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A MULTI-LABORATORY STUDY ASSESSING REPRODUCIBILITY OF A 2D-DIGE DIFFERENTIAL PROTEOMIC EXPERIMENT

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Although 2DE-electrophoresis has been long used to study differential proteomics, its reproducibility has been always a major concern. In recent years, different methodological improvements have contributed to more robust 2DE workflows: use of immobilized IEF strips, fluorescence based difference gel electrophoresis (DIGE), new software tools, etc. In order to assess the reproducibility of 2DE experiments across laboratories, we set up a multi-laboratory study, performed at 11 laboratories of the ProteoRed network (Spanish network of proteomics facilities). All participating labs received two protein extracts, prepared from cultured human adenocarcinoma MDA-MB-468 cells, treated or not with 50 ng/ml EGF (Epidermal Growth Factor) for 24h. Differential analysis was performed by a 4-gel 2D-DIGE experiment, using 4 technical replicates of each sample, with Cy dye swapping. Strictly defined 2DE conditions were followed by all labs. Each lab selected the 30 spots presenting the highest fold variations (with $p<0.05$), and attempted MS protein identification.

The results demonstrate a very good within lab and across lab reproducibility. Within labs, 75-85% detected spots present %CV <10%, and 40-60 %CV <5%. Across all labs, around 60% and 15% of spots show %CV <10% and <5%, respectively. Selection of differentially expressed spots shows good reproducibility across labs, although there is a certain degree of subjectivity in the selection, as each lab applied its own filtering criteria. Overall, 24 spots were ranked among the top-30 by at least 3 labs, and 14 by at least 4. MS protein identification was, on average, 60% successful, with 22 spots identified by at least 3 different labs. In those cases, identical gel locations corresponded to the same protein Id.

In conclusion, the results of the study show the robustness of the methodology used, and demonstrate the feasibility of across lab validation schemes, pointing towards development of inter-lab QC strategies for proteomic research.