1D and 2D Protein Electrophoresis: optimization and innovations

Reiner Westermeier, GE Healthcare Europe
Net charges on proteins

Acidic pH

pH = pI

Basic pH

Isoelectric point (pI)

net charge

pH
SDS Polyacrylamide Gel Electrophoresis
Separation according to the Molecular Weights
SDS Disc electrophoresis

Sample application and start
- Tris-glycine SDS
- Tris-Cl pH 8.8 0.375 molar
- Resolving gel
- Tris-Cl SDS

Protein concentration in the stacking gel
- Tris-glycine SDS
- SDS sample stacking gel

Separation of the proteins in the resolving gel
- Tris-glycine SDS
- pH 9.5

Silver stained SDS gel

Adapted to SDS PAGE in the year 1970 by Laemmli
Practical hints

Always use Tris base
Never titrate the running buffer (Tris-glycine)
“Cross-over” of electric parameters

- max. 35 W
- max. 50 mA
- max. 600 V

Conductivity:
- Tris-Cl
- Tris-glycine

Time:
- 30 min
- 1 h
- 1 h : 30 min
- 2 h
Disc electrophoresis in readymade gels

Tris-acetate / tris-tricine buffer system
PhastSystem™
Pre-cast PhastGel™ run in 30 minutes

SDS-PAGE of different strains of mycoplasma
SDS sample preparation

- **native**
  - Tertiary structure
  - Quaternary structure

- **nonreducing SDS treatment**
  - 1 - 2% (w/v) SDS
  - 3 min at 95°C

- **reducing SDS treatment**
  - 1 - 2% (w/v) SDS
  - + DTT, DTE or 2-mercaptoethanol
  - 3 min at 95°C

- **reducing SDS treatment and alkylation**
  - 1 - 2% (w/v) SDS
  - + DTT, DTE or 2-mercaptoethanol
  - 3 min at 95°C
  - + iodoacetamide or vinylpyridine
SDS electrophoresis

SDS is an anionic detergent, binds quantitatively to proteins: 1.4 g SDS / 1 g protein.
Molecular weight standards

Peptide Markers (P)
- 2.5 - 17 kDa

Low Molecular Weight (L)
- 14 - 94 kDa

High Molecular Weight (H)
- 53 - 212 kDa

Rainbow Markers (R)
- 10 - 250 kDa
Blotting and Specific Detection
Immunoblotting

1. Gel
2. Transfer
3. Primary Antibody Binding
4. Secondary Antibody Labeling
5. Blocking
6. Detection
Gel staining versus blotting

Identification on membranes is:

- more sensitive
- more specific

Coomassie Blue stained SDS gel

Detection of E. coli GroEL with ECL Plus
ECL semidry blotters

TE 70: up to 14 × 16 cm,
TE 77: up to 21 × 26 cm,
PWR: with inbuilt power supply
Chemiluminescent Detection - ECL

- Protein bound to blocked membrane
- Primary Ab
- Secondary Ab - HRP
- Peracid
- Oxidized product
- Light
- HRP catalyzed Oxidisation of Luminol + Proprietary Enhancer
- Signal stability 30 min-2H00

Hyperfilm
ECL / CCD
ECL Plus

Protein bound to blocked membrane

Secondary Ab - HRP

ECL Plus substrate acridinium based

Light

Acridinium esters

Signal stability 12H00-24H00

Peroxide

H₂O

Primary Ab

Hyperfilm ECL / CCD
ECL Advance

Protein bound to membrane blocked with ECL Advance Blocking Agent

Primary Ab

Secondary Ab - HRP

HRP catalyzed Oxidisation of substrate

ECL Advance Substrate

Oxidized product

Light

Signal stability 4H00-5H00

Hyperfilm ECL / CCD
ImageQuant 300, 400, and ECL

CCD-based imaging systems, which cover the full range of gel documentation, fluorescence, and chemiluminescence applications.
ECL Plex – relative quantitative Western blotting
Principles of Fluorescence Western Blotting

**ECL Plex**

1. Protein bound to blocked membrane
2. Primary antibody
3. Secondary Antibody-CyDye
4. Monochromatic light (excitation)
5. Light (emission)
6. Scanner/CCD detector
GE Healthcare Life Sciences
Imaging platform for fluorescence detection

Typhoon™

Ettan™ DIGE Imager

Storm™
ECL Plex Fluorescent Western blotting

- Primary antibody
- Secondary antibody CyDye Conjugate
- Antigen on membrane

△ = Antigen 1

△ = Antigen 2
CyDye™ Properties

Very bright
  • Coefficient d’extinction molaire
  • Quantum efficiency

Highly photostable

Wide pH range tolerance

Spectrally well resolved

Range of colors

CyDye™ Emission Spectra
Multiplexing – CyDye Fluors

- Minimal cross-talk between the fluors because the dyes are spectrally well resolved
- Typhoon™ is using single wavelength light to excite fluors
The power of relating to house-keeping protein

- Loading the exact same amount of protein is uncertain.

- Strip and re-probe from an ECL blot: new uncertainties:
  - Loss of target proteins unevenly across the blot.
  - Detect unspecific signal by poor stripping especially for proteins of the same size.

- Relating phosho-protein to total (non-phospho and phospho) protein: It can be valuable to also relate to an unrelated housekeeping protein, since you can not be sure that your total protein level is not varied in your experiment.

Use of ECL Plex eliminates all these uncertainties.
Effects of FGF-2 stimulation on total ERK1/2 expression in wild type and enzyme knock-out mouse embryonic fibroblasts

“Equal” loading estimated according to protein concentration determined by Bradford assay

Increased ERK ½ levels in -/- cells in response to FGF-2 stimulation

Data courtesy of Dr. Jin-Ping Li and Juan Jia, Department of Medical Biochemistry and Microbiology, Uppsala, Sweden.
Detection of proteins from rat brain

Data courtesy of Prof. Willard M. Freeman and Dr. Kruti Patel, Penn State College of Medicine, PA, USA.

Dilution series from 0.5 to 16 µg of total protein lysates of homogenate from the nucleus accumbens (NAc) region of the rat brain (down to 0.5µg)
Detection of low abundant phosphorylated protein

Increase in p38 activation upon TGF-β stimulation for 0, 2.5, 5 or 15 minutes in 293T cells.
ECL™ Plex products

**ECL Plex conjugates**
- ECL Plex goat-α-mouse IgG, Cy2
- ECL Plex goat-α-rabbit IgG, Cy2
- ECL Plex goat-α-mouse IgG, Cy3
- ECL Plex goat-α-rabbit IgG, Cy3
- ECL Plex goat-α-mouse IgG, Cy5
- ECL Plex goat-α-rabbit IgG, Cy5

**Membranes**
- Hybond-ECL (nitrocellulose membrane)
- Hybond-LFP (low fluorescent PVDF membrane)

**Markers**
- ECL Plex Fluorescent Rainbow markers

**Blocking agent**

**Combination packs**
- ECL Plex Western blotting combination pack (RPN998)
- ECL Plex Western blotting combination pack (RPN999)
Blue Native PAGE

For the analysis of

- Membrane proteins
- Intact protein complexes
- Intact protein super complexes

First paper:
Schägger H. and von Jagow G.
Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form.
Latest Review

Krause F.

Detection and analysis of protein–protein interactions in organellar and prokaryotic proteomes by native gel electrophoresis: (Membrane) protein complexes and supercomplexes.

Nonionic detergents for solubilization of protein complexes

Triton X-100

Digitonin

Dodecyl maltoside

From Braun, HP and Eichacker, L, Blue Native PAGE Courses
Blue Native Electrophoresis: Charge-providing reagent

SDS

Coomassie G250

From Braun, HP and Eichacker, L, Blue Native PAGE Courses
Steps after solubilization

Incubation of the samples for 0 – 30 minutes on ice
Centrifugation at 18,000 x g for 10 – 30 minutes to remove insoluble material

**BN DIGE**: Labeling of complexes with CyDyes at pH 8.5

**BN DIGE**: Mixing of differently labeled sample solutions

Addition of Coomassie-blue solution, e.g. 5 µl

Loading Coomassie-treated protein samples onto blue-native gels
Casting a porosity gradient 4 – 16 %

from the bottom

from the top
Buffer system for BN PAGE

Blue loading buffer
750 mM amino caproic acid; 5% (w/v) Coomassie G-250

6 × Gel buffer
1.5 M ε-amino caproic acid; 150 mM bis-Tris

6 × Anode buffer
300 mM Bis-Tris

5 × Cathode buffer Blue
250 mM tricine; 75 mM Bis-Tris; 0.1 % (w/v) Coomassie G-250
BN-PAGE in a 24 cm long gradient gel in the SE 660

From Braun, HP and Eichacker, L, Blue Native PAGE Courses
Blue Native-PAGE of Protein Complexes

1st Dimension

Marker | Etioplast | Chloroplast

- 669 kDa
- 440 kDa
- 232 kDa
- 140 kDa
- 67 kDa

- cpHSP60
- RCII Core (2)
- PSI-LHCl
- Rubisco
- ATPase (CF₁)
- RCII Core (1)
- LHCII (3)
- POR *
- POR

Prof. L. Eichacker, Botanik, LMU München
Connecting 1st and 2nd dimension

Method 1
- 1 mm thin cassette spacers
- 1.5 mm thick BN gel
- acrylamide monomer solution

Method 2
- 1 mm thin cassette spacers
- acrylamide monomer solution
- agarose
- 0.7 mm thin BN gel
- polyacrylamide gel
2D Blue Native/SDS-Electrophoresis

Solubilization of mitochondrial proteins by Dodecyl maltoside (DDM) and Digitonin

From Prof. H.-P. Braun, Abteilung für angewandte Genetik, Universität Hannover
Resolution of mitochondrial supercomplexes from Arabidopsis by 2D Blue-native / Blue-native PAGE

DIGE: BN-PAGE vs. IPG-DALT

8th Blue Native Electrophoresis Course

Directed by: Prof. Dr. Hans-Peter Braun and Prof. Dr. Lutz Eichacker

Where: University of Hannover

When: 26th to 28th September 2007

Costs: 780 €

Registration: at GE-Healthcare ....or....

braun@genetik.uni-hannover.de
Analysis of membrane proteins with acidic electrophoresis


Cationic Detergent PAGE
For the analysis of very hydrophobic proteins

First paper:
MacFarlane D.
Two dimensional benzyldimethyl-n-hexadecylammonium chloride – sodium dodecyl sulfate preparative polyacrylamide gel electrophoresis: a high capacity high resolution technique for the purification of proteins from complex mixtures.

Buffer system for 16-BAC acidic PAGE

Separating gel:
7.5–15% T acrylamide (3.25 %C),
0.4 M Methoxy acetic acid/KOH pH 3.0,
0.002% 16-BAC,
Catalyst system: 4 mM ascorbic acid, 8 µM FeSO$_4$·7H$_2$O,
Start of polymerization: add 2.0 mM H$_2$O$_2$.

Stacking gel:
4%T acrylamide (7.8%C),
0.4 M Acetic acid/KOH pH 4.0,
0.002% 16-BAC,
Catalyst system: 4 mM ascorbic acid, 8 µM FeSO$_4$·7H$_2$O,
Start of polymerization: add 2.0 mM H$_2$O$_2$.

Kramer ML.
A new multiphasic buffer system for benzyldimethyl-n-hexadecylammonium chloride polyacrylamide gel electrophoresis of proteins providing efficient stacking.
Cationic detergent acidic gel PAGE / SDS PAGE of hydrophobic proteins
DIGE: CTAB / SDS PAGE

of Cy™3 Fluor saturation dye-labelled proteins of mouse T cells.
**DIGE:** 16-BAC / SDS PAGE gels for hydrophobic proteins

Samples labeled with CyDyes and mixed together

From Oesterhelt *et al.*
MPI, Martinsried
Germany
Spot co-detection
Isoelectric Focusing

Separation according to the isoelectric points
Isoelectric Focusing

Migration of amphoteric compounds to isoelectric points

Focusing effect
IEF with carrier ampholytes

Pharmalytes
Ampholines

\[
\begin{align*}
\text{CH}_2 - \text{N} - (\text{CH}_2)_x - \text{N} - \text{CH}_2 - \\
(\text{CH}_2)_x - (\text{CH})_x \\
\text{NR}_2 - \text{COOH}
\end{align*}
\]

where \( R = \text{H} \)
or \(- (\text{CH}_2)_x - \text{COOH} ,\)  
\( x = 2 \) or 3

decreasing pI

gel

electric field

pH

10
9
8
7
6
5
4
3
PhastGel IEF of oligoclonal IgGs

TCA fixation and silver staining of all proteins

Immunofixation followed by silver staining

Immunofixation can be applied because gel is only 0.35 mm thin and soft (4% T)

Samples: S...serum; C...cerebrospinal fluid
**Immobilized pH Gradients (IPG)**

**Immobiline Gels**
(0.5 mm gel layers on film supports)

Acrylamide derivatives: Immobiline®
$\text{CH}_2=\text{CH-CO-NH-R}$,
R contains a *carboxylic*
or a *tertiary amino* group
Two-dimensional Electrophoresis

For separating very complex protein mixtures

The most important separation technique in Proteomics
2-D electrophoresis with IPG strips

Görg A, Postel W, Günther S.
Review. The current state of two-dimensional electrophoresis with immobilized pH gradients.
Sample preparation
Avoiding protein losses
Protein solubilization

Urea (8-9.5 M), or 7 M urea / 2 M thiourea

Detergent (CHAPS, …)

Reductant (DTT, 2-mercaptoethanol)

Carrier ampholytes (0.8 % IPG buffer)

Sonication can help solubilization

Sample can be heated only prior to addition of urea
SDS in 2-D sample preparation

- Best solubilizing agent known but not compatible with IEF unless diluted into an excess of another detergent

- Inhibits proteolysis
- Useful with lipid-rich samples

- Limited to low sample load
- Can disturb first dimension

Dilute out SDS with 9M urea (or 2M thiourea / 7Murea ) plus 4 % CHAPS to 0.1 % SDS:

CHAPS : SDS ratio >8 :1
Sample preparation is very important

- Cleanup from contaminants
- Dissolve complexes completely
  - Protein-protein
  - Protein-polysaccharides
- Stop protein activities (protease, phosphatases)

Precipitation is the most efficient.....
Protein precipitation

Clean-up from lipids, nucleic acids, polysaccharides, polyphenols, salts

Concentration of proteins

Irreversible inhibition of proteases

Prevention and dissolution of complexes

For DIGE: removal of endogeneous peptides
Cancer cell extract

Crude extract

After cleanup

Rat tongue tissue (26ug)

pH 3-10, 13cm

Without treated with clean up kit.

Bromophenol dye doesn’t move during 1st Dimension IEF.

Contributed by Janice Cheung, GE-Healthcare Malaysia

24.01.2005
Rat tongue tissue (26ug)

-pH 3-10, 13cm

-Sample treated with Clean Up Kit.

-Gel stained with Plus One Silver Staining kit

Contributed by Janice Cheung, GE-Healthcare Malaysia

24.01.2005
Resolubilization possibilities

- Pellet must not become completely dry!
- Pipette repeatedly lysis solution over the pellet (do not vortex!)
- Rehydration can take several hours (or over night) @ RT (do not vortex!)
- Carefully sonicate (avoid heating of sample)
- Use PlusOne Molecular grinding kit
- Freeze pellet with lysis solution at 20 °C
- Use SDS solution (2 % SDS, hot) and then dilute with 9 M urea / 4 % CHAPS
New flexible tools for sample preparation using immunospecific enrichment techniques
The Trap platform

Scale down from mg to μg
Enrichment of proteins with immunospecific affinity
Protein Enrichment products

- NHS HP SpinTrap™
- Streptavidin HP SpinTrap
- Streptavidin HP MultiTrap™
- Protein A HP SpinTrap
- Protein A HP MultiTrap
- Protein G HP SpinTrap
- Protein G HP MultiTrap
Protein Enrichment products

**MultiTrap™ and SpinTrap™:**
- flexible, prepacked 96-well filter plate and spin column for micro preparations

**Prepacked gives:**
- ease of use
- high reproducibility performance run for run

**Proven high throughput protocols for:**
- sample enrichment with immunospecific affinity tools
- optimized for different analyses, e.g. electrophoresis and LC-MS
Make your own protein enrichment tool
– couple your antibody to enrich for your protein of interest

Pre-activated NHS HP SpinTrap™ for covalent coupling of proteins or antibodies through primary amines

Streptavidin HP SpinTrap and MultiTrap™ for coupling of biotinylated molecules e.g. antibodies or proteins.

Protein A HP SpinTrap and MultiTrap or Protein G HP SpinTrap and MultiTrap for

- High specificity of the ligands for coupling
- Enrichment of the target proteins with immunocapture methods
- Simple protocols with elution procedures both for electrophoresis and LC-MS analysis
Enrichment of plasminogen from human plasma

Sample loading volumes

<table>
<thead>
<tr>
<th></th>
<th>100 µl</th>
<th>50 µl</th>
<th>20 µl</th>
<th>100 µl control</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT</td>
<td>W1</td>
<td>W3W5E1</td>
<td>E2</td>
<td>E3</td>
</tr>
<tr>
<td>FT</td>
<td>W1W3W5E1</td>
<td>E2 E3</td>
<td>FTW1W3W5E1</td>
<td>E2 E3</td>
</tr>
<tr>
<td>FT</td>
<td>W1W3W5E1</td>
<td>E2 E3</td>
<td>FTW1W3W5E1</td>
<td>E2 E3</td>
</tr>
</tbody>
</table>

Column: NHS HP SpinTrap™ columns coupled with monoclonal mouse anti-plasminogen

Sample: Human plasma

Sample volumes: 100, 50 and 20 µl

Lanes
- FT: flowthrough
- W1-5: five wash steps
- E1-E3: three elution steps
Repeatability study: enrichment of a model protein with Streptavidin SpinTrap

- 12 Streptavidin HP SpinTrap™ columns
- Antibody coupled- biotinylated polyclonal rabbit anti-human albumin
- Sample - 200 μl *E.coli* protein extract, 5 mg/ml containing 7.5 μg/ml spiked HSA
- Recovery varied 6% RSD between the 12 columns
Classic protocol with Protein A HP SpinTrap: enrichment of a model protein

Column Protein A HP SpinTrap™

[Graph showing theoretical vs measured ratios for differential analysis (E1) and (E2)]

Calculated vs Measured values for differential analysis (E1)

Calculated vs Measured values for differential analysis (E2)
Isoelectric Focusing

Separation according to the isoelectric points
Running IPG strips
Principle of 2-D electrophoresis

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

2. Second dimension: SDS electrophoresis separation according to the molecular weight

2-D electrophoresis resolves a few thousand protein spots.
Basic gradients: Cup-loading on pre-rehydrated IPG strips

Basic gradients: E. coli protein extract in IPG pH 6-11
Sample loading on pre-rehydrated IPG strip

Cup-loading

Multiphor: max 100 µl

Paperbridge-loading

max 500 µl
The new IPGphor III and Manifold
IPGphor run recording graphs

- Good run
- Bad run
Some hints for running basic IPG strips

Use correct IPG buffer

Minimize rehydration time:
- Start rehydration as late in the day as possible
- Start separation as early as possible next day

Cup-loading at anodal side

Minimize separation time
- Minimum volthours
- High voltage and short time
**Streaking in basic pH region**

DTT and DTE become deprotonated;

Cysteins are not protected, cause:
- Backfolding
- Aggregation of subunits
- Reaction with urea

resulting in horizontal streaks.

Alternative reductants like TBP, TCEP, THPP disturb separation

Alkylation prior to IEF causes artifactual spots
“DeStreak” approach

Rehydrate IPG-strips in solution containing *Hydroxyethyl disulfide* (DeStreak™) instead of DTT.

Apply the samples to anodic end of IPG-strips. DeStreak™ converts cysteiny1 groups in the samples to “mixed disulfides”.

Micropreparative focusing with DeStreak™

Immobiline DryStrip pH 6-9, 18 cm

Sample:
2 mg mouse liver proteins
in 500 µl solution with paper bridge loading
IPG-Dalt of Mouse Liver Proteins

DTT

DeStreak™

from Prof. Angelika Görg, Technical University Munich
Basic gels: pH 7-11 NL

New proprietary Immobiline reagent:
Increased buffering capacity
Stabilizes protein patterns

Mouse liver extracts
SDS polyacrylamide gel electrophoresis

2nd Dimension:
SDS PAGE chambers for Proteomics

- 25 x 19 cm Ettan™ DALT twelve
- 25 x 19 cm Ettan™ DALT six
- 14 x 14 cm SE 600 Ruby
Casting SDS gels – important points

HQ reagents: PlusOne labelled chemicals are a benchmark

TEMED not too old

Freshly made APS

Precool monomer solution mix (containing the TEMED)

Degas the solution for >15 minutes

Add APS short before use

Pour solutions quickly in one go
Casting SDS gels and overlay gel edges

Pre-cool monomer solutions in refrigerator to delay onset of polymerization
Optimized running conditions

First phase: 0.5 W (10 mA) per gel for 1 hr

Second phase: 17 W per gel for 4 hours 30 min

or

1 W per gel overnight
Wide pH gradient: 3 – 11 NL

Mouse liver extract
IPG 24 cm, pH 3-11

From A. Görg
Proteomics Department
Technische Universität
Munich
Protein Detection
Staining – Scanning – Image analysis
“The red blobs are your red blood cells. The white blobs are your white blood cells. The brown blobs are coffee. We need to talk.”
## Protein Detection Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity Limit</th>
<th>Quantitative</th>
<th>Living cells</th>
<th>Linear Dynamic Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coomassie Blue staining</td>
<td>20 ng</td>
<td>+++</td>
<td>no</td>
<td>7</td>
</tr>
<tr>
<td>Negative staining</td>
<td>15 ng</td>
<td>+</td>
<td>no</td>
<td>3</td>
</tr>
<tr>
<td>Silver staining</td>
<td>200 pg</td>
<td>++</td>
<td>no</td>
<td>3</td>
</tr>
<tr>
<td>Fluorescent staining</td>
<td>400 pg</td>
<td>++++</td>
<td>no</td>
<td>$10^4$</td>
</tr>
<tr>
<td>Fluorescent labelling</td>
<td>a few pg</td>
<td>++++++</td>
<td>no</td>
<td>$10^4$</td>
</tr>
<tr>
<td><strong>Radioactive labelling:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray film</td>
<td>1 pg</td>
<td>+++</td>
<td>yes</td>
<td>20</td>
</tr>
<tr>
<td>Phosphor-Imager plates</td>
<td>0.2 pg</td>
<td>++++</td>
<td>yes</td>
<td>$10^5$</td>
</tr>
<tr>
<td>Stable isotope labelling</td>
<td>&lt; 1 pg</td>
<td>+++</td>
<td>yes</td>
<td>?</td>
</tr>
<tr>
<td>(with MS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fluorescent staining and labeling

- General protein stains:
  - Nile red
  - Sypro® Orange, Red, Rose Plus
  - Sypro® Ruby
  - Deep Purple™

- General protein labels:
  - CyDyes: Cy2, Cy3, and Cy5 (for lysine and cystein)

- Specific stains for glyco- and phosphoproteins
Deep Purple Staining – fluorescent but not Heavy Metal
Deep Purple™ Total Protein Stain

Naturally occurring fluorophore free from heavy metals:

- Easy to dispose of and environmentally friendly
- Reversible binding: highly compatible with mass spectrometry

Deep Purple™ - applications

Deep Purple is suitable for:

• 1 & 2D SDS PAGE Gels
• Native gels
• Electro-blotting on Nitrocellulose or PVDF
• IEF gels
Trouble shooting

2-D electrophoresis
Carbamamylation trains

Impurities in urea.
Use better urea or
Remove isocyanate with
Amberlite IRN-150L
Effect of salt

*E. coli* extract pH 4-7

no salt

30 mM NaCl
Salt in the gel

Cell washing with PBS
IPG 3-10
Poor chemical quality

TEMED old
Equilibration with SDS buffer

15 min equilibration with DTT

15 min equilibration with DTT plus
15 min equilibration with IAA
Equilibration time

15 min DTT / 15 min IAA

20 min DTT / 30 min IAA
2D Electrophoresis


2-D Electrophoresis Discussion Group
http://amersham.zeroforum.com

Angelika Görg’s manual on her Website:
http://www.weihenstephan.de/blm/deg
Thank you

reiner.westermeier@ge.com
Amersham Biosciences AB, a General Electric Company, going to market as GE Healthcare.

GE Healthcare
Amersham Biosciences AB
Björkgatan 30
SE-751 84 Uppsala, Sweden.
Sweden

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2-D Fluorescence Difference Gel Electrophoresis (Ettan DIGE) technology is covered by US Patent Numbers 6,043,025, 6,127,134, 6,426,190 and foreign equivalents and exclusively licensed from Carnegie Mellon University.

Amersham Biosciences has patent applications pending relating to its DeCyder software technology, including European patent application number EP1,234,280.

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