Rapid Dispatches Highlights

Protein's new norm

Normalization is an important tool when attempting to make meaningful comparisons between differing samples. Protein microarrays can be used for quantitative protein analysis from a wide number of samples, including those with limited starting material. The challenge when using approaches such as reversed phase protein microarrays (RPMA) is the inconsistency in cell numbers that often exists between spots on the same array. This is where a robust and simple normalization procedure could have a huge impact on the uptake of RPMA in proteomics labs. However, up to this point, very few standardized normalization approaches for RPMA have been detailed. This month in *Rapid Dispatches*, Antonella Chiechi and colleagues from Istituto Ortopedico Rizzoli in Bologna, Italy, and the Center for Applied Proteomics and Molecular Medicine at George Mason University in Manassas, Virginia, tackle this issue head-on as they describe an RPMA normalization process that results in higher resolution when identifying differences between tissue groups that might have otherwise been obscured. In their improved RPMA normalization method, the authors took a cue from the existing gene microarray normalization algorithms (including those found in the popular geNorm and NormFinder programs) to identify normalization parameters with the lowest variability between all samples. Single-stranded DNA (ssDNA) was found to be directly correlated with cell number. To initially measure ssDNA in samples, the authors took advantage of physiochemical properties of nitro-cellulose, which is capable of binding ssDNA owing to the negatively charged phosphate groups but not double-stranded DNA. Antibodies against ssDNA were then used to demonstrate that the ssDNA staining intensity was directly proportional to the number of cells in a sample. This information enabled Chiechi and his co-authors to take advantage of ssDNA in normalization of their RPMA. Proteins were also assessed using gene microarray algorithms to identify additional RPMA normalization factors. In the end, the approach described in this Report provides researchers a new method for normalizing their protein microarray datasets to get the most robust and accurate protein data. **See “Improved data normalization methods for reverse**

Bottom feeders

While the transfection of adherent cells is pretty standard (assuming all parameters are optimized), transfection of cells in suspension can be a more challenging process. One possible reason for this difficulty could lie in the need to bring the transfection complex consisting of DNA and carrier molecules in close contact with the cell surface to facilitate uptake. In the case of adherent cells, the transfection complex can be placed directly on top of the cells, whereas with suspension cells, this interaction is harder to achieve. Julia Schumann and her colleagues at the University of Leipzig in Germany considered this dilemma and decided the best solution was to actually make suspension cells look more like adherent cells during transfection. Reporting in *Rapid Dispatches*, Schumann's group describes how employing a cell culture dish coated with chicken egg white can lead to high-efficiency transfection of suspension cell lines. Using a canine mastocytoma C2 cell line, the authors first examined whether or not a chicken egg white-coated dish promoted attachment of cells to the plate surface and whether or not this attachment resulted in altered cell function. Cell function was tested by assessing degranulation upon C2 cell stimulation. The results clearly demonstrated that there was no alteration of mast cell degranulation because of the egg white coating. After determining that cell function was not altered with egg white binding, the authors next determined the transfection efficiency of suspension cells on the coated dish. In 20 independent experiments, transfection efficiencies of only 5% were observed for suspension cells in uncoated dishes, while a 50% transfection efficiency could be obtained when using egg white-coated dishes. Similar efficiency increases were also observed when the authors tried a second cell line (HL-60). In the end, egg white-coated dishes should prove to be an extremely cost effective, efficient approach to transfecting various types of suspension cell lines. **See “High-efficiency transfection of suspension cell lines.”**

Category: Molecular Biology

Chicken egg whites coated onto plastic cell culture dishes can enhance transfection efficiency for suspension cells.

phase protein microarray analysis of complex biological samples.”

Category: Proteomics

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