Taking DIGE to the Next Level: A Novel Platform for Biomarker Discovery

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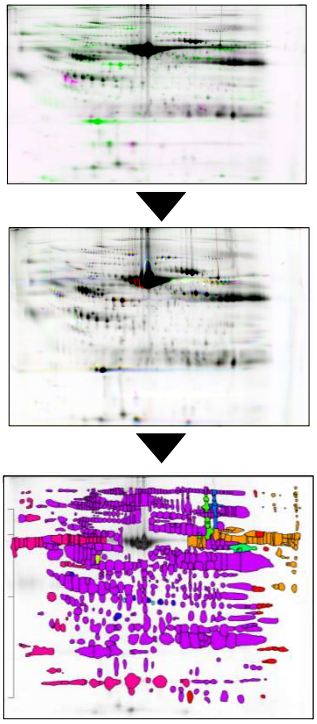
Introduction

- Modern, optimised DIGE is ideal for simultaneous analysis of multiple protein isoforms within the human proteome.
- Our next generation platform employs methodological and technological improvements in combination with our proprietary AI system to mine proteomic data from large clinical populations for accurate proteoform analysis.
- We can utilise this platform to drive hypothesis-free biomarker discovery and validation.

Quality controls for accurate and precise proteomic analysis

- Protein imaging is performed according to a system of strict quality controls including visual checks, multivariate software analysis and statistical process control (Jackson & Bramwell, 2013; Bramwell, 2013).



Cy5-labelled sample and Cy3-labelled QC standard are run in parallel to allow specific sample alignment. Use of the international standard, ERM DA 470k, as an internal control enhances alignment, normalisation and QC procedures.

Sample triplicates show uniform alignment when performed over time and with the use of different reagent batches. Comparison across sample and biological replicates is thus possible to increase the power of biomarker identification and analysis studies.

Statistical process control automatically flags common running errors to ensure all sample separation and gel images are of sufficient quality for thorough analysis via the AI system.

Figure 1: Human plasma samples (2µl) were analyzed using our optimized DIGE platform. Optimised 2D electrophoresis combined with our quality control software systems result in a highly sensitive and reproducible assay, to allow simultaneous analysis of multiple protein isoforms throughout the plasma proteome.

Biomarker identification from clinical populations

Our AI system mines clinical proteomic data from large patient cohorts, using multivariate analysis of clustered factors to identify disease signatures that correlate patients with specific diagnostic and prognostic risks.

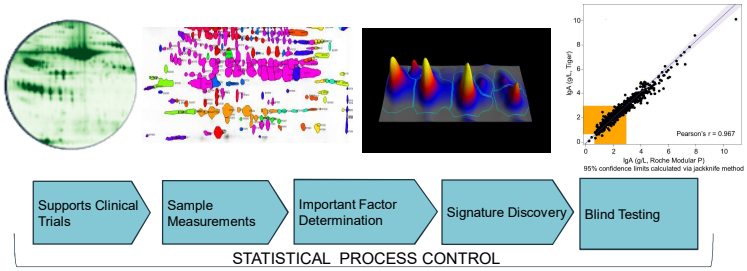


Figure 2: Machine learning systems analyse the plasma proteome across all patient samples concurrently to identify verifiable biomarkers from clinical populations.

- The technology is multi-modal; combining standard measures (age, weight etc) with both established measures (PSA for prostate cancer) and new measures from analytical assays.
- Use of 2D electrophoresis allows analysis and identification of modified proteins and proteoforms that are inaccessible via other analytical techniques.

Correlation of Tiger platform with clinical immunoassay

- Blood samples were collected from patients as part of a biomarker discovery study and standard clinical biochemical analytes measured via immunoassay in parallel with analysis via the Tiger platform.
- High numbers of biological replicates (n=706) were analysed via single stain experiments. DIGE was subsequently used to reduce technical noise on a further dataset (n=238).
- Stratified 10-fold cross validation robustly demonstrated the ability to identify and accurately quantify specific analytes of interest.

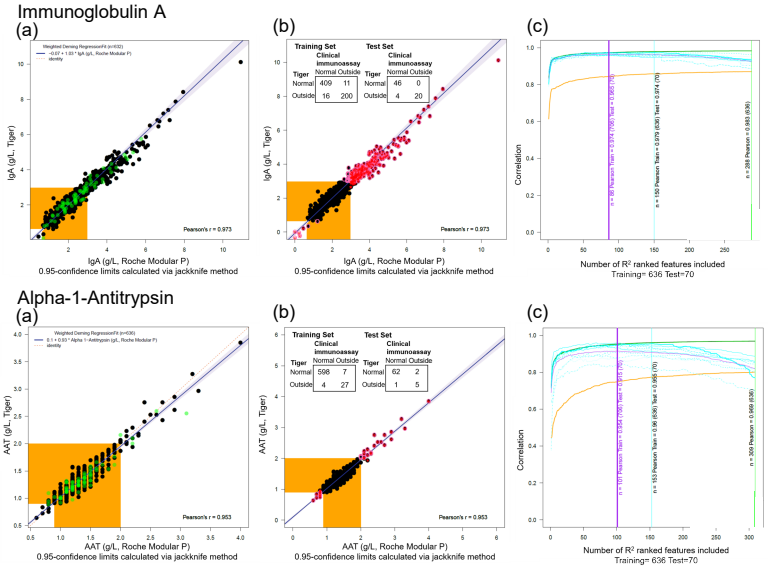


Figure 3: Tiger biomarker measurement of clinical analytes shows good correlation with immunoassay. (a) Correlation of Roche modular P and Tiger analyte quantity. Orange shading represents normal clinical range for analyte. (b) Accuracy of the Tiger platform in predicting patients outside the normal clinical range (red data points). (c) Number of features identified in 2DE image analysis used to predict analyte levels. Vertical purple line shows optimal number of features used.

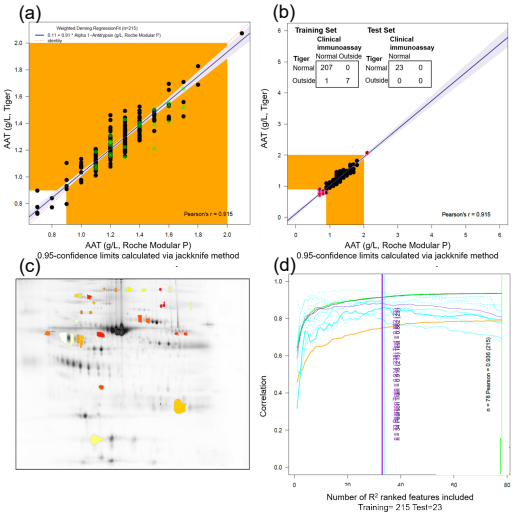


Figure 4: Tiger biomarker analysis of AAT using DIGE. (a) Correlation of Roche Modular P with Tiger. Orange shading represents normal clinical range. (b) Accuracy of Tiger in predicting patients outside normal clinical range (red data points). (c) Sample gel showing features used for quantification. (d) Number of features used to predict AAT levels.

Summary

- For a fixed cost, the greatest accuracy in biomarker quantification is achieved through increasing the number of biological replicates rather than decreasing technical noise through DIGE analysis.
- Multiple key clinical analytes can be accurately quantified from 2µl sample of patient plasma and show high correlation with standard immunoassay.
- Patient outliers of the standard clinical range can be accurately identified using the specific biomarkers discovered for each clinical analyte.
- Our platform offers step-change improvements over traditional proteomic approaches in reliability, processivity and cost, whilst enabling AI-based protein biomarker discovery and quantification.

For further information regarding Biosignatures Tiger technology platform, please contact info@biosignatures.com or visit www.biosignatures.com