‘OMICS – APPLICATION IN FOOD AND NUTRITION

M. John Dennis

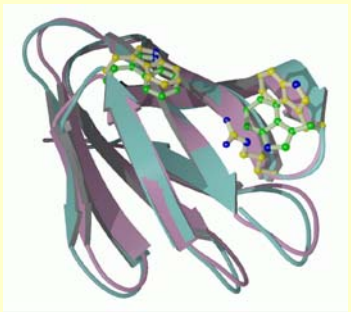
Central Science Laboratory, UK

Genomics

DNA

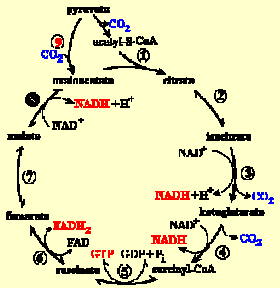
Transcriptomics

RNA



Proteomics

Proteins



Metabolomics

Metabolites

‘OMICS?

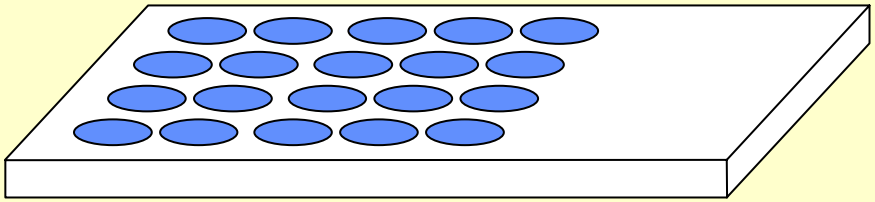
- Genomics, Proteomics, Metabolomics (Metabonomics)
- Functionally linked
- Comparative technologies devoted to identifying significant differences
- High throughput methods
- Applicable to population studies or low frequency events

Genomics applications

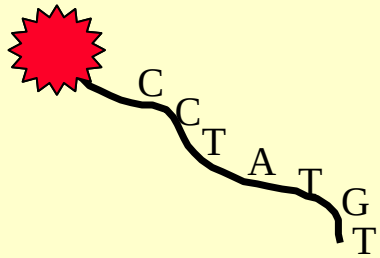
- Concept of Microarrays invented in 1989
- Widely used in the field of human and animal genomics; Affymetrix leader
- Microarrays enable detection of large numbers of DNA (and RNA) signatures
- Applications to species identification
- Differential expression
- Single Nucleotide Polymorphisms (SNP’s)

Microarray detection

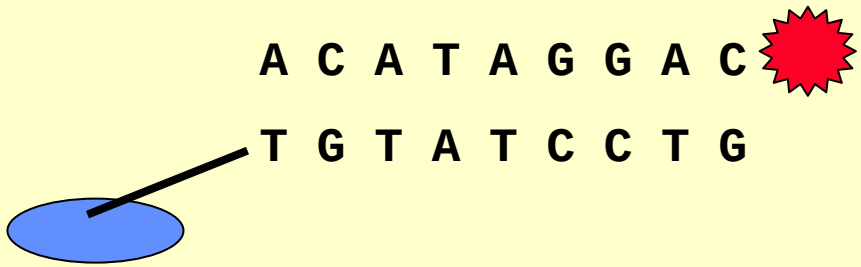
DNA capture probes held on solid support



Fluorescent labelling of sample nucleic acid

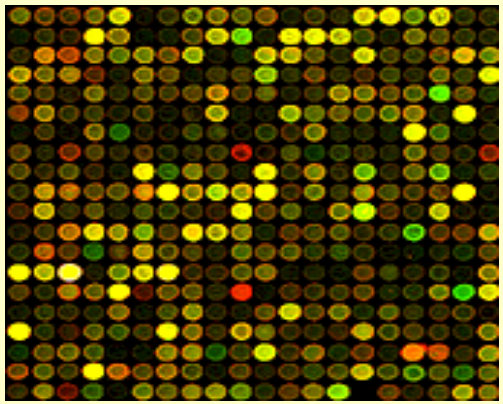


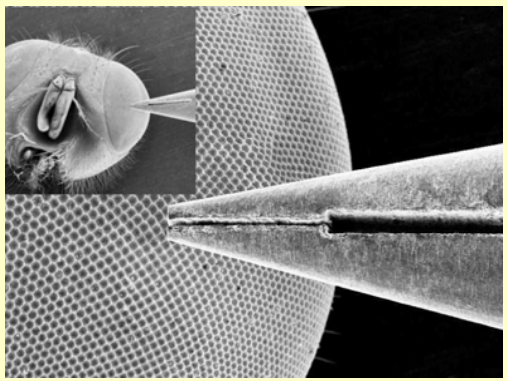
Hybridisation to the array



What is a microarray?

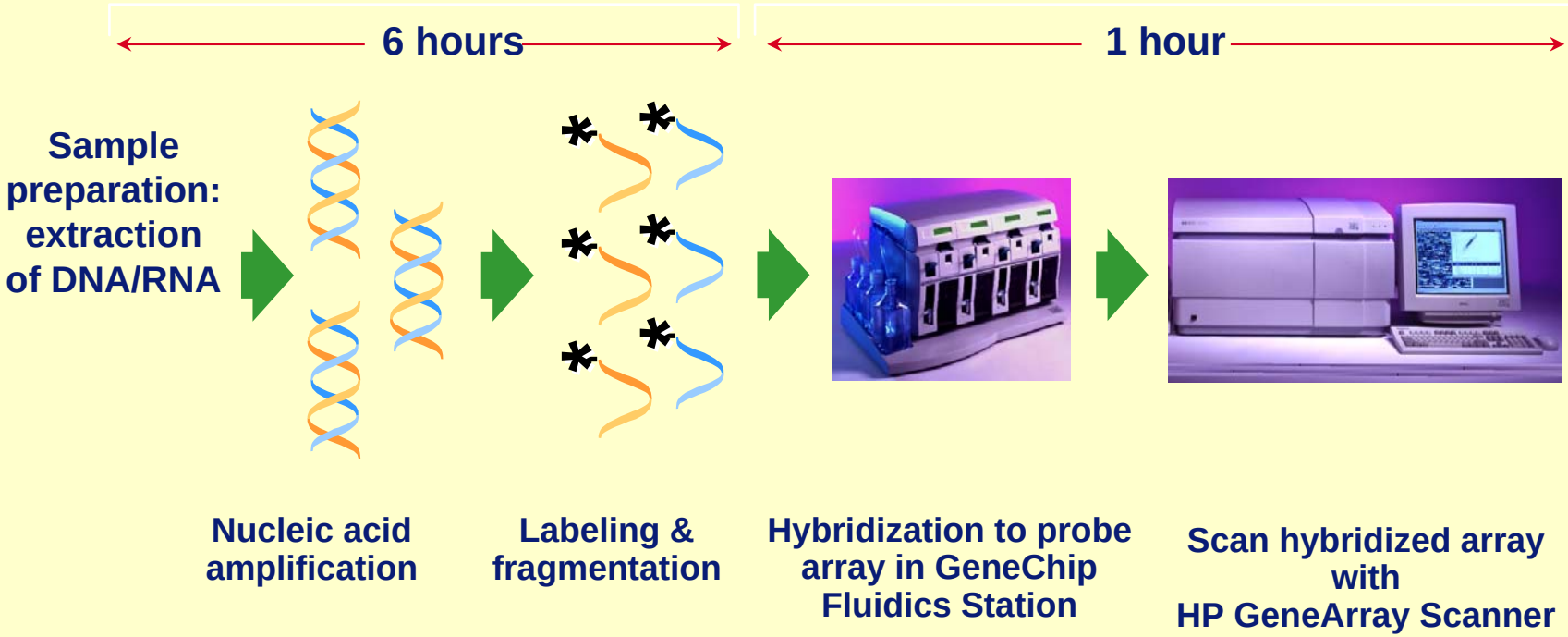
- ‘Ultimate’ generic technology
  - One technology for all targets
- Can test > 30,000 DNA targets at once
- Term can be applied to protein or DNA based analyses



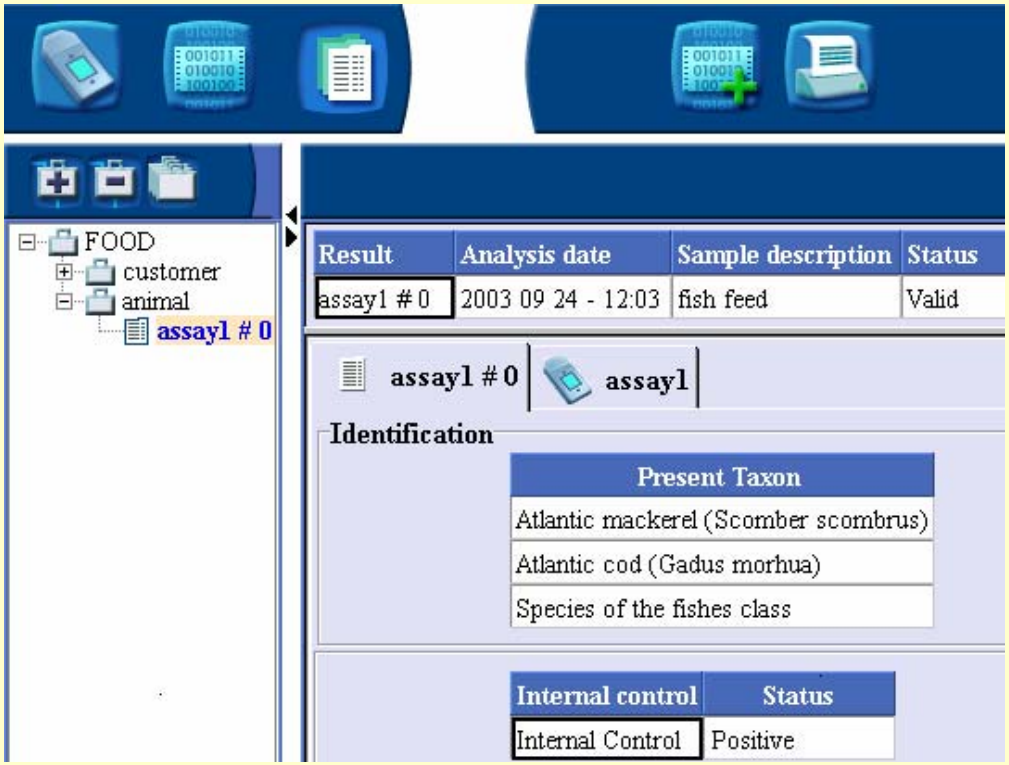


cytochrome B Microarray format

GeneChip Probe Array : Process Overview



bioMérieux FoodExpert-ID®
Food and Animal Feed Identity Card



- ✓**1 cm²**
- ✓**80 000 probes**

Species Identified

Avian species

- Chicken (*Gallus gallus*)
- Goose (*Anser anser*)
- Guinea fowl (*Numida meleagris*)
- Ostrich (*Struthio camelus*)
- Turkey (*Meleagris gallopavo*)

Fish species

- Arctic char (*Salvelinus alpinus*)
- Atlantic bonito (*Sarda sarda*)
- Atlantic cod (*Gadus morhua*)
- Atlantic mackerel (*Scomber scombrus*)
- Atlantic salmon (*Salmo salar*)
- Brook trout (*Salvelinus fontinalis*)
- European eel (*Anguilla anguilla*)
- European hake (*Merluccius merluccius*)
- Greenland cod (*Gadus ogac*)
- Japanese eel (*Anguilla japonica*)
- Mozambican eel (*Anguilla mossambica*)
- Rainbow trout (*Oncorhynchus mykiss*)
- Sea trout (*Salmo trutta*)
- Skipjack tuna (*Euthynnus pelamis*)
- Spotted tunny (*Euthynnus alleterattus*)

Mammalian species

- Beef (*Bos taurus*)
- Cat (*Felis catus*)
- European hare (*Lepus europaeus*)
- Goat (*Capra hircus*)
- Human (*Homo sapiens*)
- Mouse (*Mus musculus*)
- Mule deer (*Odocoileus hemionus*)
- Pig / Boar (*Sus scrofa*)
- Rabbit (*Oryctolagus cuniculus*)
- Rat (*Rattus norvegicus*)
- Reindeer (*Rangifer tarandus*)
- Sheep (*Ovis aries*)

Tracing and Identifying animal tissues

Feed Industry

- Identify species composition of animal feed (eg. MBM)
- Verify absence of regulated materials (eg. Bovine CNS)
- Customer & regulatory compliance, product marketability,
- Quality assurance & safety (eg. BSE), brand protection

Food Industry

- Verify compliance with labelling regulations
- Verify component authenticity of supplies & ingredients
- Quality assurance; brand protection

An application of expression array technology

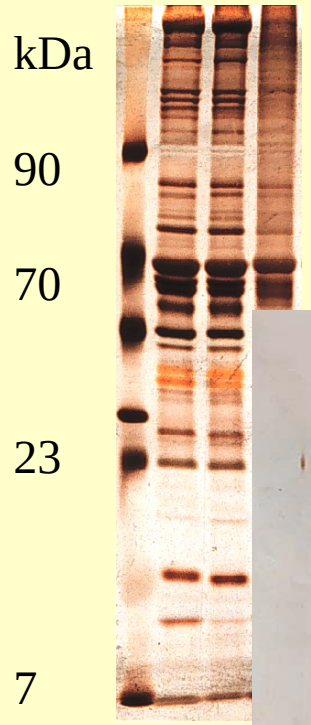
- Mice fed n-18 PUFA ± n-6 or n-3 PUFA
- Liver and hippocampus examined
- 12000 genetic elements examined using array
- 300 genes in target tissues differed in their expression.
- Fish oils act by activating multiple nuclear transcription factors
- *German et al, J Nutrition 2078S-2083S, 2003*

Proteomic approaches

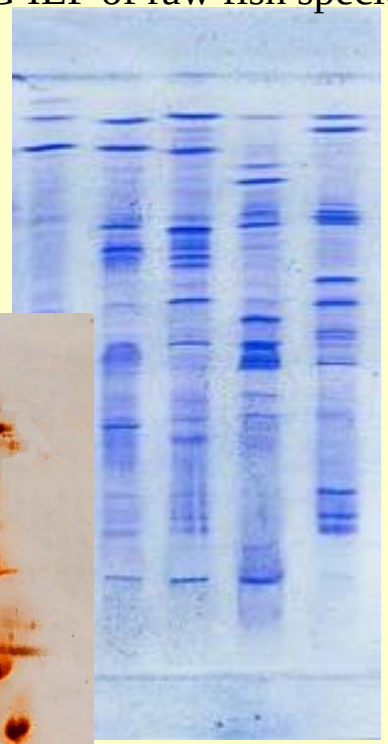
- Linked to genomics through power of databases
- Technologies based on separation from complex mixtures
- Identification from protein or peptide masses
- Protein- antibody arrays enable immuno identification of large numbers of targets

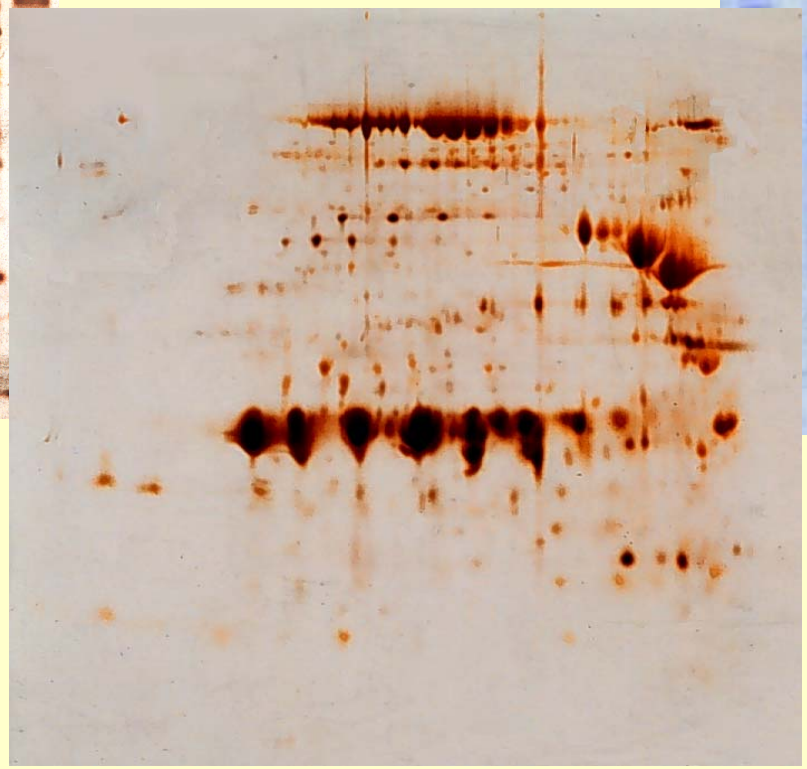
Protein electrophoretic techniques used in proteomics

Silver stained SDS gel of animal muscle



PAG IEF of raw fish species



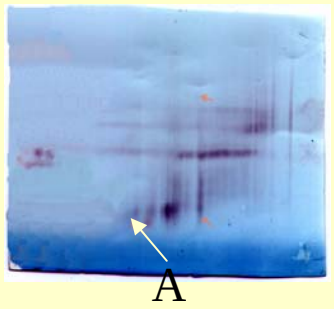


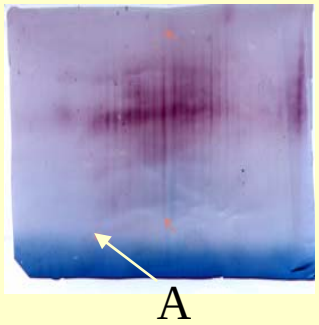
2D profile of GM pea leaf protein

Western blot analysis of Peanut and Hazelnut 2D electrophoretograms

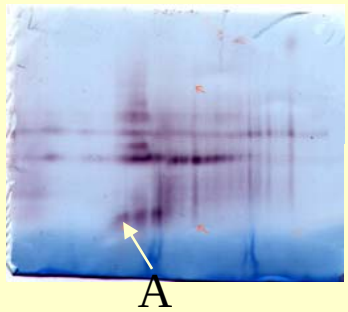
peanut

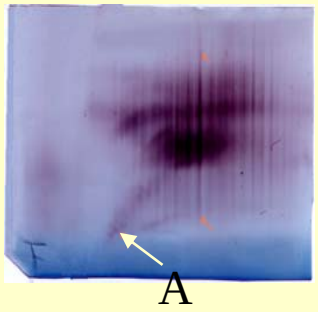
hazelnut



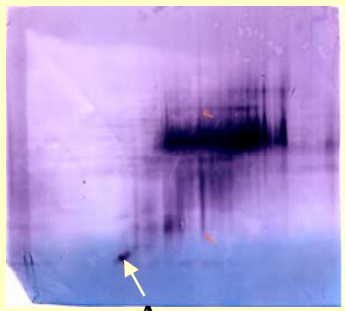


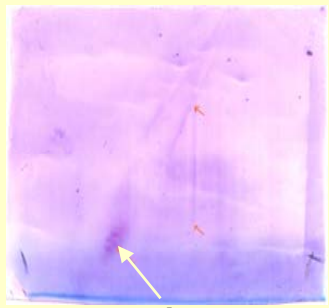
Patient 7





Patient 11





Patient 5

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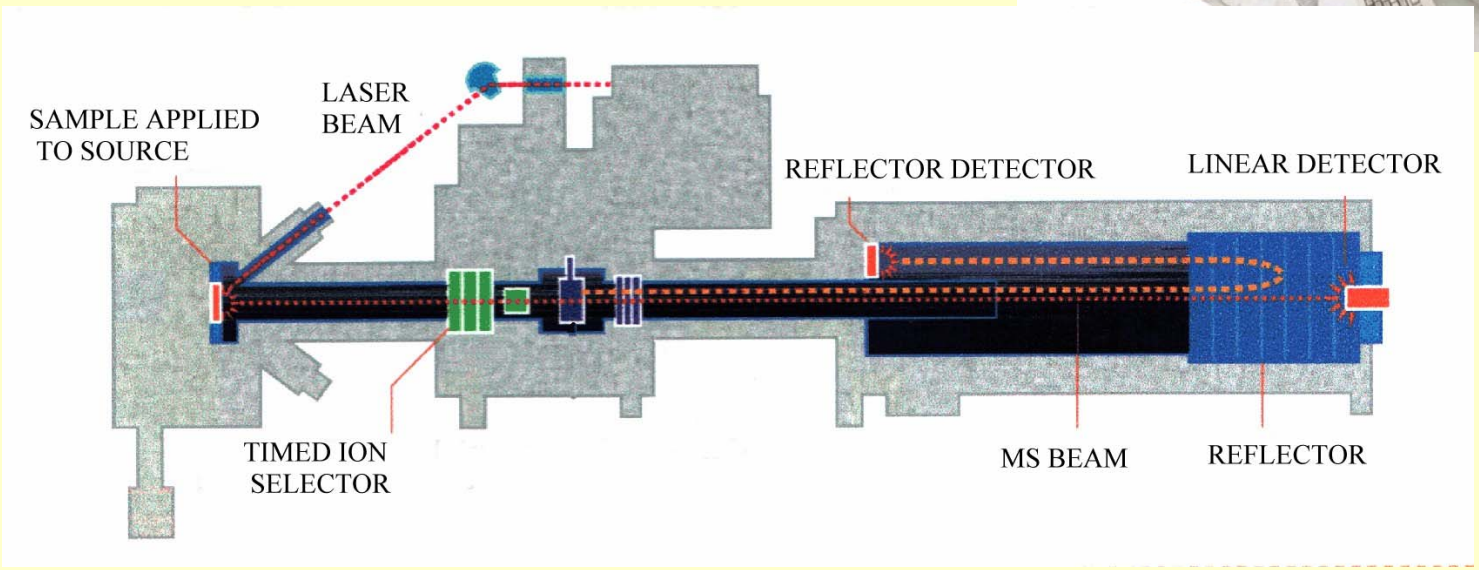
Hird, H., Pumphrey, R., Wilson, P., Sunderland, J. and Reece, P. (2000). *Electrophoresis* **21**:2678-2683.

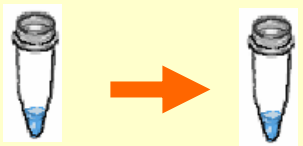
The MALDI TOF

Principle



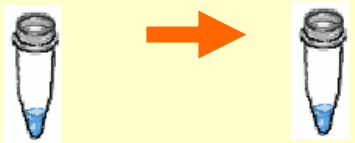
In-gel trypsin digestion

Spot excised from 2D gel



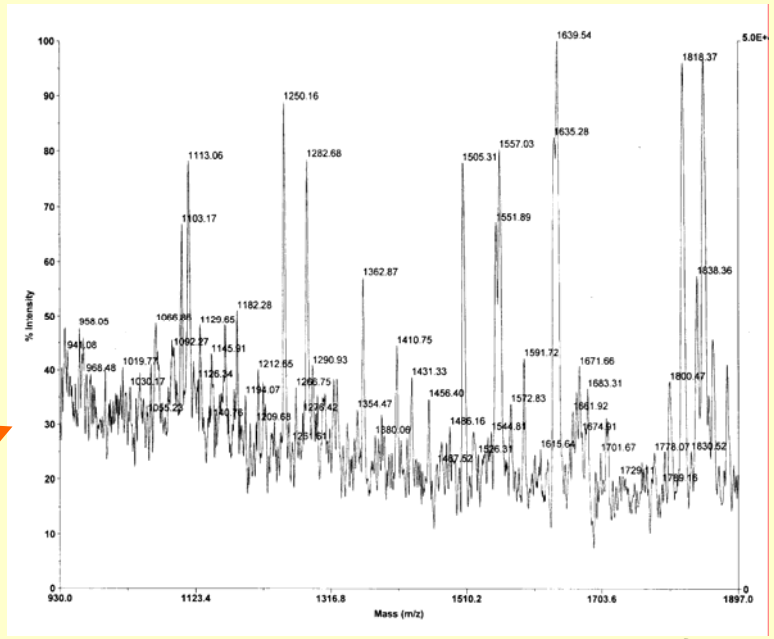
Destain

Reduce
and alkylate



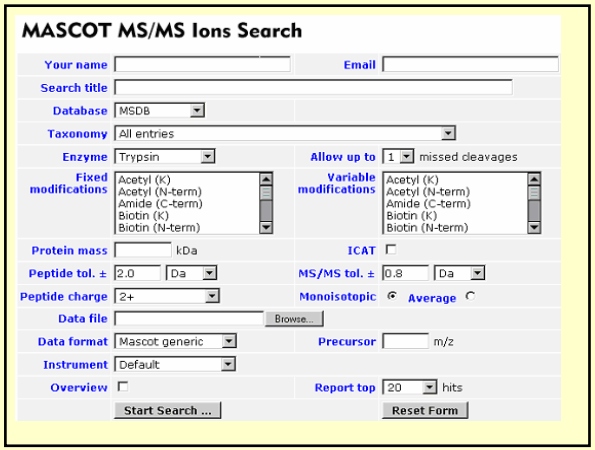
dry

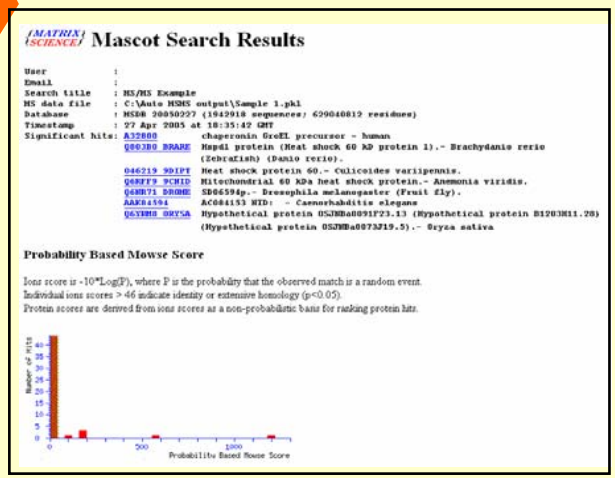
Digest in
trypsin 24hr

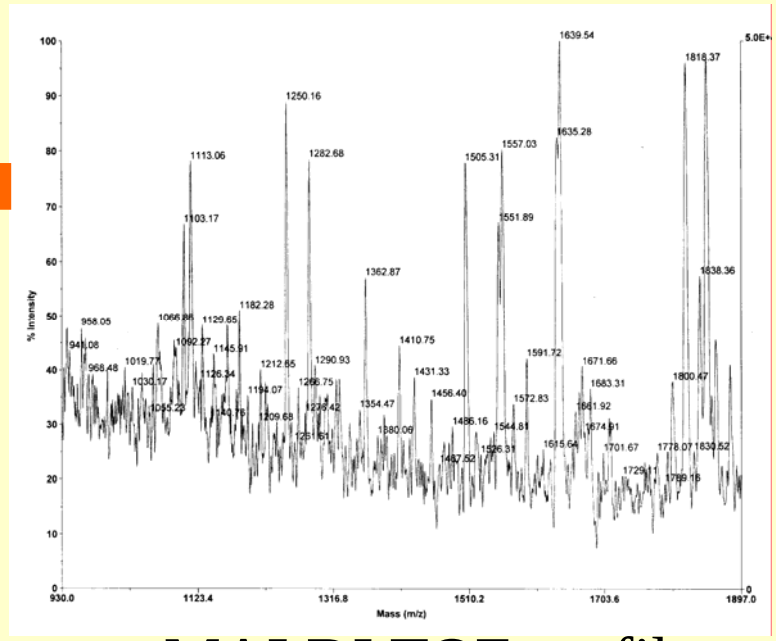


MALDI TOF profile

In-gel trypsin digestion





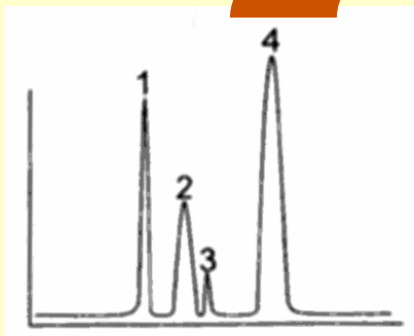


MALDI TOF profile

LC Tandem MS of peanut allergen Ara h1

Sample
ultrafiltration

Trypsin
digestion

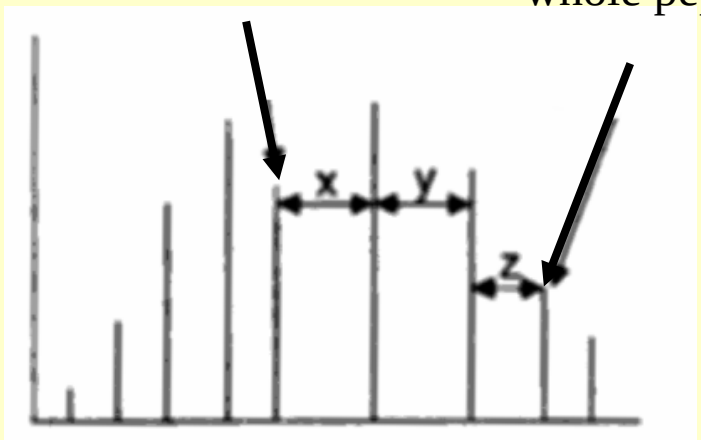


HPLC of protein digest

MS spectrum of peak 2

Mass of peptide
minus 3 amino acids

Mass of
whole peptide



Start mass,
x,y,z,
end mass,
(peptide sequence
Tag)

Database
search

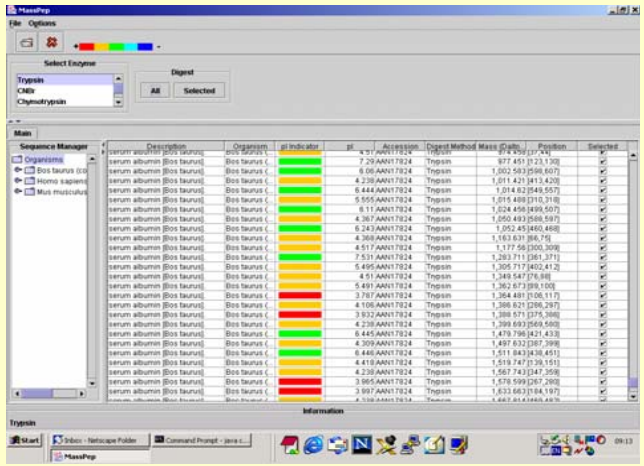
Identification
of protein

SHEFCHECK K.J. and STEVEN M MUSSER J.AG.FOOD CHEM. (2004) 52,2785-2790

Application of protein databases to the production of immunotests for specific food risk materials

Monoclonal antibody production

Synthesis of peptide



In Silico digestion of tissue proteins and identification of tissue specific peptides

Preparation of assay format

Cooked sample

Trypsin digestion

Confirmation of tissue

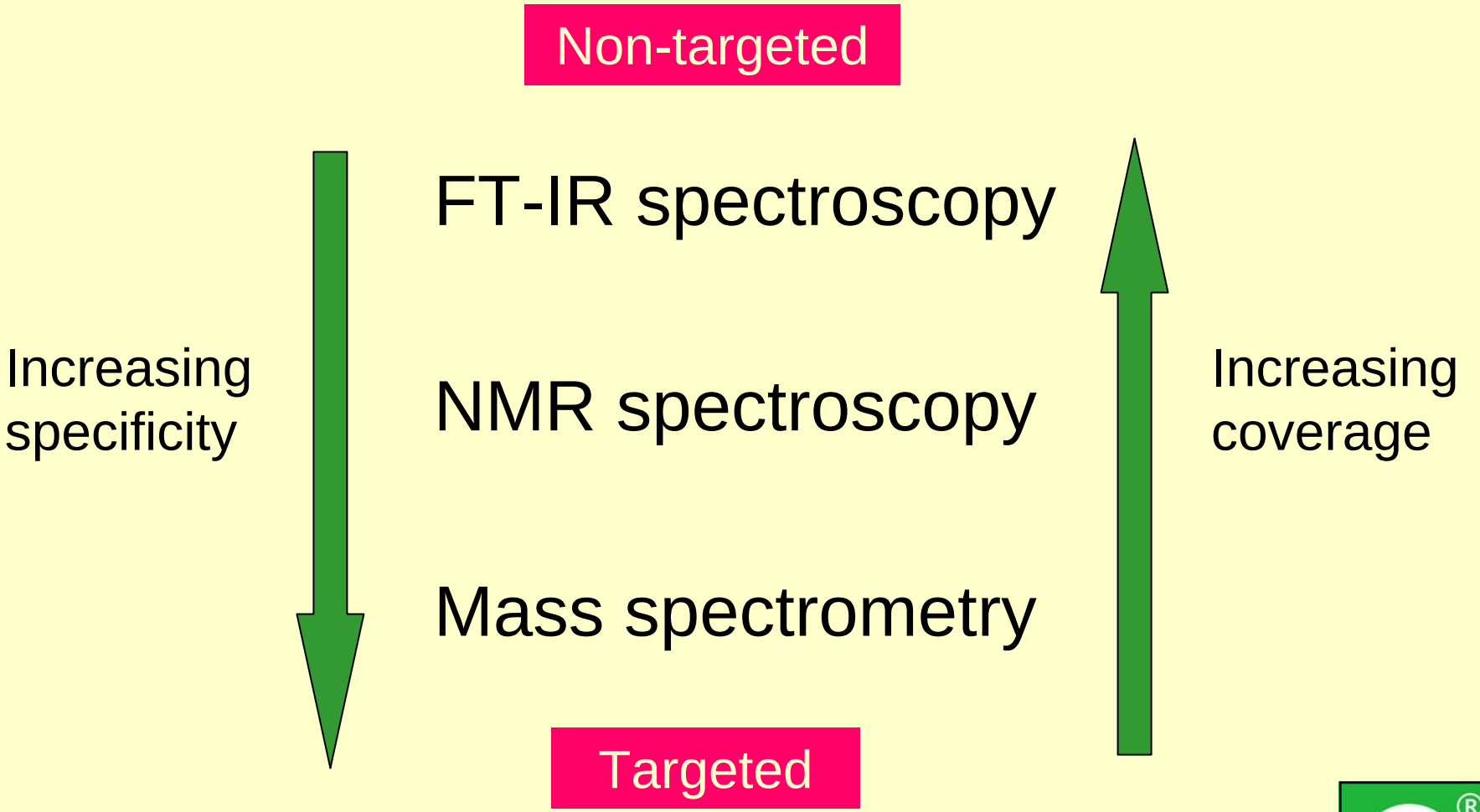
WWW.SRMTEST.INFO

Proteomics and food quality

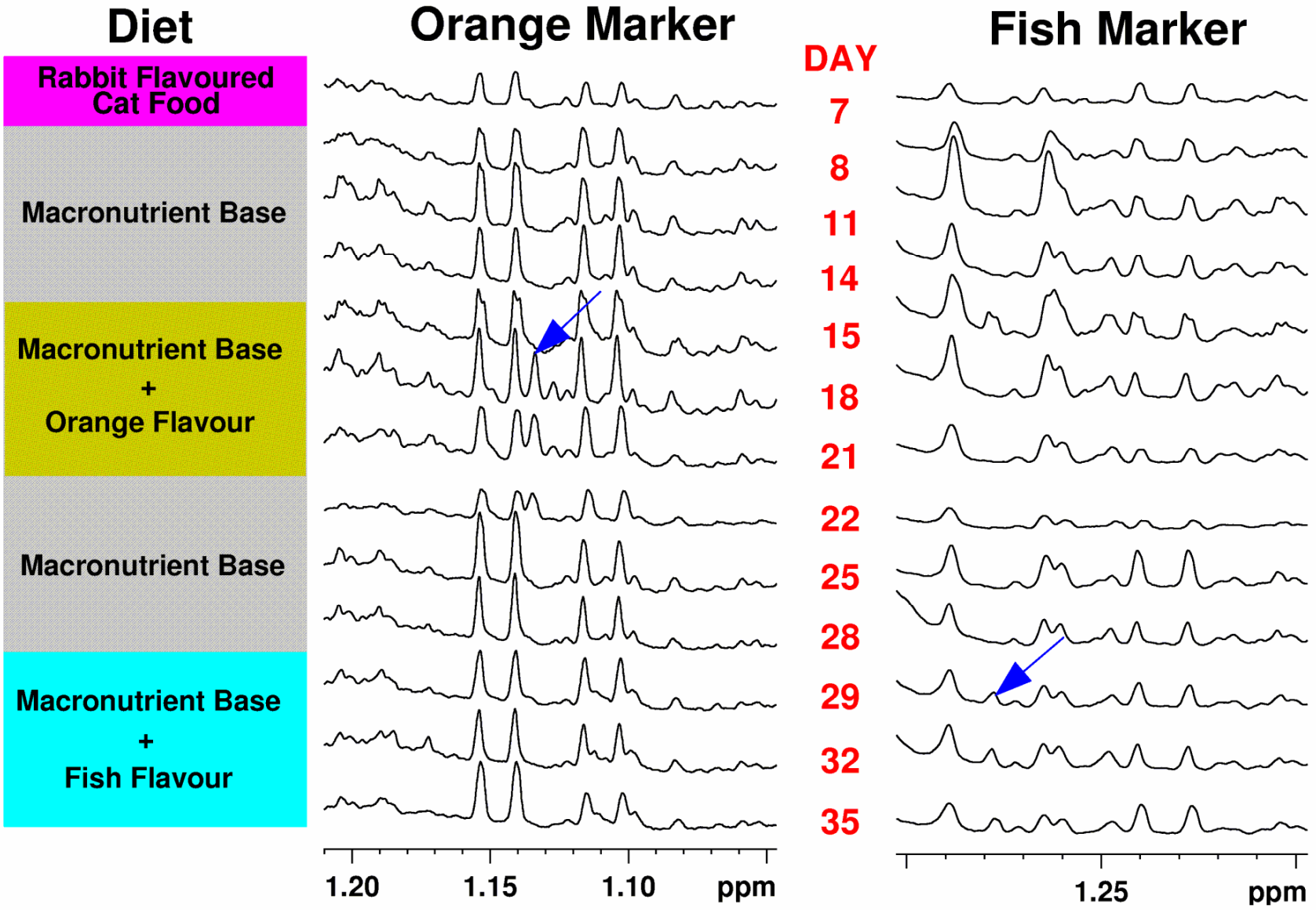
- *Post mortem* changes in pork meat
- Comparison of meat species
- Identification of allergens
- Quality of fermentation starter cultures
- Markers of origin and quality

- *Carbonaro et al Trend in Food Science and Technology (2004) 15 209-16*

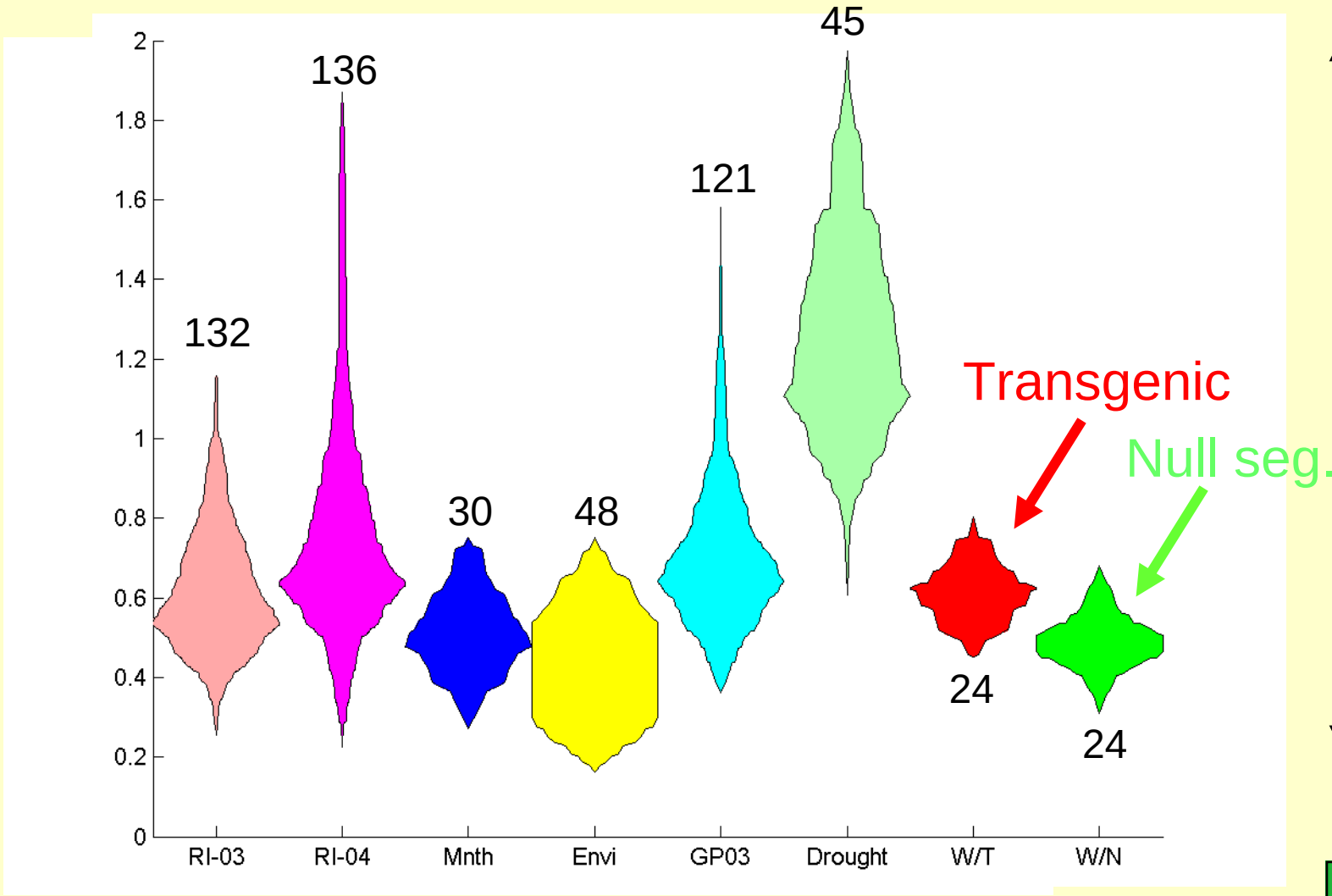
Spectroscopic approaches to metabolomics



^{1}H NMR Analysis of Cat Urine

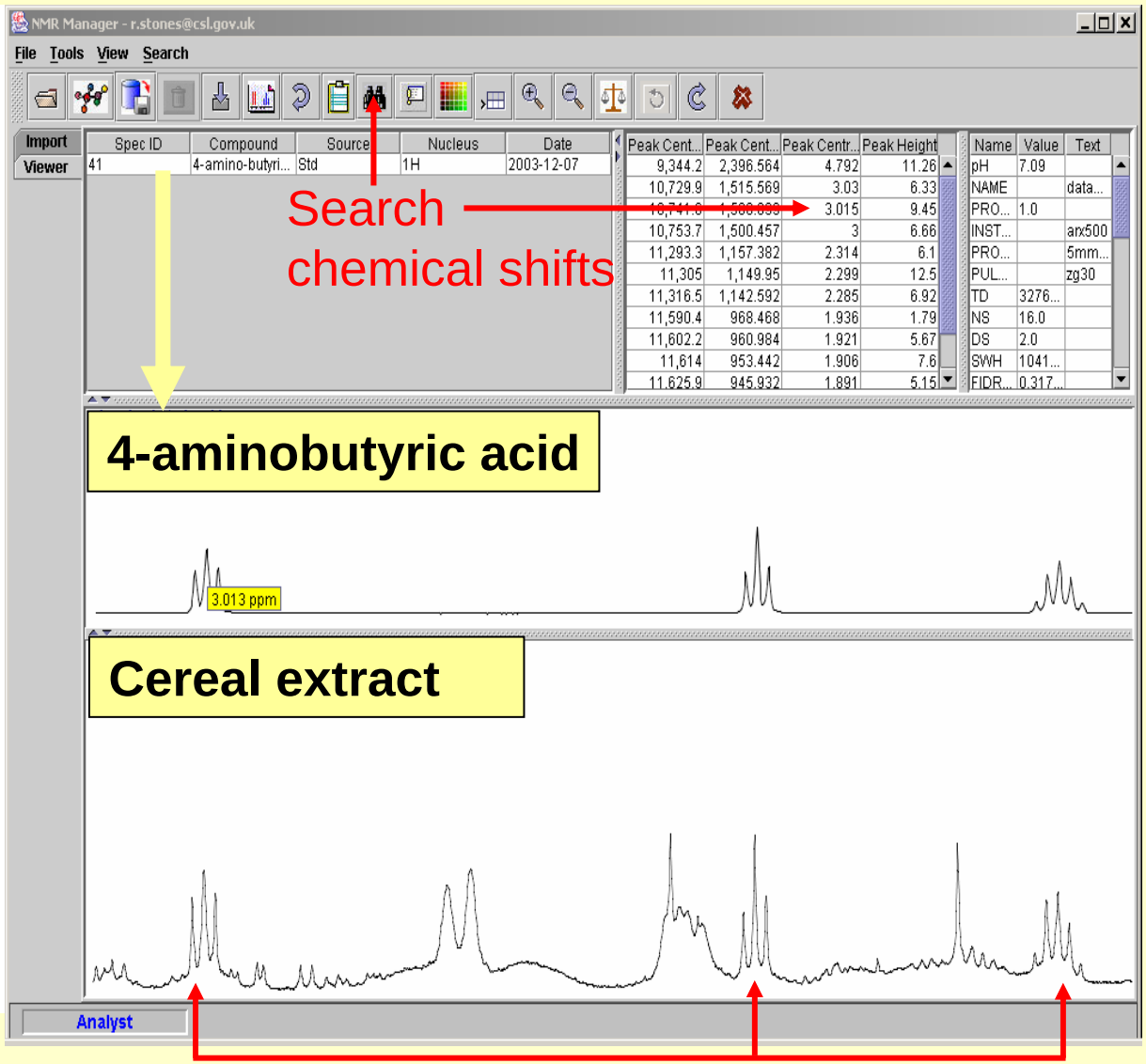


Natural variation



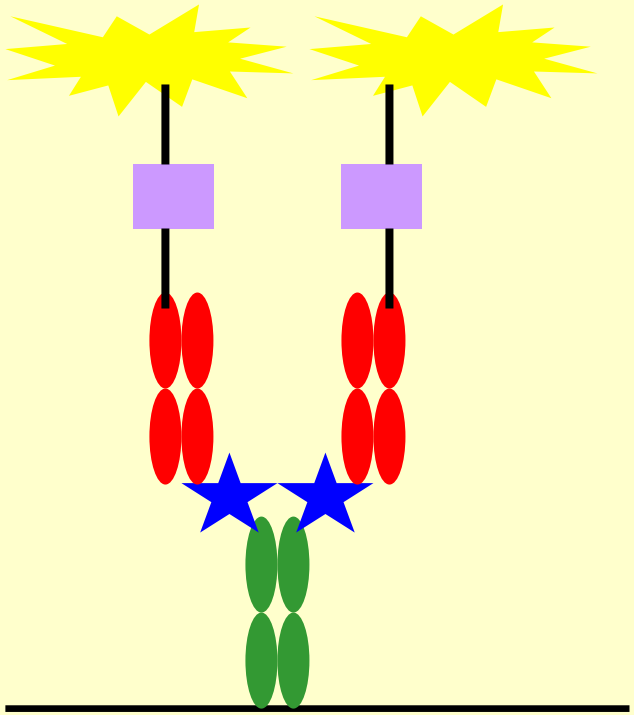
- Most significant differences between GM and NS are within the range of natural variation.

Identification of metabolites



- 2D homo and heteronuclear NMR investigation to measure correlations
- Determine structure of compounds without reference materials
- In-house database of metabolites (>100 to date)

Proteoplex™ array for cytokines



SensiLight dye

Biotin

Secondary antibody

Cytokine

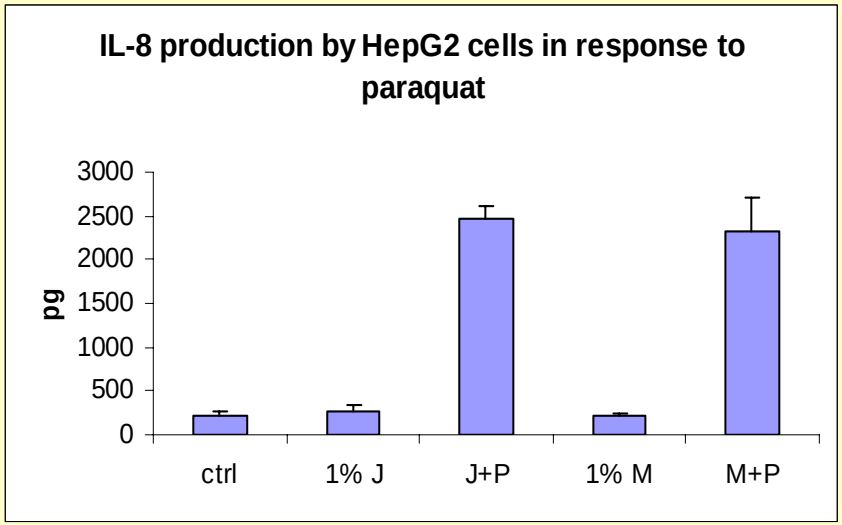
Primary antibody

Array slide

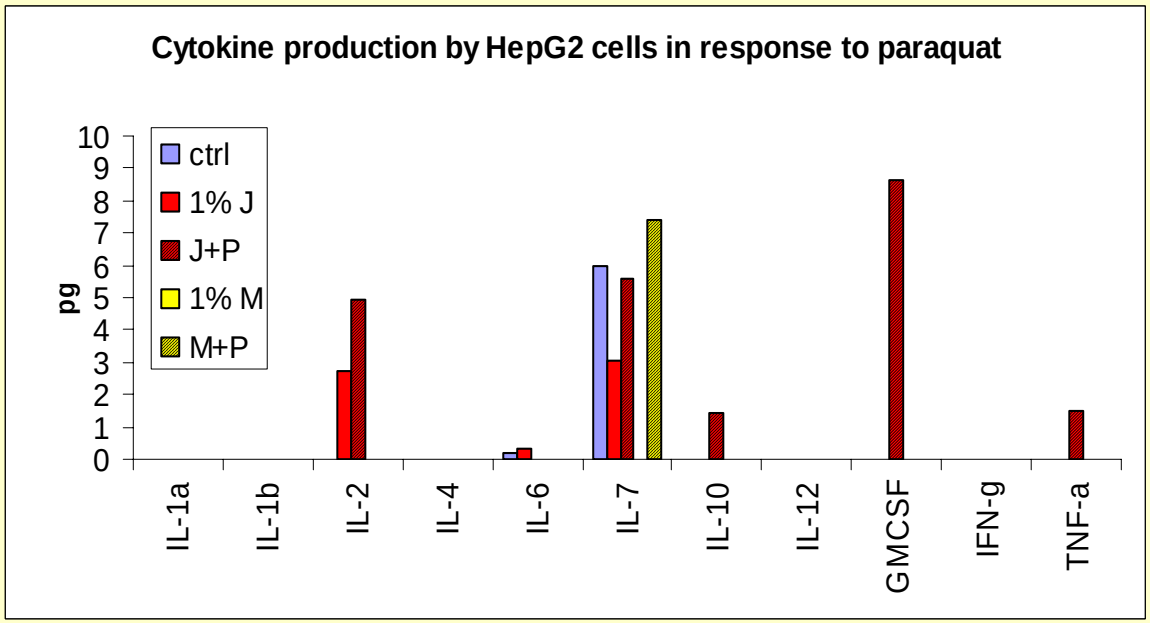
Model experiment

- Liver cell line (C)
- Milk (M) and Apple Juice (J) food extracts
- Paraquat (P) model contaminant
- Analysis of 12 cytokines in cell supernatant after 24 hr exposure to 1% food extract +/- paraquat.
- Mean data from three experiments.

Cytokine expression

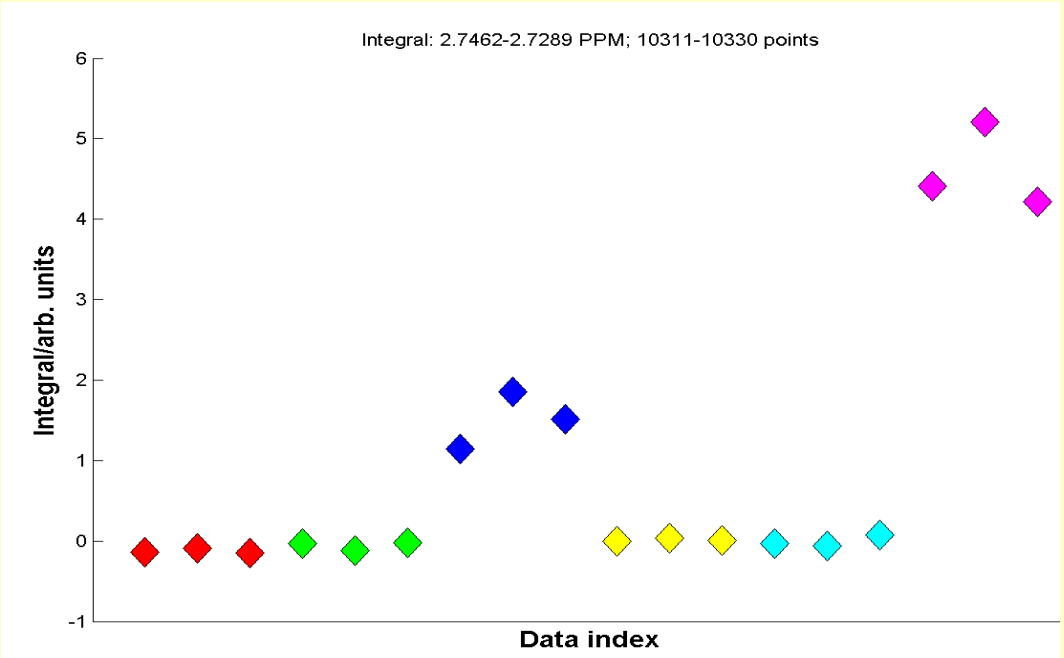


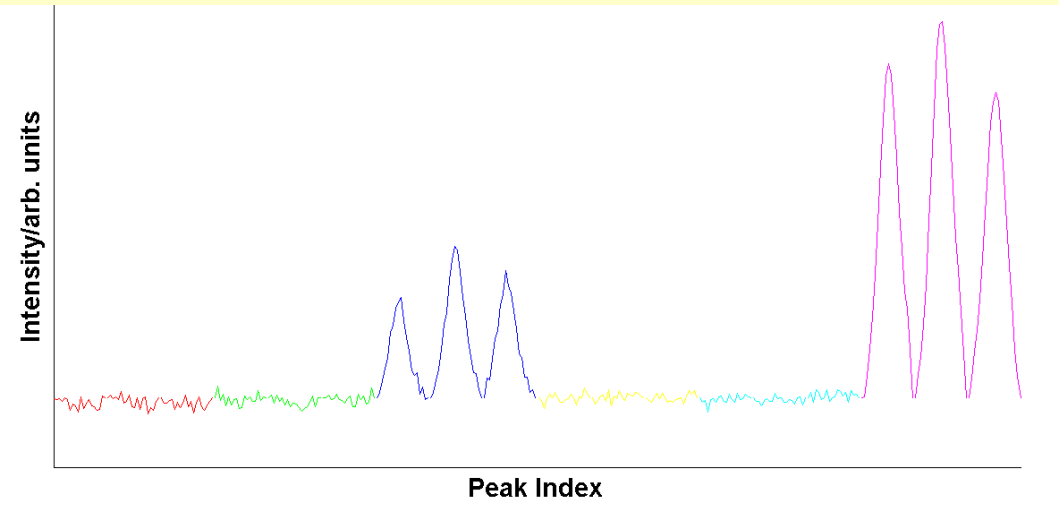
10-fold increase in IL-8 production in presence of paraquat



Induction of IL2, IL7, IL10, GM CSF, TNFa by combination of apple juice and paraquat

NMR detects the metabolic change





- All experiments performed in triplicate (cultured separately).
- Repeat batches measured several weeks apart.
- Amplified marker for paraquat in milk

Single or multiple biomarkers?

- A clear link needed between a potential biomarker and the human disease outcome
- 40+ Nobel laureates for work on cholesterol
- ‘omic technology enables move from population to individual recommendations
- Single biomarker possible with diseases having cause/effect relationship
- Complex multifactorial metabolic diseases need holistic approach provided by ‘omic technologies
- *German et al Journal of Nutrition, vol. 133 (6, Suppl. 1): p.2078S-2083S, 2003*

Systems biology - combining the ‘omics

- Complementary data from each of the ‘omics
- Metabolism. (SNP variations may form the basis for diabetes, obesity …)
- Personalised Health. (improved knowledge of individual responses give rise to intervention through diet etc)
- Prevention. (maintain health through disease prevention rather than cure)
- But lowering 1 risk at expense of disrupting metabolism may not improve health.

Bio-guided food processing

- Optimising composition of food commodities used for food processing
- Defining and optimising and biological materials used in processing, starter cultures, enzymes etc,
- Food pathogen control
- *German, Aust J. Dairy Technol. **58** 82-88*