Technology Offer

Ref.-No. M0109

CoFGE – Comparative Fluorescence Gel Electrophoresis

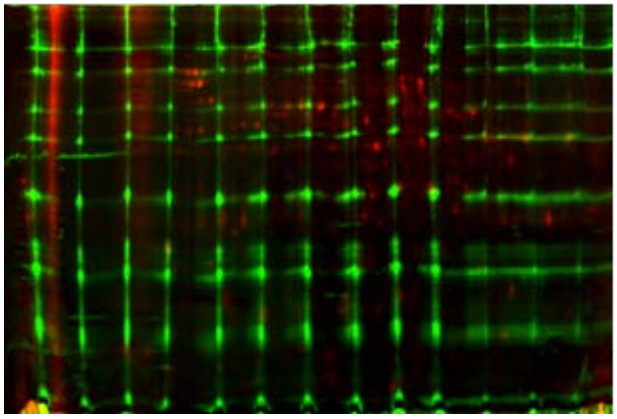
Introduction

Functional proteomics requires reproducible comparison of proteins separated by two-dimensional gel electrophoresis (2-DE). This is however difficult due to a number of method-inherent factors such as gel-to-gel variability. An excellent solution for the investigation of similar proteomes (e.g., normal and stimulated cells) was introduced with the DIGE system (Differential fluorescence Gel Electrophoresis). Unfortunately, this method is not suitable for small proteomes which vary considerably such as protein aeroallergens isolated from dust. Despite widespread use of 2-DE, a method to reproducibly assign x,y-coordinates to protein spots suitable for storage in a 2-DE database is still lacking.

Invention

The present invention provides a means to correct coordinates in such a way that the comparison of gels run in different laboratories at varying time points finally becomes feasible. The only requirement is the use of identical equipment and reference.

- The method ties sample proteins spots to a references protein grid run on the same gel.
- The use of fluorescence stains allows the separation of both protein mixtures (sample and reference marker, respectively) on the same gel.
- A special gel assembly (Gel-Strip-Sandwich, GSS) holds both pI-strip and a comb for the generation of sample wells for the reference proteins.
- The comb has V-shaped teeth in order to produce wells which support the formation of protein spots rather than bands during gel electrophoresis.
- A mixture of pure standard proteins (Grid-Mix) is used as reference.
- Commercially available 2-DE analysis software is capable of gel warping and correction of protein spot coordinates using a pre-determined protein grid.



New aspects of the invention

CoFGE combines aspects of 1/2-DE and DIGE. Proteome spots are referenced to a standardized marker grid overlaying the sample image. Thereby, the running behaviour of proteins in the electric field is accurately reflected. The use of commercial pI-strips in GSS keeps the potential influence of protein pI negligible.

Advantages of the invention

For a set of six gels of Escherichia coli lysate run a week apart the average deviation of x,y-coordinates of 47 well defined samples spots was improved from 7% to less than 1%. This result shows that correct spot assignment based on standardized tools and protocols is possible.
The method can easily be adapted in proteomic laboratories.

Patent situation

A European and PCT patent application have been filed.

Für weitere Informationen wenden Sie sich bitte an:

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