

Proteomics

Application in NIPD – (and a bit of transcriptomics as well)

New and developing technologies for genetics diagnosis NGRL

July 7th 2008

Neil Avent



Biomarker discovery

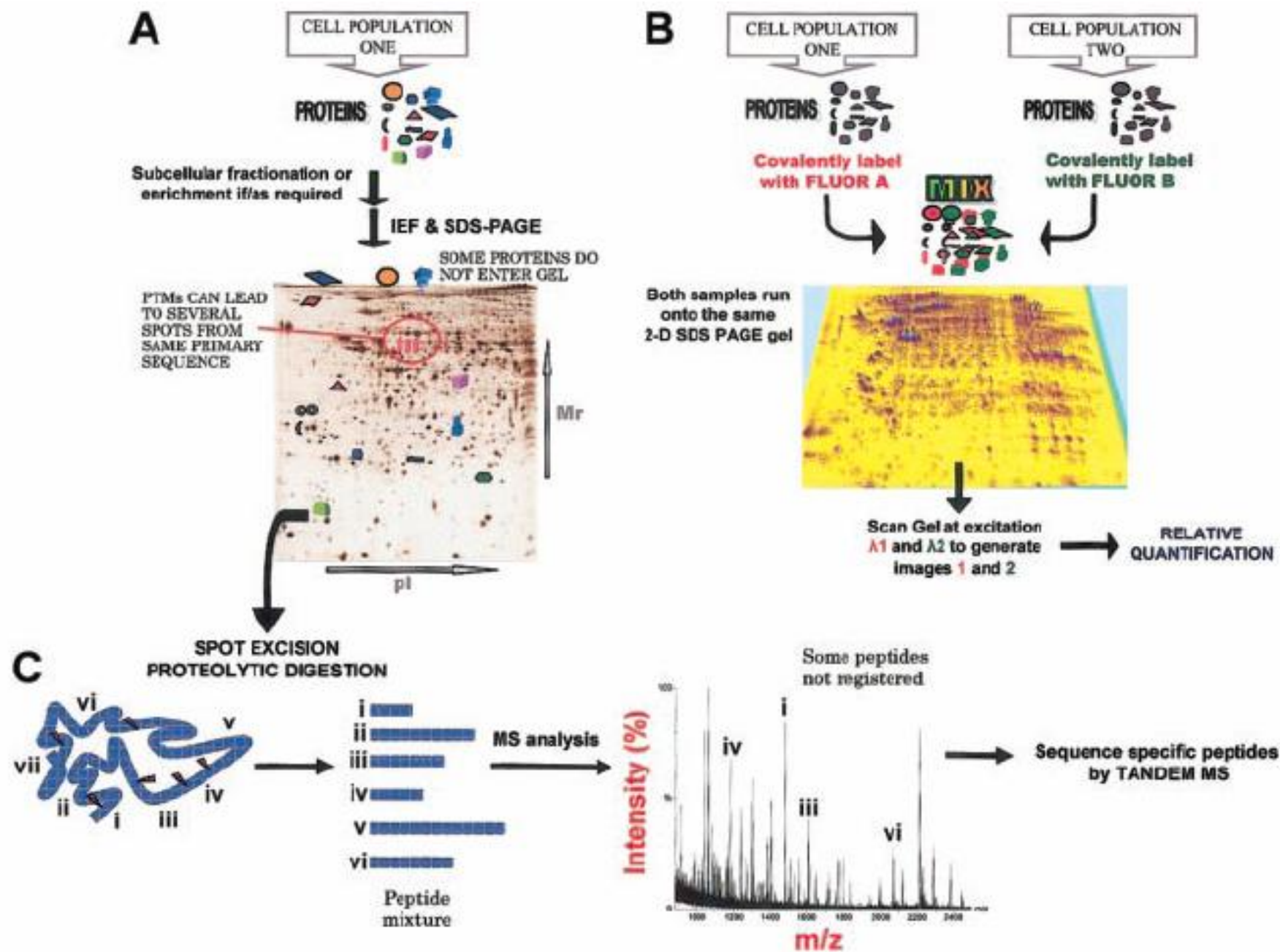
- Major focus of Post genome sequence activity
- Protein markers of disease for diagnostics
- Plasma proteins major consideration

WP3B SAFE Network of excellence activity – Biomarker discovery for :

- Aneuploidy
- Pre-term labour
- Preeclampsia

Proteomics work flow

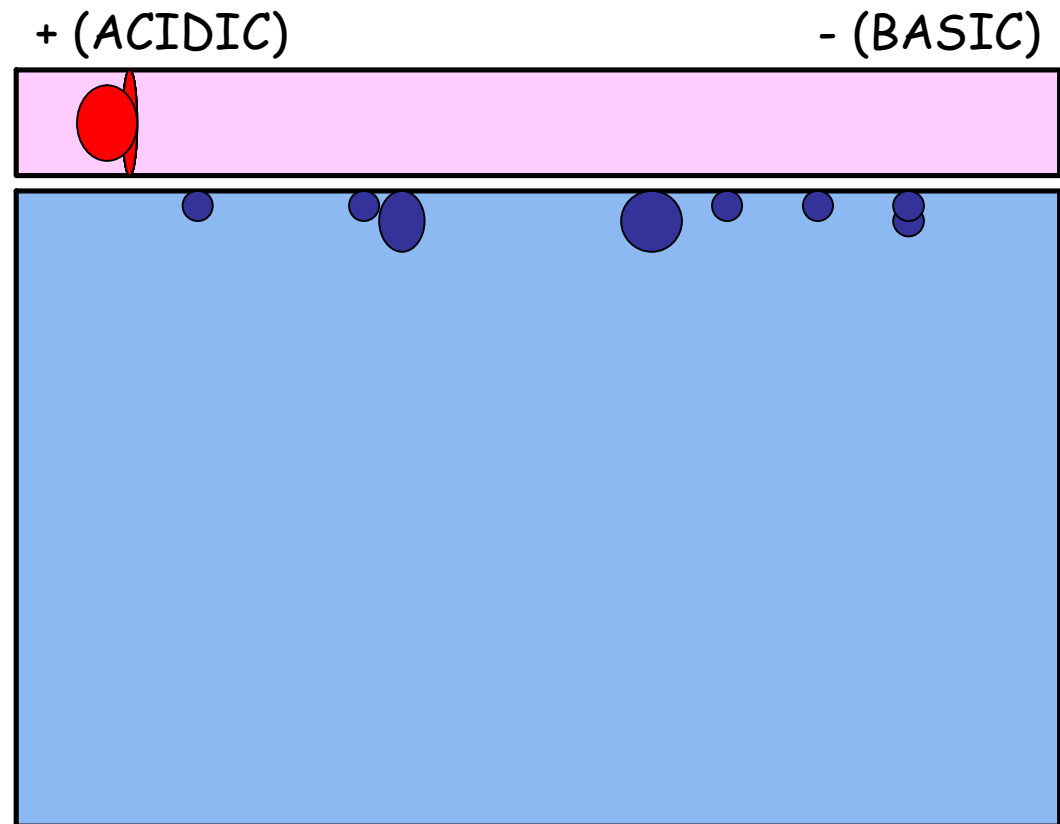
- Protein isolation
- Protein separation
- Protein Identification – staining
- Protein Identification – Excision and trypsin digestion
- Protein Identification – Mass spectrometry



Most Proteomic Investigations involve
Two-dimension gel electrophoresis
(2D-GE)



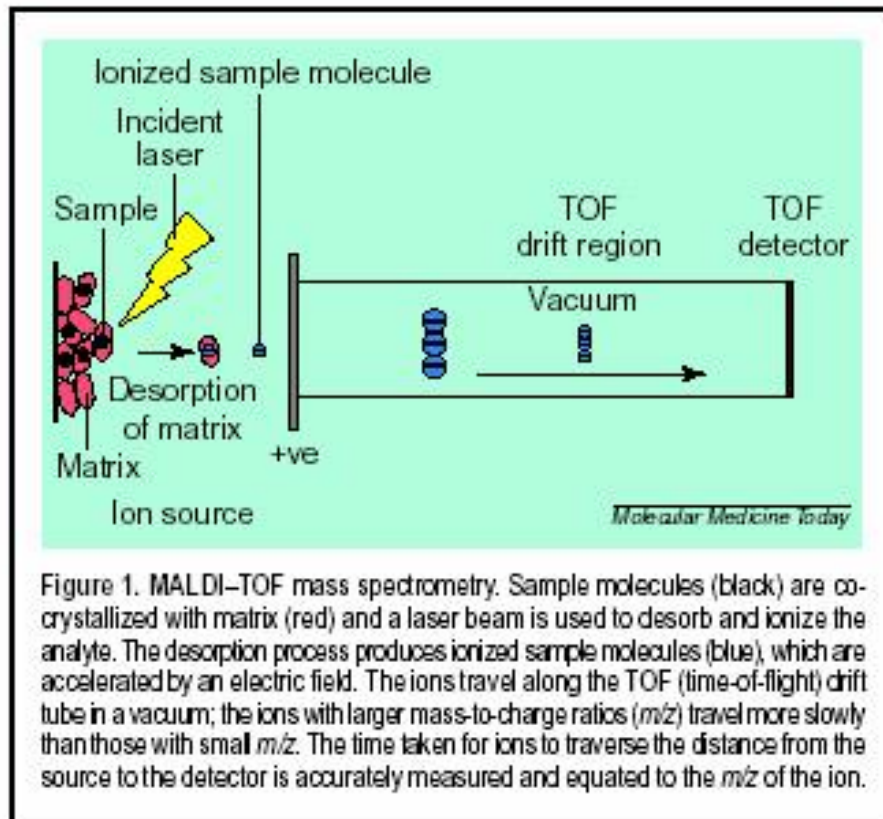
FIRST DIMENSION SEPARATION
ISOELECTRIC FOCUSSING



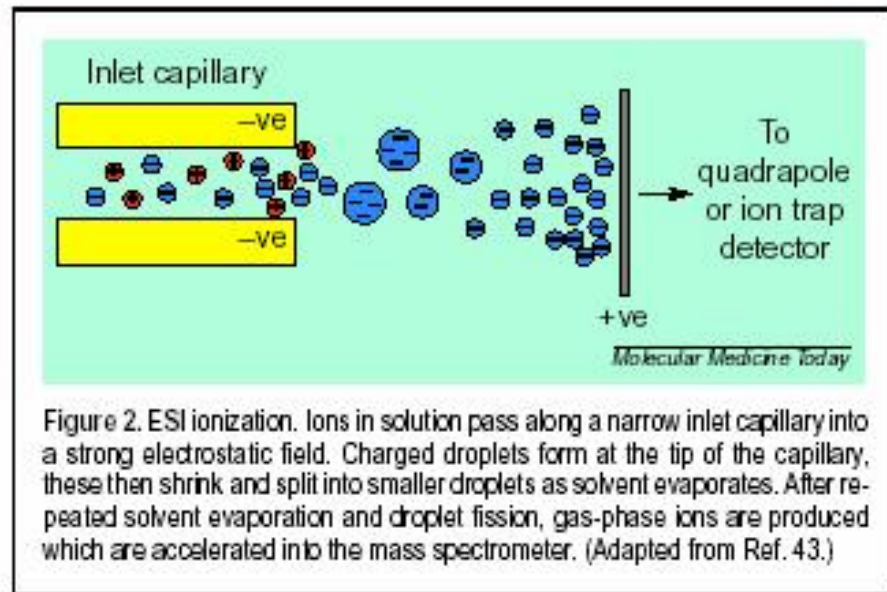
SECOND DIMENSION SEPARATION
SDS-PAGE MOLECULAR WEIGHT

Proteomics work flow

- Protein isolation
- Protein separation
- Protein Identification – staining
- Protein Identification – Excision and trypsin digestion
- Protein Identification – Mass spectrometry



ESI-MS



The resulting fragmentation pattern can yield structural information about the parent ion. This leads to an increase in the specificity of detection as two parent ions with the same mass (for example, two oligonucleotides with the same base composition but different sequence) produce different daughter ions following fragmentation.

Mass spectrometry of peptide species following 2D-GE

Review see : Cristea IM (2004) Proteomics techniques and their application to Hematology. *Blood* 103: 3624-3634.

Better Down plasma biomarkers ?

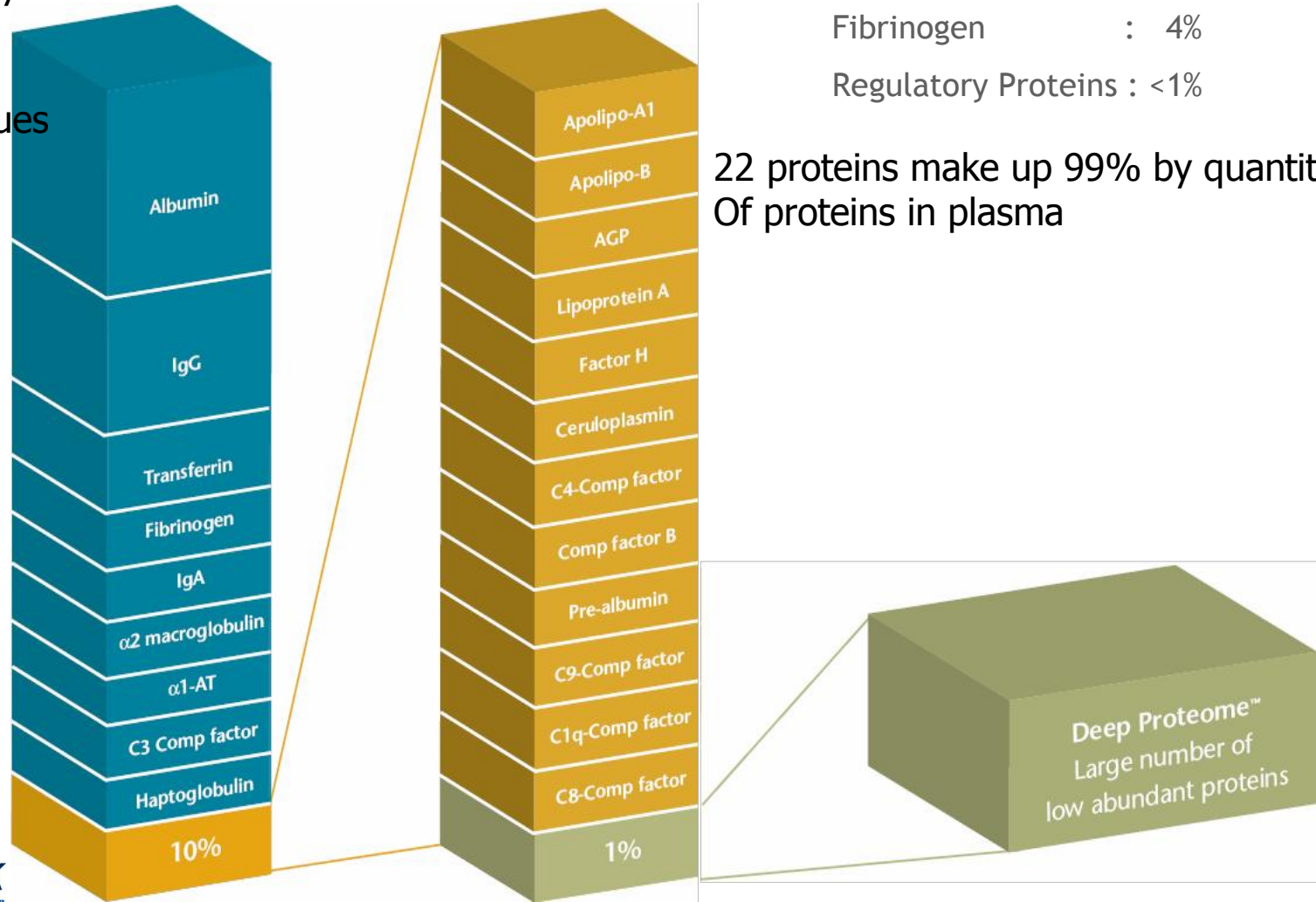
- New generation screening tests
- Replace those currently used? AFP, β -HCG, PAPP-A, Inhibin-A
- 99%+ accurate?
- Would genetic diagnosis then be necessary?
- Almost certainly for confirmation

Plasma Proteomics: High complexity

Plasma most complex proteome-
 Coagulation cascade
 Complement
 Humeral immune system
 Cytokines

Plus bathes all tissues

Albumins	: 60%
Globulins	: 35%
Fibrinogen	: 4%
Regulatory Proteins	: <1%



Low abundance proteins make up less than 1% of protein content.



University of the
West of England

CRIB

Centre for Research in Biomedicine

SAFE Plasma Proteomics (and transcriptomics)



Dr Lyn Chitty
Dr Wendy Heywood
Dr Kevin Mills



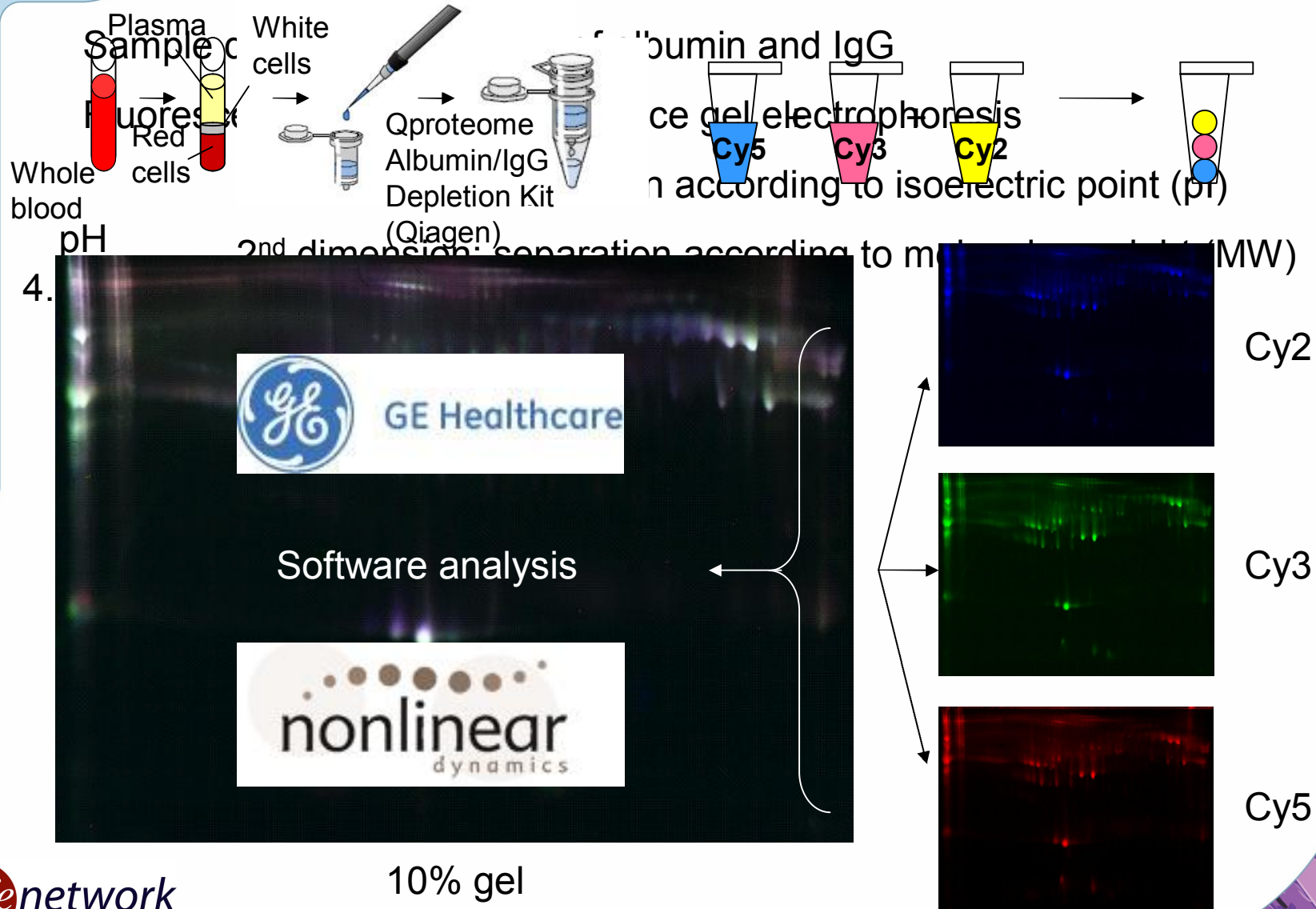
University of the
West of England

Prof Neil Avent
Dr Tracey Madgett
Dr Debbie Maddocks



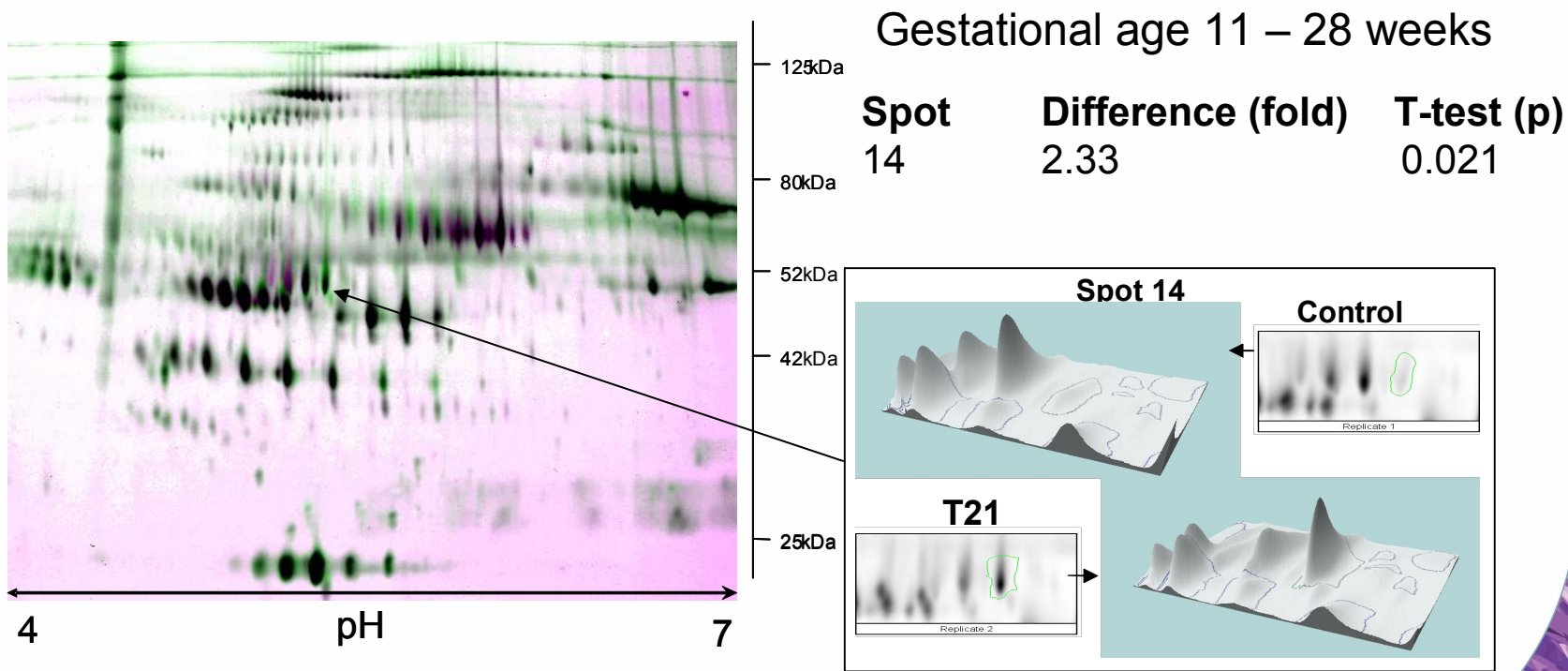
Prof Peter Soothill
Kin Choi

Sample Depletion and DIGE protocol



T21 samples:

- 6 pairs of age- and sex- matched control and T21 samples
- Study performed at UCL and UWE



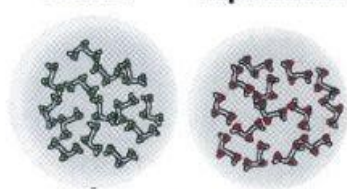
T21 samples: pH ranges

- *safe* UCL – 1st trimester (<14 weeks)
- *safe* UWE – 2nd trimester (14-32 weeks)
- *safe* pH 4.5 – 5.5 (2nd trimester ongoing)
- *safe* pH 5.3 – 6.5 (complete)
- *safe* pH 6.0 – 9.0 (complete)
- *safe* In total, 11 to 14 pairs of control/T21 maternal plasma samples per pH range and trimester
- *safe* Lots of candidate biomarkers identified!

Normal **Trisomy 21**

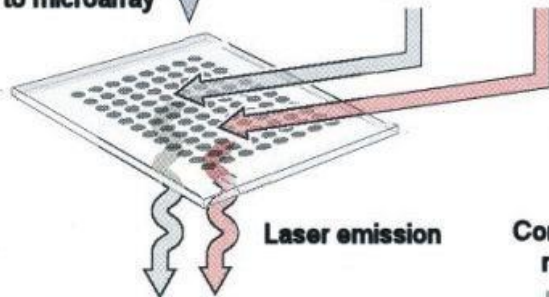
Make cDNA reverse transcript
Label cDNAs with fluorescent dyes

Control Experimental



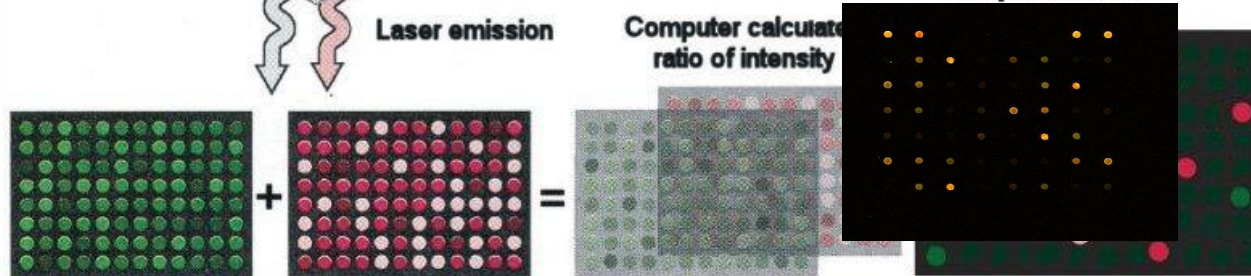
Hybridization
to microarray

Laser excitation
at dye-specific Hz

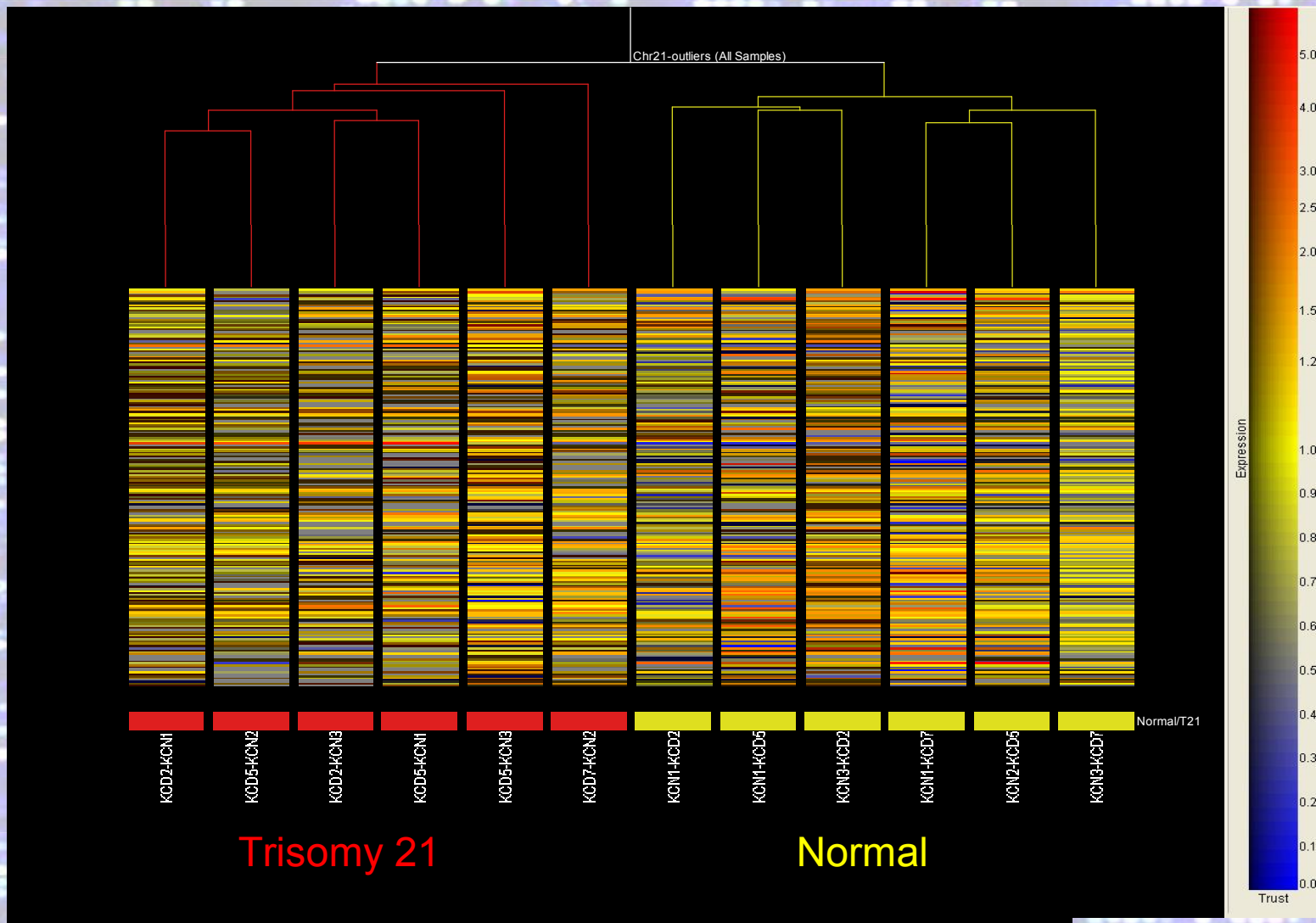


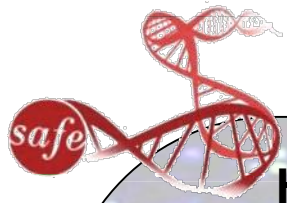
Principle of cDNA microarray
assay for gene expression

Red = "up-regulation"
Green = "down-regulation"
**Black = constitutive
expression**



Hierarchical Clustering - Gene Condition tree





Hierarchical clustering – Gene condition tree

Gene tree
Spearman corr.

Clear differences
between norm v
T21 at genome
level

Distance

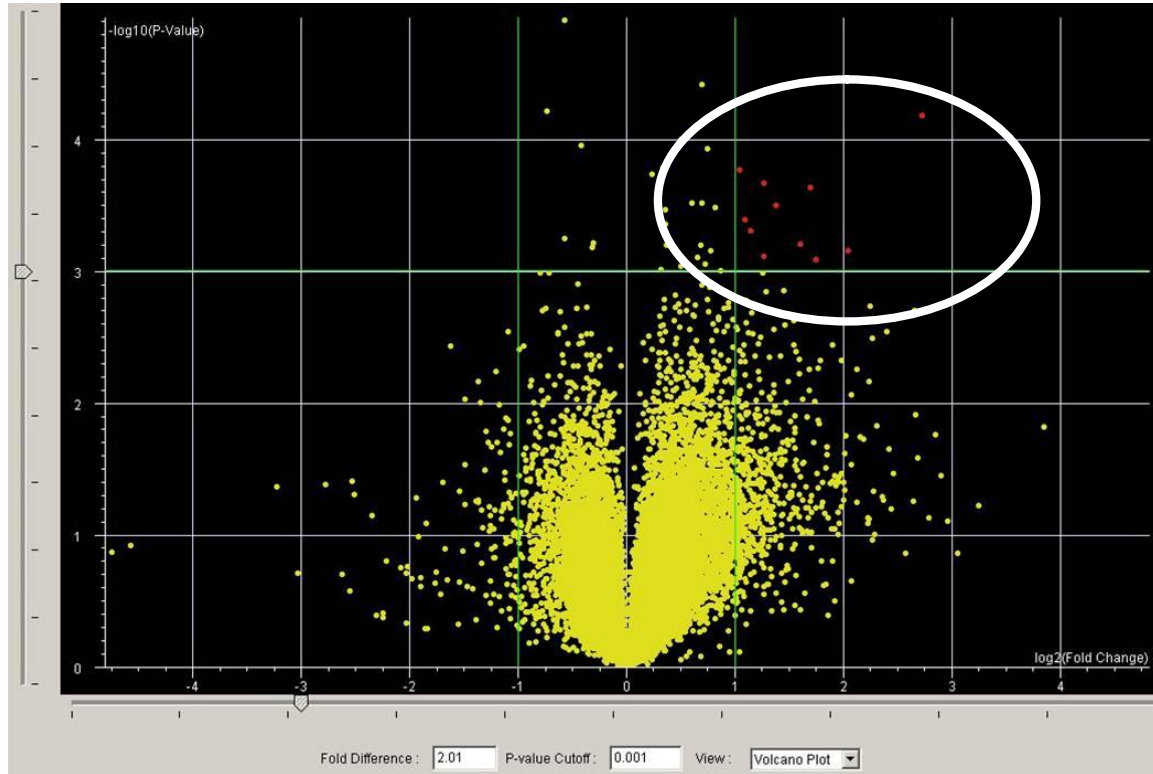
T21- 0.82

Norm – 0.85



Trisomy 21

Normal



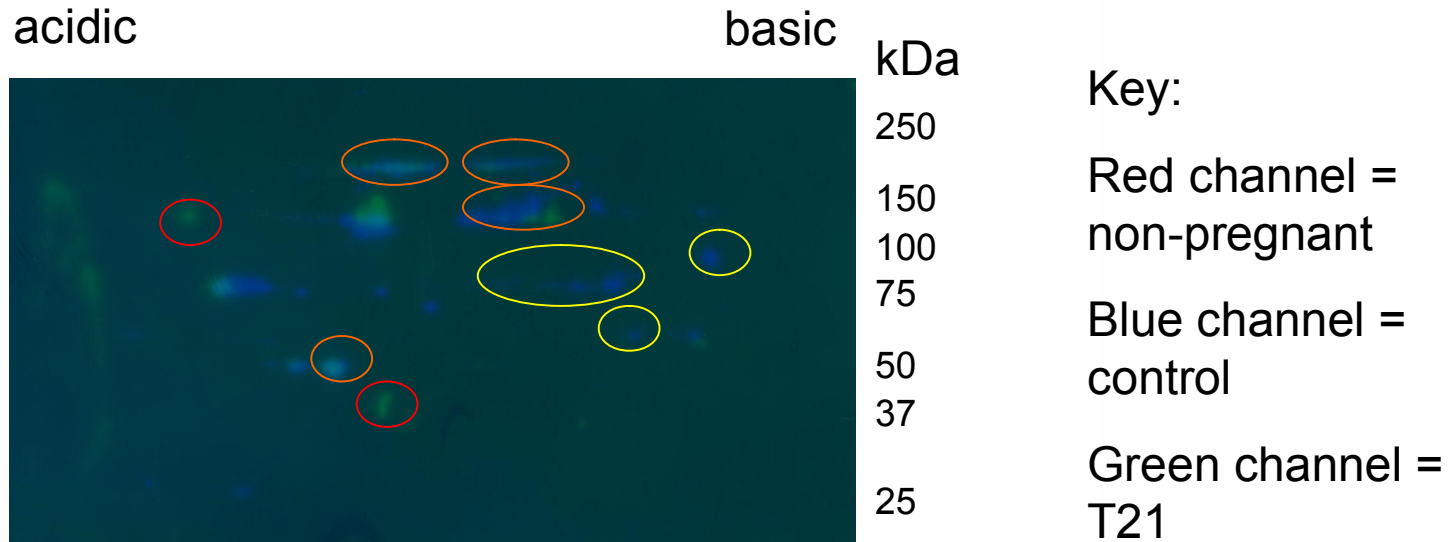
Placental Transcriptomics – Affymetrix U0133 arrays

11 genes with $p > 0.0001$

3 1st trimester placentas Normal; 3 1st trimester placentas, Downs

Results: 2D Western Blotting

Polyclonal antibody to protein of interest – possibly protein of interest is bound to other proteins



present in T21, non-pregnant, control and T21

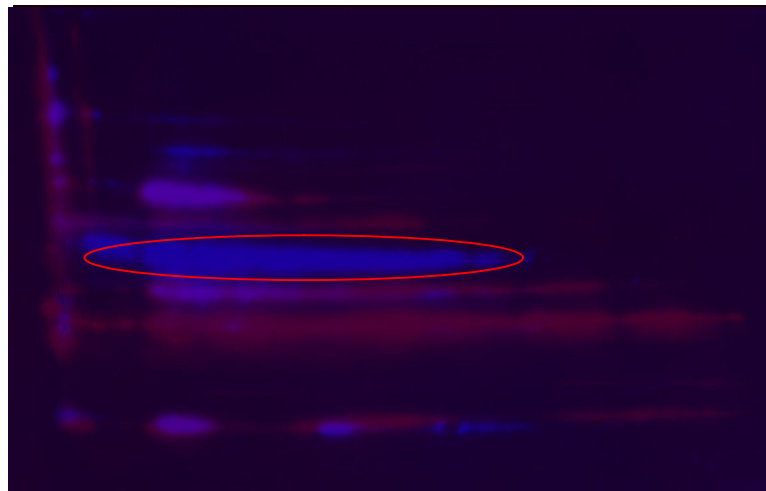
Western blot films have been scanned and then converted to single channel images (RGB) using Adobe Photoshop

Results: 2D Western Blotting

Polyclonal antibody to protein of interest – possibly protein of interest is bound to other proteins

acidic

basic



Key:

Red channel =
non-pregnant

Blue channel =
control

Green channel =
T21

present in T21 and not in control

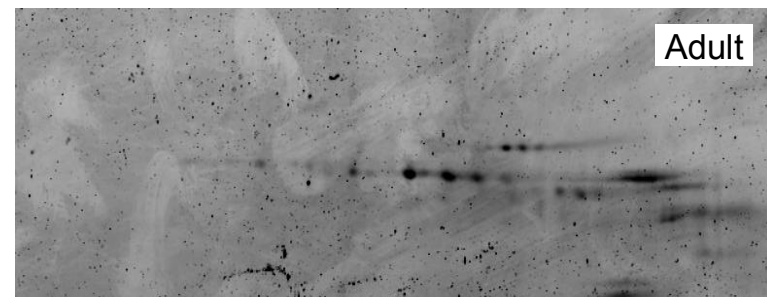
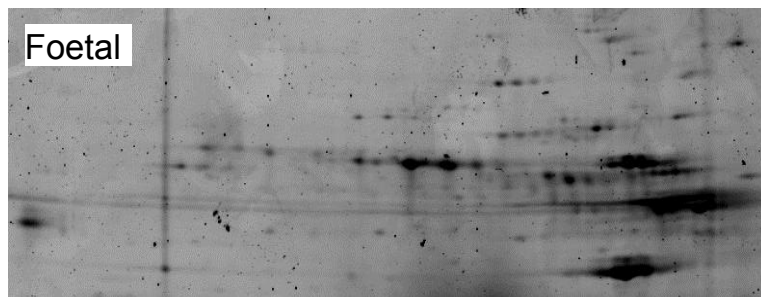
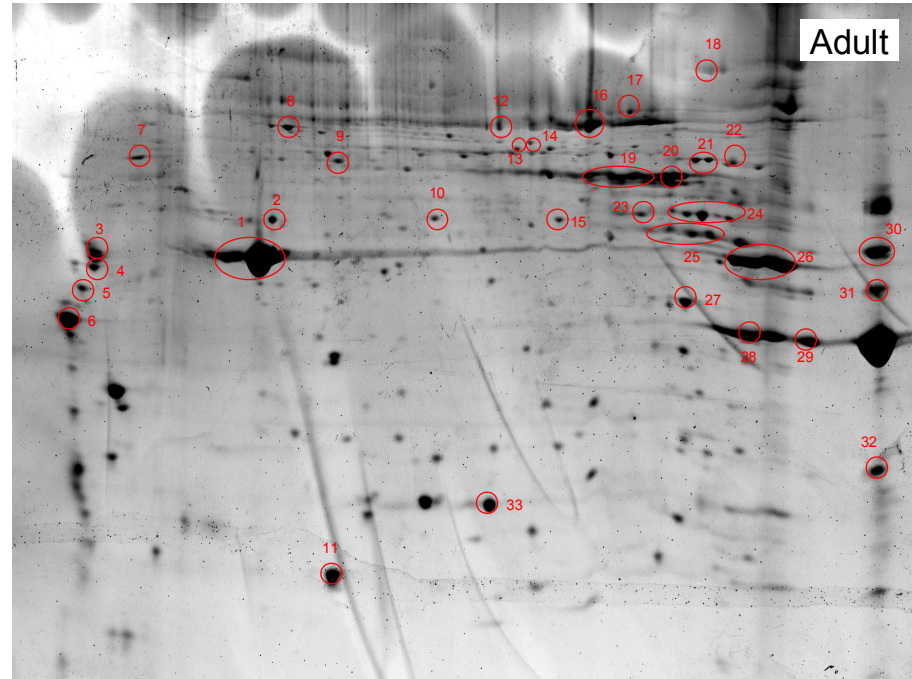
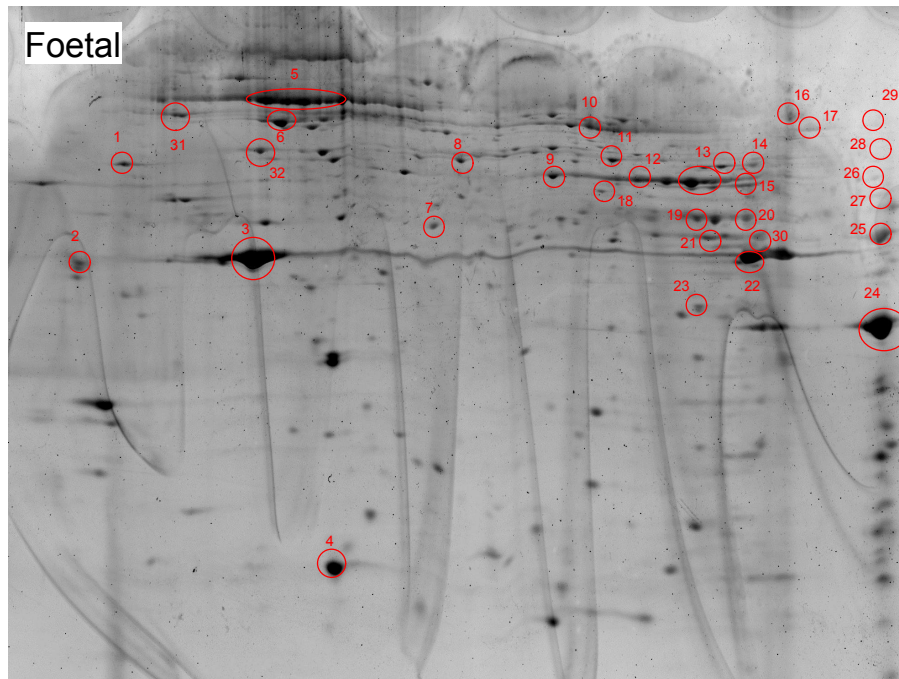
Western blot films have been scanned and then converted to single channel images (RGB) using Adobe Photoshop

Proteomics of fetal erythroblasts

- Funded by National Blood service and SAFER Strep FP6 project (SAFE spin-out project)
- Identification of fetal-erythroblast specific markers
- Comparison of cordocentesis and adult erythroid cells
- Provide better targets for fetal cell isolation

Protein Mapping & Marker Discovery

2D Electrophoresis (2DE): Erythroid & Erythroblast membranes

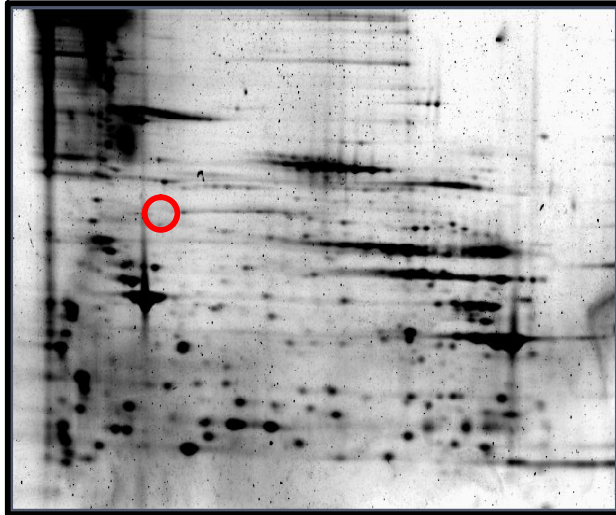


Analysed by PDQuest computer software – Many up and down regulated foetal proteins – 21 patented (Submitted, August 2007)

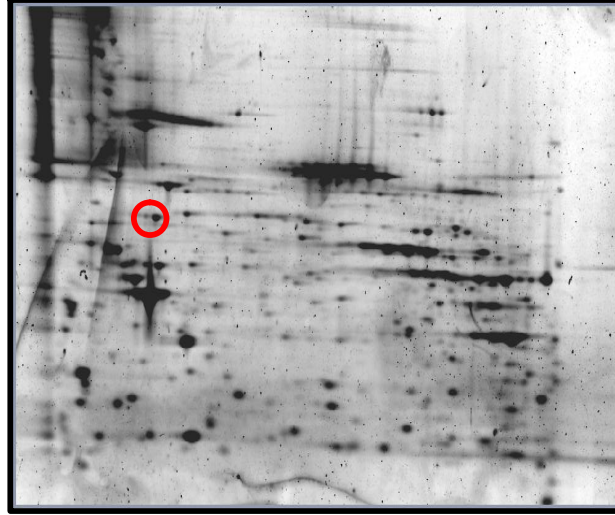
Marker Specificity – Hsp60

2D Electrophoresis (2DE) and Fluorescent 2DE of erythroid membranes

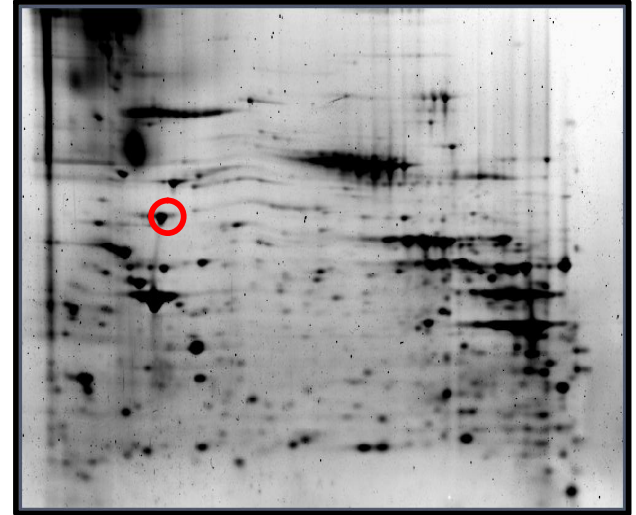
Adult



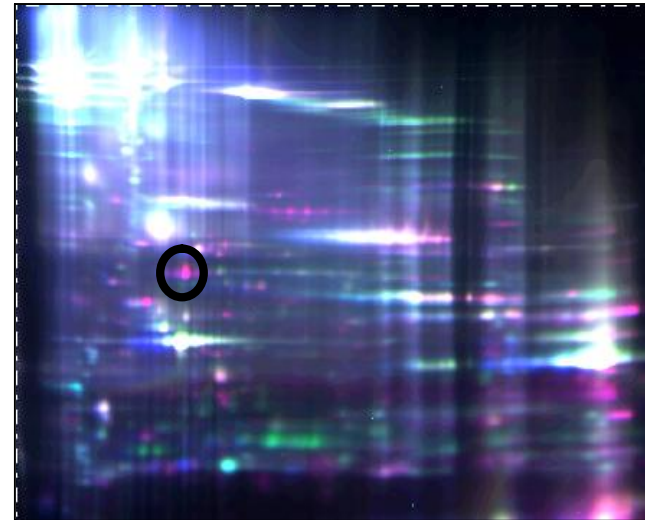
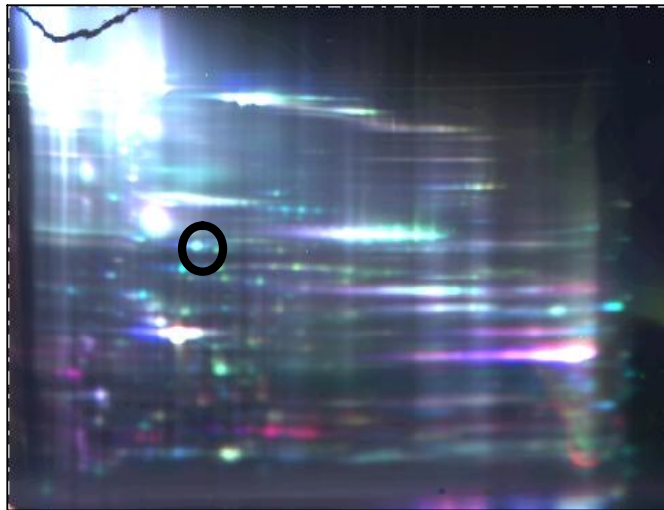
Cordocentesis (26 wks)



Cordocentesis (22 wks)



Foetal specific:
Blue



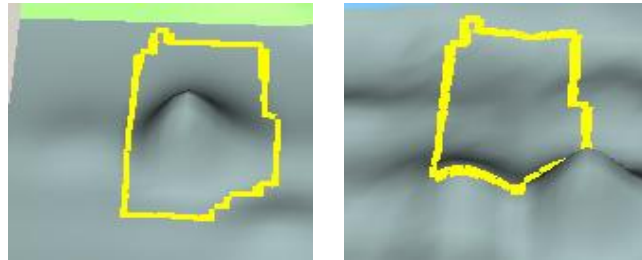
Foetal specific:
Pink

Foetal Vs Adult

Marker specificity – Hsp60

2DE software analysis, western blotting and immunofluorescence

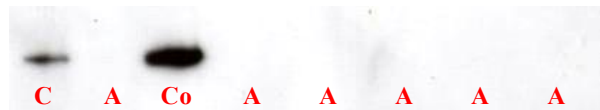
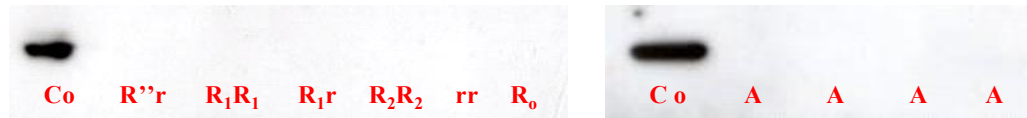
DeCyder Software analysis of 2DE Maps



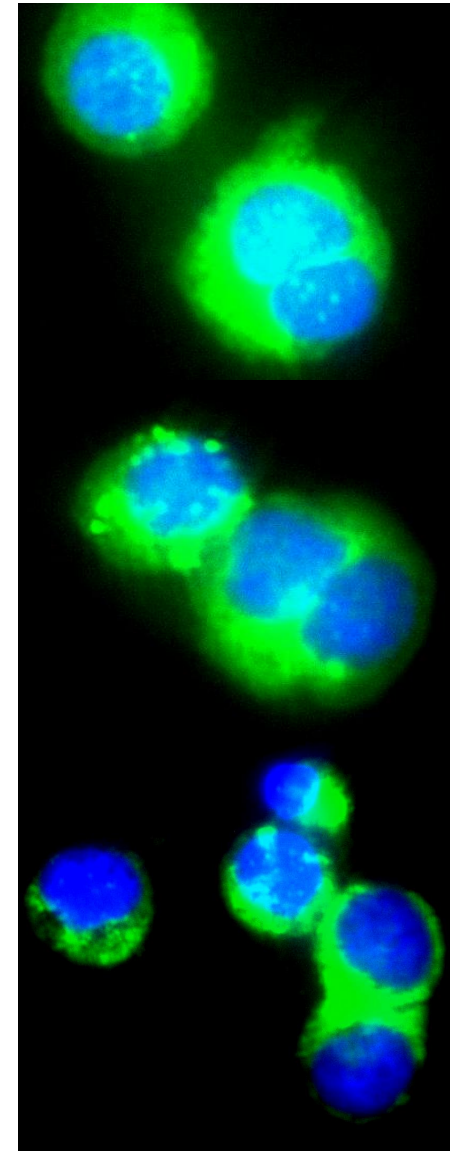
Foetal

Adult

Western blots



G3PDH



Analysis of Hsp60 cellular localisation in foetal cultured erythroblasts

Hsp60: Green (FITC)

Nucleus: Blue

(Alexa 488)

Fetal erythroblast markers

- New generation fetal erythroid markers determined, and patent submitted
- Some expressed on other peripheral blood mononuclear cells
- Requires parallel development of microfluidics (SAFER project objective)
- Requires further funding (thanks Wellcome trust!)

Conclusions

Plasma proteomics- huge logistic exercise to identify candidate biomarkers (Placental origins)

Transcriptomics of target tissue (Placenta) more productive approach?

New candidate biomarkers being validated

New markers of fetal erythroblasts may improve fetal cell isolation techniques?