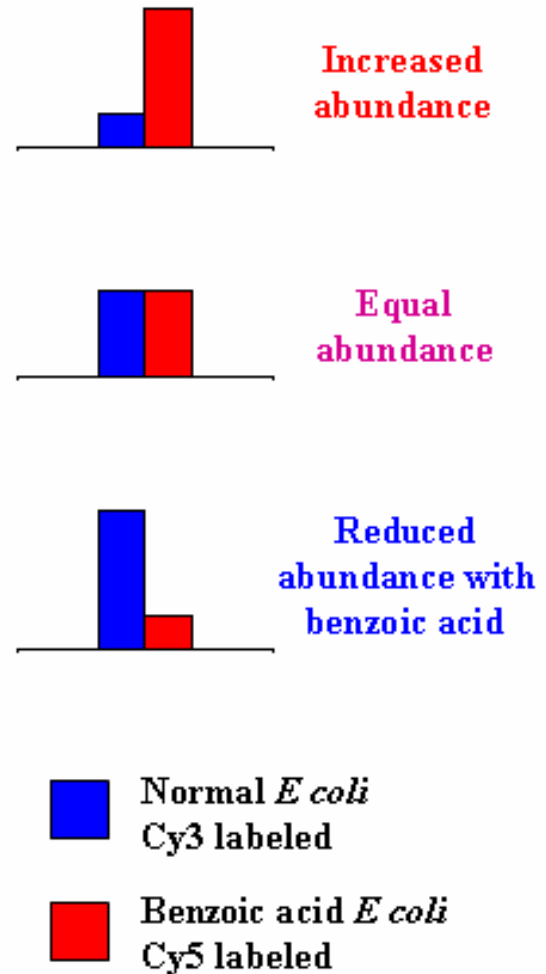
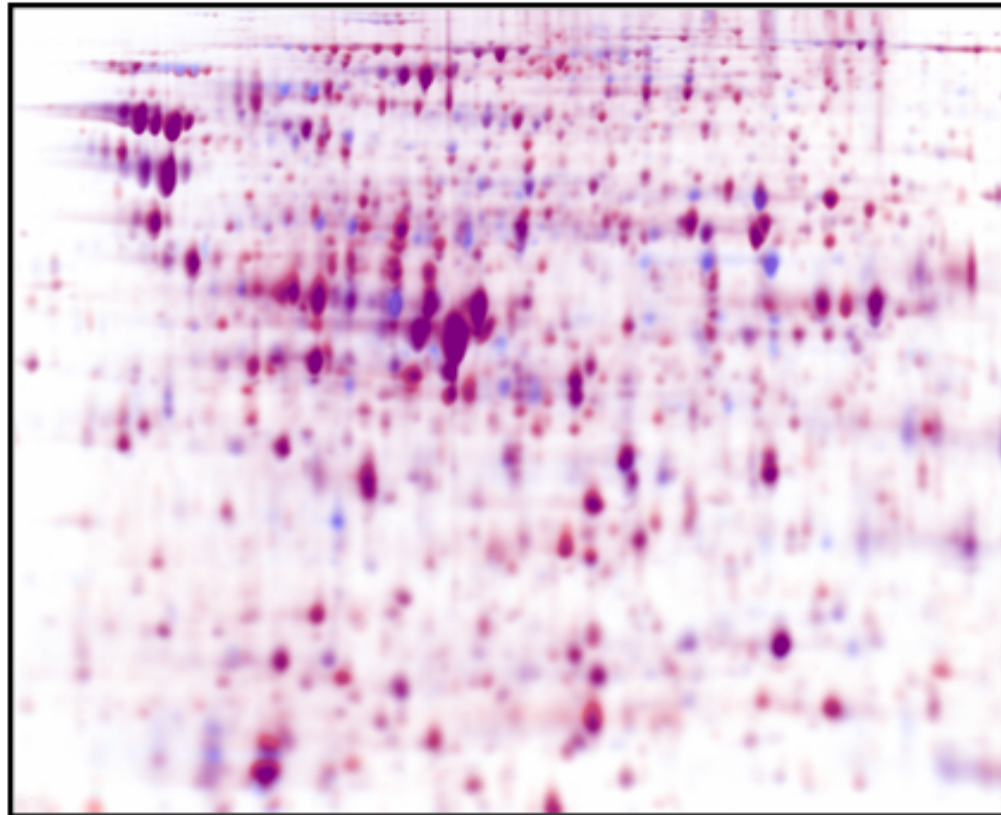
Outline of Approach

- 1. Extract proteins from control and experimental cells/tissues.**
- 2. *In vitro* label control with Cy-3 and experimental proteins with Cy-5 dyes which have been matched with respect to charge and mass - with single positive charge of the dye replacing the charge lost by the modified lysine or N-terminus of protein.**
- 3. Cy-3 and Cy-5 labeled proteins co-migrate - with the dye label adding approximately 580 Da to proteins in each sample.**
- 4. Mix control and experimental samples together and subject to 2D gel electrophoresis. Replicate samples required for statistical analysis of results.**
- 5. Optional (recommended) use of a third dye (Cy-2) as an internal (pooled control + experimental) standard to permit normalization of multiple gels and for internal normalization.**
- 6. Use Amersham Typhoon 9400 Imager to scan gels (while still held between glass plates) at 2-3 wavelengths.**
- 7. Use Amersham DeCyder software package to quantify ratio of expression of proteins from control versus experimental samples.**
- 8. DeCyder software automatically outputs a listing of statistically significant differences in protein expression including t-test values, using the Cy-2 internal standard.**
- 9. A *major* advantage of use of fluorescence dyes is their 4-order of magnitude linear response range - which compares to only about 1 order of magnitude range for silver or CB staining.**

Additional Information on the DIGE Approach to Protein Profiling

Limit of detection of differential protein expression	1.3 fold above background
Dye linkage chemistry	N-Hydroxy-succinimide (NHS) linkage to primary amines
Approximate MW of CyDye DIGE fluors	580 Da
Labeling stoichiometry	On average, 3% of protein will have one dye molecule attached
Limit of detection for quantifying protein ratios	Similar to silver staining, from 0.25 - 0.95 ng or 5 -19 fmol of a 50 kD protein^{a, d}
Dynamic range	10^4 as compared to only about 10^1 for CB of silver staining^a
Maximum number of spots likely to be quantified/gel with high protein load	>1,050^b
Approximate system cost with gel electrophoresis set-up, scanner, gel picker, start-up reagents, and one software license	\$300,484
Additional software license cost	\$22,000 each license
Service contract cost/year	\$29,490
Supply cost/gel assuming 3 dye labeling and post-staining of 26 x 20 cm gels with SYPRO Ruby	\$149/gel
Supply cost/gel assuming 2 dye labeling and post-staining of 26 x 20 cm gels with SYPRO Ruby	\$120/gel

Overlay of Normal and Benzoic acid-treated *E. coli*



Blue = more highly expressed in control *E. coli* = repressed by treatment with benzoic acid

(Above figure kindly provided by Amersham Inc.)

Recent References Using 2D Differential Fluorescence Gel Electrophoresis (DIGE) to Quantify Relative Protein Expression

- a) Tonge, R., Shaw, J., Middleton, B., Rowlinson, R., Rayner, S., Young, J., Pognan, F., Hawkins E., Currie, I., Davison, M. (2001) "Validation and development of fluorescence two-dimensional differential gel electrophoresis proteomics technology." *Proteomics* **1**, 377-96.
- b) Zhou, G., Li, H., DeCamp, D., Chen, S., Shu, H., Gong, Y., Flaig, M., Gillespie, J., Hu, N., Taylor, P., Emmert-Buck, M., Liotta, L.A., Petricoin, E.F., Zhao, Y.. (2002) "2D differential in-gel electrophoresis for the identification of esophageal scans cell cancer-specific protein markers." *Molecular & Cellular Proteomics*. **1**(2), 117-24.
- c) Ruepp, S.U., Tonge, R.P., Shaw, J., Wallis, N., Pognan, F. (2002) "Genomics and proteomics analysis of acetaminophen toxicity in mouse liver." *Toxicological Sciences*. **65**(1), 135-50.
- d) Gharbi, S., Gaffney, P., Yang, A., Zvelebil, M., Cramer, R., Waterfield, M., and Timms, J., (2002) "Evaluation of two-dimensional differential gel electrophoresis for proteomic expression analysis of a model breast cancer cell system." *Molecular and Cellular Proteomics* **1**, 91-98.
- e) Macdonald, N., Chevalier, S., Tonge, R., Davison, M., Rowlinson, R., Young, J., Rayner, S., and Roberts, R. (2001) "Quantitative proteomic analysis of mouse liver response to the peroxisome proliferator diethylhexylphthalate (DEHP)", *Arch. Toxicol.* **75**, 415-424.
- f) Kernec, F., Unlu, M., Labeikovsky, W., Minden, J., and Koretsky, A., (2001) "Changes in the mitochondrial proteome from mouse hearts deficient in creatine kinase." *Physiol. Genomics* **6**, 117-128.