

Seamless Integration of 2D Difference Gel Electrophoresis (2D DIGE) with the Investigator ProPic and ProPic II

Proteomic Technical Note 2111

OVERVIEW

Two-dimensional gel electrophoresis has been the workhorse of proteomics for decades, its unique resolving power outweighing many of its imperfections, like limited dynamic range and gel to gel variations. A relatively new technique, 2D Difference Gel Electrophoresis (2D DIGE), overcomes some of these limitations. DIGE was first described in 1997¹, has been recently commercialized, and is now evolving to become one of the most powerful techniques used in gel-centric proteomic studies.

Digilab has introduced a DIGE Upgrade Kit for seamless integration of 2D DIGE with the ProPic II spot picking robots. This allows the user to combine the power of 2D DIGE with superior protein picking accuracy.

The DIGE Upgrade Kit eliminates the need for post-staining or the use of a specific picking gel, dramatically streamlining the workflow and saving both time and expense.

The 2D DIGE Experiment

2D DIGE involves the covalent labelling of sample proteins with the CyDyes™ Cy3 and Cy5. A mixture of all samples in an experiment is labelled with Cy2 and serves as an internal standard minimizing inter- and intra-gel variations. Two samples and the internal standard are combined (multiplexed) and separated within a single gel.

Proteins exhibiting expression differences can be identified using the application-specific image analysis software DeCyder™ (2D Differential Analysis Software, GE Healthcare Bio-Sciences Ltd.). The list of spot coordinates of interesting proteins generated with DeCyder can then be used to drive automated spot picking.

ProPic II DIGE Upgrade Kit for Accurate Picking from DIGE Gels

The DIGE Upgrade Kit has been developed to integrate the Ettan™ 2-D DIGE system (GE Healthcare Bio-Sciences Ltd.) with the Investigator ProPic II spot picking robots². It includes software that translates spot coordinates from DeCyder, as well as a specific gel holder.

The DIGE software has an intuitive, wizard-driven user interface (Figure 1). It enables the automatic translation of spot coordinates created with DeCyder into ProPic II coordinates for accurate spot excision directly from a DIGE gel without the need for post-staining. A custom algorithm is used to correlate the locations of the reference spots from the DIGE image (analyzed using DeCyder) and the ProPic II image of the DIGE gel. The software uses this correlation to calculate the location of the gel spots in the ProPic II image and generates a compatible pick list.

The ProPic II DIGE software maintains the original DeCyder spot number during translation of coordinates to ensure effortless tracking.

The gel holder is designed to hold the (glass-backed) DIGE gel in the exact orientation needed for accurate spot excision using patented picking technology.

Streamlined Workflow

The typical workflow for the identification of interesting proteins from a 2D DIGE experiment (see Figure 2) contains numerous labour-intensive and time-consuming steps. These involve a mandatory post-staining procedure of the DIGE gel (with total protein stains like Sypro® or Deep Purple®) to be used to create a pick list. The whole procedure can easily take more than one week for completion.

The advent of the ProPic II DIGE software with its capability to translate the DIGE-specific application software eliminates the need for many of these steps. Specifically, there is no need for post-staining the DIGE gel, imaging/analyzing it and comparing the analysis to the DeCyder analysis. This results in a much shorter and streamlined workflow with the ProPic II (see Figure 2).

Summary

The DIGE Upgrade kit enables seamless integration of 2D DIGE with ProPic II, with the benefit of giving the the DIGE user access to highly accurate and reliable spot picking and a much streamlined workflow.

Reference

1. Ünlü, M., Morgan, M.E. and Minden, J.S. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis* **18**:2071-2077 (1997).
2. Mahnke, R.C., Corzett, T.H., McCutchen-Maloney, S.L. and Chromy, B.A. An integrated proteomic workflow for two-dimensional differential gel electrophoresis and robotic spot picking. *J. Proteome Research* (2006); ASAP Article, <http://pubs.acs.org/cgi-bin/abstract.cgi/jprobs/asap/abs/pr050465u.html>

Figure 1a.

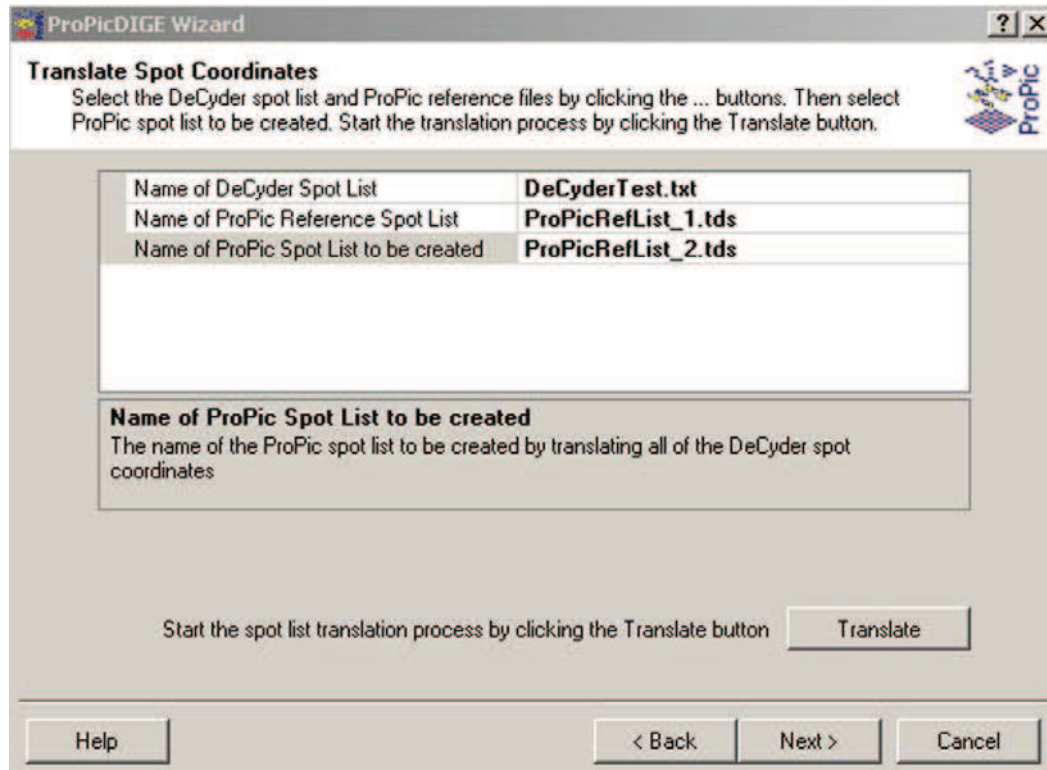


Figure 1b.

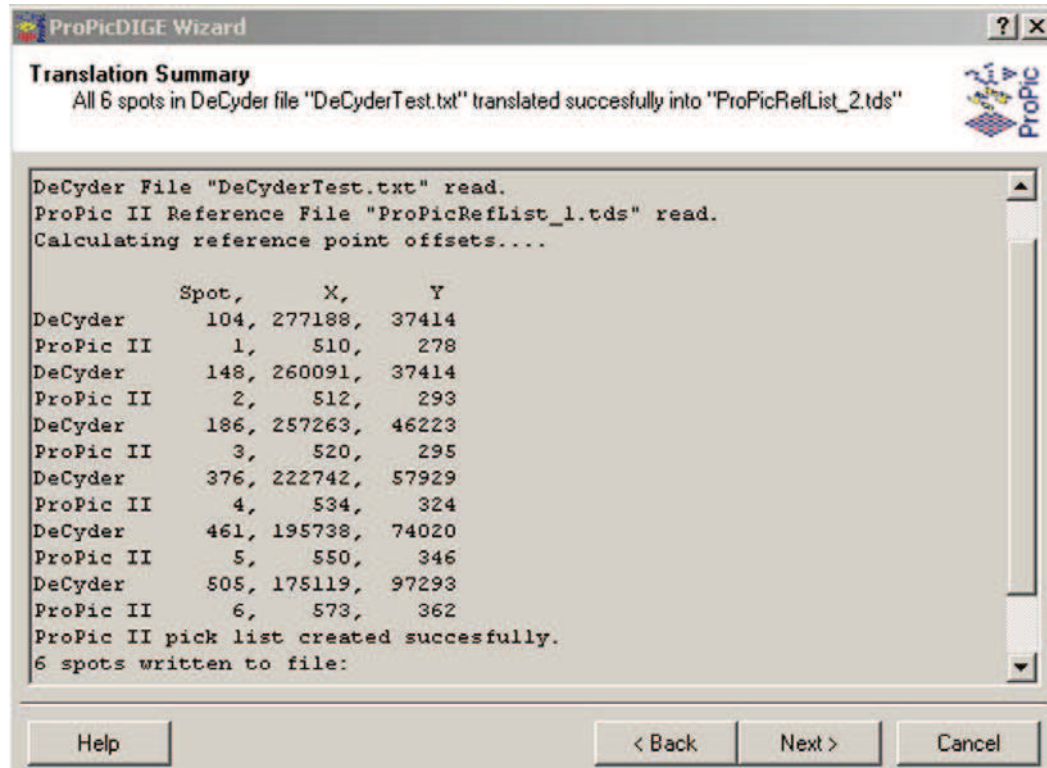


Figure 1a/b. DIGE software automatically translates the Decyder derived x,y, spot coordinates into ProPic coordinates for accurate spot excision

Figure 2.

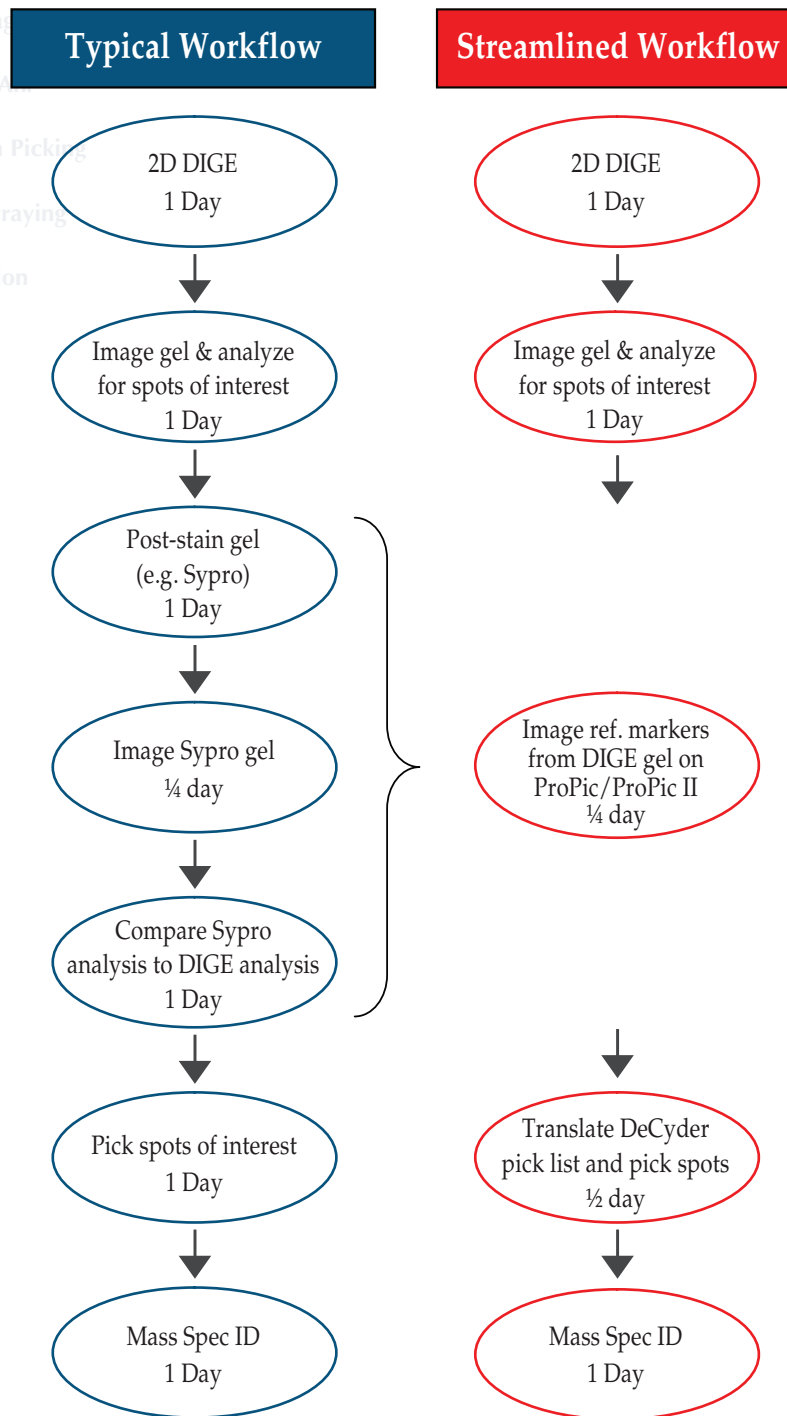


Figure 2. Comparison of the typical and the streamlined spot picking workflow for a 2D DIGE experiment (including duration of each step).

Worldwide Headquarters

Digilab, Inc.
84 October Hill Road
Holliston, MA 01746
USA

Phone: (508) 893-3130
Toll Free: (800) 935-8007
Fax: (508) 893-8011
E-Mail: info@digilabglobal.com

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