

Overview:

- Complex top-down proteomics methods like 2DE benefit from rigorous approaches to ensure reliability and reproducibility of methodologies and results¹
- SpotCheck provides a quantitative analysis of your 2D gel running quality.
- Here it is applied to set 2D gel quality within a lab and teach novices how to confidently attain this quality before running large experiments using limited sample.

Sample: HeLa Cell Lysate (prepared by CilBiotech)



Suspend in lysis buffer and run to a basic, generic protocol². In summary,

- First-dimension IEF Analysis – 24cm IPG strip, pH 4-7.
- Second-dimension – 12% TG/SDS Gels (20 x 25cm, 1.5mm thick)



Stained and imaged

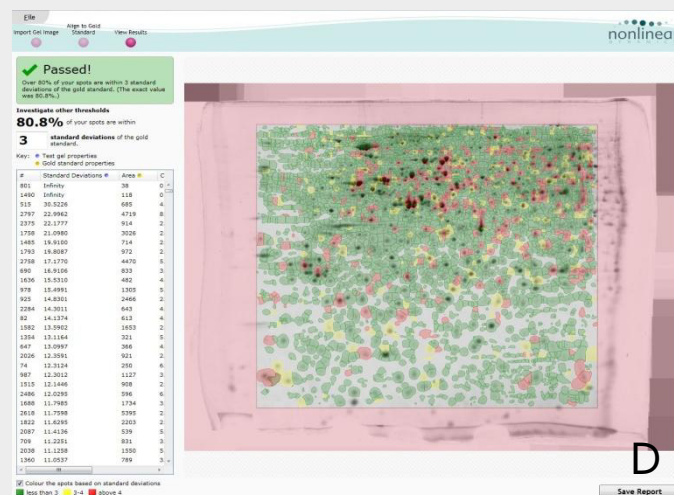
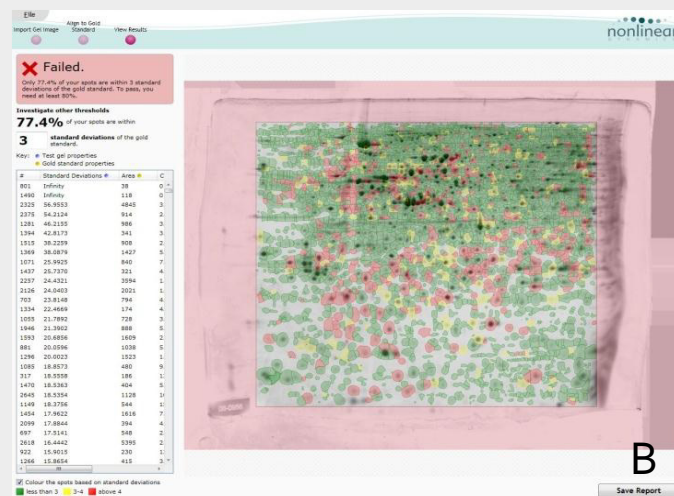
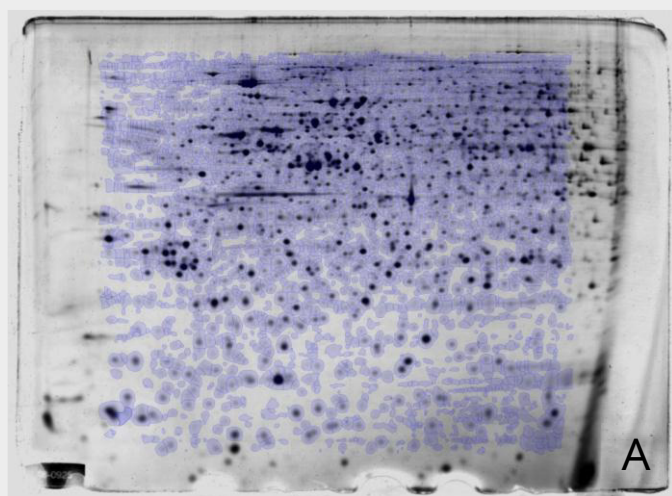


Image alignment and quantification by SameSpots SpotCheck workflow



Figure 1: Quantifying 2D gel quality using SpotCheck
Four gels of the same sample, run to the quality required by the lab supervisor, were analysed using SameSpots. The analysed images were used to create a SpotCheck gold standard with a defined quality measure. In this study 80% of spots in gels compared to the gold standard must be within 3 standard deviations (3SD) to be considered of the same or better quality.

A novice user ran gels following the same protocol with and without known changes made to the method above. The quality of these gels were measured against the gold standard. A pass was reported if >80% of spots quantified on a test gel lie within 3SD of mean normalised volumes for the same spots on the gold standard. A visual display showed the distribution of similarity measurements across the gel image.



- Test gel spots <3 SD vs. gold standard
- Test gel spots 3-4 SD vs. gold standard
- Test gel spots >4 SD vs. gold standard

- Areas excluded from comparison on gel images
- Spot detection pattern on gold standard

Figure 2: Results of quantifying gel quality from a novice attempting to run the same protocol² as a lab supervisor with and without known changes introduced. (A) One of a set of four 2D gel images run and analysed by the lab supervisor, indicated by blue outlined spots, used to set up a SpotCheck Gold Standard. To be considered similar >80% of spots in the novice gels must be within 3SD of the mean values on the gold standard. Gel edges can contain a lot of noise so these areas, shown in pink, have been excluded from spot detection and quantification. (B) Novice **failed** to achieve the 2D gel quality limit after using a different first dimension, IEF, step (C) Novice **failed** to achieve the 2D gel quality limit after using a buffer of higher salt conc. (D) Novice **passed** the 2D gel quality target after applying identical 1st and 2nd dimension steps as the lab supervisor.

Conclusion:

SpotCheck gives you an objective way to measure and maintain 2D gel quality within your lab. This gives you confidence in applying 2D gels for proteomics. A display showing the regions of highest variation helps you to optimise 2D gel running protocols and improve results.

References:

1. Healing the Achilles Heel of Proteomics – 2DE Reproducibility Study poster, HUPO Annual Congress, Sept 2010
2. Basic, generic 2DE gel running protocol from www.fixingproteomics.com/protocols available free to download and use