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…….Mapping new directions

in Proteomics……..

Problem:

Can a better more efficient Protein Profiling scheme be designed to map the myriad of "intact" proteins in complex samples required in clinical research?

Eprogen's Approach:

To develop fully automated all-liquid phase techniques based on HPLC to replace older gel based techniques.

Eprogen's Goal:

To produce a protein profiling technique that will interface with all current techniques use in Proteomics Research including microarray development.

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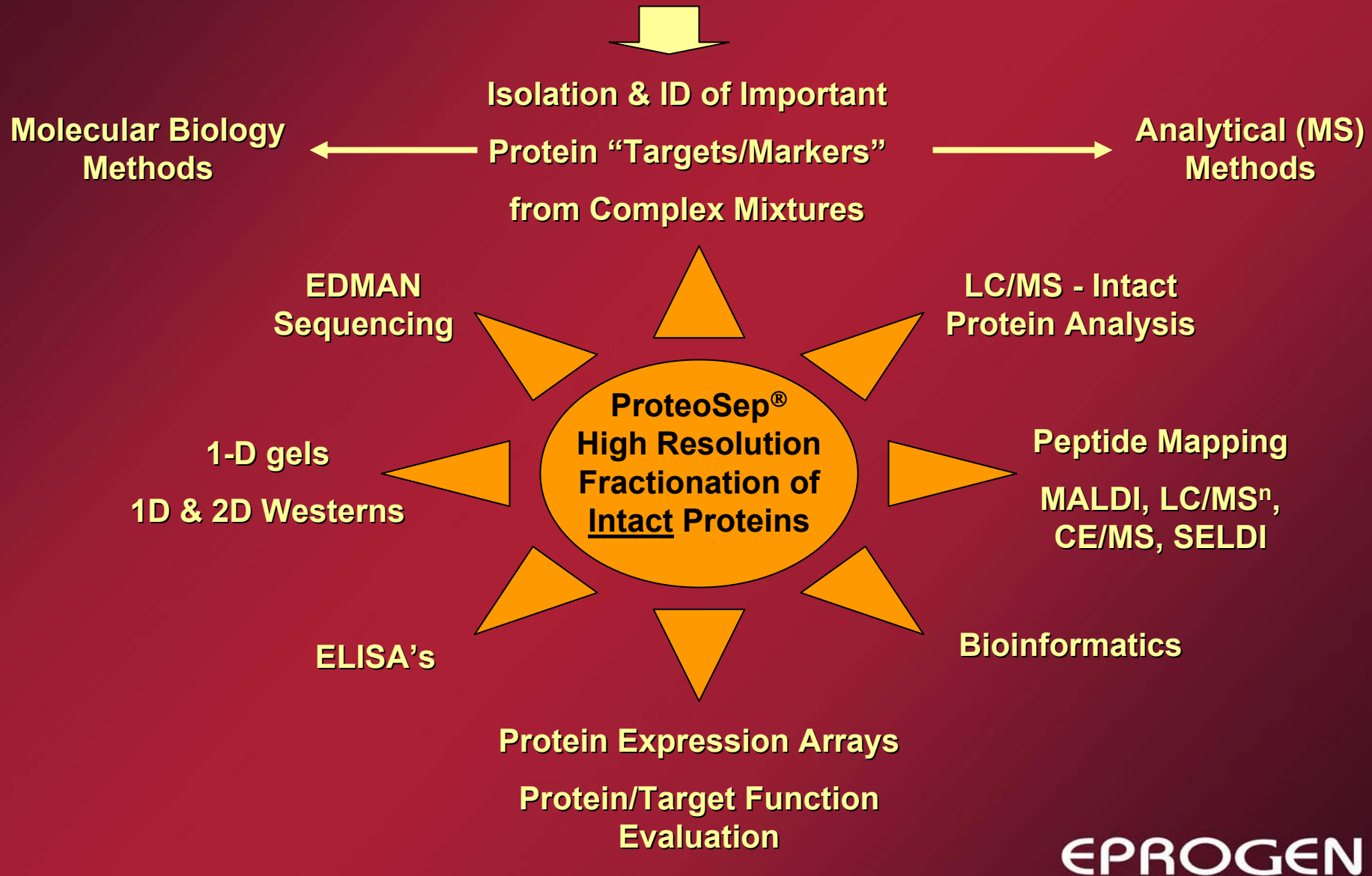
ProteoSep® 2D Intact Protein HPLC Concept

New Gel-Free Methodologies for Proteomics



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Intact Protein Fractionation Analytical Flow-Scheme

1st Dimension
pI – (Chromatofocusing)

Liquid CF Fractions
Every 0.2 – 0.3 pH units

~ 20 Liquid CF Fractions
100 ug – 5 mg protein loadings

2nd Dimension
Hydrophobicity (RPHPLC)

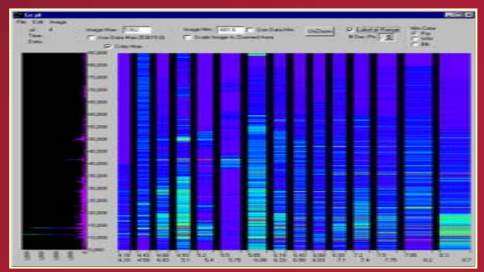
Liquid Fractions in
Multi-well plates

UV
Detector

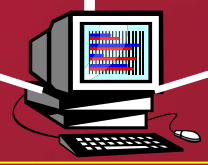
~ 1000 Liquid Protein fractions for MS analysis
or traceable "Array of Proteins" for analysis

Mass
Analyzer

2D (pI/Mass) Map

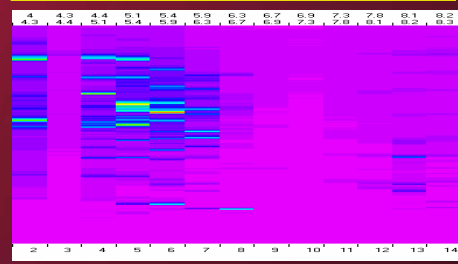


ProteoVue® Software Suite



Bioinformatics

2D (pI/Hyd) Map



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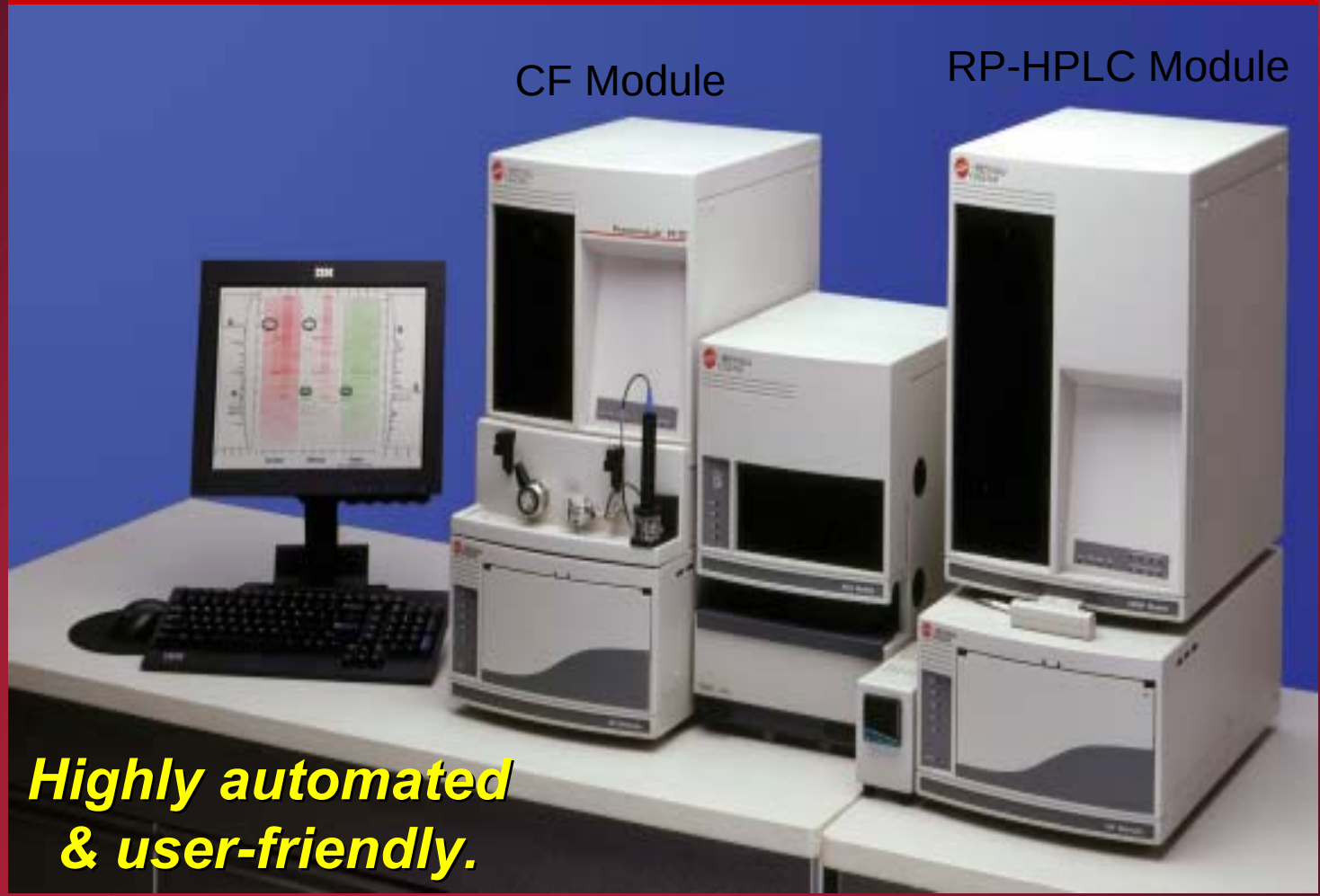
Chromatofocusing (pI) – RP-HPLC (Hydrophobicity) 2D Maps

ProteomeLab® PF2D System from Beckman Coulter



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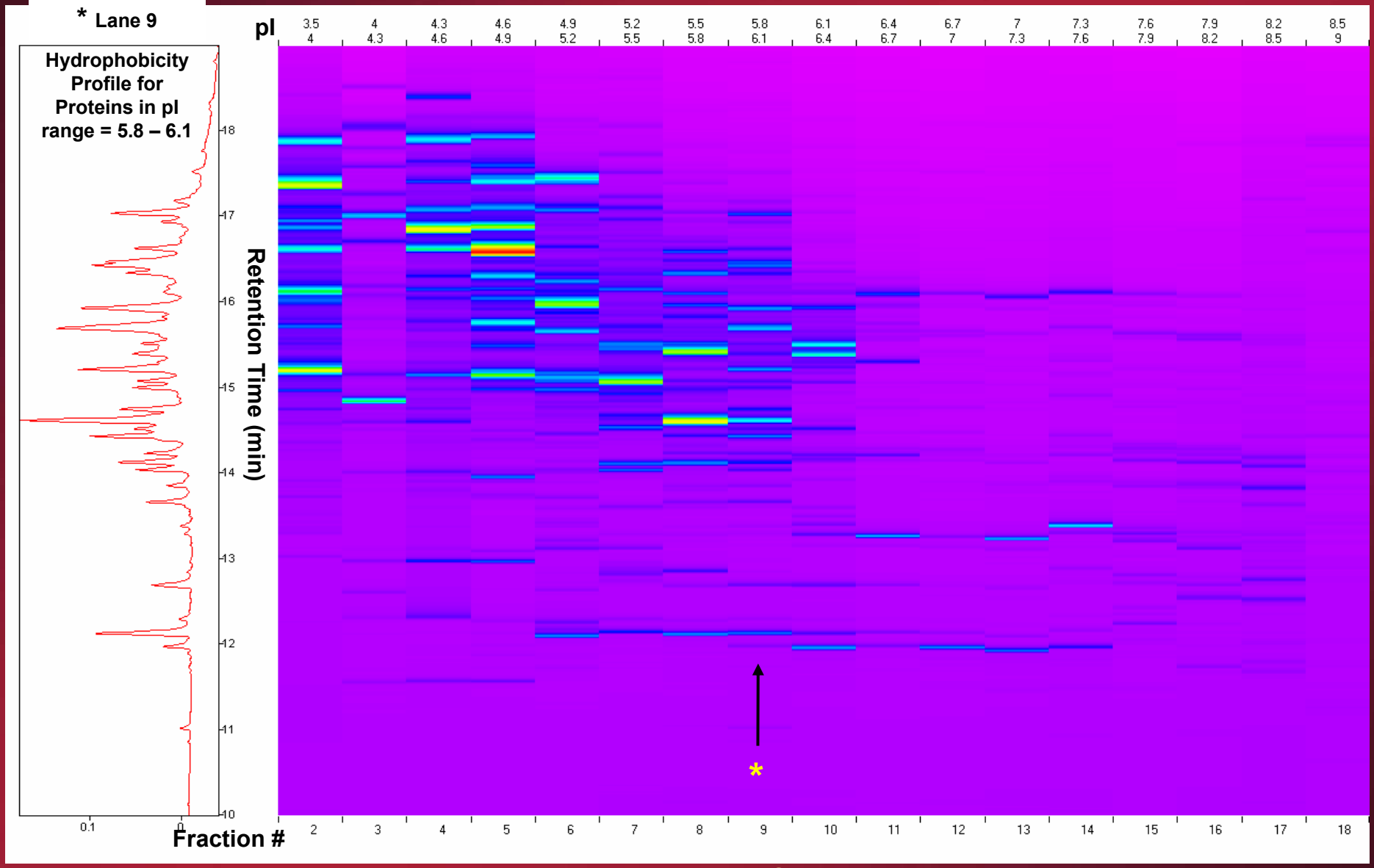
ProteoVue pI/Hydrophobicity 2D Protein Expression Map

E. Coli O157:H7 Whole Cell Lysate



Each pI Fraction analyzed by NPS® RP-HPLC

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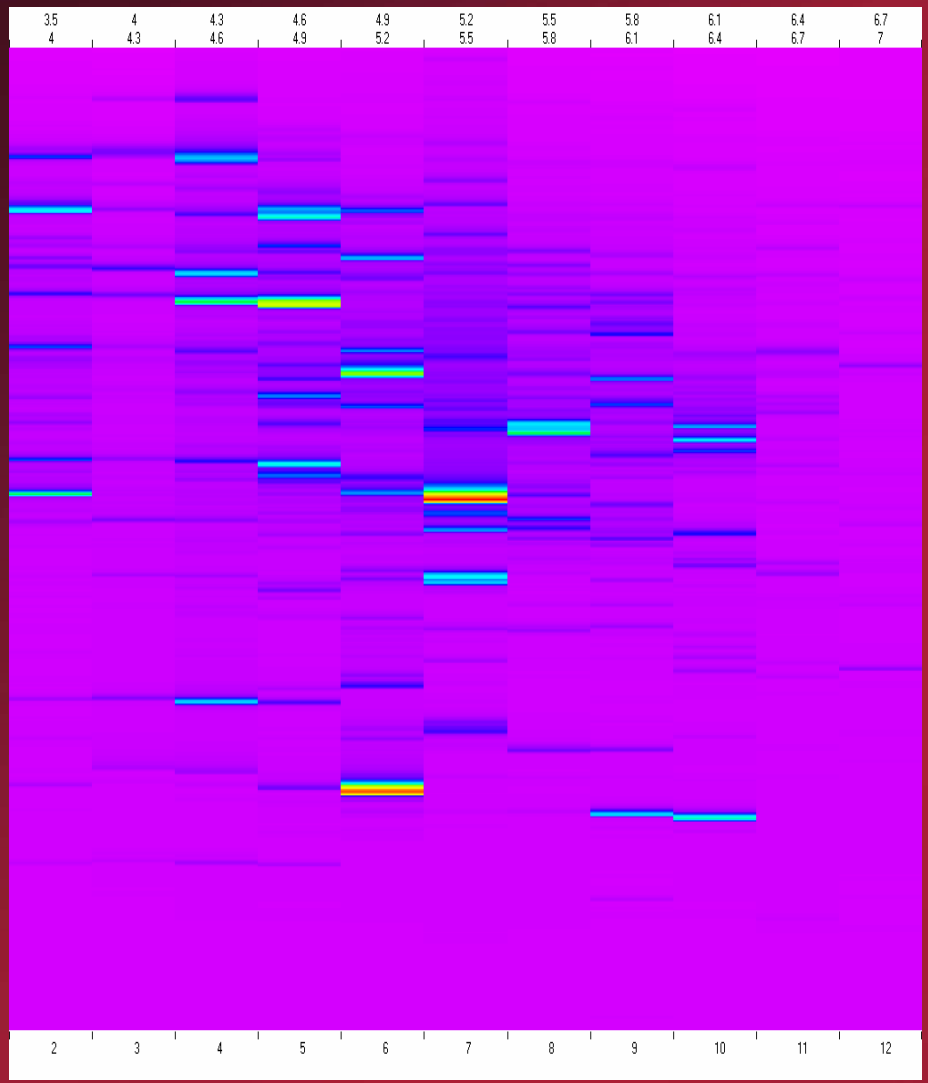
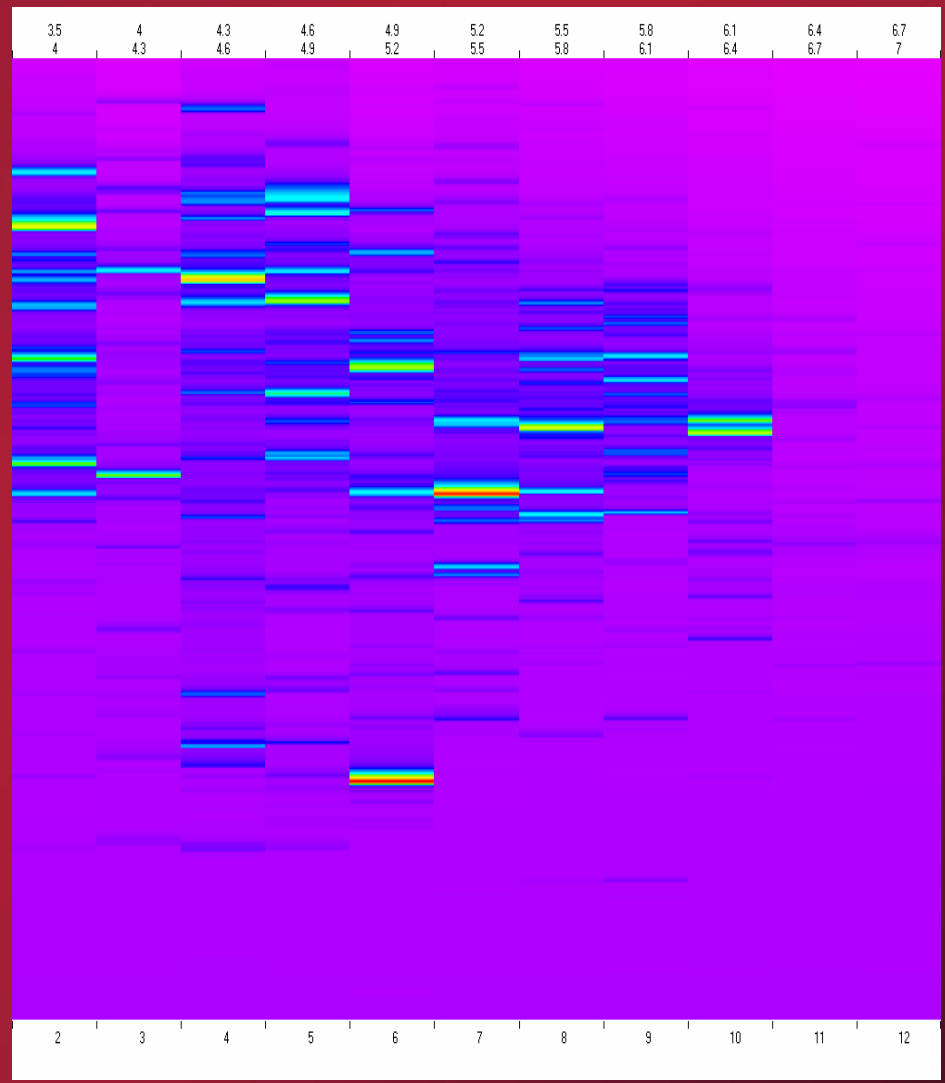
Protein “Fingerprinting” using ProteoVue 2D maps





E. coli O157:H7

E. coli O157:H32

Two Different E. coli O157 strains from Penn State
pI range 3.5 – 7; Hydrophobicity range 10 – 19 min

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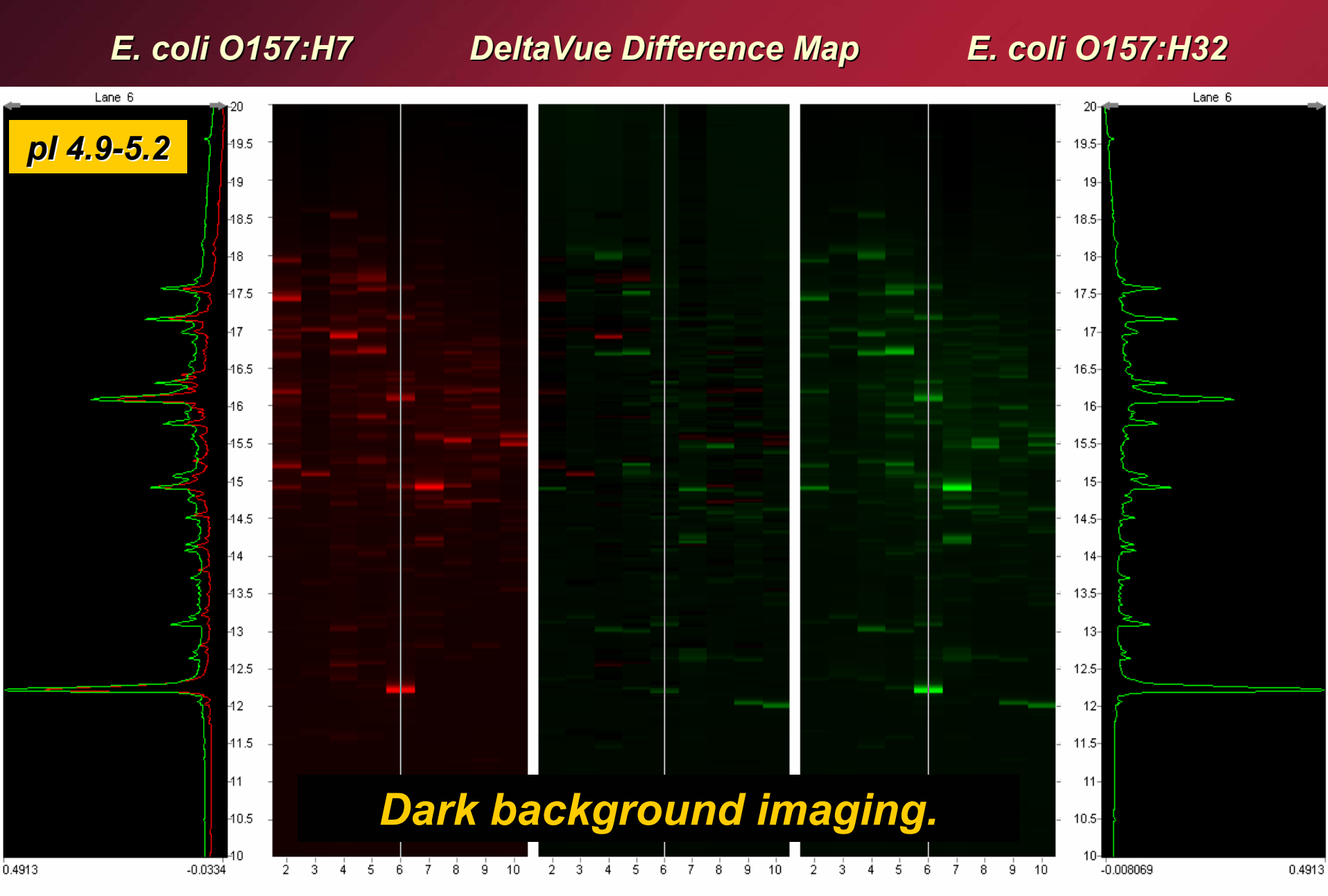

DeltaVue Maps Protein Expression Pattern Differences & Similarities

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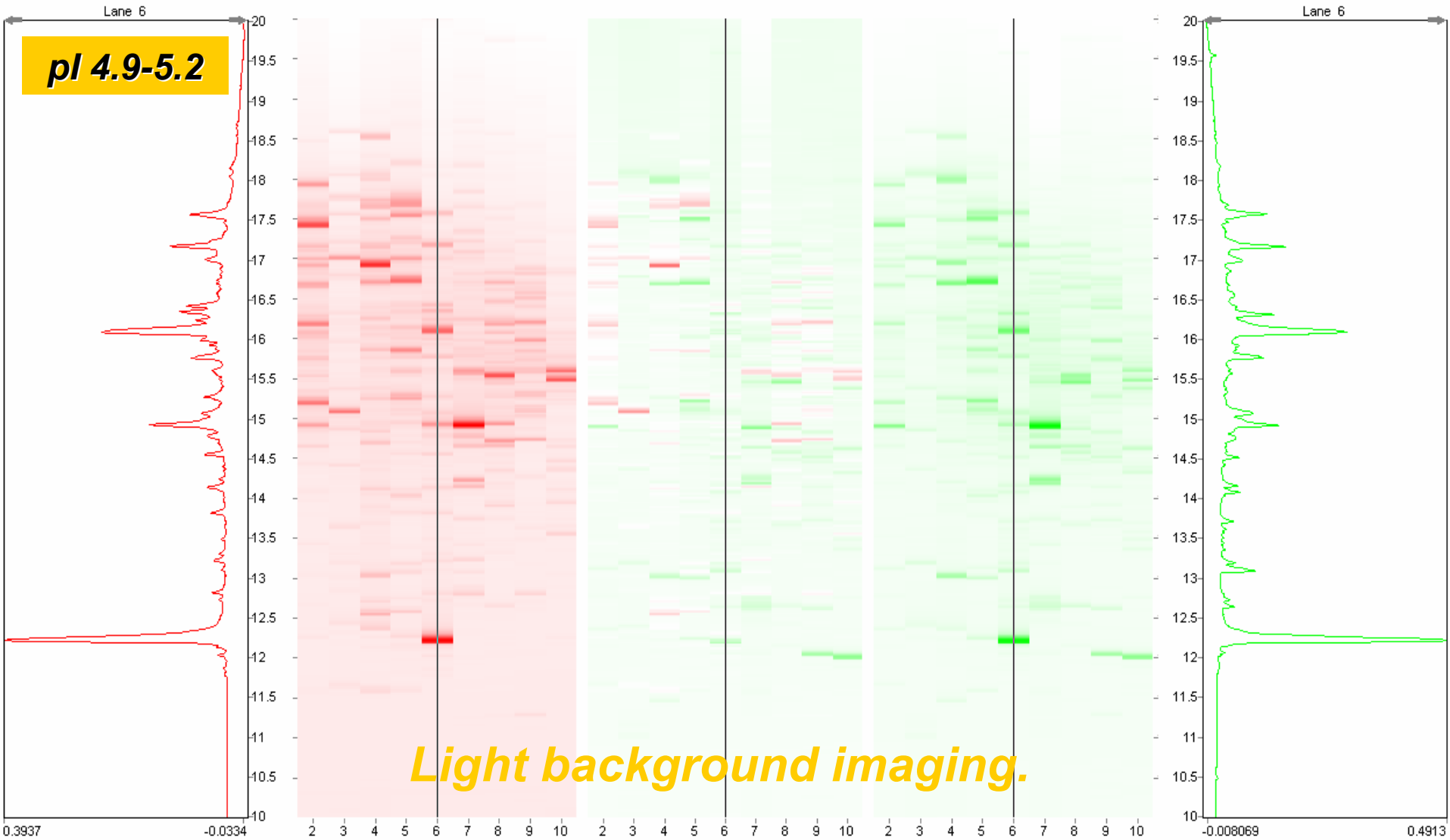
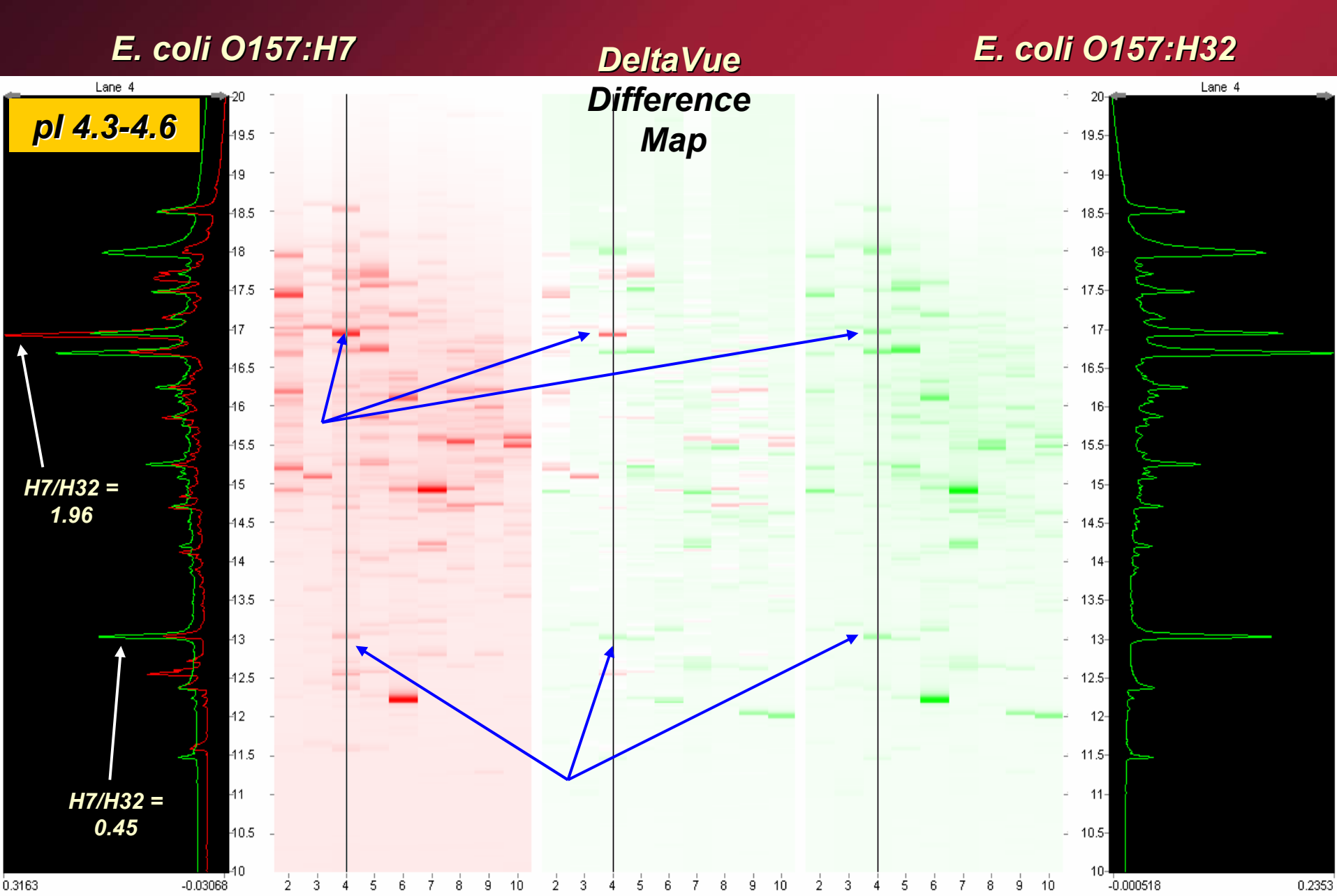
E. coli O157:H7 DeltaVue Difference Map E. coli O157:H32





DeltaVue Measures Protein Expression Level Differences

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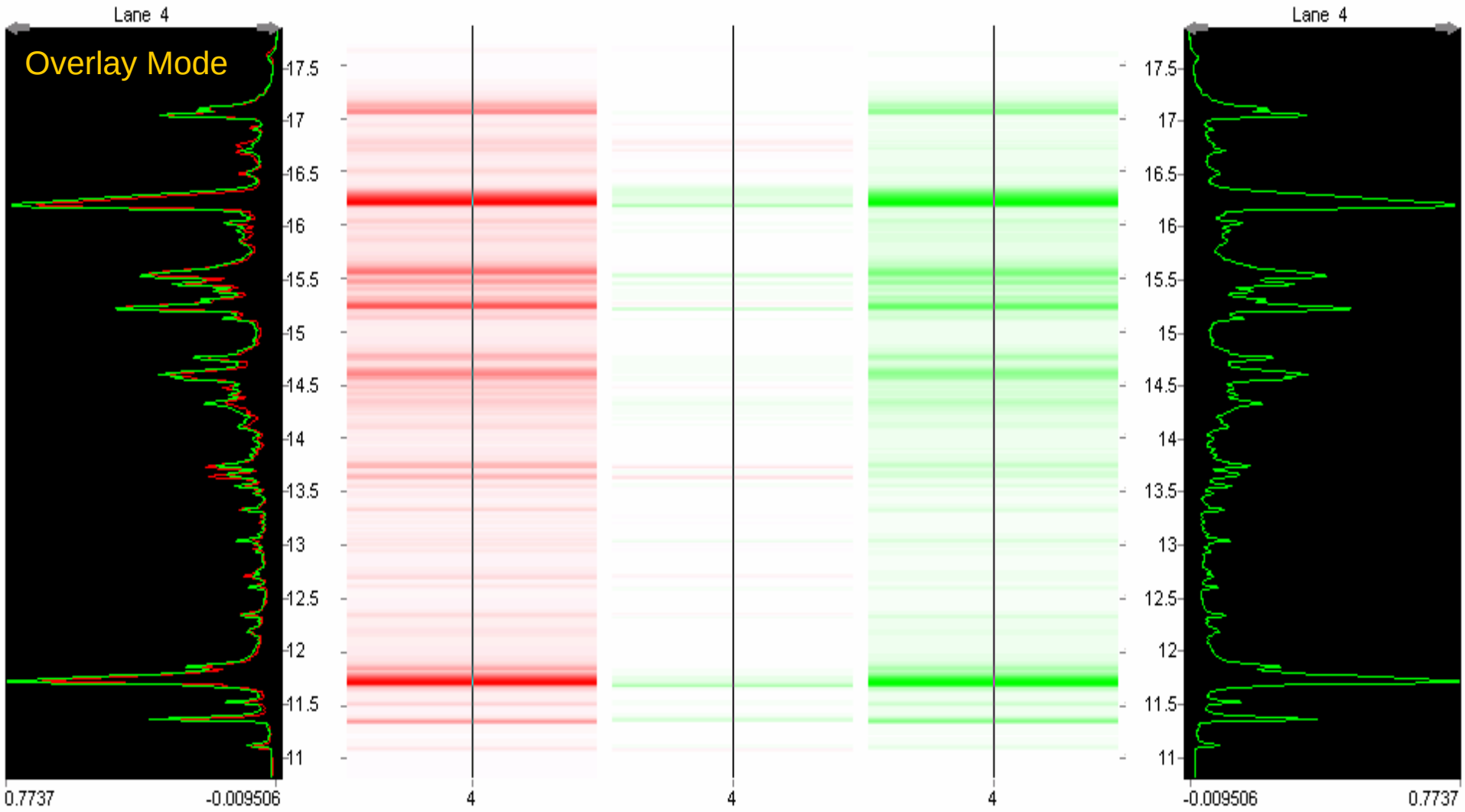
Reproducibility of Protein Expression Patterns: Same E. coli O157:H7 sample Run 2 times through ProteoSep



Differential Proteomic Analysis of Ovarian Cell Lines

Ovarian clear cell adenocarcinoma (ES2) cell line & HPV16 E6/E7 ovarian surface epithelial (OSE) cell line

Significant advantages of liquid phase fractionation of proteins from cell lysates for direct interface to MS.

Reference: Maureen T. Kachman, Haixing Wang, Donald R. Schwartz, Kathleen R. Cho, and David M. Lubman, Analytical Chemistry. Vol. 74, No. 8 (2002) 1779-1791.

Yanfei Wang, Rong Wu, Kathleen R. Cho, Kerby A. Shedden , Timothy J. Barder and David M. Lubman; To be published

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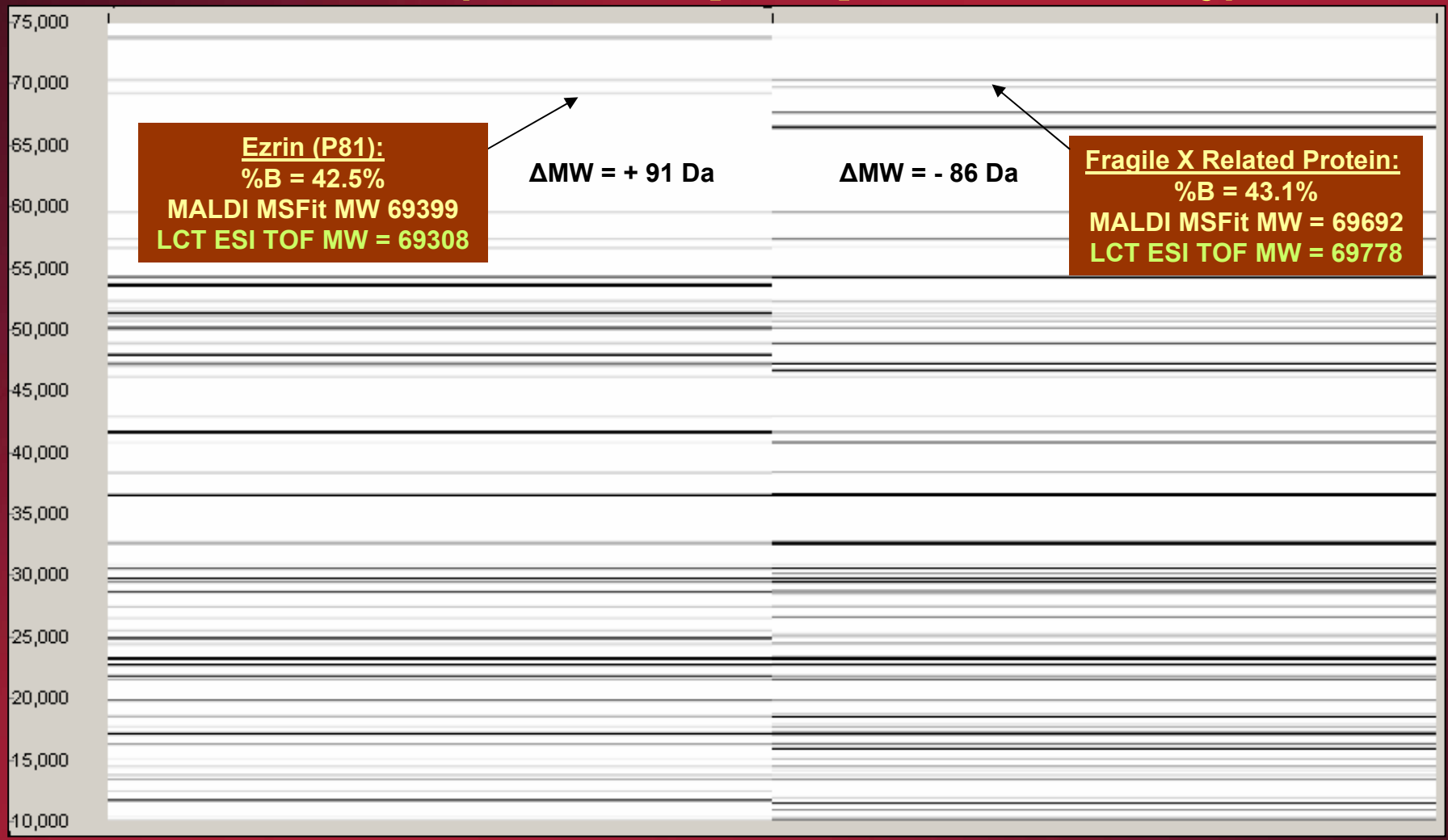
ProteoVue Mass Maps of pI 6.4 fraction for Ovarian Cell Lines

Database MW – Intact protein MW [Δ MW] values indicate type of PTM



Micromass LCT ESI-TOF analysis of comparative pI fraction

Kachman, M T.,et. al, Analytical Chemistry. Vol. 74, No. 8 (2002) 1779-1791.

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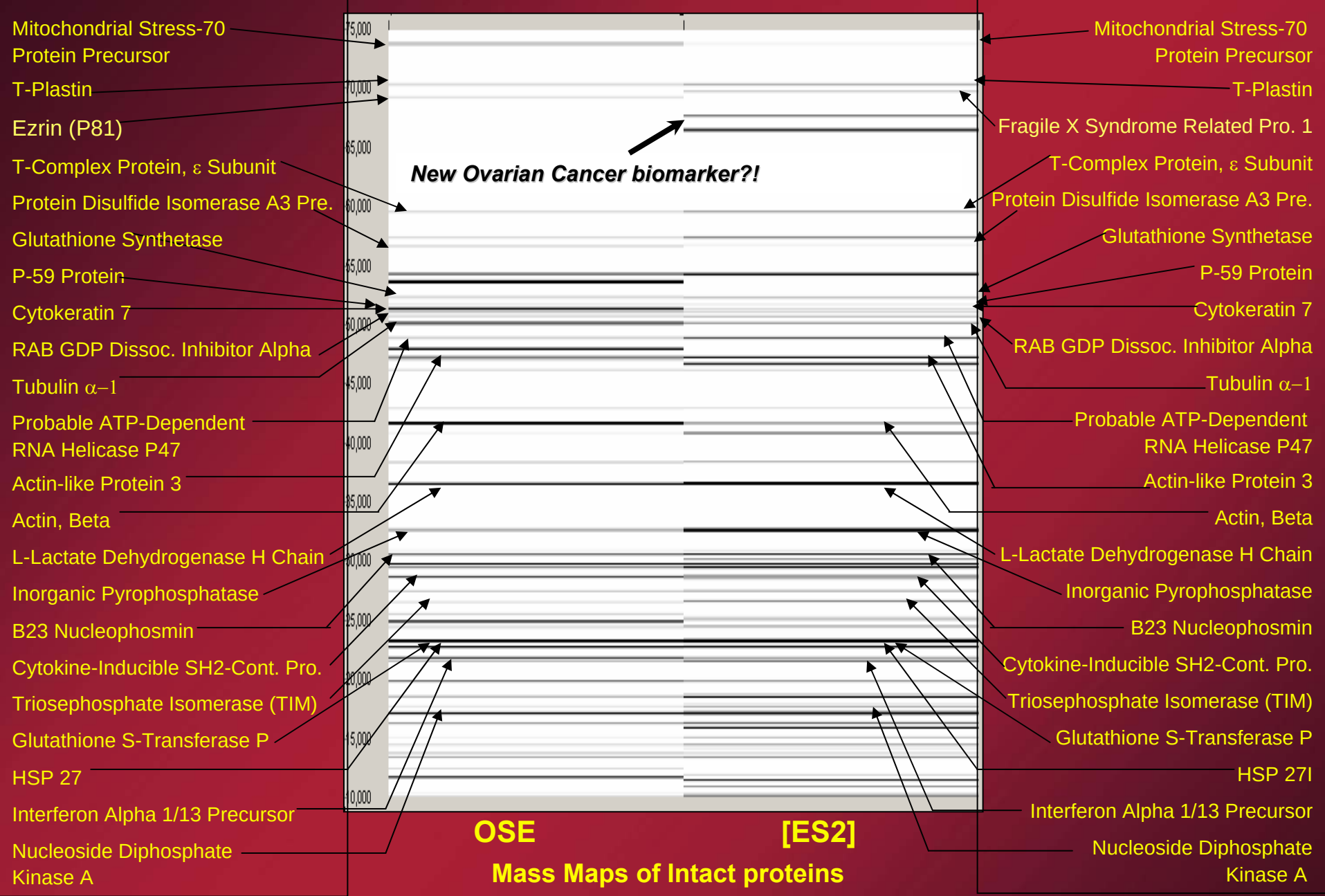

Voyager-STR MALDI TOF peptide mapping to ID proteins

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Advantage of Intact Protein Fractionation & Mass Mapping for Whole Cell Lysates

- **Direct PTM Information (MW and pI shifts) accessible.**
- **3D mapping possible to resolve similar pI/MW proteins.**
- **Direct isolation of important expressed proteins intact for further analysis.**
- **Deeper proteomics mining possible with larger sample loadings.**

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Novel Cancer Protein Arrays from Established Cell lines

Protein Expression Patterns and Biomarker Mining Using Autoantibodies.

Fang Yan, Arun Sreekumar, Bharathi Laxman, Arul Chinnaiyan, David Lubman, Timothy Barder, ***Proteomics* 3, 1228-1235** *(2003)*

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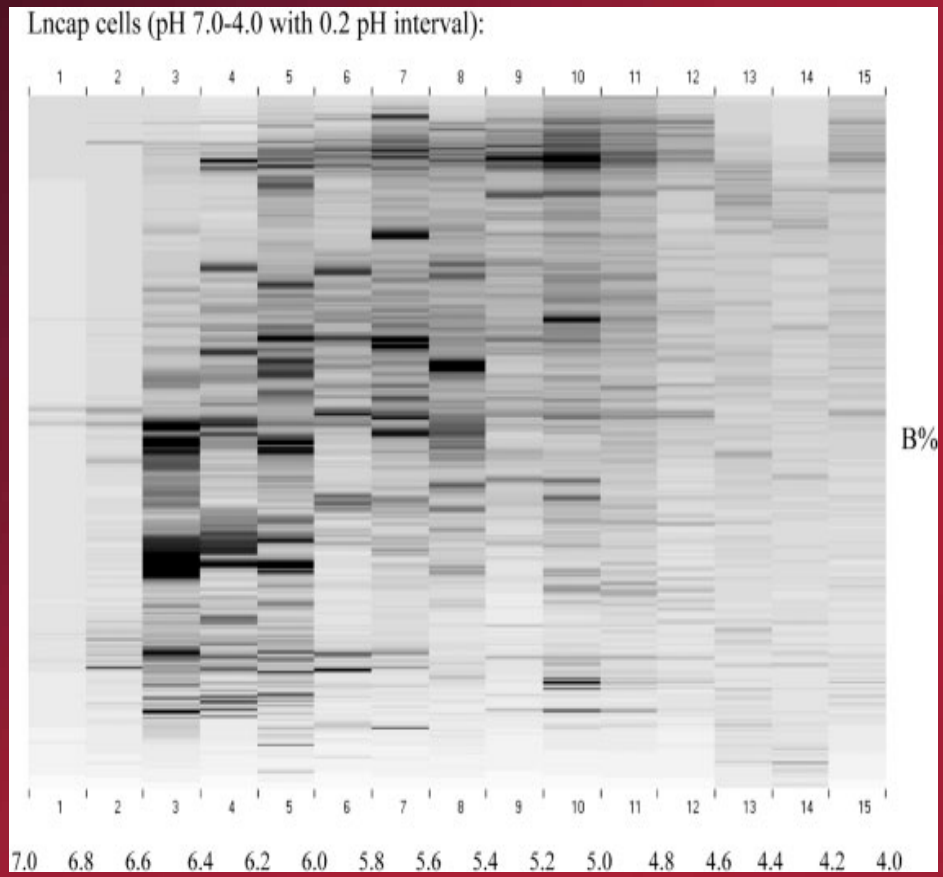
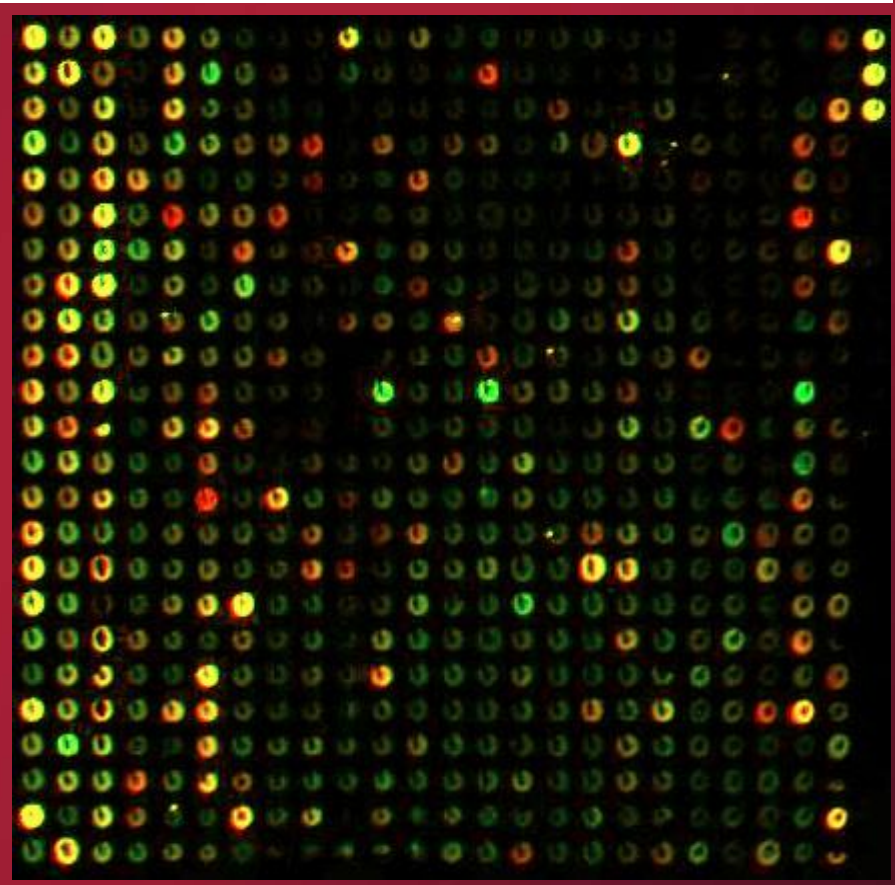
One Very Unique Advantage of ProteoSep!

ProteoSep Liquid phase mapping can produce a spotted Microarray!





Microarrays are key to the future of Clinical Diagnostics!

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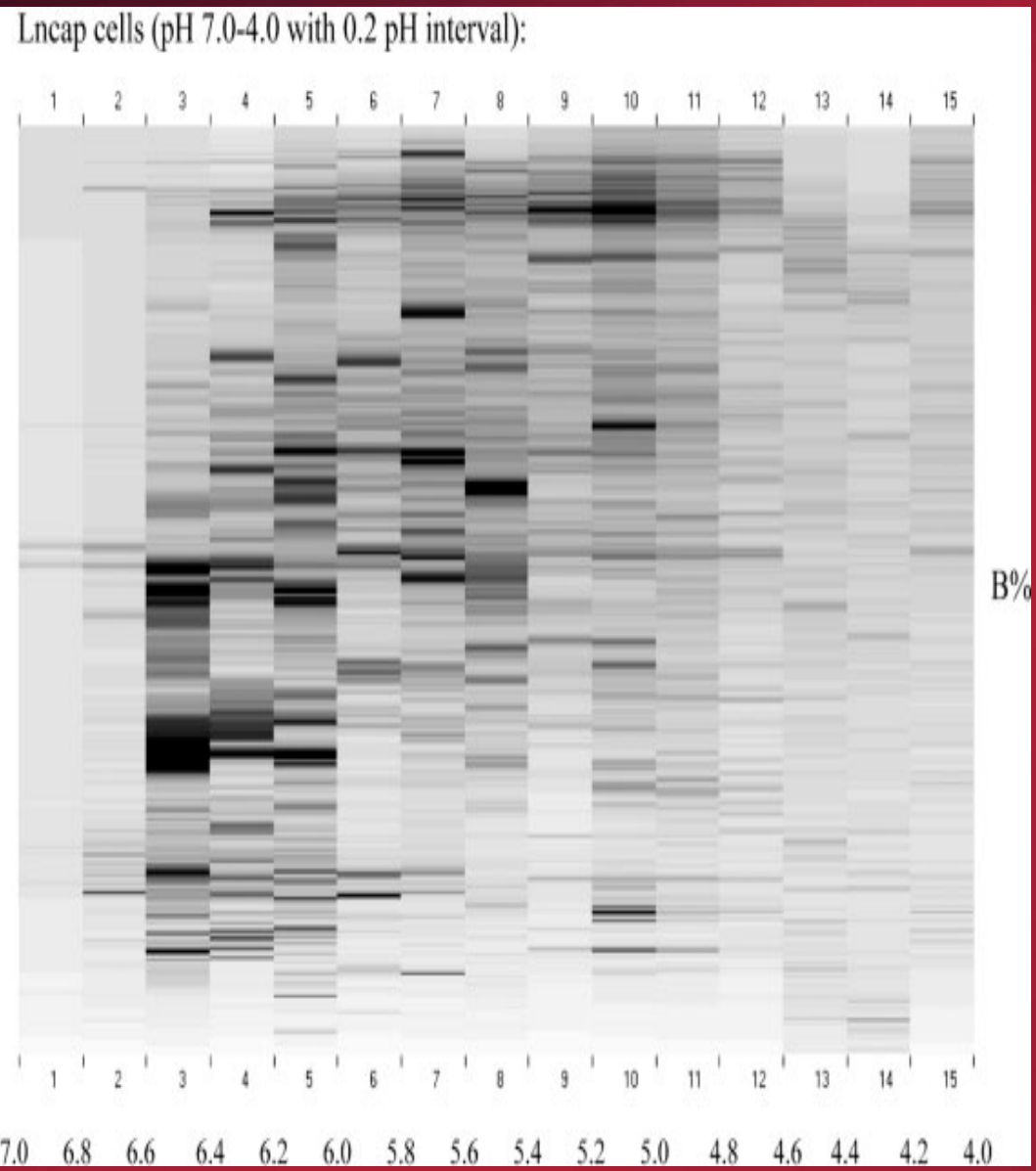
2-D pI - hydrophobicity map of LnCAP cells



- Think of this 2D map as an "array" of Intact proteins.
- Subdivide each pI fraction using RPHPLC into multi-well plate.
- Denature and spot individual wells onto nitrocellulose slides.
- Bands now contain protein(s) that can act a "bait" for auto-antibody (humoral) response from patient sera.

F. Yan *et al. Proteomics* 2003, *3,* 1228–1235

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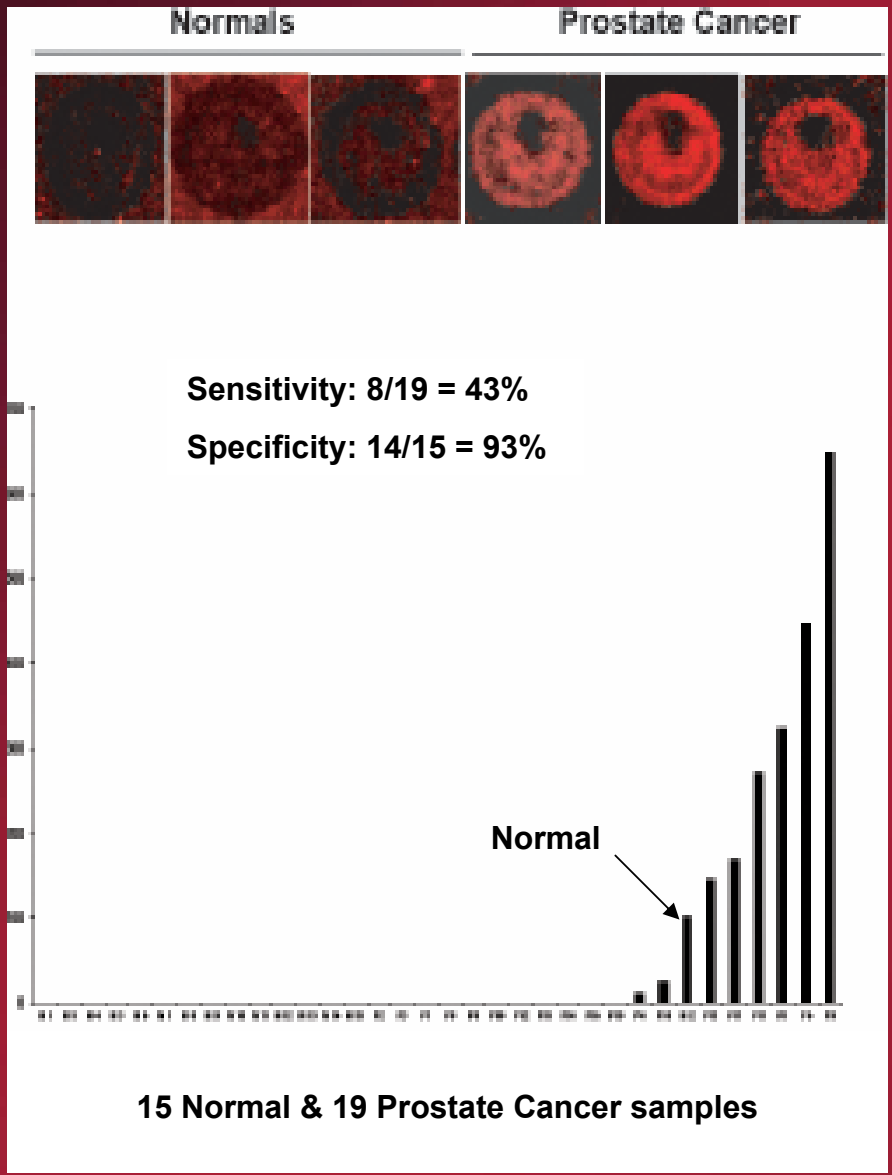
Results for 2nd dimension Well B6 for NC slide of arrayed proteins having pI's in the 6.8 – 7.0 range.



- Prepare a number of slides using 96 well fractions from 2nd dimension fractionation of proteins with pI 6.8 – 7.0.
- Interrogated 34 separate slides with serum from 19 PCA patients and 15 normal individuals.
- Fraction B6 shows high specificity for PCA samples.
- Unable to ID any protein in Fraction 6 with MS.

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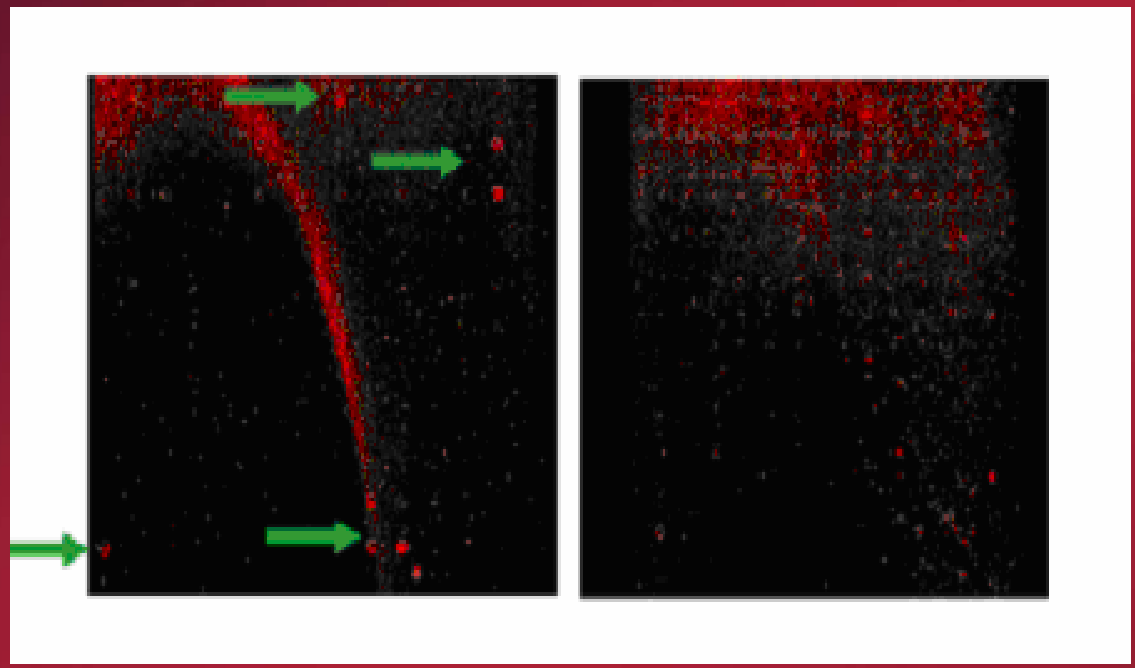
Slides for the arrayed proteins having pI's in the 6.0 – 6.2 range.

A B



- Interrogate separate slides with serum from 12 PCA patients (A) and 12 normal individuals (B).
- Green arrows highlight specific fractions showing immuno-reactivity with sera that was not seen with serum from normal individuals.
- Were able to ID proteins in 2 of the fractions using MS.

F. Yan *et al. Proteomics* 2003, *3,* 1228–1235

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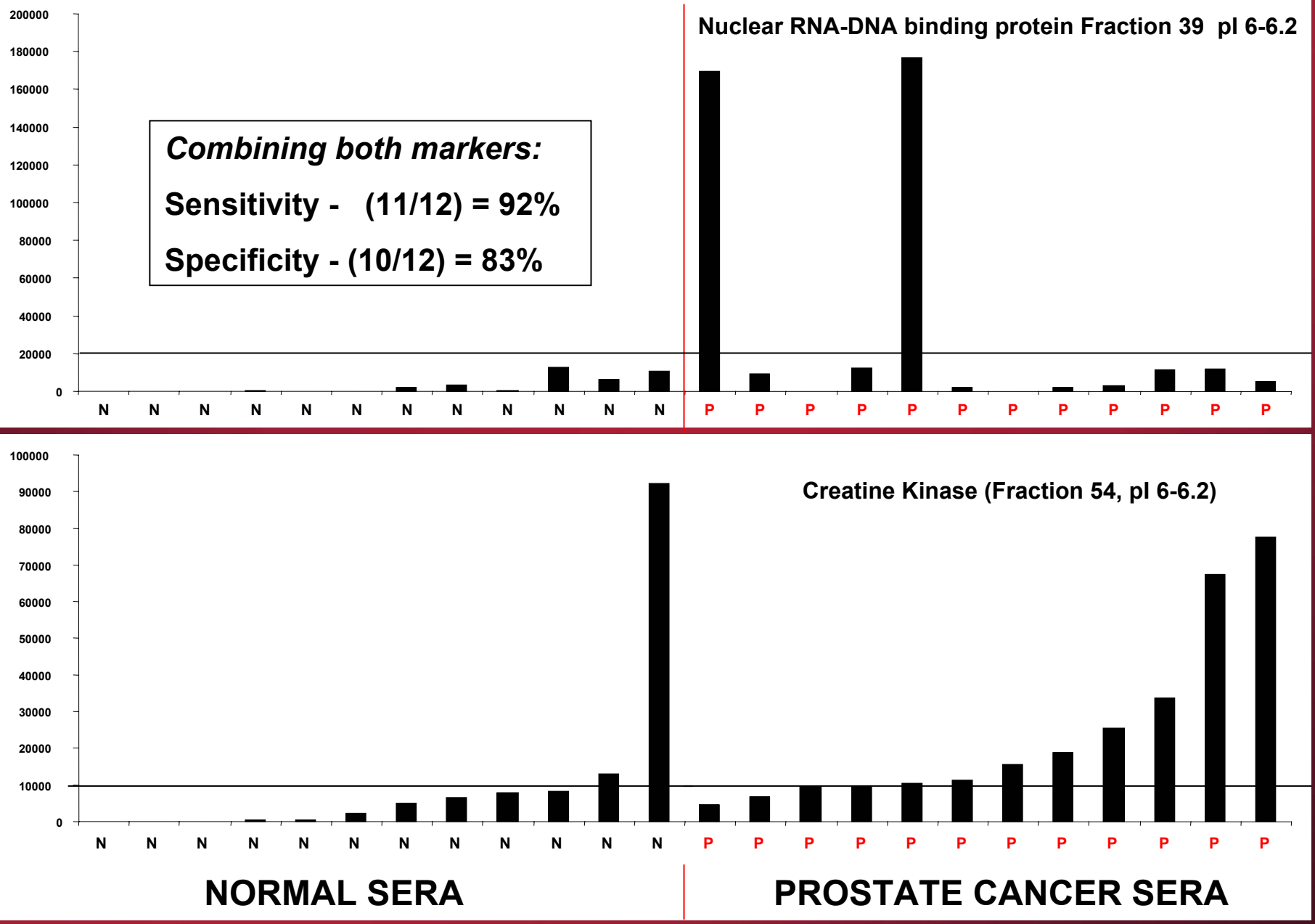
Nuclear RNA-DNA binding protein Fraction 39 pI 6-6.2
Combining both markers:
Sensitivity - (11/12) = 92%
Specificity - (10/12) = 83%
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
N N N N N N N N N N N N
P P P P P P P P P P P P
Creatine Kinase (Fraction 54, pI 6-6.2)
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
N N N N N N N N N N N N
P P P P P P P P P P P P
NORMAL SERA
PROSTATE CANCER SERA

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Auto-antibodies and Established Cell Lines as a Means of ID'ing New Diagnostic Markers and/or Patterns.

- **Using this 2D liquid fractionation technique, one can "mass-produce" disease specific intact protein arrays from well known cell lines for reasonably fast interrogation with patient sera.**

- **These protein arrays slides could also be used to establish unique expression patterns relevant to various aspects of the disease (stage, grade, clonogenicity, early detection, etc.)**

- **Working with a "complete set" of proteins as bait for auto-antibodies may help develop for more comprehensive understanding of the interplay of important disease related proteins (parallel vs. linear diagnostic approach).**

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ProteoSep®:

A New Multipurpose Proteomics Platform

- Is an all liquid-phase 2 dimensional mapping platform for separating and imaging intact proteins present in complex protein expression systems such as cells, tissues and biofluids like sera and plasma.
- Is the first the first fully automated commercial alternative to 2D gel technology.
- Can easily be directly interfaced to other technologies like MS for further protein characterization.
- Can be used to "fingerprint" complex protein expression patterns to aid in the identification of sets or groups of proteins unique to the sample being analyzed.
- Can produce novel kind of total protein microarray
  - for use in drug development screening and diagnostics;
  - in the search for new important disease biomarkers and biomarker patterns;
  - to provide for a more complete understanding of all the pathways influenced by drug candidates in the cell, tissue or biofluid sample being surveyed.

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ProteoSep; the liquid phase alternative to 2D Gels!

2D Liquid Phase Separation and Mapping System for Intact Protein Expression Analysis.

1st Dimension; pI information

- HPLC - Chromatofocussing => Direct Reference to 2-D Gels
- Liquid pI fractions for direct analysis in second dimension HPLC

2nd Dimension; MW Information

- RP-HPLC => Orthogonal to pI & analogous to MW
- Multi-well Fractionation for highly resolved liquid protein fractions

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Biological Samples Analyzed Using the ProteomeLab™ PF2D Instrument Platform

Whole Cell Lysates

Hepatocytes, Breast Cancer, Colon Cancer, Ovarian Cancer, Mouse embryonic stem cells, Yeast, E. coli, Staph Bacteria, Rat Brain Tissue, PBMC's, Flow cytometry samples.

Protein Fluids

Secreted Proteins (conditioned media), Sera, Plasma, Amniotic Fluid, Ascites, Saliva, Urine, Various Lavages, CSF.

Misc. Protein Samples

Veterinary Vaccines, Bacterial Antigens, Bacterial spores and extracts, Plant extracts, GMO samples, Meat Product extracts, Milk/Cheese Extracts

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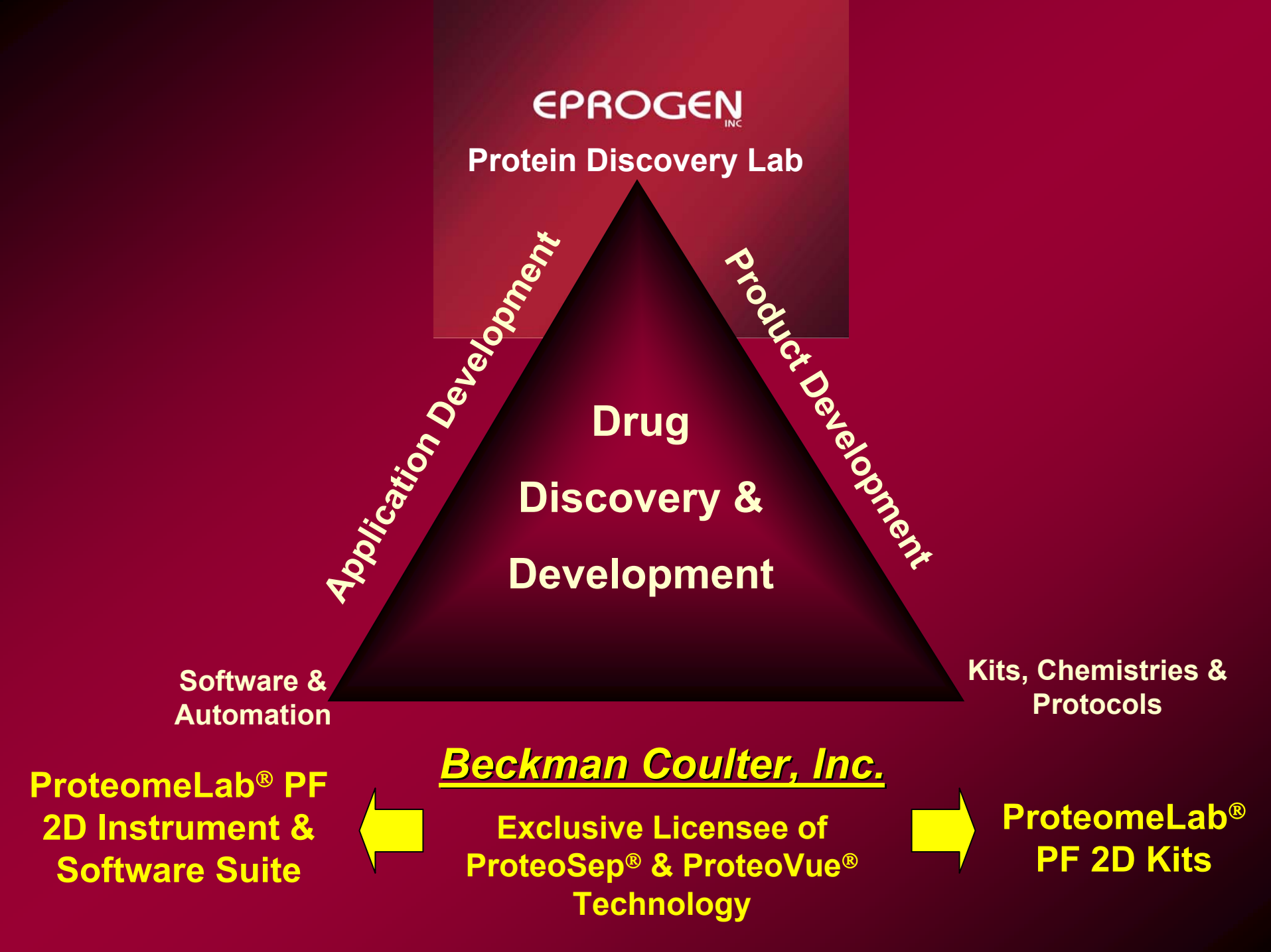
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Protein Discovery Lab
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Product Development
Drug
Discovery &
Development
Software &
Automation
Kits, Chemistries &
Protocols
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Exclusive Licensee of
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in Proteomics……..