

Two-Dimensional Differential Gel Electrophoresis (2D-DIGE)

Background

High resolution 2-Dimensional Gel Electrophoresis (2DGE) is a key analytical method in many areas of proteome research [1]. Since the original introduction of 2DGE as a method for the resolution of complex protein mixtures, the technique has evolved to be capable of generating reproducible and statistically relevant data on cellular proteomes.

Progress has been made in obtaining reproducible 2D protein profiles that, with appropriate analytical software, can be reliably compared with each other. However, further improvements are possible through the use of Differential Gel Electrophoresis (2D-DIGE) a method originally introduced by Minden and colleagues [2] to overcome the inherent variability of 2D gels when comparing large data sets. Fundamental to 2D-DIGE is the use of fluorescent protein labels that have minimal effect on protein electrophoretic mobility and allow the co-electrophoresis of multiple samples on a single gel. The combination of commercially available optimised fluorescent labelling protocols along with precast gels provides a reliable route to the application of 2DGE for proteomic studies.

Experimental Protocol

The protocol adopted at **Aberdeen Proteomics** for 2D-DIGE combines commercial kits for labelling protein samples with gel electrophoresis on precast 1st and 2nd dimension gels to provide good inter-experiment reproducibility. The analytical workflow is summarised below.

1. Samples are prepared using standard protocols for protein analysis by 2DGE and the protein concentration determined. The proteins are precipitated and resuspended, at a concentration of approximately 6 µg/µl, in a compatible buffer for labelling.
2. Individual protein samples are labelled using the Cy3 or Cy5 dyes in a minimal labelling protocol; 50 µg of protein are labelled according to the manufacturer's protocol. A pooled sample, prepared by mixing equal amounts of protein for all samples in the experiment, is labelled with the Cy2 dye.
3. Following labelling, the samples are loaded to immobilised pH gradient (IPG) gel strips. Specifically, Cy3- and Cy5-labelled samples along with an aliquot of the Cy2-labelled pool sample are loaded to each IPG gel.
4. The IPG gel strips are focused on an IPGPhor instrument (GE Healthcare) before being transferred to a Criterion precast slab gel (Bio-Rad Ltd) for the second dimension electrophoresis step using our standard equilibration buffers [4].
5. Finally, the gels are scanned using an Ettan DIGE Imager (GE Healthcare) to image each gel to detect the Cy2-, Cy3- and Cy5-labelled proteins. The image files are transferred to Progenesis SameSpots, Version 4.2 (Nonlinear Dynamics) for gel alignment, spot detection and statistical analysis. Data processing uses the in-built routines available within the Progenesis SameSpots software.

Representative Data

Representative data are shown in Figures 1 to 3 which are part of an ongoing study of *Escherichia coli* proteomics in relation to urinary tract infections. Cellular proteins of clinical *E. coli* isolates were prepared as described by Smith *et al* [3] and labelled for 2D-DIGE as described. The protein samples were analysed on pH4-7 Linear IPG gel strips in the first dimension and Criterion slab gels (AnyKd) in the second dimension. Gels were imaged and subsequent analysis carried out using Progenesis SameSpots.

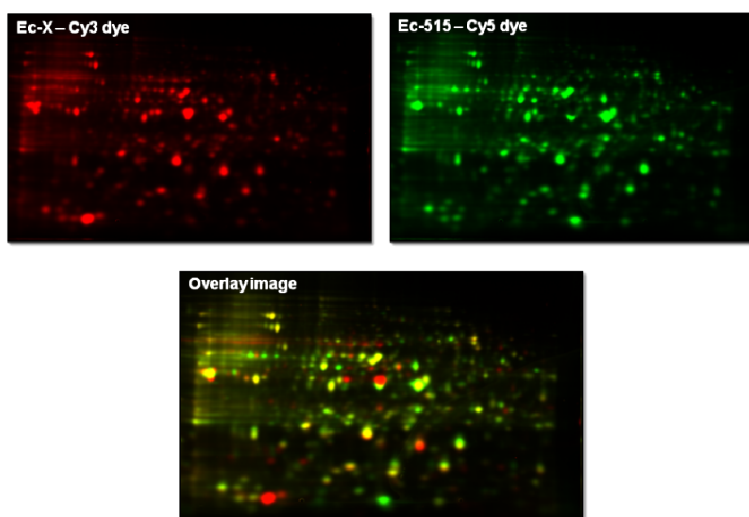


Figure 1: Multiple images for one 2D gel analysing two *E. coli* isolates, *Ec-X* (Cy3 labelled) and *Ec-515* (Cy5 labelled). Images are shown for individual profiles as well as an overlay image. Pseudo-colour images are generated using ImageQuant software (GE Healthcare)

Figure 2: Quantitative analysis of selected protein spots compared between *E. coli* isolates Ec-X and Ec-515.

Panels show (Left to Right): Enlarged region of pseudo-coloured overlay image; montage of gel region from replicate gels of each isolates; 3-D profiles of protein spot across replicate gels; quantitative analysis of normalised volumes for protein spot.

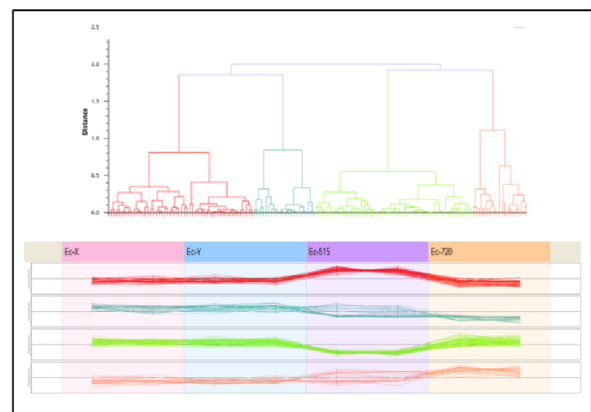
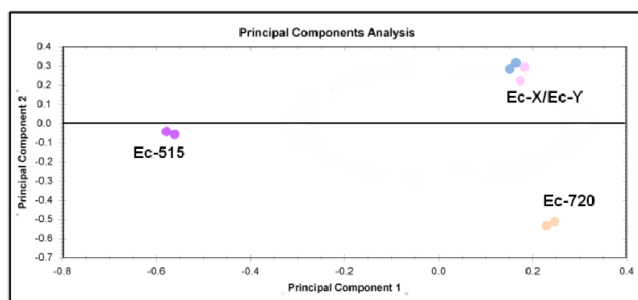
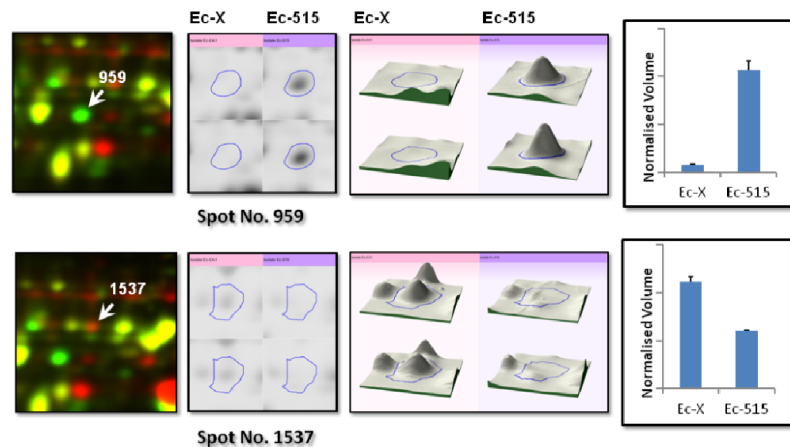


Figure 3: Global analysis of protein expression for 4 *E. coli* isolates (Ec-X, Ec-Y, Ec-515 and Ec-720). The displays are derived from the analysis of proteins showing significant differences in expression (ANOVA <math><0.05</math>) between the 4 bacterial isolates. **Left:** Principal component analysis illustrating the clustering of the replicate 2D protein profiles for the four bacterial isolates. Ec-X and Ec-Y are known to be closely related based on the clinical histories of the samples [3] but distinct from the other two *E. coli* isolates. **Right:** Display of co-ordinated expression profiles of significant proteins across the four bacterial isolates.

Contact Details

For further information on the processing of protein samples using 2D-DIGE at **Aberdeen Proteomics** in proteomic studies please contact:

Phil Cash (p.cash@abdn.ac.uk) or Evelyn Argo (e.argo@abdn.ac.uk)

Aberdeen Proteomics

Aberdeen Proteomics provides an analytical service to the research community covering a wide range of gel- and MS-based proteomic analyses. Samples are processed either individually or as part of large-scale collaborative projects. For further information visit the web site

www.abdn.ac.uk/ims/proteomics/

References

1. Sa-Correia, I., Teixeira, M. C., 2D electrophoresis-based expression proteomics: a microbiologist's perspective. *Expert Rev. Proteomics* 2010, 7, 943-953.
2. Unlu, M., Morgan, M. E., Minden, J. S., Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis* 1997, 18, 2071-2077.
3. Smith, A., van Rooyen, J. P., Argo, E., Cash, P., Proteomic analysis of *Escherichia coli* associated with urinary tract infections. *Proteomics* 2011, 11, 2283-2293.
4. Cash, P., Argo, E., Analysis of bacterial proteins by 2DE. *Methods Mol. Biol.* 2009, 519, 131-144.