TotalLab
LIFE SCIENCE ANALYSIS

UNIVERSITY OF LIVERPOOL

CLIQS - 1D Gel Analysis in Proteomics

Summary

- Objectively quantify signals on Western blots or gels, rapidly, reliably and with confidence to validate proteomics discovery e.g. by iTRAQ.
- Using Western blot data and TotalLab software interpret subtle, rapid changes in post -translational modifications (PTMs) with limited sample and time, while accounting for inter -experimental variation.

How TotalLab software benefits proteomic research:

- Effortless lane and band detection
- Precise background subtraction allowing accurate quantitation of band material
- Estimating is absolute rather than relative quantities
- Analysis of blots in approximately 30 seconds!
- New users trained in 5 minutes
- Analysis of 16 blots completed in a quarter of the time, compared to competitor software

University of Liverpool

The research emphasis of the University of Liverpool's laboratory is the analysis of the effect of cell shape on calcium signalling and cell cycle progression in mammalian cells.

A number of different experimental approaches were employed to determine shape-dependent signalling events in cultured fibroblasts including cDNA array hybridisation, laser scanning confocal microscopy, flow cytometry and Western blotting. The laboratory also employed quantitative protein expression analysis by iTRAQ and validated results using Western blot.

Challenges faced in gel image analysis

The quantities of material available were frequently limiting, with studies typically performed over short time courses. Differential protein expression levels and rapid changes in PTMs of proteins were of particular interest to its research. Western blot data generated from such samples, where observed changes in expression/modification of the antigens of interest are often subtle and subject to inter experimental variation. This required the researcher to interpret the results as objectively as possible. Image analysis software packages for analysis of 1D gels must therefore be able to quantify signals on blots or gels with the minimum of input from the user and do this rapidly, reliably and consistently.

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How TotalLab's software delivered rapid and reliable image analysis

CLIQS fulfils these requirements extremely well. A TIFF image of the blot is required so that files from various image acquisition devices may readily be analysed. Through a guided workflow, lanes and bands were detected and the background was subtracted automatically. Using CLIQS, it is not necessary to draw around the band of interest, to guess at which part of the blot best represents the background signal or to be overly concerned about smearing of bands. There are options for determining the molecular weight of bands of interest from standards run on the gel and for normalising to a particular band to estimate absolute rather than relative quantities. The quantitative data is then presented in a spreadsheet that can be exported to Excel for manipulation and analysis.

Analysis in practice

The analysis of a set of 16 blots that had previously been analysed using an alternative package was repeated using TotalLab. The analysis was completed in approximately a quarter of the time and, since there was little user influence on the band detection, there was a greater degree of confidence in the statistical significance of the resulting data.

> Analysis of blots may be performed in approximately 30 seconds using the fully automated process. The program is easy to navigate; analyses were performed with confidence by myself and by graduate students after a five minute demonstration.
>
> Dr. Roz Jenkins
> – Principal Experimental Officer, University of Liverpool

Intuitive algorithms supporting complex image analysis

Imperfect or complex images can be difficult for any software to handle. However, the semi-automated process of lane and band detection, as well as background subtraction, requires little more input than the fully automated process. The user is prompted to set parameters such as sensitivity and background subtraction method and is able to visualise in real time whether these settings enable all bands to be detected correctly. For instance, there is a sliding scale for sensitivity of band detection, which identifies all the bands of interest. A specific band may be displayed graphically and by hovering the cursor over each peak in turn. The band that each peak represents may be highlighted in green on the blot image and the synthetic lane image. This is particularly useful when each lane contains multiple bands or the bands are obscure due to noisy edges. While viewing the peaks and the synthetic lane it is possible to easily remove and refine bands and their edges.

Find out more and request a free trial visit: totallab.com/products/cliqs

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