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Review

2D Gels and Bioinformatics – An Eye to the Future

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ABSTRACT: 2-D Gel Technology has had profound impact on proteomic research over the years. Informatics support brought a new dimension to 2D gels and associated technologies. But with advent of new and emerging technologies, it will be interesting to observe the trends of 2D gel technology in the years to come. Here we review 2D gel technology and its applications besides looking at the future scope of 2D gels in the post genome era.

KEYWORDS: proteomics, 2D gel databases, 2D gel technology, bioinformatics, protein chips

INTRODUCTION

It was predicted way back in 1975 [1] that 2D gel systems in its current form would be able to resolve 5,000 species in a single gel. 2D gels in proteomics have been a success and have lived up to that prediction. The technology has been successfully supported by informatics to present the information lucidly in the form of 2D gel databases. The longevity of this technology has been mainly due to its unparalleled use in studying protein modifications. But with the advent of protein chips and weaknesses to overcome, it will be interesting to observe its trends over the coming years.

2D GEL TECHNOLOGY – YESTERDAY AND TODAY

2-D Gel technology [1,2] has long found use in proteomics and much research has been done on it over the years. Considerable progress has been made in staining and visualising methodologies [3,4] as well as improving image analysis techniques. However, despite recent advances, some areas are definitely found wanting. There is still much reliance on the human eye to make statistical sense out of an image spot. There are still no fully automated publicly available image analysis systems and analysis is often a time consuming process. The amount of protein observed may vary from gel to gel as a result of various discrepancies (for instance over or under staining, or variation in gel size depending on the solution in which the gel has been stored). Such discrepancies may induce serious analysis problems especially when it involves complicated examples like that of heart proteins [5–7]. In such a case, a good laboratory could

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come up with a reproducible 2D gel database but without standard sample data and running conditions another laboratory may present gels which could carry different information altogether. This calls for standardisation of 2D electrophoresis protocols, which is difficult to propose without narrowing down all the possible sources of error.

Characterisation of low abundance proteins and separating proteins at extremes of both isoelectric point and molecular weight have historically been a problem when it comes to 2D gels. There are signs that 2D gel image analysis can be used to map proteins with varying abundance [6] but a fully automated system could give further insights on protein expression in different cell types, stages of development and various associated disease states. However, there have been recent advances. For instance, Tecan has come up with ProTeam FFE [8], which is a device combining free flow electrophoresis and 2D gels to improve separation. Therefore better characterisation and visualisation of low abundant proteins can be achieved. Techniques like non-equilibrium pH gradient electrophoresis [9] and strategic modifications to 2D electrophoresis (2DE) [10] have been reported to give better resolution of basic proteins.

IS THE BEST YET TO COME?

Recently, much effort has gone into the concept of 'Lab-on-a-chip' [11]. These chips involve micron-sized channels embedded in glass or chips. Attempts have been made to carry out two-dimensional gel based experiments on chips. Microchips that are able to carry microfluidic experiments are being developed (Nanogen Inc., DiagnoSwiss, Caliper Technologies) which are faster and more accurate than the conventional gel technology. If such technologies were made 2DE compatible then it would offer immense research potential. A recent review [12] elucidates the latest improvements in the experimental features of 2D gel technology. Especially promising are advancements in detecting low-abundance proteins [13] and the study of heart proteins [14]. The best of 2D gel technology may be yet to come.

2D GEL DATABASES – PROBLEMS GALORE

There are a number of 2D gel databases of proteins from wide ranging species, both quantitative and annotative [15–17]. Current 2D databases [5,15–17] being flatfile formatted offer limited information. They do not offer much information regarding sample running conditions, pre-treatment, number of replicates used for the composite gels and similar issues. Public utility tools such as Flicker [18] help in comparing images from different sources over a common interface. But considering the fact that there is a huge degree of uncertainty and variability in the 2DE images due to the differences in the condition of the run, comparing images from different sources does not seem to be reasonable. It might be of value if the running conditions could be optimised. There are companies developing integrated solutions for 2DE data analysis. For instance BioRad has introduced PDQuest [19] which can analyse around 100 gels at a time (Figure 1), but they have very limited abilities when it comes to project management. But it is only fair to say that there are no commercially available solutions that do everything from sample management to handling experimental data whether it is image analysis or mass spectrometric data.

There has been some progress towards achieving this with BioRad coming up with an integrated bioinformatics platform called WorksBase^{TM} [20], which integrates proteomics laboratory management system and facile bioinformatics support. Commercial bioinformatics, especially when it involves such issues, require commitment to the use of proprietary software and hardware which is often unreasonable since the preferred equipment rapidly changes in a field whose growth is at its peak.

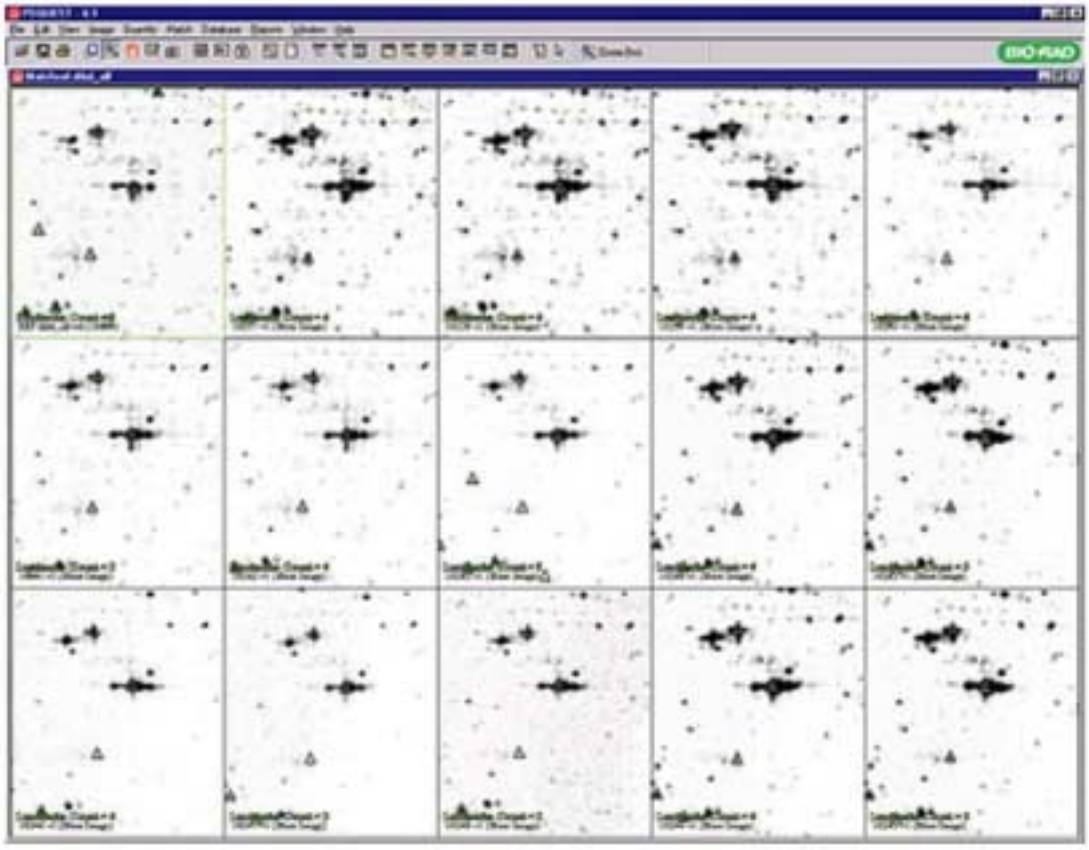

Fig. 1. BioRad's PDQuest. Simultaneous analysis of 100 gels or composite gels using BioRad's PDQuest. (Courtesy: BioRad laboratories)

OUTLOOK

2-D Gel technology has found many applications in proteomics over the years. There are some challenges that can be overcome, such as the informatics design as well as improvements in the database system. But there are also other issues to be addressed, like standardising the 2D gel protocols, which would be a difficult proposition in spite of recent advancements. With protein chips attracting much attention, 2D gels can be expected to play a complementary role. Possibly, by merging features of these two technologies, new horizons could open up. 2D Gel technology might have more to offer.

REFERENCES

[1] O'Farrell, P.H. (1975). High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**, 4007–4021.

[2] Klose, J. (1975). Protein mapping by combined isoelectric focusing and electrophoresis in mouse tissues. A novel approach to testing for induced point mutations in mammals. Humangenetik **26**, 231–243.

[3] Langen, H., Takacs, B., Evers, S., Berndt, P., Lahm, H.W., Wipf, B., Gray, C. and Fountoulakis, M. (2000). Two-dimensional map of the proteome of *Haemophilus influenzae*. Electrophoresis **21**, 411–429.

[4] Pitarch, A., Pardo, M., Jimenez, A., Pla, J., Gil, C., Sanchez, M. and Nombela, C. (1999). Two dimensional map of the proteome of *Haemophilus influenzae*. Electrophoresis **20**, 1001–1010.
[5] Jungblut, P., Otto, A., Zeindl-Eberhardt, E., Pleissner, K.P., Knecht, M., Regitz-Zagrosek, V., Fleck, E. and Wittmann-Liebold, B. (1994). Protein composition of the human heart: The construction of a myocardial two-dimensional electrophoresis database. Electrophoresis **15**, 685–707.
[6] Arnott, D., O'Connell, K.L., King, K.L. and Stults, J.T. (1998). An integrated approach to proteome analysis: Identification of proteins associated with cardiac hypertrophy. Anal. Biochem. **258**, 1–18.
[7] Pleissner, K.P., Sander, S., Oswald, H., Regitz-Zagrosek, V. and Fleck, E. (1996). The construction of the World Wide Web-accessible myocardial two-dimensional gel electrophoresis protein database "HEART-2DPAGE": A practical approach. Electrophoresis. **17**, 1386–1392.
[8] Tecan Proteome FFE. http://www.tecan.com.
[9] Yamaguchi, Y. and Pfeiffer, S.E. (1999). Highly basic myelin and oligodendrocyte proteins analyzed by NEPHGE two-dimensional gel electrophoresis: Recognition of novel developmentally regulated proteins. J. Neurosci. Res. **56**, 199–205.
[10] Rabilloud, T., Valette, C. and Lawrence, J.J. (1994). Sample application by in-gel rehydration improves the resolution of two-dimensional electrophoresis with immobilized pH-gradients in the first dimension. Electrophoresis **15**, 1552–1558.
[11] Mouradian, S. (2002). Lab-on-a-chip: applications in proteomics. Curr. Opin. Chem. Biol. **6**, 51–56.
[12] Lilley, K.S., Razzaq, A. and Dupree, P. (2002). Two-dimensional gel electrophoresis: recent advances in sample preparation, detection and quantitation. Curr. Opin. Chem. Biol. **6**, 46–50.
[13] Hoving, S., Voshol, H. and van Oostrum, J. (2000). Towards high performance two-dimensional gel electrophoresis using ultrazoom gels. Electrophoresis **21**, 2617–2621.
[14] Westbrook, J.A., Yan, J.X., Wait, R., Welson, S.Y. and Dunn, M.J. (2001). Zooming-in on the proteome: very narrow-range immobilised pH gradients reveal more protein species and isoforms. Electrophoresis **22**, 2865–2871.
[15] Celis, J.E., Ostergaard, M., Jensen, N.A., Gromova, J., Rasmussen, H.H. and Gromov, P. (1998). Human and mouse proteomic database: novel resources in the protein universe. FEBS Lett. **430**, 64–72.
[16] Proteome Inc. http://www.incyte.com/sequence/proteome/index.shtml.
[17] World 2-D PAGE. http://www.expasy.ch/ch2d/2d-index.html.
[18] Lemkin, P.F. Comparing two-dimensional electrophoretic gel images across the Internet. Electrophoresis **18**, (1997) 461–470.
[19] BIORAD (PDQuest). http://www.proteomeworks.bio-rad.com/html/pdquest.html.
[20] BIORAD (WorksBase). http://www.biorad.com/LifeScience/pdf/Bulletin_2637.pdf.