

# Development of Protein Biomarker Identification Protocols

## A.S. Leaflet R2940

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### Summary and Implications

Protein biomarkers can be used to predict biological traits or diseases. There is a need for robust methods to identify protein biomarkers that are linked to livestock production traits. Serum from blood is often used for detecting and screening for biomarkers. Albumin is a protein that comprises 50% or more of the total content of serum, and has historically been removed from serum before biomarker development and testing. We have shown that it is possible to successfully identify potential biomarkers without albumin removal, thus reducing steps and potentially cost in both development and routine testing phases.

### Introduction

Identification of biomarkers for production traits in animal agriculture will create new approaches to improving livestock performance. Biomarker identification is a well established field in biomedicine, yet has seen limited use on agriculture. Serum from blood has been a major source of biomarkers, as it is easily accessed and is comprised of thousands of proteins in a wide range of protein concentrations. Albumin often accounts for more than 50% of the total protein in the serum, while useful protein biomarkers are generally not high abundance proteins like albumin. Albumin, due to its high concentration relative to

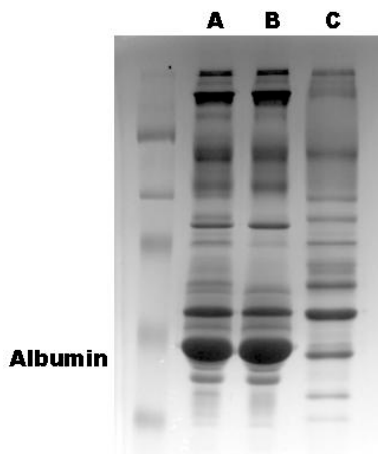
most other proteins, could mask some potential biomarkers during the discovery process. The objective of this project was to identify methods that would allow biomarker identification without albumin removal.

### Materials and Methods

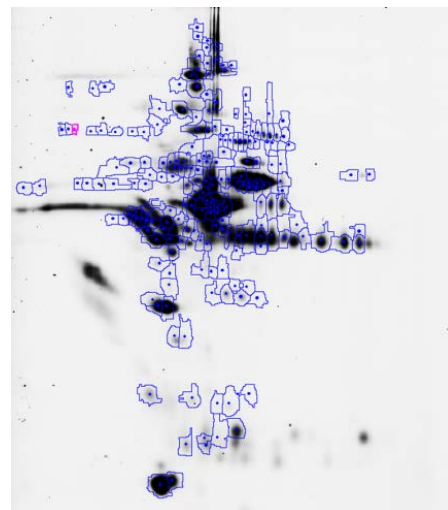
Eight serum samples collected from 5-week-old piglets were used for this project. Albumin was removed from half of each sample using a commercially available kit designed for serum albumin depletion. To confirm albumin removal, samples were run on a one-dimensional SDS-PAGE gel separating proteins by molecular weight (Figure 1). Then two-dimensional difference in-gel electrophoresis was used to globally determine the difference in proteins between whole serum and albumin-depleted serum. The use of two-dimensional electrophoresis allows the separation of individual proteins by charge and molecular weight. Difference in-gel electrophoresis allows the comparison of multiple samples within a single gel via labeling of each protein sample with different fluorescent dyes. In this case, serum samples from the same animal p were compared before and after albumin depletion. Statistical analysis was conducted to determine the impact albumin depletion has on the variation in the protein abundance.

### Results and Discussion

A total of 239 protein spots were found across all the two dimensional difference in-gel electrophoresis gels (Figure 2). In a comparison between albumin depleted serum and whole serum, 179 of these spots had a change in abundance of over 50%. Eighty-six spots were increased in abundance in the albumin-depleted serum and 118 were



**Figure 1:** Representative one-dimensional SDS-PAGE gel electrophoresis image showing A) Whole serum, B) Albumin enriched, and C) Albumin depleted serum.



**Figure 2:** Representative two-dimensional difference in gel electrophoresis image showing all 239 protein spots resolved in the gel.

decreased. Additional statistical analyses showed depletion of albumin in these samples increased the variation of the abundance of many individual proteins. As albumin is known to interact with many proteins in the blood, thus during albumin removal other proteins could be removed with albumin. Such an increase in variation in many proteins is undesirable. Further, the resolution of albumin to a small portion of the gel shows that many proteins can be detected and measured even in the presence of albumin.

These data show that albumin may not need to be removed from the blood to successfully identify protein biomarkers.

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