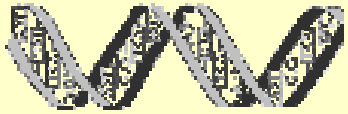


'OMICS – APPLICATION IN FOOD AND NUTRITION

M. John Dennis

Central Science Laboratory, UK



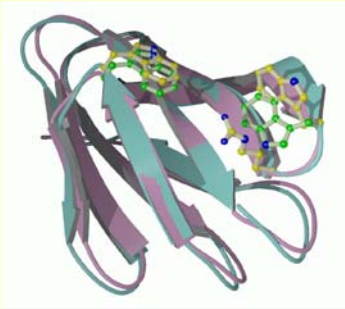
Genomics

DNA



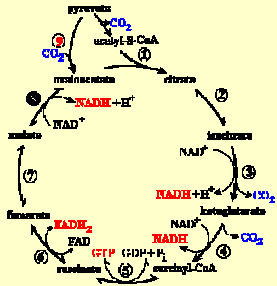
Transcriptomics

RNA



Proteomics

Proteins



Metabolomics

Metabolites



'OMICS?

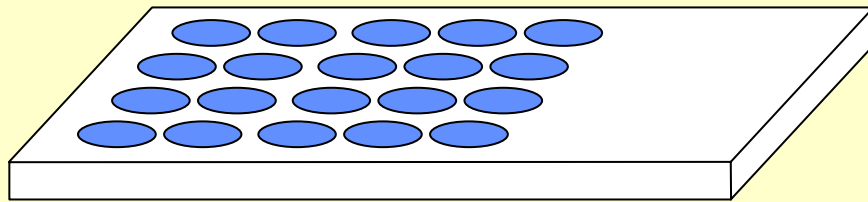
- Genomics, Proteomics, Metabolomics (Metabonomics)
- Functionally linked
- Comparative technologies devoted to identifying significant differences
- High throughput methods
- Applicable to population studies or low frequency events

Genomics applications

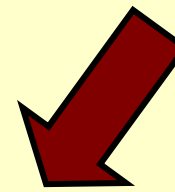
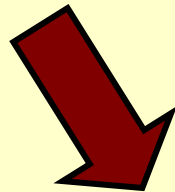
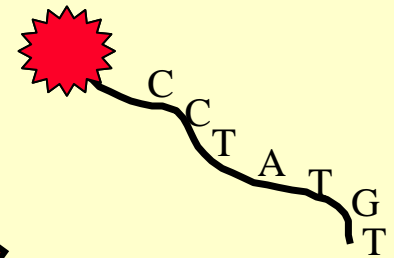
- Concept of Microarrays invented in 1989
- Widely used in the field of human and animal genomics; Affymetrix leader
- Microarrays enable detection of large numbers of DNA (and RNA) signatures
- Applications to species identification
- Differential expression
- Single Nucleotide Polymorphisms (SNP's)

Microarray detection

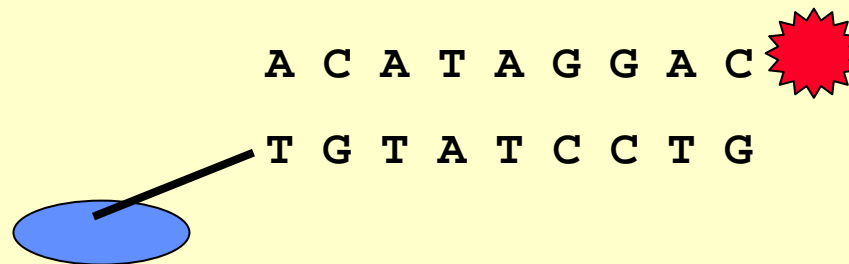
DNA capture probes held on solid support



Fluorescent labelling of sample nucleic acid

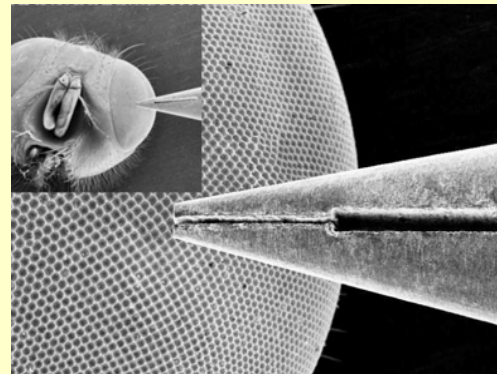
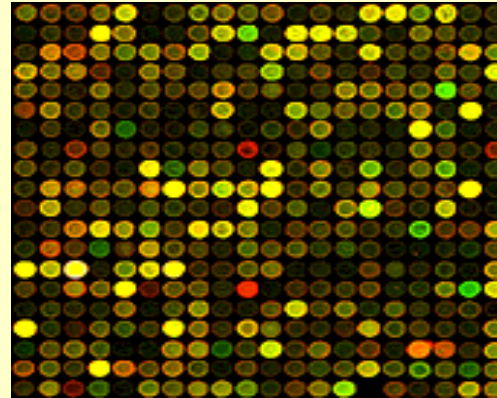


Hybridisation to the array



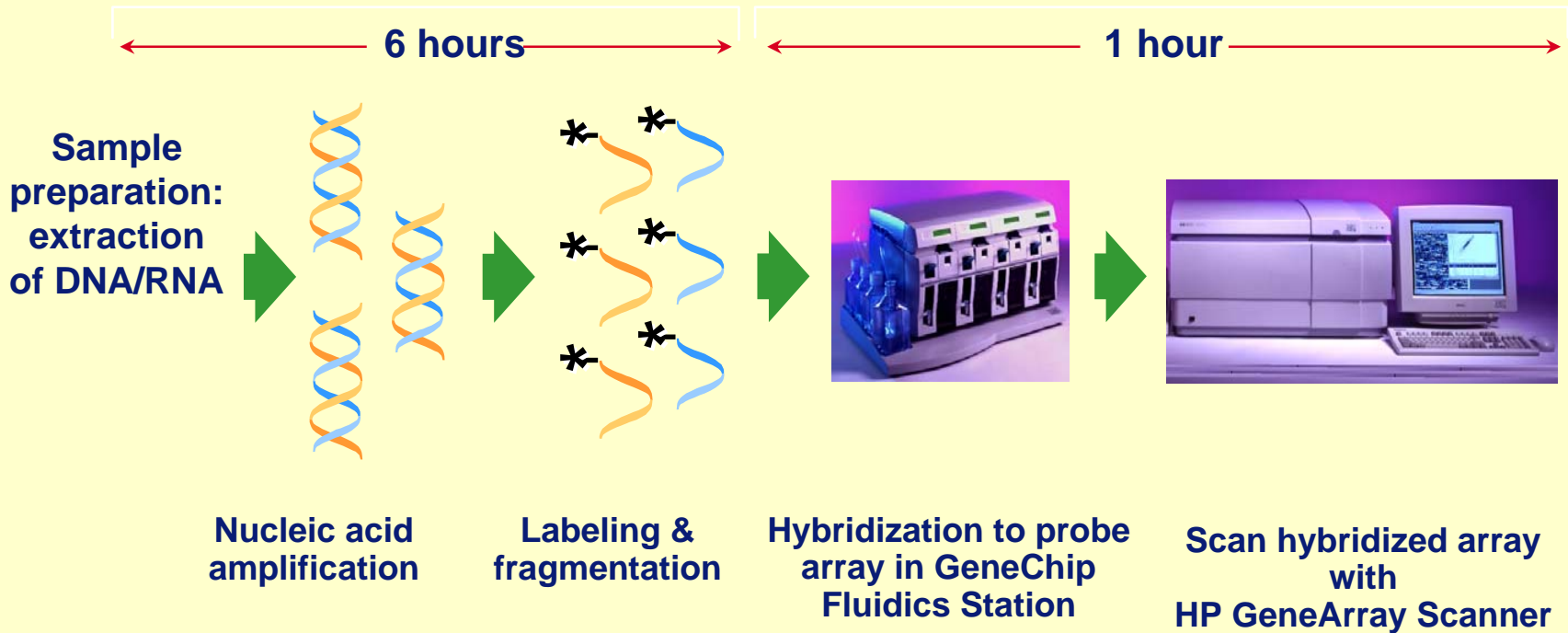
What is a microarray?

- ‘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

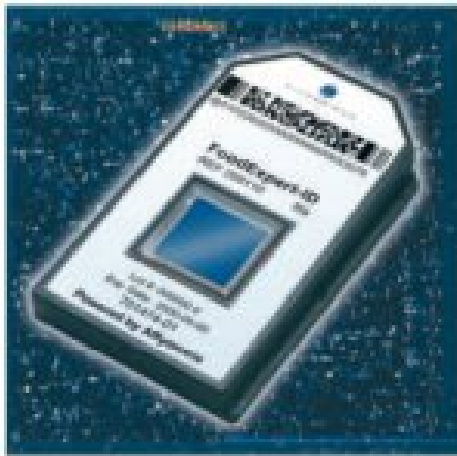


cytochrome B Microarray format

GeneChip Probe Array : Process Overview



bioMérieux FoodExpert-ID® *Food and Animal Feed Identity Card*



Result	Analysis date	Sample description	Status
assay1 # 0	2003 09 24 - 12:03	fish feed	Valid

assay1 # 0

Identification

Present Taxon
Atlantic mackerel (<i>Scomber scombrus</i>)
Atlantic cod (<i>Gadus morhua</i>)
Species of the fishes class

Internal control	Status
Internal Control	Positive

✓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

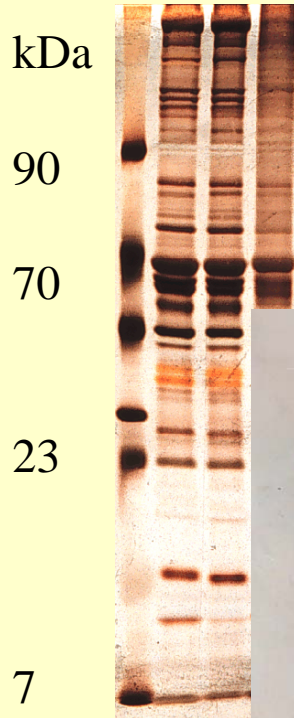
- Mice fed n-18 PUFA \pm n-6 or n-3 PUFA
- Liver and hippocampus examined
- 12000 genetic elements examined using array
- 300 genes in target tissues differed in their expression.
- Fish oils act by activating multiple nuclear transcription factors
- *German et al, J Nutrition 2078S-2083S, 2003*

Proteomic approaches

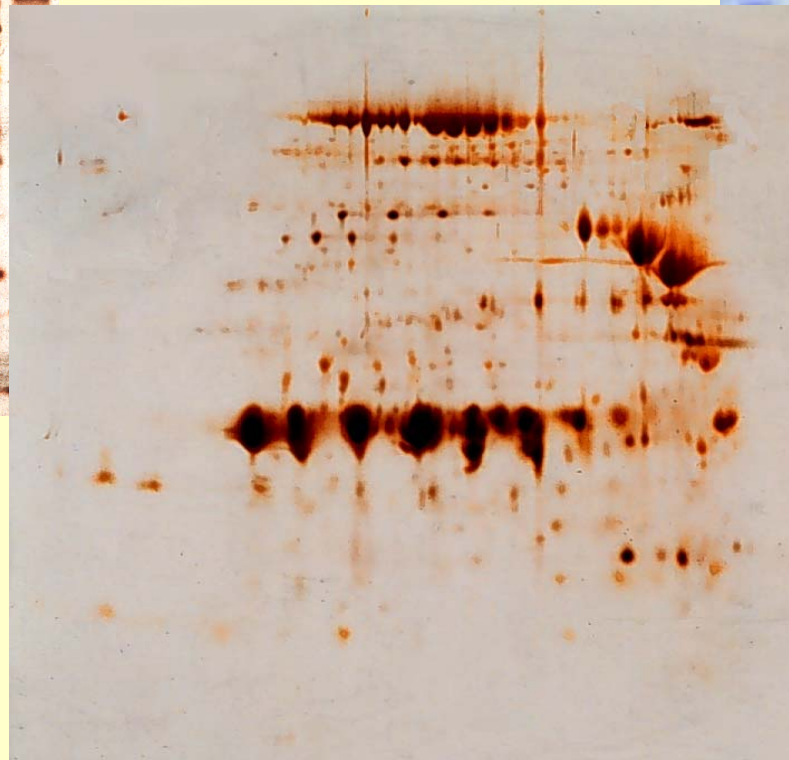
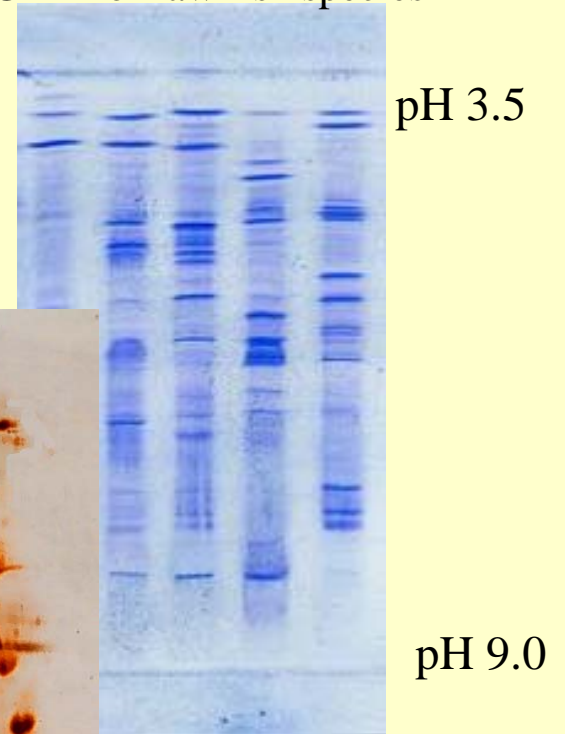
- Linked to genomics through power of databases
- Technologies based on separation from complex mixtures
- Identification from protein or peptide masses
- Protein- antibody arrays enable immuno identification of large numbers of targets

Protein electrophoretic techniques used in proteomics

Silver stained SDS gel of animal muscle



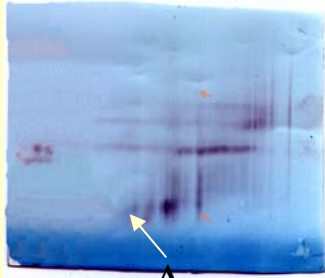
PAG IEF of raw fish species



2D profile of GM pea leaf protein

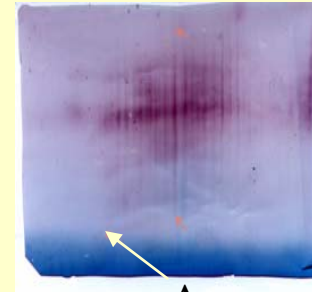
Western blot analysis of Peanut and Hazelnut 2D electrophoretograms

peanut



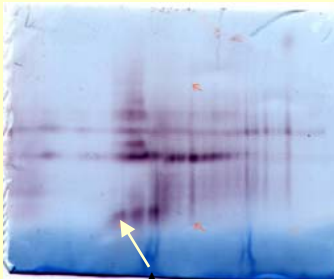
A

hazelnut

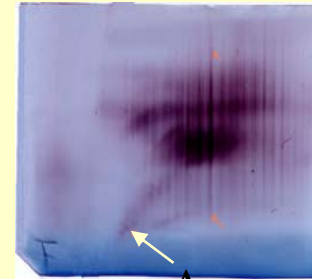


A

Patient 7

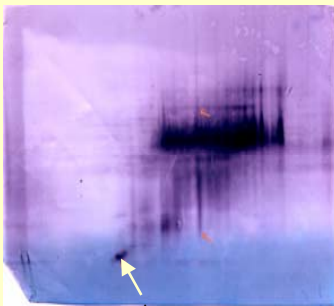


A

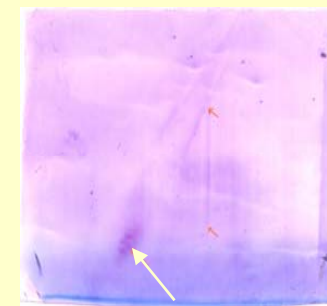


A

Patient 11



A

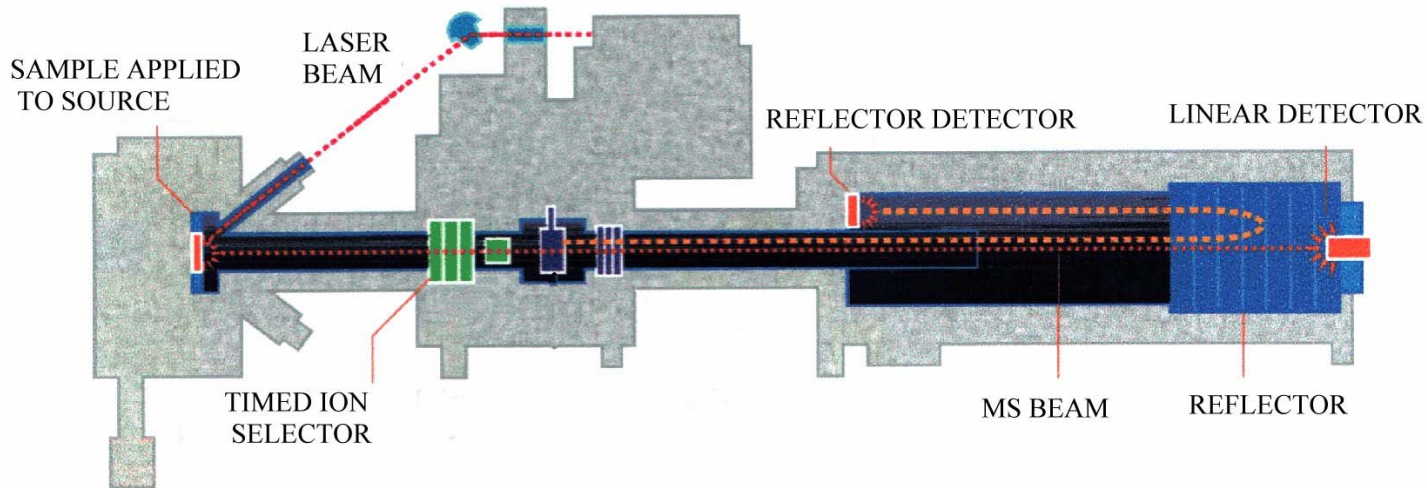


A

Patient 5

The MALDI TOF

Principle



In-gel trypsin digestion



Spot excised from 2D gel



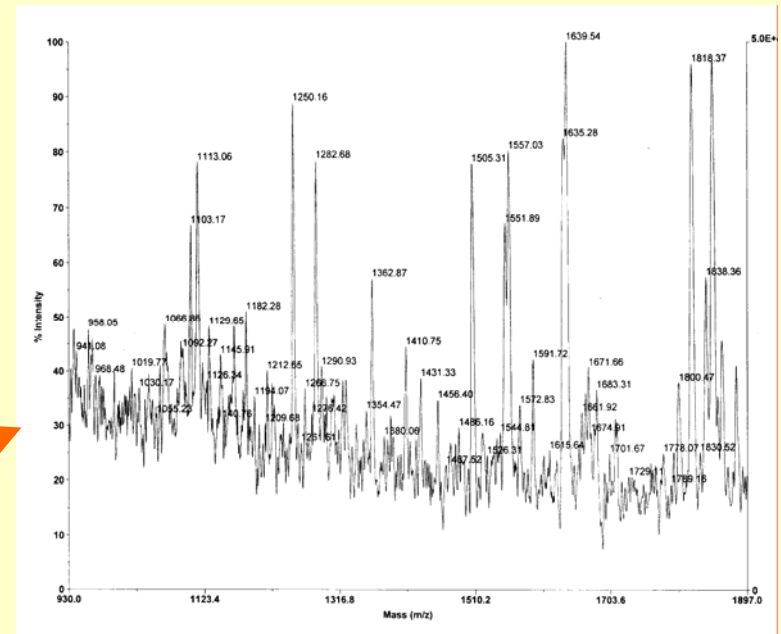
Destain

Reduce and alkylate



dry

Digest in
trypsin 24hr



MALDI TOF profile

In-gel trypsin digestion

MASCOT MS/MS Ions Search

Your name: Email:

Search title:

Database: MSDB

Taxonomy: All entries

Enzyme: Trypsin Allow up to: 1 missed cleavages

Fixed modifications: Acetyl (K), Acetyl (N-term), Amide (C-term), Biotin (K), Biotin (N-term)

Variable modifications: Acetyl (K), Acetyl (N-term), Amide (C-term), Biotin (K), Biotin (N-term)

Protein mass: kDa ICAT:

Peptide tol. \pm : 2.0 Da MS/MS tol. \pm : 0.8 Da

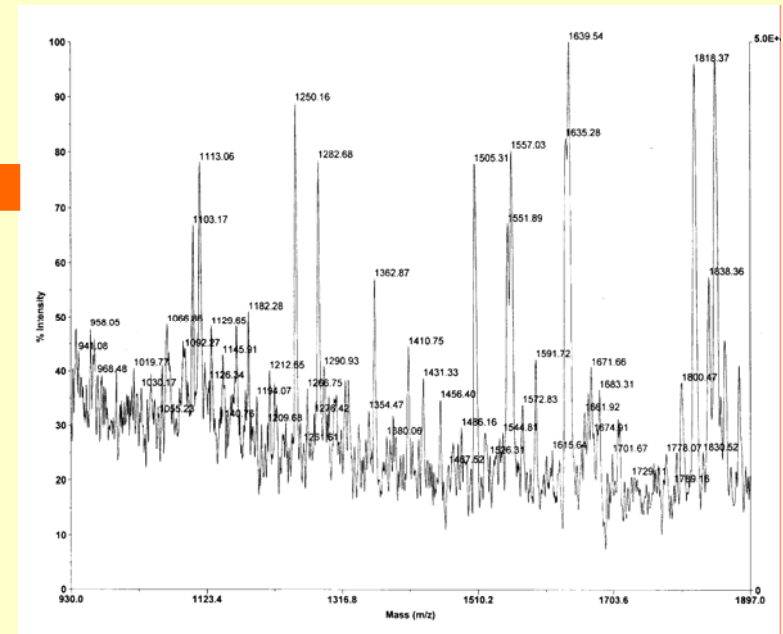
Peptide charge: 2+ Monoisotopic: Average:

Data file: Browse...

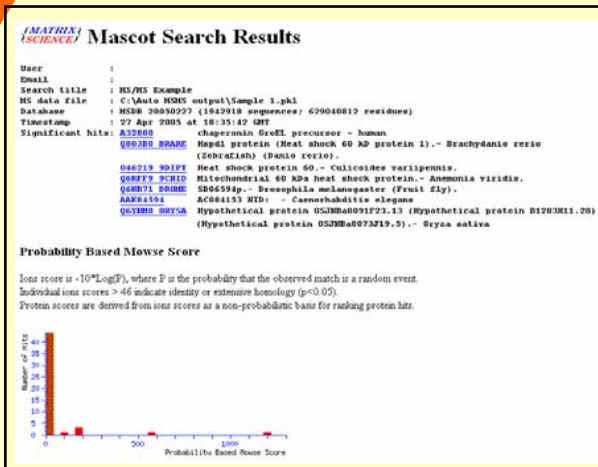
Data format: Mascot generic Precursor: m/z

Instrument: Default

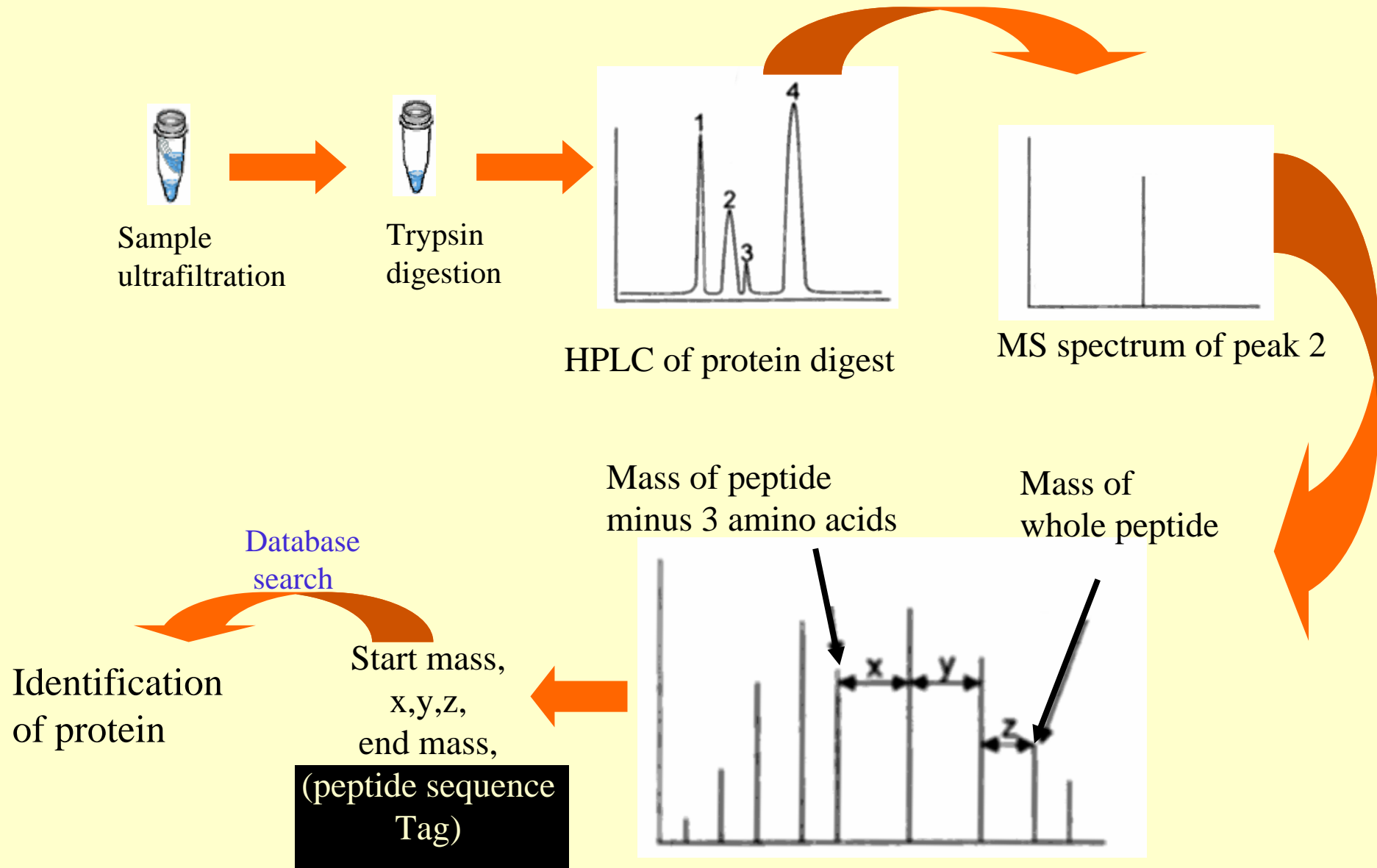
Overview: Report top: 20 hits



MALDI TOF profile



LC Tandem MS of peanut allergen Ara h1



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

Monoclonal antibody production

Synthesis of peptide



Preparation of assay format



Confirmation of tissue

Trypsin digestion



Cooked sample

SeqName	Protein	Org	pt	Abundance	Digest Method	Mass (Da)	Position	Selected
1017	serum albumin [Bova taureau]	Bova taureau	7.29	1000000	Trypsin	677455	[121,300]	<input checked="" type="checkbox"/>
1018	serum albumin [Bova taureau]	Bova taureau	6.96	1000000	Trypsin	1002563	[266,807]	<input checked="" type="checkbox"/>
1019	serum albumin [Bova taureau]	Bova taureau	4.23	1000000	Trypsin	1011421	[413,420]	<input checked="" type="checkbox"/>
1020	serum albumin [Bova taureau]	Bova taureau	6.44	1000000	Trypsin	1014822	[548,557]	<input checked="" type="checkbox"/>
1021	serum albumin [Bova taureau]	Bova taureau	5.55	1000000	Trypsin	1015489	[110,318]	<input checked="" type="checkbox"/>
1022	serum albumin [Bova taureau]	Bova taureau	6.11	1000000	Trypsin	1024498	[499,507]	<input checked="" type="checkbox"/>
1023	serum albumin [Bova taureau]	Bova taureau	4.28	1000000	Trypsin	1056493	[266,807]	<input checked="" type="checkbox"/>
1024	serum albumin [Bova taureau]	Bova taureau	6.24	1000000	Trypsin	1092451	[440,448]	<input checked="" type="checkbox"/>
1025	serum albumin [Bova taureau]	Bova taureau	4.28	1000000	Trypsin	1163631	[86,76]	<input checked="" type="checkbox"/>
1026	serum albumin [Bova taureau]	Bova taureau	4.51	1000000	Trypsin	117756	[300,308]	<input checked="" type="checkbox"/>
1027	serum albumin [Bova taureau]	Bova taureau	7.51	1000000	Trypsin	1291711	[181,371]	<input checked="" type="checkbox"/>
1028	serum albumin [Bova taureau]	Bova taureau	5.49	1000000	Trypsin	1305717	[421,42]	<input checked="" type="checkbox"/>
1029	serum albumin [Bova taureau]	Bova taureau	4.51	1000000	Trypsin	1348547	[76,88]	<input checked="" type="checkbox"/>
1030	serum albumin [Bova taureau]	Bova taureau	5.49	1000000	Trypsin	1362672	[106,110]	<input checked="" type="checkbox"/>
1031	serum albumin [Bova taureau]	Bova taureau	3.78	1000000	Trypsin	1364481	[106,117]	<input checked="" type="checkbox"/>
1032	serum albumin [Bova taureau]	Bova taureau	4.10	1000000	Trypsin	1368621	[296,297]	<input checked="" type="checkbox"/>
1033	serum albumin [Bova taureau]	Bova taureau	3.52	1000000	Trypsin	1368971	[127,368]	<input checked="" type="checkbox"/>
1034	serum albumin [Bova taureau]	Bova taureau	4.23	1000000	Trypsin	1399693	[369,468]	<input checked="" type="checkbox"/>
1035	serum albumin [Bova taureau]	Bova taureau	4.46	1000000	Trypsin	1478798	[421,438]	<input checked="" type="checkbox"/>
1036	serum albumin [Bova taureau]	Bova taureau	4.30	1000000	Trypsin	1497632	[187,398]	<input checked="" type="checkbox"/>
1037	serum albumin [Bova taureau]	Bova taureau	8.44	1000000	Trypsin	1511843	[438,451]	<input checked="" type="checkbox"/>
1038	serum albumin [Bova taureau]	Bova taureau	4.41	1000000	Trypsin	1518747	[128,151]	<input checked="" type="checkbox"/>
1039	serum albumin [Bova taureau]	Bova taureau	4.23	1000000	Trypsin	1567743	[147,358]	<input checked="" type="checkbox"/>
1040	serum albumin [Bova taureau]	Bova taureau	3.86	1000000	Trypsin	1578599	[247,268]	<input checked="" type="checkbox"/>
1041	serum albumin [Bova taureau]	Bova taureau	3.99	1000000	Trypsin	1633663	[184,197]	<input checked="" type="checkbox"/>
1042	serum albumin [Bova taureau]	Bova taureau	4.10	1000000	Trypsin	1648144	[418,419]	<input checked="" type="checkbox"/>

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

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

Non-targeted

FT-IR spectroscopy

NMR spectroscopy

Mass spectrometry

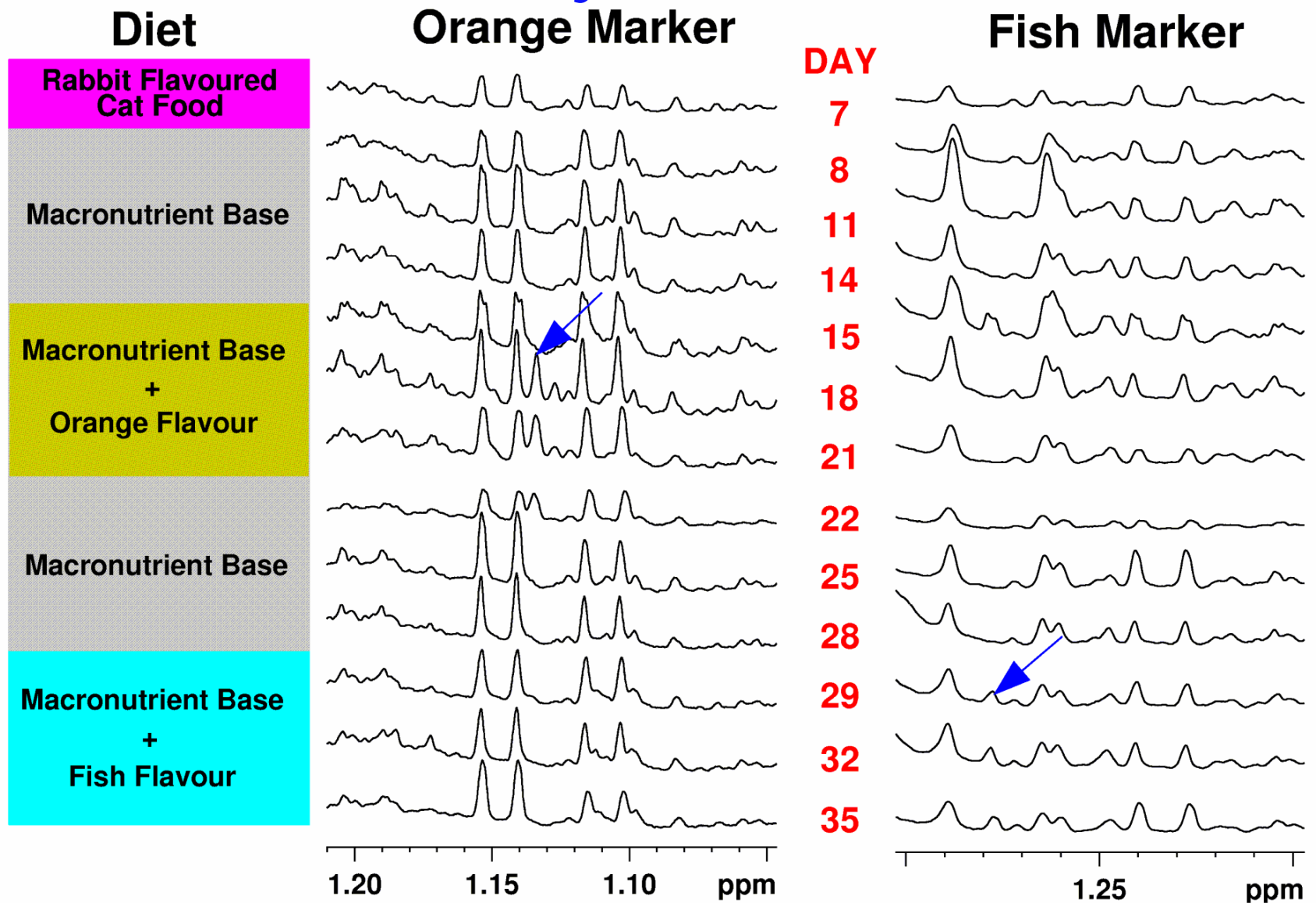
Targeted

Increasing
specificity

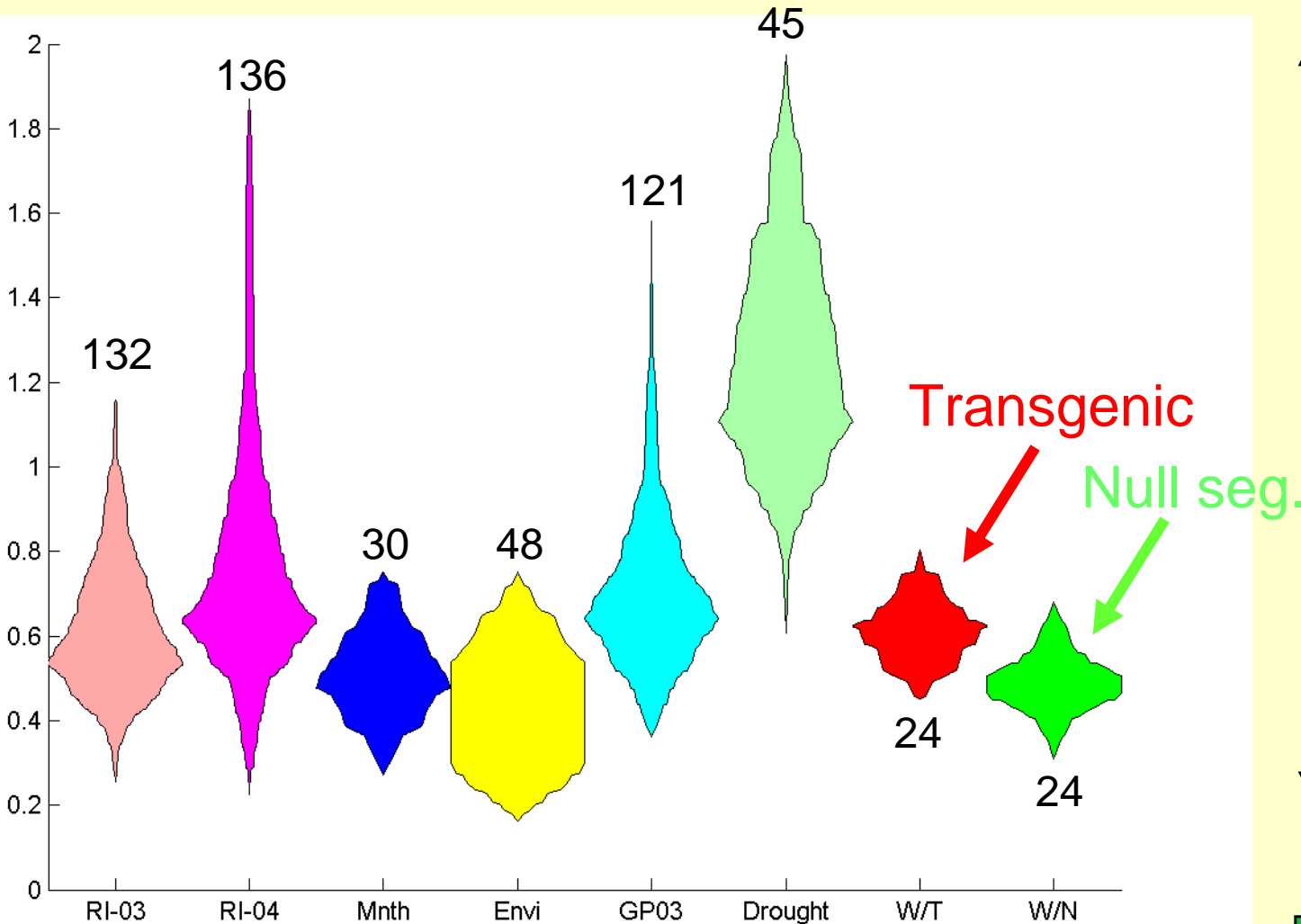
Increasing
coverage



^1H NMR Analysis of Cat Urine



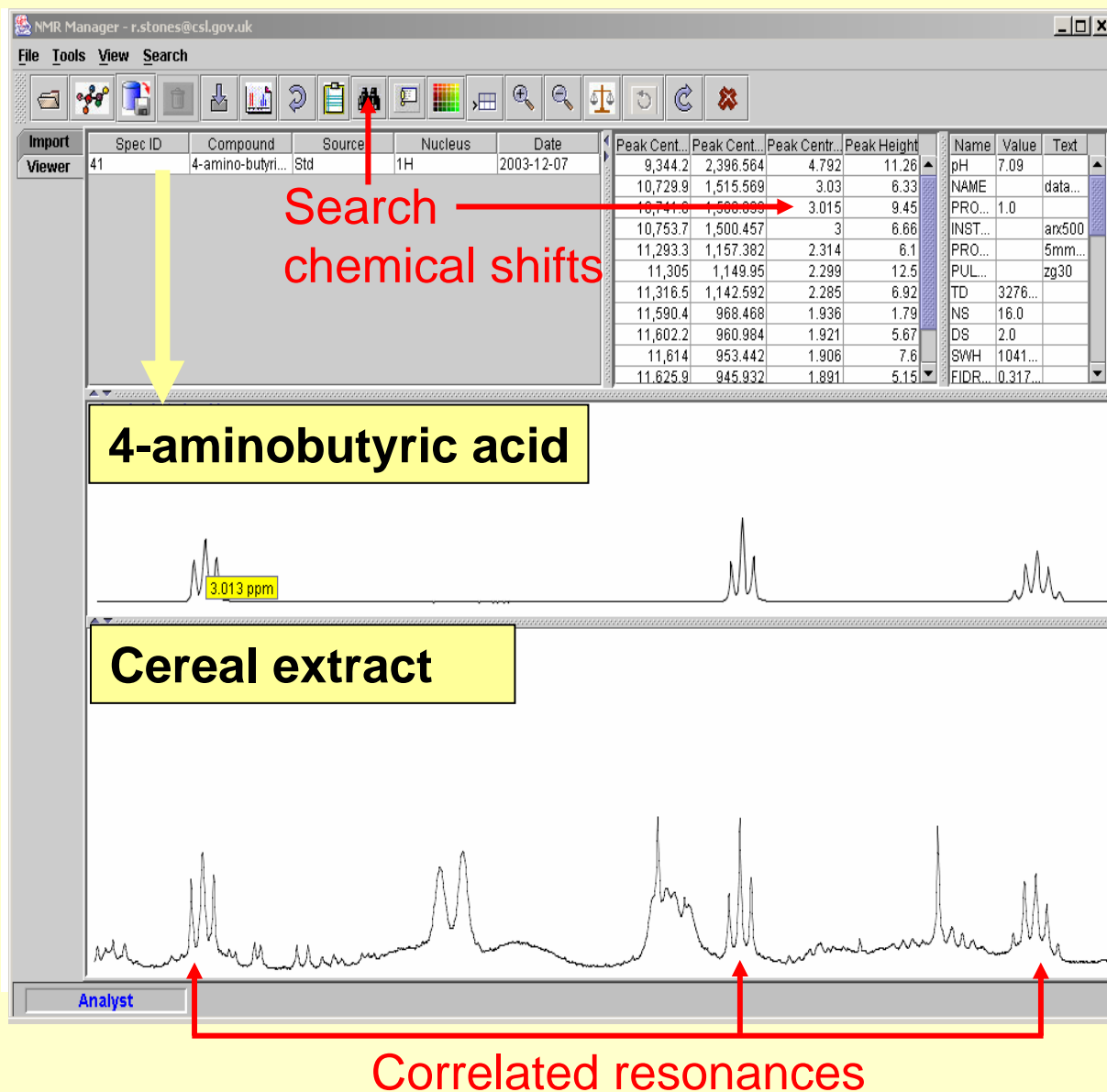
Natural variation



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



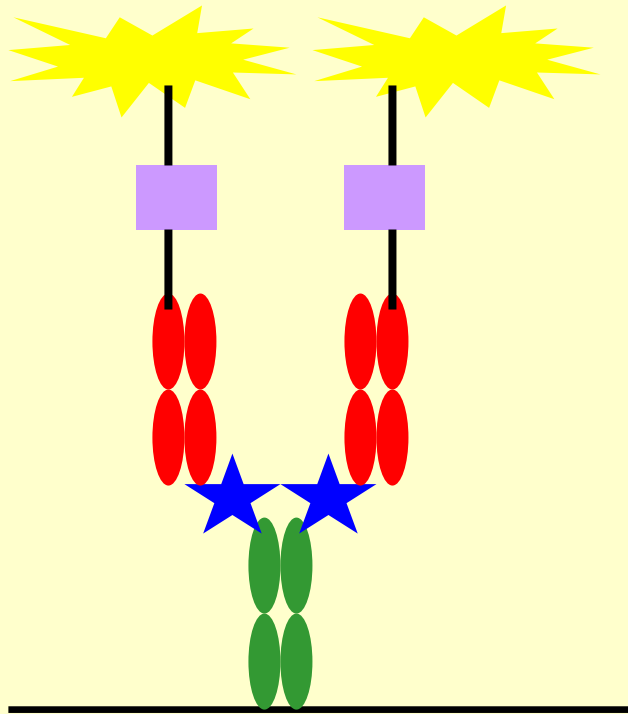
Identification of metabolites



- 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

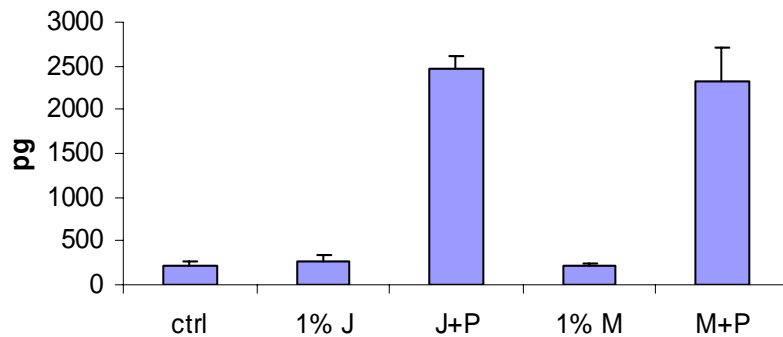
Array slide

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.

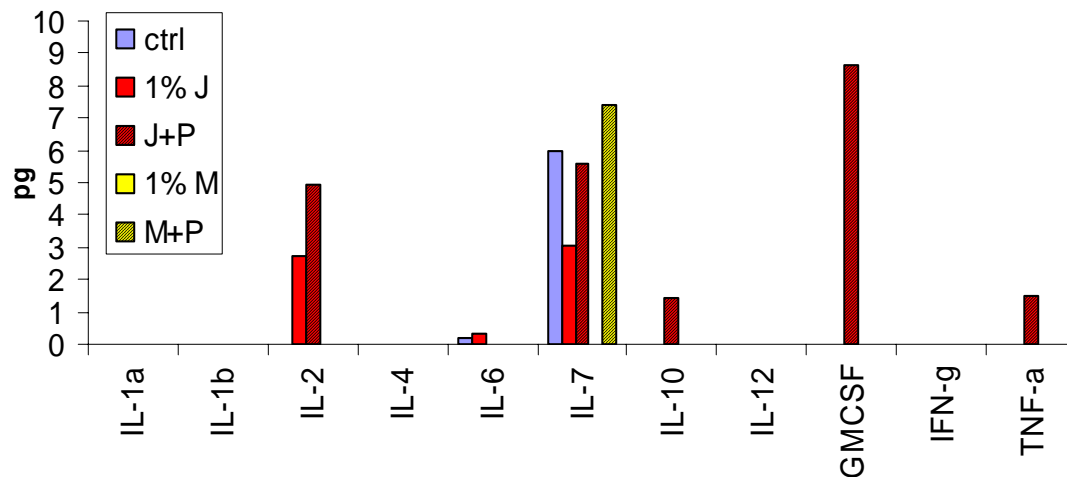
Cytokine expression

IL-8 production by HepG2 cells in response to paraquat



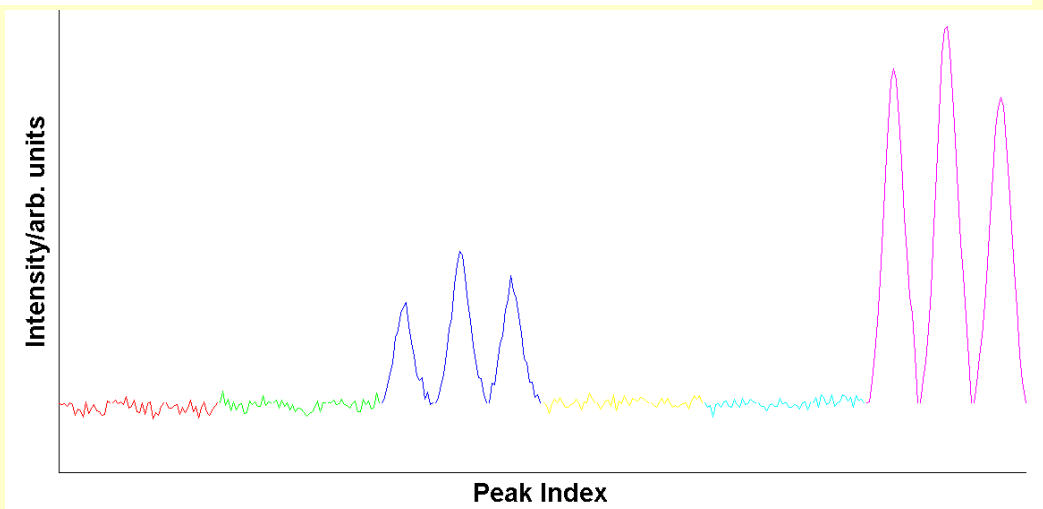
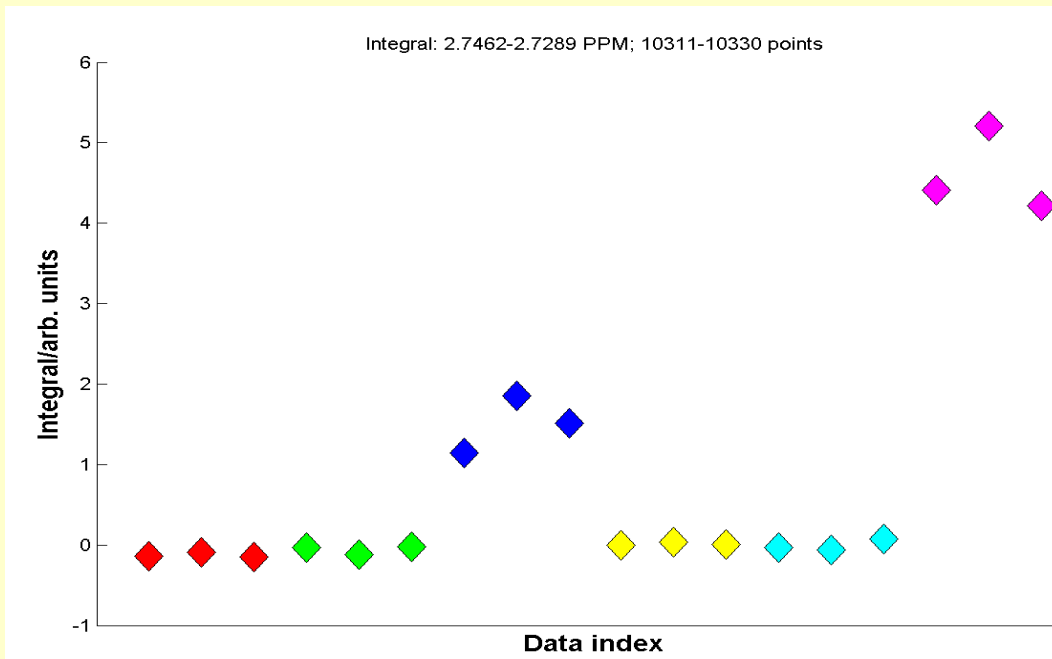
10-fold increase in IL-8 production in presence of paraquat

Cytokine production by HepG2 cells in response to 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*