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# Prefractionation of *E. coli* lysates by free-flow electrophoresis for subsequent analysis by two-dimensional electrophoresis

## *Introduction*

Two-dimensional electrophoresis (2-DE) is the method of choice for the analysis of complex protein mixtures, e.g. whole cell lysates, but the sheer number of proteins expected to be present within a cell clearly pinpoints the limitations of 2-DE: First of all the resolution of the method can't cope with the enormous complexity of the samples and secondly, low abundant proteins are very likely to be covered by highly expressed species. Free-flow electrophoresis (FFE) is perfectly suited to solve these drawbacks. It allows to reduce the sample complexity very efficiently by the fast prefractionation of the samples with high resolution and great reproducibility in combination with nearly complete sample recoveries. To demonstrate the pre-fractionation qualities of FFE, this application note describes the fast pre-fractionation of E. coli lysate by isoelectric focusing to yield fractions with highly reduced complexity that show only little overlap of their 2-DE patterns as well as highly enriched protein spots.

## *Methods*

**Sample preparation**

Culture medium was centrifuged and the pellet was washed with a low salt washing buffer and stored at -20°C. The sample was resuspended in lysis buffer containing Urea (9 M), CHAPS (2 % w/v), DTT (1%), and Servalyte 3-10 (2 % v/v). Cells were sheared by passing through a syringe needle and sonication for 15 min. After centrifugation at 10000 g for 1h, the supernatant was diluted 1:2 with glycerol (25%), Urea (8M), DTT (30mM) to reach a final protein concentration of 0.45 mg/ml. Traces of the red, acidic dye 2-(4-Sulfophenylazo)- 1,8-dihydroxy-3,6-naphthalenedisulfonic acid (SPADNS, Aldrich) were added to ease the optical control of the migration of the sample within the separation chamber.

**Free-flow electrophoresis** FFE separations were conducted at 17°C using the following media: The experiments were run in a horizontal separation using a 0.4 mm spacer. The separation chamber was artificially narrowed from 10 cm to approximately 3 cm by the application of the stabilization media via inlets I1, I2, I5, I6, and I7 instead of merely inlets I1 and I7. A flow rate of ~ 65 g/h (Inlet I1-7) was used in combination with a voltage of 400 V which resulted in a current of 20 mA. Samples were perfused into the separation chamber using the middle inlet at ~ 5.2 g/h. Residence time in the separation chamber was ~ 20 min. Fractions were collected in polypropylene minititer plates, numbered 1 (anode) through 96 (cathode).

**Anodic stabilization medium (I1+2)** 17.5% (w/w) glycerol; 42% (w/w) 8M urea; 0.03% (w/w) HPMC; 150 mM $H_2SO_4$

**Separation medium (I3+4)** 14.5% (w/w) glycerol; 42% (w/w) 8M urea; 0.03% (w/w) HPMC; 1% (w/w) Servalyt® 3-10, 10 mM DTT, 1% (w/w) CHAPS

**Cathodal stabilization medium (I5-7)** 17.5% (w/w) glycerol; 42% (w/w) 8M urea; 0.03% (w/w) HPMC; 150 mM NaOH

**Counterflow medium** 14.5% (w/w) glycerol; 42% (w/w) 8M urea

**Anodic circuit electrolyte** 100 mM $H_2SO_4$

**Cathodic circuit electrolyte** 100 mM NaOH

**Data analysis** The pH-values of the individual microtiter plate fractions were measured manually. Subsequently, the protein fractions were analysed by SDS-PAGE using an XCell SureLock™ Mini-Cell (Novex) in combination with precast NuPAGE® Novex 4-12% Bis-Tris gels. Silver-staining of the proteins was carried out using the SilverQuest' kit (Novex). 2-DE analysis was performed according to Görg et al. using an IPGphor (Amersham Biosciences) and multiple SDS-PAGE on a vertical system.

## *Results*

FFE being understood as a pre-fractionation technology prior to 2D electrophoresis should provide a reduced number of fractions. Thus, the actual width of the separation chamber was reduced artificially by using 5 out of 7 inlets for stabilization media which results in a separation of the samples into approximately 30 fractions.

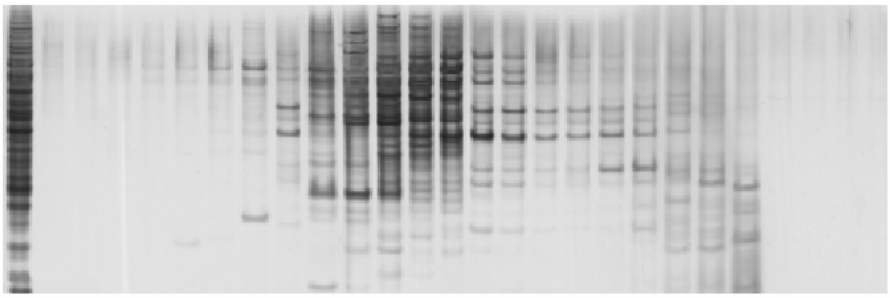

*Figure 1: Silver-stained SDS-PAGE analysis of IEF-FFE-fractionated E. coli lysate. Lanes from left to right: Unprocessed E. coli lysate; fraction 31 (pH = 3.6); 32; ...; 55; 56 (pH = 10.1).*

The silver-stained SDS-gel in Figure 1 exemplifies the fast (residence time only 20 min!) fractiona tion of the E. coli lysate proteins by isoelectric focusing FFE. The fact that a lot of protein bands just occur in one or two lanes already indicates the high resolution of the FFE fractionation. The most complex fractions according to Figure 1 were analysed by 2-DE. The corresponding areas of the 2-D gels depicted in Figure 2 clearly demonstrate that the individual, adjacent fractions have protein patterns that show only little overlap which reflects the high resolution of the method. In addition, virtually every single protein that was observed for the unfractionated sample could also be found in one of the fractions indicating the great sample recovery. Furthermore, several protein spots have been observed that were highly enriched in the individual fractions as compared to the unfractionated sample, e.g. the upper two spots in the grey circle of fraction 40, all spots in the black circle of fraction 41, the upper right spot in the magenta circle of fraction 42).

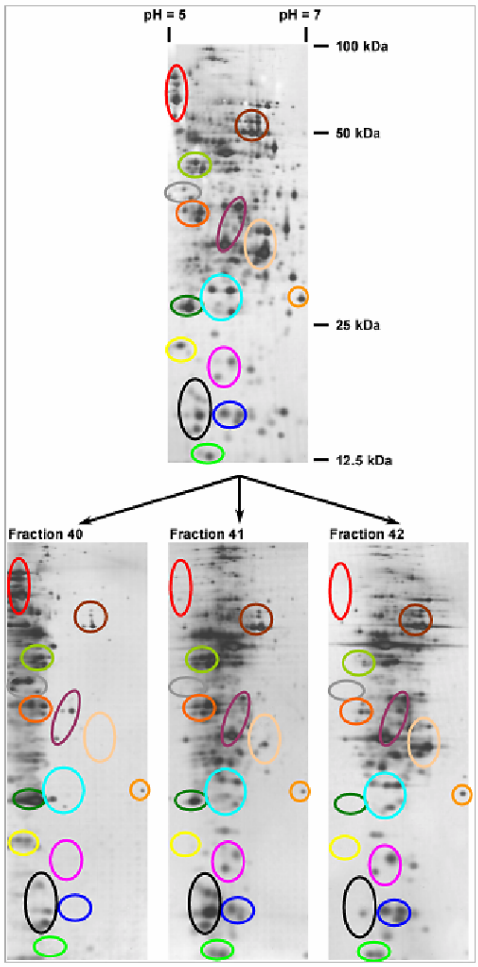


*Figure 2: Silver-stained 2-DE analysis of crude E. coli lysate (top; slightly acidic region) compared to three samples of the same origin that were pre-fractionated by isoelectric focusing FFE. For better comparison, several regions were highlighted by coloured circles.*

## Conclusion

The complexity of protein mixtures can be reduced efficiently by isoelectric focusing FFE in a fast and straightforward manner. Besides high resolution and great reproducibility, the method provides nearly complete sample recoveries and can be even used for the enrichment of proteins. This predestines FFE as an indispensable pre-fractionation technology for subsequent 2-DE analyses.

## References

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3) Görg, A., Obermaier, C., Boguth, G., Harder, A., Scheibe, B.,Wildgruber, R. and Weiss,W. (2000) The current state of two-dimensional electrophoresis with immobilized pH gradients, Electrophoresis 21(6) 1037-1053.