Conference abstract PO-11

Comparison of Different Staining Methods and Software Evaluation Tools for 2DE Gel Electrophoresis

E. STRIZSIK, M. STESSL, J. WINKLER, B. LACHMANN, C. R. NOE

Department of Medicinal Chemistry, University of Vienna, Althanstraße 14, 1090 Vienna, Austria

E-mail: christian.noe@univie.ac.at (C. R. Noe)

Sci Pharm. 2009; 77: 210 doi:10.3797/scipharm.oephg.21.PO-11

Various staining methods for protein staining acrylamide gels exist. But as they all differ in their mechanism of action, sensitivity, linear range and the software tool required for analysis, not all of them can be used for the same issue of research and therefore need to be evaluated.

In contrast to the pre-labelling DIGE technique silver staining and Sypro® Ruby staining are classical post staining methods. Although silver staining possesses a high sensitivity, it only works in a relatively low linear range. Therefore it is not recommended for quantitative proteomic approaches. The fluorescent dye Sypro® Ruby possesses a broad linear dynamic range and high sensitivity. By using different cye dyes of the DIGE technique for different samples, multiple samples can be run on one gel, detecting them at different wavelengths of light.

Silver staining, Sypro® Ruby staining and the DIGE technique were used to analyse off-target effects of an antisense oligonucleotide against the protein Bcl-2 in the human melanoma cell line 607B.

607B cells were transfected with phosphorothioate oligonucleotides and the inhibition of Bcl-2 was demonstrated by western blotting. Gels of lysates of transfected cells and untreated cells were run separately for post-staining first with silver nitrate and afterwards with Sypro® Ruby. Silver stained gels were scanned, Sypro® Ruby stained gels were detected with the Bio-Rad Imager ChemiDoc XRS, both were analysed using the 2DE-image-analysis program PD-QUEST by Bio-Rad. For the DIGE techniques both samples were run on one gel. DIGE gels were detected with the Ettan DIGE Imager and analysed with the DeCyder 2D software.

The analysis of the differently stained 2DE gels with different software tools showed immense differences in number and position of off-target effects.

[1] Lagoja IM., Herdewijn P. Use of RNA in drug design. Expert Opin Drug Discov. 2007; 2: 889–903. doi:10.1517/17460441.2.6.889

[2] White IR, Pickford R, Wood J, Skehel MJ *et al.* A statistical comparison of silver and SYPRO Ruby staining for proteomic analysis. Electrophoresis. 2004; 25: 3048–3054. doi:10.1002/elps.200405947

[3] Timms JF, Cramer R. Difference gel electrophoresis. Proteomics. 2008; 8: 4886–4897. doi:10.1002/pmic.200800298