

Healing the Achilles Heel of Proteomics – 2-DE Reproducibility Study

BIO-RAD

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Introduction

The Achilles Heel of proteomics as highlighted at HUPO 2007, is the reproducibility of data irrespective of the methodology - top-down or bottom-up. This leads to a unified cry in the proteomics community — “Don’t publish if you can’t reproduce your data”. In response, recently reports have been published on 3 key global initiatives and here we highlight the outcome from one of them — a Fixing Proteomics Initiative.

The Fixing Proteomics Campaign is a noncommercial, vendor-independent campaign dedicated to solving the experimental challenges that prevent proteomics from delivering on its potential. Encouraging reproducibility of results has emerged as the key factor toward achieving this end. The organization’s website www.fixingproteomics.org provides background on the importance of and tips for applying quantitative proteomics techniques in a reproducible manner.

The goal of this project is to facilitate the introduction to and use of 2-DE by providing reference protocols, images, image analysis tools and samples. This poster describes the experience with those reference materials, which were distributed to around 20 labs worldwide with the request to produce at least three gels and submit the images.

Materials & Methods

Sample: HeLa Cell lysate (Prepared by CiiBiotech)

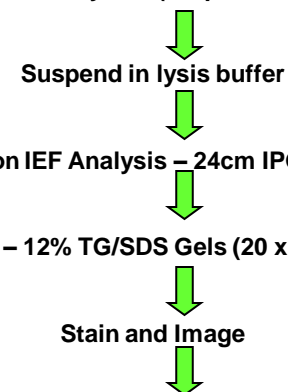


Image alignment and objective: Quantitative analysis of test images compared to gold standard image set

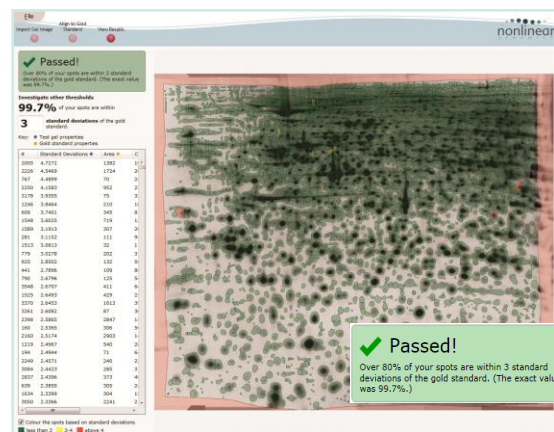


Fig.1. Progenesis SpotCheck assessment of the reproducibility of your gel running.

- Using a single sample, run a set of gels to the standard you want to maintain and enter the SpotCheck QC workflow within Progenesis SameSpots v4.1 (Nonlinear Dynamics)
- Convert this analysis into a SpotCheck gold standard, specifying the required similarity in subsequent runs of the same sample. In this study 80% of spots had to be within 3 standard deviations (3SD) to be considered similar.
- Compare a test gel of the same sample run to the same protocol as your gold standard image set. The end result is a pass-or-fail verdict, indicating whether the gel has met your quality requirements.

The reference sample consisting of a HeLa cell lysate in a standard 2-DE lysis buffer was provided to 20 labs (see supplementary information). Details on the sample preparation, gel running, detection and imaging can be obtained by visiting www.fixingproteomics.org. Submitted gel images were compared to gold standard images (Fig. 2 A and B) using Progenesis SpotCheck (Nonlinear Dynamics). In summary, test images were cropped and aligned to the gold standard so all data are in the same coordinate space. Normalized spot volumes on the test image were measured against average spot volumes from the gold standard. The pass criteria was chosen when 80% of spots on the test gel had a normalized volume within 3SD of the average spot volumes on the gold standard. Intra-lab and inter-lab quality measurements were made against gold standards consisting of an Intra-lab gold standard and an Inter-lab gold standard.

Example of Gels Run by Participating Labs Compared to Gold Standard Gels

Figure 2 shows Gold Standard gel image (A) compared to a gel image from a participatory lab (B). Study participant followed the protocol and generated the image that is compared to the gold standard. Highlighted region in the Red Box is for visual reference.

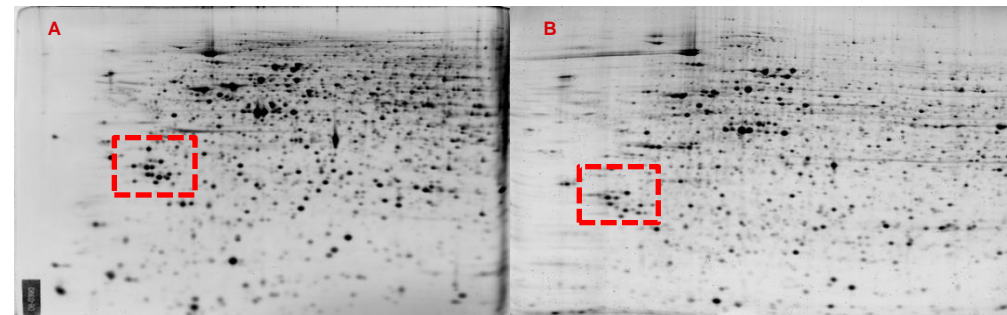


Fig.2. Comparison between Gel A (representative gold standard image) and Gel B (test image from a study participant).

Intra-lab and Inter-lab QC Analyses

Figure 3 shows the comparison of four test gel images to a gold standard made up of seven gel images from running the same sample within the same lab over several days. All images show they passed the objective which is the statistical measure of gel similarity.

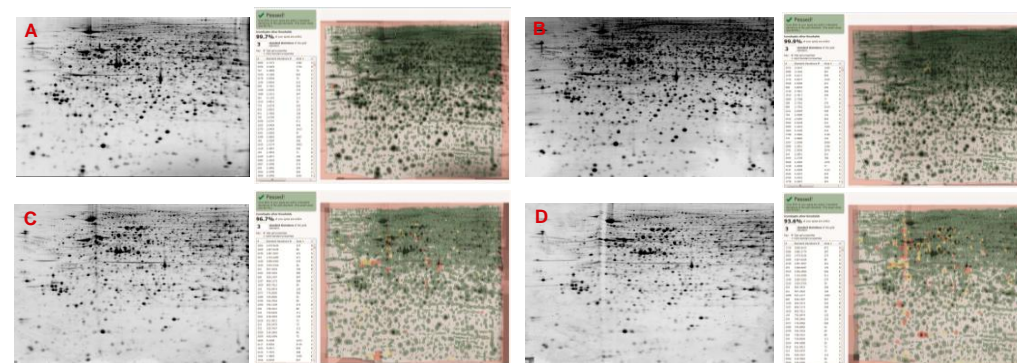


Fig.3. Intra-lab test gel images (A,B,C,D) compared to a gold standard made up of seven gel images. The screenshots on the right of each image show that each “passed” the similarity measure. (A) 99.7% (B) 99.9% (C) 94.7% and (D) 93.6% of test image normalized spot volumes were within 3SD of gold standard average normalized spot volumes.

Figure 4 shows the comparison of four test gel images to a gold standard made up of ten gel images representing accepted limits of gel running variation from different labs around the world. Three gel images (A,B,C) passed the objective, the statistical measure of gel similarity, while the fourth gel image (D), with clear distortions failed.

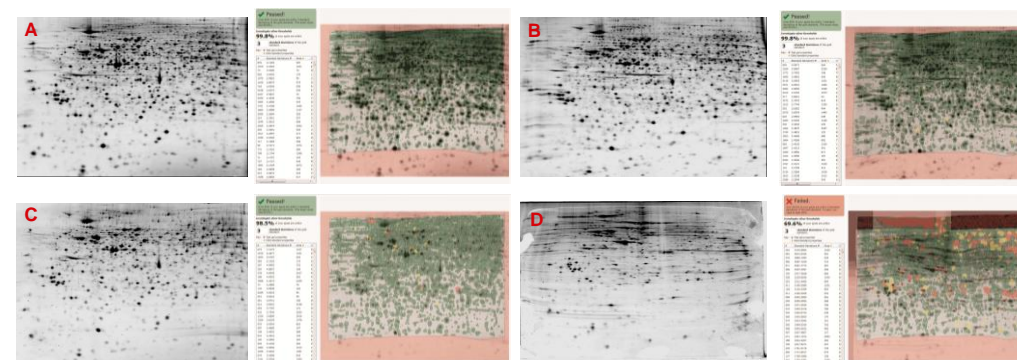


Fig.4. Inter-lab test gel images (A,B,C,D) compared to a gold standard made up of ten gel images. The screenshots on the right of each image show that image A, B and C “passed” the similarity measure and image D failed. (A) 99.8% (B) 99.8% (C) 98.5% and (D) 69.6% of test image normalized spot volumes were within 3SD of gold standard average normalized spot volumes.

In this case we chose a pass/fail criteria of 80% of spot measurements from test gel to fall within 3 SD of gold standard spot measurements, which represents 99.6% of the data range. You can create gold standards and pass/fail criteria to be more or less stringent.

Reference Materials Lead to Quality Improvements

Initially several participants had difficulties reaching an optimal result with the reference sample (Fig. 5. Gel 1). In several cases, the observed inconsistencies immediately led to suggestions towards improvement (Fig. 5. Gel. 2) and subsequent attempts.

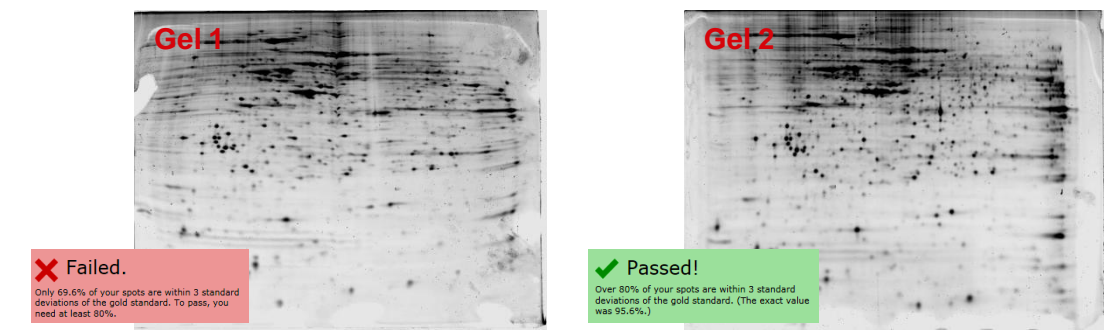


Fig.5. Improvement in quality following feedback. User X submitted an initial gel (Gel 1) that failed the study QC criteria. More gels were run following feedback from study participants and the person responsible for running the gold standard gels. Gel 2 passed the study QC threshold. This illustrates the value of feedback and a consultative approach to improve 2-D gel quality.

Results of Inter-lab QC Analysis

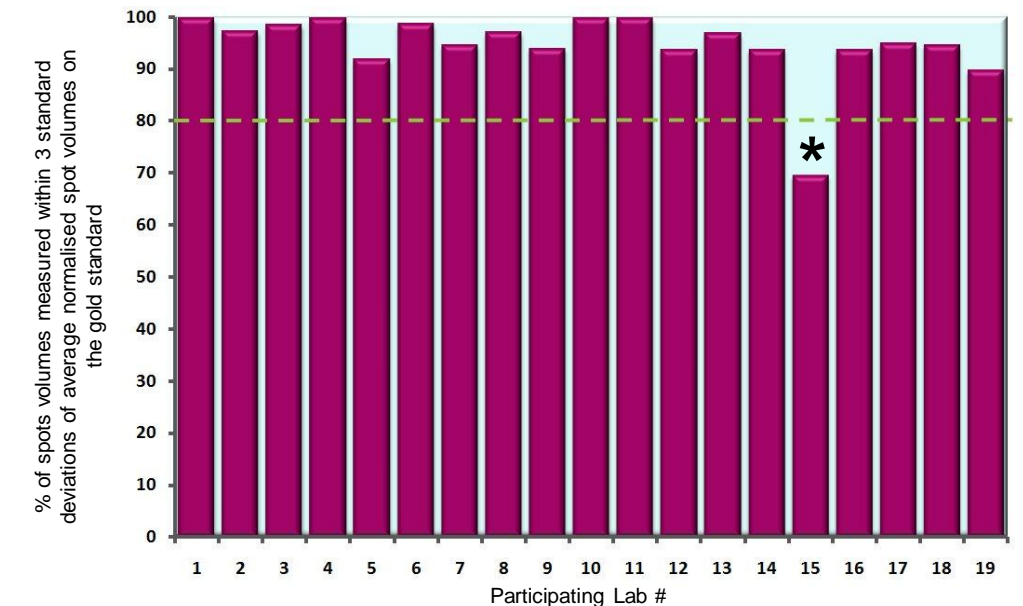


Fig.6. Measurement of the quality of 2-D gels run by each of the 19 participating labs compared to a gold standard. By following a well-defined protocol and using a standardized complex sample, all labs expect one, were able to produce images that passed the accepted criteria of 80% of spots within 3SD of average normalized spot volumes from the gold standard (indicated by ---). The gold standard contained ten images representing the accepted limits of cross-lab gel running. * Lab 15 failed the QC criteria with the first gels run. Based on feedback the gel running was optimized and subsequent gels passed the criteria (see Fig.5)

Conclusions and Lessons Learned

- This project illustrates that a complex top-down proteomics method such as 2-DE can benefit greatly from the availability of reference materials and a standardized approach
- Participants achieved a high degree of both Intra- and Inter-lab reproducibility using a standardized approach. This study highlights that 18/19 labs produced data that fell within 3SD
- Feedback on gel quality and an interface that shows where the most variable spots appear on a 2D gel image means users can gain confidence in generating reproducible data with their precious samples
- Progenesis SpotCheck supports reproducible results for proteomics and gives you rapid feedback to help optimize 2D gel running

Acknowledgment

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