Two-dimensional Difference Gel Electrophoresis: 2D DIGE

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Covalent derivatization of proteins with fluorophores in complex protein mixtures prior to IEF and SDS-PAGE allows detection and quantification of differences in protein abundance between different biological samples within one single gel (Ünlü *et al.*, Tonge *et al.*). Special size- and charge-matched cyanine CyDye™DIGE Fluor **minimal dyes** (Cy™3, Cy5, Cy2) that react over an NHS-ester group with ε-amino residues of lysine enable co-electrophoresis of up to three different samples in one approach with a dynamic range up to five orders of magnitude. Approximately 3% of the available proteins are labelled and then only on a single lysine per protein whereas the rest remains unlabelled. This makes the technique robust and labelling optimization is usually not necessary. The three different CyDye tags add approximately 450 Da to the protein's mass when coupled to the protein. The resulting image patterns are comparable to silver stained gels. Sensitivity of the minimal dyes is similar to most sensitive silver staining. With a dedicated fluorescent imager (Typhoon™, Ettan™DIGE Imager) the dye images are acquired without crosstalk. A pooled **internal standard** can be created by mixing aliquots of all samples to be analysed. The use of such an internal standard on each gel that comprises equal quantities of each of the samples in the experiment allows to calculate ratios for the same protein spot within one gel as well as between gels. This largely removes experimental gel-to-gel variation leading to improved accuracy of protein quantification between samples from different gels. In the setup of two samples plus internal standard per gel, renunciation of gel repetitions and emphasis on biological repetitions brings down the total number of gels per experiment necessary for quantitative statistics (Alban *el al.*). The bottlenecks of classical 2-D electrophoresis like methodical variation, laborious image analysis and restricted quantification are therefore minimized in the Ettan DIGE workflow. For special applications e.g. samples from microdissection, CyDye DIGE Fluor **saturation dyes** (Cy3, Cy5) enable the complete 2D analysis and quantification of protein abundance changes in scarce sample amounts (5 μg protein/image). The dyes react over a maleimide group with all available cysteine residues in the protein sample, giving a high labelling concentration. Because they have a net zero charge, there is no charge alteration to the labelled protein. As with all 2D DIGE experiments an internal standard sample containing an equal amount of each

sample is run on each gel. Sensitivity is twenty-fold higher than with silver staining. In contrast to minimal labelling with saturation dyes a labelling optimization is necessary to determine the right amount of dye (Sitek *et al.*). Dedicated image analysis software (DeCyder™2D, ImageMaster™Platinum) utilizes a proprietary spot co-detection algorithm (up to triple detection) that permits automatic detection, background subtraction, quantification, normalization and inter-gel matching of fluorescent images. The experimental design using the internal standard, effectively eliminates gel-to-gel variation, allowing detection of small differences in protein levels. System variation as well as inherent biological variation arising from patient-to-patient, culture-to-culture etc. can be clearly differentiated from induced biological changes using the DIGE system. Further statistical software (DeCyder EDA) working with standardized log abundance data allows e.g. unsupervised principle component analysis and hierarchical clustering of the individual DIGE proteome expression maps provided independent confirmation of distinct expression patterns from the individual experiments and demonstrated high reproducibility between replicate samples (Friedman *et al.*).

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