Journal of Proteomics & Bioinformatics - Open Access
www.omicsonline.com ***Application Note*** **JPB/Vol.1/April 2008**

AC2DGel: Analysis and Comparison of 2D Gels

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Abstract

Two-dimensional gel electrophoresis can retrieve information regarding thousands of different proteins from a crude protein sample. One of the major challenges in field of proteomics is to extract maximum information from 2D gels. In this study, we developed a web server for the analysis and comparison of 2D gels, which consists of three major modules. The first module allows the analysis of gels on the basis of molecular weight and pH. This module assists in calculating molecular weight and pH of a protein by clicking on corresponding spot at 2D gel image. The second module allows the comparison of two gels and presents the result as a superimposed image where spots/proteins on two gels can be examined. The useful feature of this module is that it allows the comparison of whole gel images or user specified areas or spots of gels. Besides this, it also allows zooming and other image transformations such as brightness and contract enhancement. The third module is an interface to the database of 2-D gel images maintained locally. The database consists of information about more than 3500 well annotated 2-D gel images obtained from public databases and literature. The server allows searching of gels from the database by keyword. Web server AC2Dgel is available for public from http://www1.imtech.res.in/raghava/ac2dgel/.

Introduction

In the post-genomic era, proteomics has emerged as a new and powerful tool for the analysis and annotation of protein data (Ong S.E. and Pandey, A. 2001). Despite of many pitfalls, 2-D gel electrophoresis (O'Farrell, P.H. (1975) has proved to be the most comprehensive technique for the analysis of proteome. It is popular in proteomics due to its ability to separate proteins on the basis of their molecular weight and pH gradient. The two-dimensional gel electrophoresis is also useful in identifying the differential expression of genes under different conditions. In 2-D gels a complex pattern of protein spots is usually observed due to unique behavior of proteins when they are separated on the basis of pH and molecular weight simultaneously. A single 2-D gel have large number of protein spots differing slightly in their concentration which make the visual analysis of these gels very time consuming and cumbersome task. So, there is a great demand for computation tool for the automated analysis of gels. This motivated computer biologists to develop tools for the analysis of virtual 2-D gel images.

In the past, several computation packages have been developed for the analysis of 2-D gels such as Melanie, Delta 2D and Z3. These packages provide number of options for manipulation and analysis of gels. Melanie (Appel RD et al, 1997) is a comprehensive package for the analysis of multiple 2-D gel images. This software facilitates analysis of protein spots in virtual 2-D gel images. Similarly, Delta 2D and Z3 are other packages for the manipulation and comparison of virtual 2-D gel images. The major limitation of these packages is that they are available only for Microsoft Windows and require large memory for processing. These requirements restrict their usage on the personal computers. In order to overcome the

limitations of these packages, many dynamic web servers have been developed for the manipulation of images. The Flicker and Carol are two major online web servers for the analysis of 2-D gel images. The Flicker compares the two gel images using flicker method, in which two aligned gel images are shown simultaneously in the same visual space one by one (Lemkin, P.F. 1997). On the other hand, Carol web server analyzes the gel images on the basis of pattern matching algorithm (Pleissner KP, 1999). These servers run with high speed internet connectivity and Java enabled browsers. Thus, all the packages available for 2-D gel analysis have few merits and few demerits. In order to complement the existing 2-D gel analysis packages, we developed a new web server. This server allows the comparison and analysis of 2-D gel images. The server also provides an interface to a database of locally maintained 2-D gels. The server is platform independent and freely available for academicians from http://www2.imtech.res.in/raghava/ac2dgel/.

Description of the Web Server

The server has been systematically developed by constructing three modules for analysis and comparison of 2-D gel images. Following is the brief description of modules.

Analysis of Gels

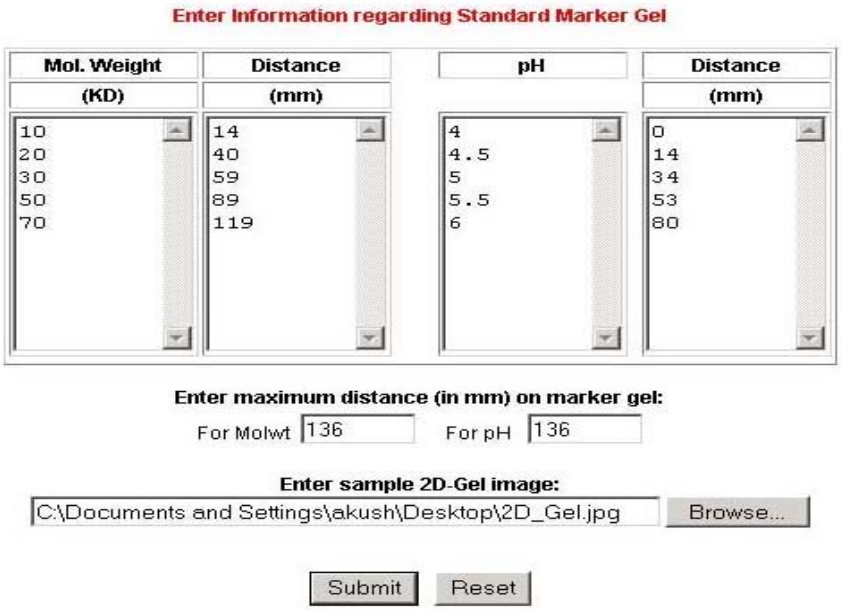
This module allows annotation of proteins based on their molecular weight and pH. The user can compute the molecular weight and pH of protein spots on gel by clicking on the corresponding spot. In order to compute molecular weight and pH of protein, user need to specify the molecular weight and pH with their scales corresponding to standard or known markers of spots (Figure 1a). The user can upload a virtual image of gel in any standard format. The module uses the linear square curve fitting technique to fit markers data (e.g. molecular weight *vs* distance of spots from bottom and pH *vs* distance of spots from left) for computing linear equations, which serves as the internal calibration for determining the molecular weight and pH of the experimental proteins on the gel. The module calculates pH and molecular weight using the linear equations derived from marker data corresponding to the spot on gel by clicking on it (Raghava, G.P.S. 1994) (Figure 1b). Thus, user can choose any point on the image to calculate the molecular weight and pH of protein of specified location. The tool also allows to further search the protein of similar molecular weight and pH from the database as chosen by user. The list of all proteins of related molecular weight can be displayed in the result. The user can choose any gel from the result to compare it with his gel of interest. In summary, this module allows assignment of pH and molecular weight of protein of interest. The combination of this tool with gel comparison module allows functional annotation of protein.

Comparison of Gels

This module facilitates the comparison of two images corresponding to 2-D gels. One of the advantages of this module is that it allows user to upload/get images to be compared, directly from various resources. That includes uploading of images from local disk and downloading from Internet by providing the URL of image. It also allows the comparison of images stored in local databases maintained by user. The method can accept 2-D gel images in any standard image format such as Joint Photographic Experts Group (jpeg), Graphics Interchange Format (gif) and Tag Interchange File Format (tiff). All the input images from different sources are first converted to a uniform format using the 'convert' subroutine of ImageMagick library. The images are then processed through 'gifinter' function of the giflib library to convert the interlaced images to non-interlaced. These images are finally converted to gray scale tiff images for further manipulation. This is done using functions of giflib and tiflib libraries respectively for further manipulation of images. After processing of images, server presents two images as shown in Figure 2a. The user can perform the transformation of these images in term of varying contrast or brightness of both images. This is carried out using the 'histex' digital image manipulation tools (Seul, M et al, 2000) and 'convert' subroutine. The images can then be compared as a whole or the location of any specific spot or protein to be compared can be specified. The specified area of image is obtained using 'gifclip' function of giflib library for the comparison. In last, the images are compared by inverting one of the two images using the 'imgarith' function (Seul, M et al, 2000) and superimposing it over the first one. The resultant image (figure 2b) is zoomed up using the 'xscale' function (Seul, M et al, 2000) and displays gray and white areas where gray specifies areas common in between two images and white specifies the areas, which differ in two images. This module can be used to identify the change in protein profile under various psychological conditions.

Module for Data Storage and Retrieval

This module has been developed as an interface for the database of 2-D gels. The database has more than 3500 well annotated 2-D gels. These images were obtained from various databases and literature, which are available free for academic users. The



(b)

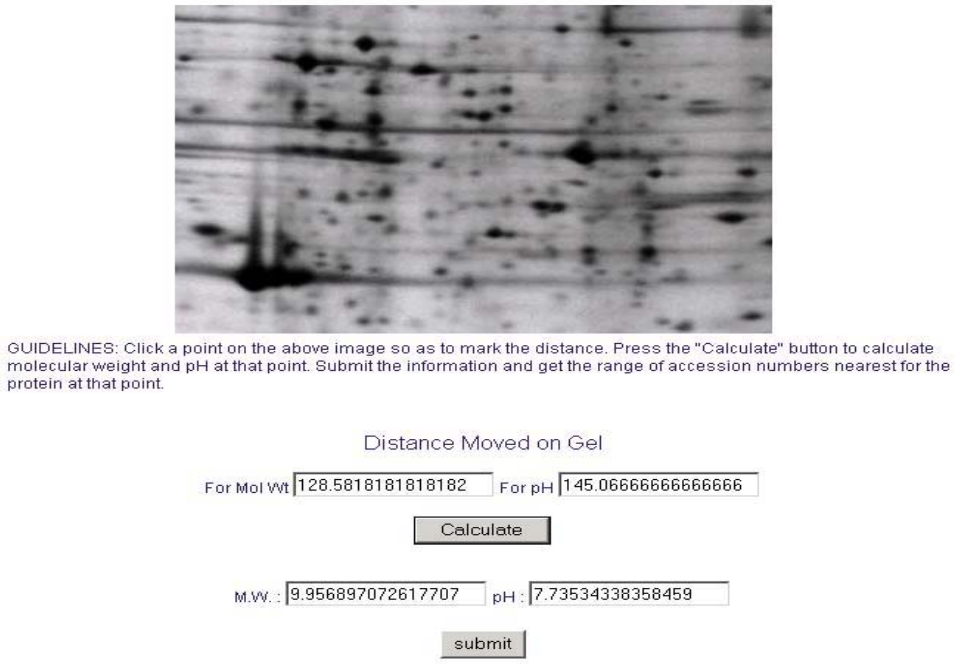
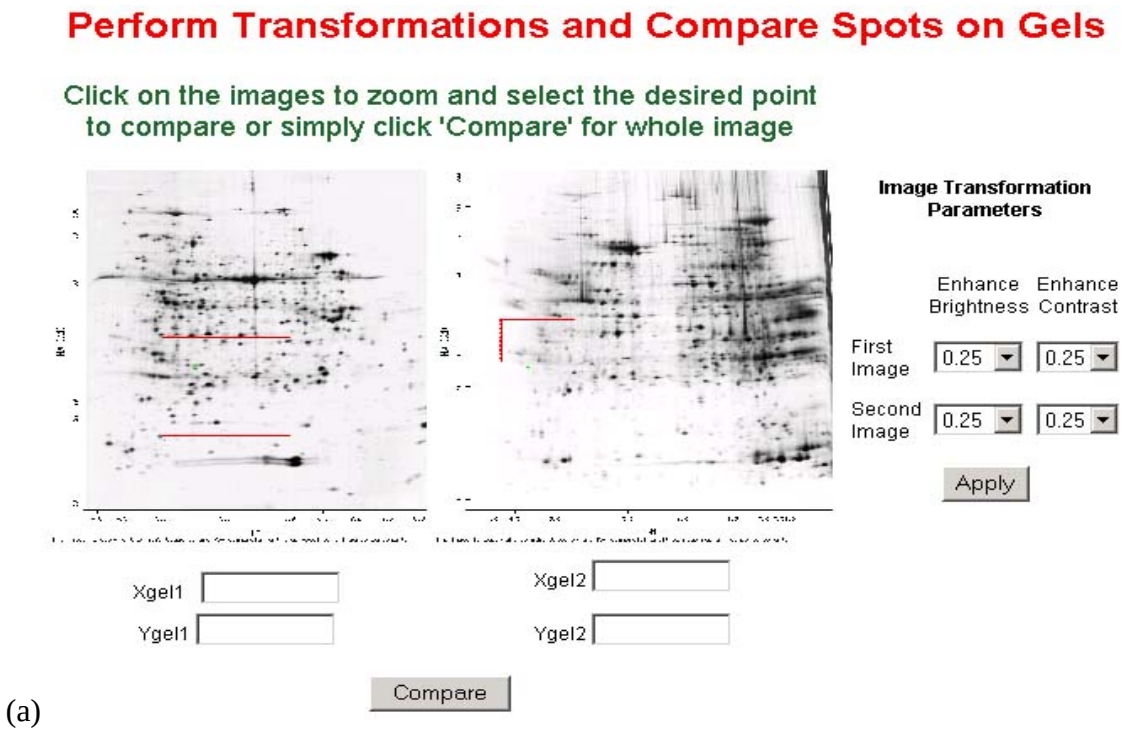


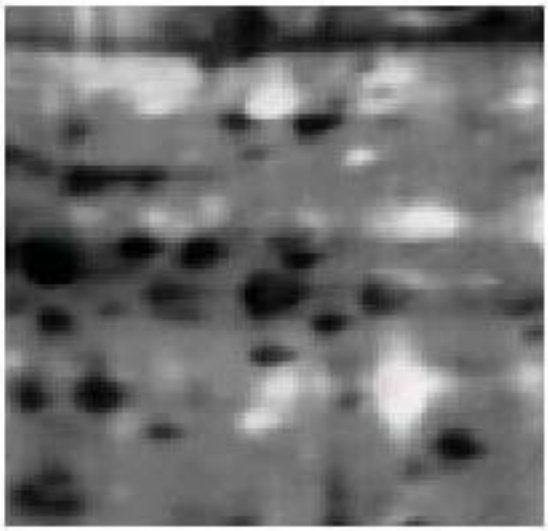
Figure 1: Demonstration of application of 'Gel Analysis' module; a) example submission form to input information about standard markers and b) demonstration of calculation of molecular weight and pH by clicking on any spot.

module allows the keyword search on fields of database such as accession number, master name or molecular weight. It displays the complete information about gels like molecular weight, pH, SWISS-PROT accession number of the 2-D gels that matched to users query. The results also display an annotation virtual 2-D image and the information regarding the source information and source organelle from which the gel is generated.

(a)



(a)



(b)

Figure 2: Example displays obtained from server which demonstrate its functions; (a) presents the images after processing, allowing zooming of image and applying transformations and (b) the resultant superimposed image.

AC2DGel Description

The server has been launched on World Wide Web by implementing the Apache web server (http://www.apache.org/) on a Red Hat Linux 7.2 operating system (http://www.redhat.com/). The

database is maintained locally using PostgreSQL (http://www.postgresql.org/), one of the most advanced open source database system available. The algorithms are implemented as CGI scripts in Perl (http://www.perl.com/) language. Several in-between calculations and validations in the HTML forms have been worked out using Javascripts. Several image analysis libraries such as libtiff (http://www.libtiff.org/), giflib and ImageMagick (http://www.imagemagick.org/) are used for image handling and manipulation. In addition to these, some advanced digital image manipulation and analysis tools (Seul, M et al, 2000) have been used. These tools have been utilized for determining the image size, conversion of images among different formats, image clipping, image scaling, image superimposition, determining intensity histogram, contrast enhancement and brightness control. The information of 2-D gel images stored locally has been drawn mainly from SWISS-PROT database (http://www.expasy.ch/) Gasteiger E et al, 2001; O'Donovan Cet al, 2002; Junker V.L et al, 1994)

Acknowledgement

The authors are thankful to Ms. Amita Bansal and Ms. Manjeet Kaur for initiating this project and developing various subroutines. We are also thankful to Mr. Manoj Bhasin for the critical proofreading of the manuscript and his valuable suggestions.

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