Proteomics Workflows & Technologies
Proteomics

"The analysis of the entire PROTEin complement expressed by a genOME, or by a cell or tissue type."
Wasinger VC et al, Electrophoresis 16 (1995)

“Proteomics is the study of quantitative changes of protein expression levels and their application to drug discovery, diagnostics and therapy.”
Key Challenges in Proteomics

- Post-translational modifications
- Sample complexity
- Sensitivity
- Membrane proteins
- Throughput and automation
- Dynamic range

![Graph showing dynamic range and biomarkers](image)
Proteomics: An Integrated Approach

“Researchers can benefit from using two or more different techniques to analyze the same samples.”

- Marc Wilkins

The Practice of Proteomics: LC - MS/MS

In-liquid digestion provides high sensitivity for peptide detection
Less limitations for hydrophobic proteins solubilization
Extreme basic proteins detected
Direct interface between sample separation and mass spec for identification
PTMs isolated via specialty columns and recognized via software algorithms
DeCyder MS allows comparison of disease states, etc.
The Practice of Proteomics: 2DE - MS

Protein isoforms and PTM‘s are easily visualized
Extremely high resolution
Tolerant to crude sample loads
Proteins are protected within gel matrix
DIGE allows comparison of disease states, etc.
Simplified downstream workflow
Complimentary Workflows

Sample(s)
- Sample prep
- Digestion
- Imaging
- Picking
- Digestion
- Spotting
- MALDI PMF
- DIFFE
- METHODS

LC workflow
- Gel workflow
- Comparative Analysis

MALDI-MS^n
- Differential Analysis DeCyder MS

ESI-MS^n
- Differential Analysis DeCyder 2D

Differential Analysis DeCyder 2D
Sample Preparation
Principle of 2-D Electrophoresis

First dimension: denaturing isoelectric focusing separation according to the isoelectric point

Second dimension: SDS electrophoresis separation according to the molecular weight

2-D electrophoresis resolves a few thousand protein spots
Sample Preparation

Cell disruption
Protein precipitation
Solubilization
Protection against protease activities
Removal of

• nucleic acids • lipids • salts, buffers,• ionic small molecules • insoluble material
Sample Preparation Kits

- Ettan™ Microdialysis Kit
- Ettan™ Molecular grinding Kit
- Ettan™ 2-D Quant Kit
- Ettan™ Clean up Kit
- Ettan™ Sample Fractionation Kit
- Ettan™ Albumin/IgG Removal Kit
- Ettan™ DeStreak™ Kit
Cell disruption methods

- Freeze-thaw or osmotic lysis
- Detergent lysis
- Sonication
- Enzymatic lysis
- French pressure cell
- Grinding (mortar and pestle)
- Mechanical homogenization
Ettan Molecular Grinding Kit

for rapid cell
and tissue disruption

Microcentrifuge tubes
Abrasive grinding resin
Pestles
Protein solubilization

Urea (8-9.8 M) or 7 M urea / 2 M thiourea (recommended)
   – for protein solubility
   – prevents protein aggregates and hydrophobic interactions

Detergent (CHAPS,…)
   – for protein solubility

Reductant (DTT, DTE, . . .)
   – prevents different oxidation steps

Carrier ampholytes (0.8 % IPG buffer)
   – for protein solubility
   – raises the conductivity of the DryStrips

Sonication can help solubilization
   – Sample can be heated only prior to addition of urea
Extraction: Comparison Urea vs Urea/Thiourea

- 8 M urea
- Rat liver 7 M urea / 2 M thiourea
Effect of salt

_E. coli_ extract pH 4-7

no salt

30 mM NaCl
Ettan Microdialysis Kit     (1 & 8 kDa cutoff)
3 h to overnight
## Protein precipitation

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium sulfate (salting out)</td>
<td>Not efficient, de-salting necessary</td>
</tr>
<tr>
<td>TCA precipitation</td>
<td>Can be hard to re-solubilize</td>
</tr>
<tr>
<td>Acetone and/or ethanol</td>
<td>Leaves SDS behind, but many proteins not precipitated</td>
</tr>
<tr>
<td>TCA plus acetone</td>
<td>More effective than either alone, good for basic proteins</td>
</tr>
</tbody>
</table>
2-D Clean-Up Kit for first-dimension IEF
Acidic precipitation with detergent co-precipitant

Washing of pellets with addition of organic solvents

Resuspension of pellets in sample solution
2-D Clean-Up Kit for first-dimension IEF
Rat liver extracted with 4 % SDS, 40 mM Tris base

Before Clean-up

After Clean-up
DeStreak Reagent

This will reduce streaking in the basic part of the pH gradient

IPG-strip pH 7.5 - 9.5, 24 cm.
80 µg mouse liver proteins applied anodic.
Focusing for 80 kVh, 16 hours.
Comparison: Conventional technique v. DeStreak

First dimension: Immobiline DryStrip pH 6-9, 24 cm rehydrated in 8 M urea, 0.5% CHAPS, 0.5% Pharmalyte pH 3-10, 0.5% IPG-Buffer pH 6-11 and either 10 mM DTT (left) or 100 mM disulfide (right); Anodic sample application of 80 µg mouse liver proteins. Sample solution: 8 M urea, 0.5% CHAPS, 1 % Pharmalyte pH 8-10.5 and either 10mM DTT (left) or 100 mM disulfide (right)
Ettan Albumin/IgG Removal Kit
Ettan 2-D Quant Kit

Compatible with...

<table>
<thead>
<tr>
<th>Compound tested</th>
<th>concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>2% (w/v)</td>
</tr>
<tr>
<td>CHAPS</td>
<td>4% (w/v)</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>1% (w/v)</td>
</tr>
<tr>
<td>Pharmalyte™ pH 3–10</td>
<td>2% (v/v)</td>
</tr>
<tr>
<td>IPG Buffer pH 3-10 NL</td>
<td>2% (v/v)</td>
</tr>
<tr>
<td>Tris</td>
<td>50 mM</td>
</tr>
<tr>
<td>EDTA</td>
<td>10 mM</td>
</tr>
<tr>
<td>DTT</td>
<td>1% (65 mM)</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>1% (v/v)</td>
</tr>
<tr>
<td>Urea</td>
<td>8 M</td>
</tr>
<tr>
<td>Thiourea</td>
<td>2 M</td>
</tr>
<tr>
<td>Glycerol</td>
<td>30% (w/v)</td>
</tr>
</tbody>
</table>
General DIGE Sample Prep Workflow

2D Quant kit
2D Clean up Kit
2D Quant kit
Sample labeling
Sample preparation for 1D analysis
2D Electrophoresis
Ettan IPGphor II

Platform accommodates up to 12 strip holders
Programmable “delayed start” rehydration period or use active rehydration
10 possible programs, 10 phases each (ramp or step)
7, 11, 13, 18 and 24 cm strip holders
Built-in power supply delivering 10,000 V, 1.5 mA
Manifold
Analytical & Cup-loading stripholders for all lengths
Built-in Peltier cooling, 18 - 25 °C
Safety lid
Cup Loading and Analytical Strip Holders

Analytical

manifold

Cup-loading
Highly Abundant Proteins

Analytical Strip Holder

Cup-loading Strip Holder / Multiphor

Paraffin oil
Effect of Rehydration under Low Voltage

Mouse liver proteins
18 cm IPG strips

A. No voltage applied
B. 30 V applied during rehydration

from A. Görg et al. Life Science News 1 (1998) 4-6
Wide and Narrow pH Gradients

Wide gradients are applied for:
entire protein spectrum

Narrow gradients are applied for:
increased resolution
increased loading capacity
to detect and analyse more proteins
Increased Resolution: Blow-Ups of Spots

Mouse liver proteins

IPG 4-7

IPG 5-6

IPG 5,5-6,7

From A. Görg et al. (1999)
Mouse liver proteins

From A. Görg et al. (1999)
Equilibration of IPG Strips

Prior to SDS electrophoresis the strips are treated with equilibration solution:

- 2 % SDS, 50 mM Tris-HCl pH 8.8,
- 6 M urea, 30 % glycerol
- (1 x 15 min) 1 % DTT
- (1 x 15 min) 4 % iodoacetamide

SDS provides a charge to polypeptides allowing them to migrate based on size.

Glycerol and urea for minimizing EEO effects.

DTT reduces disulfides to ensure all cystines are converted to cysteines.

Iodoacetamide ensures cysteines are not re-oxidized.
2nd Dimension: Ettan DALT Systems

DALT Twelve
- Capacity of 1 - 12 gels
- Large format system, 25.5 x 20 cm
- Lab cast gels, 1 mm and 1.5 mm
- Ettan DALT non-DIGE precast gels

DALT Six
- Capacity of 1 - 6 gels
- Large format system, 25.5 x 20 cm
- Lab cast gels, 1 mm and 1.5 mm
- Ettan DALT non-DIGE precast gels
Summary

2DE is a complimentary workflow to LC in proteomics.

Sample preparation is a key step in any analytical process.

Good sample preparation will lead to good DIGE results.