

PRACTICALS: USING DATABASES FOR IDENTIFICATION OF PROTEINS ON 2D-GELS

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060203

METHODS:

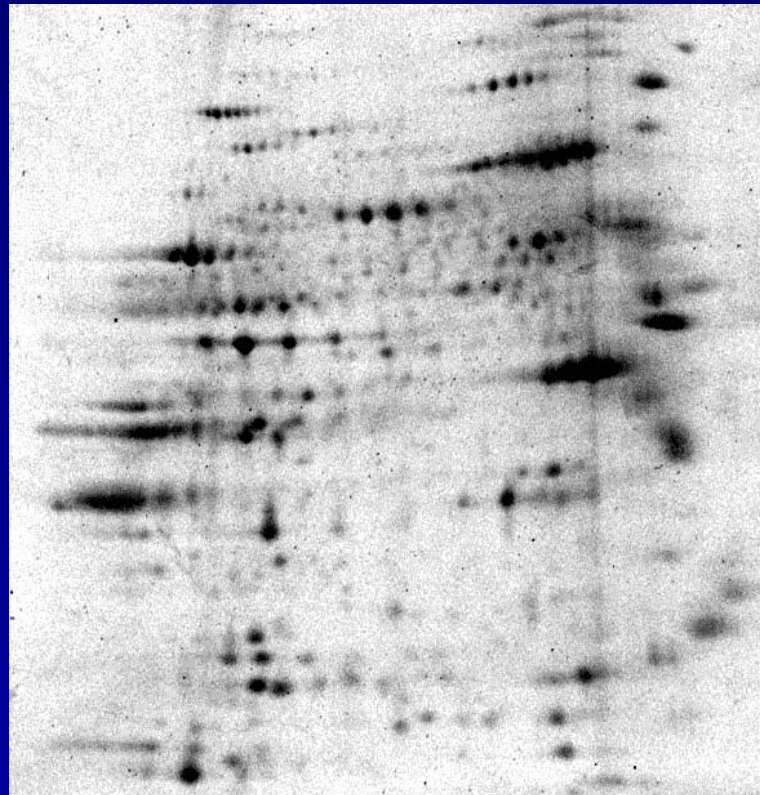
sample preparation: Solubilize proteins, prefractionation...

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2D gel electrophoresis



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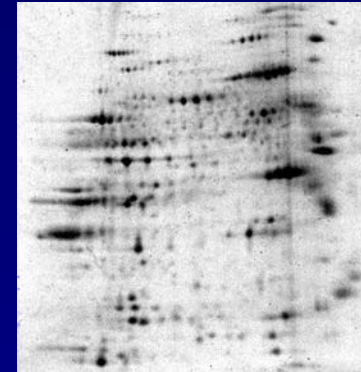
sample preparation: Solubilize proteins, prefractionation...



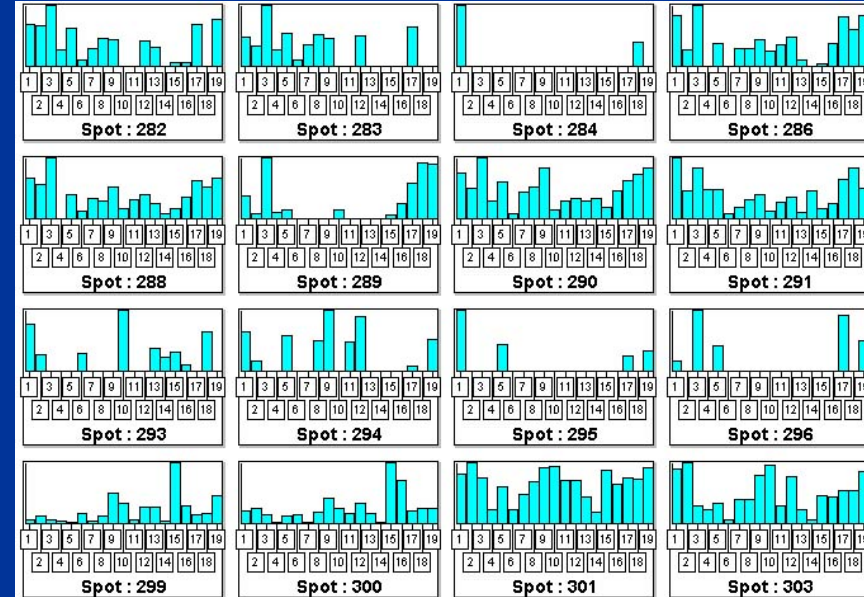
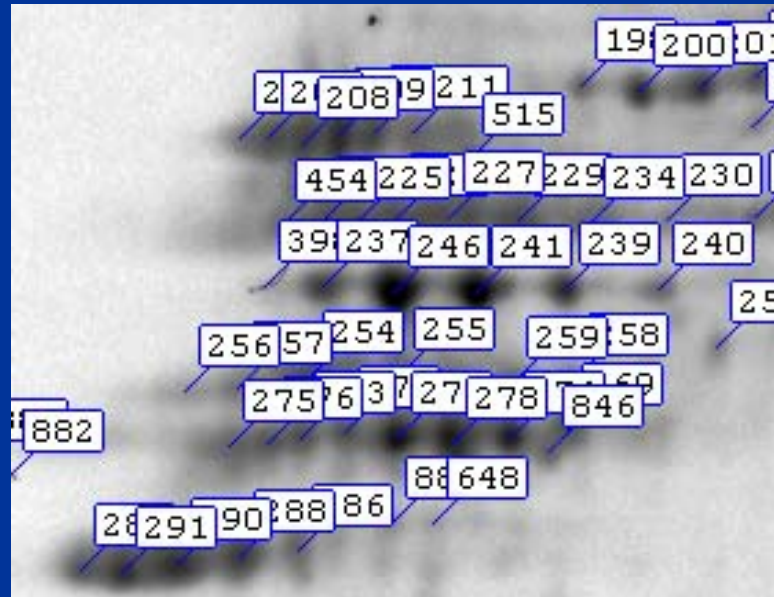
2D gel electrophoresis



protein staining



spot detection, matching, quantification...



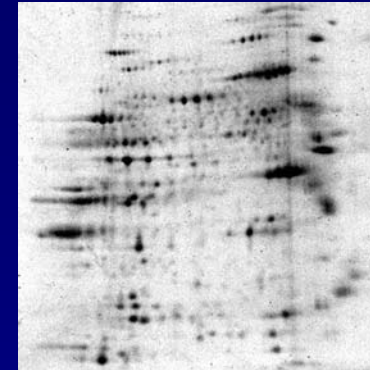
2D image analysis
and statistics

METHODS:

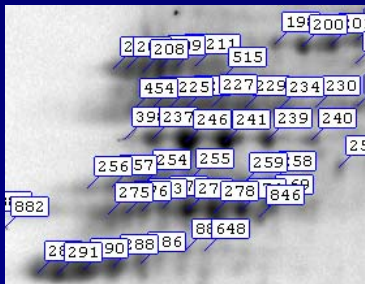
sample preparation: Solubilize proteins, prefractionation...



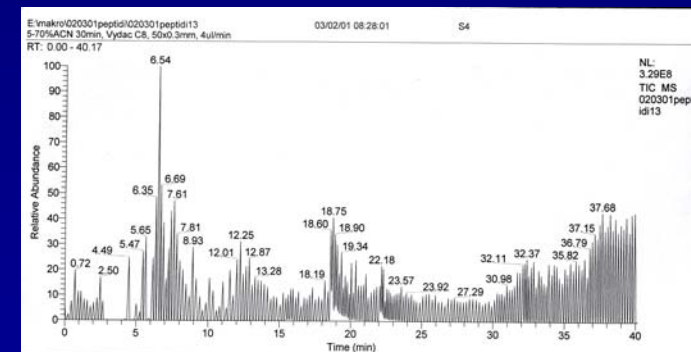
2D gel electrophoresis



protein staining



2D image analysis
and statistics



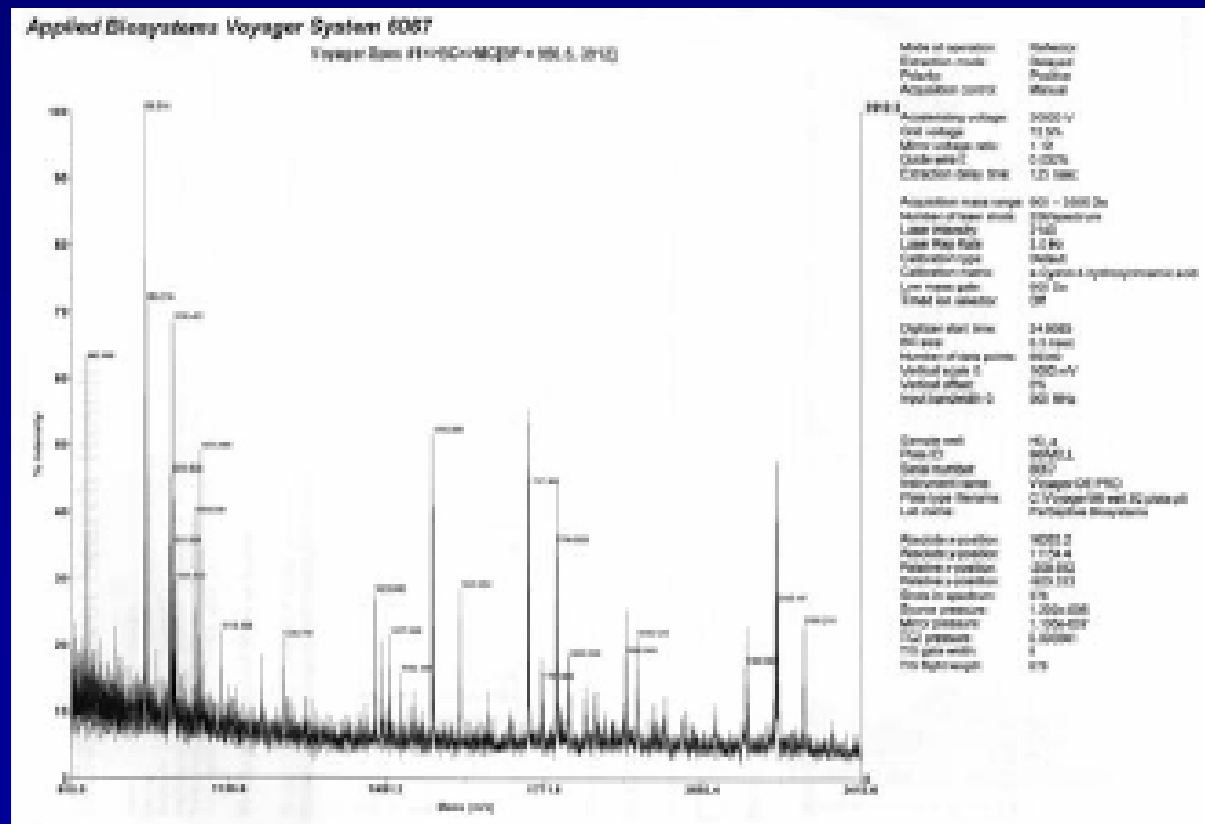
In-gel trypsin digestion and
identification of proteins
by mass spectrometry and
database searches

METHODS:

For the identification of proteins:
[http://www3.btk.utu.fi:
8080/Genomics/Proteomics/Protocols](http://www3.btk.utu.fi:8080/Genomics/Proteomics/Protocols)

PEPTIDE MASS FINGERPRINTING:

Identification of proteins according to molecular weights of peptides of the protein created after in-gel digestion



PEPTIDE MASS FINGERPRINTING:

Database:

Mascot search (www.matrixscience.com)

Mascot > Peptide Mass Fingerprint

MASCOT Peptide Mass Fingerprint

Your name Minna

Email Minna.Korolainen@uku.fi

Search title

Database NCBIInr

Taxonomy All entries

Enzyme Trypsin

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

Allow up to 1 missed cleavages

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

Protein mass kDa

Peptide tol. \pm 1 Da

Mass values MH⁺ M_r M-H⁻

Monoisotopic Average

Data file

Browse...

Query 956.51
NB Contents 963.51
of this field 1011.53
are ignored if 1196.65
a data file 1425.65
is specified. 1543.86

Overview

Report top 20 hits

Start Search ...

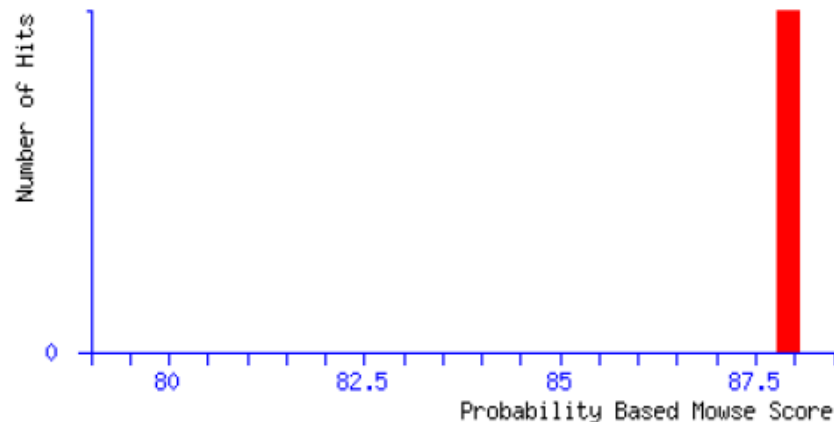
Reset Form

{MATRIX} Mascot Search Results *{SCIENCE}*

User : Minna
Email : Minna.Korolainen@uku.fi
Search title :
Database : NCBIInr 20060128 (3245378 sequences; 1113347412 residues)
Timestamp : 30 Jan 2006 at 12:24:34 GMT
Top Score : 88 for **gi|20151194**, Chain F, Structure Of Human Glutamate Dehyd

Probability Based Mowse Score

Protein score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event. Protein scores greater than 78 are significant ($p < 0.05$).



Concise Protein Summary Report

Format As

Concise Protein Summary

[Help](#)

Significance threshold $p < 0.05$

Max. number of hits 20

Re-Search All

Search Unmatched

- | | | | | |
|---|-------------|-----------|----------------|----------------|
| gi 20151194 | Mass: 55973 | Score: 88 | Expect: 0.0053 | Queries matc |
| Chain F, Structure Of Human Glutamate Dehydrogenase-Apo Form | | | | |
| gi 51863477 | Mass: 55946 | Score: 88 | Expect: 0.0053 | Queries matc |
| glutamate dehydrogenase [Cercopithecus aethiops sabaeus] | | | | |
| gi 14575683 | Mass: 56533 | Score: 87 | Expect: 0.006 | Queries match |
| glutamate dehydrogenase [synthetic construct] | | | | |
| gi 55957359 | Mass: 61359 | Score: 86 | Expect: 0.0076 | Queries matc |
| glutamate dehydrogenase 1 [Homo sapiens] | | | | |
| gi 62897195 | Mass: 61301 | Score: 86 | Expect: 0.0076 | Queries matc |
| glutamate dehydrogenase 1 variant [Homo sapiens] | | | | |
| gi 24849930 | Mass: 57853 | Score: 83 | Expect: 0.017 | Queries match |
| TAT-human glutamate dehydrogenase [synthetic construct] | | | | |
| gi 30749718 | Mass: 54916 | Score: 72 | Expect: 0.22 | Queries matche |
| Chain F, Crystal Structure Of The R463a Mutant Of Human Glutamate Dehydroge | | | | |

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 8
Number of mass values matched: 8
Sequence Coverage: 17%

Matched peptides shown in **Bold Red**

1	MYRYLGEALL	LSRAGPAALG	SASADSAALL	GWARGQPAAA	PQPGLALAAR
51	RHYSEAVADR	EDDPNFFKMV	EGFFDRGASI	VEDKLVEDLR	TRESEEQKRN
101	RVRGILRIIK	PCNHVLSLSF	PIRR DDGSWE	VIEGYRAQHS	QHRTPCKGGI
151	RYSTDVSVDE	VKALASLMTY	KCAVVDVPPG	GAKAGVKINP	KNYTDNELEK
201	ITRRFTMELA	KKGFIGPGID	VPAPDMSTGE	REMSWIADTY	ASTIGHYDIN
251	AHACVTGKPI	SQGGIHGRIS	ATGRGVFHGI	ENFINEASYM	SILGMTPGFG
301	DKTFVVQGFG	NVGLHSMRYL	HFRGAKCIAV	GESDGSIWNP	DGIDPKELED
351	FKLQHGSILG	FPKAKPYEGS	ILEADCILIL	PAASEKQLTK	SNAPRVKAKI
401	IAEGANGPTT	PEADKIFLER	NIMVIPDLYL	NAGGVTVSYF	EWLKNLNHVS
451	YGRLLTFKYER	DSNYHLLMSV	QESLERKFGK	HGGTIPIVPT	AEFQDRISGA
501	SEKDIVHSGL	AYTMERSARQ	IMRTAMKYNL	GLDLR TAAYV	NAIEKVFKVY
551	NEAGVTFT				

{MATRIX} Mascot Search Results

Protein View

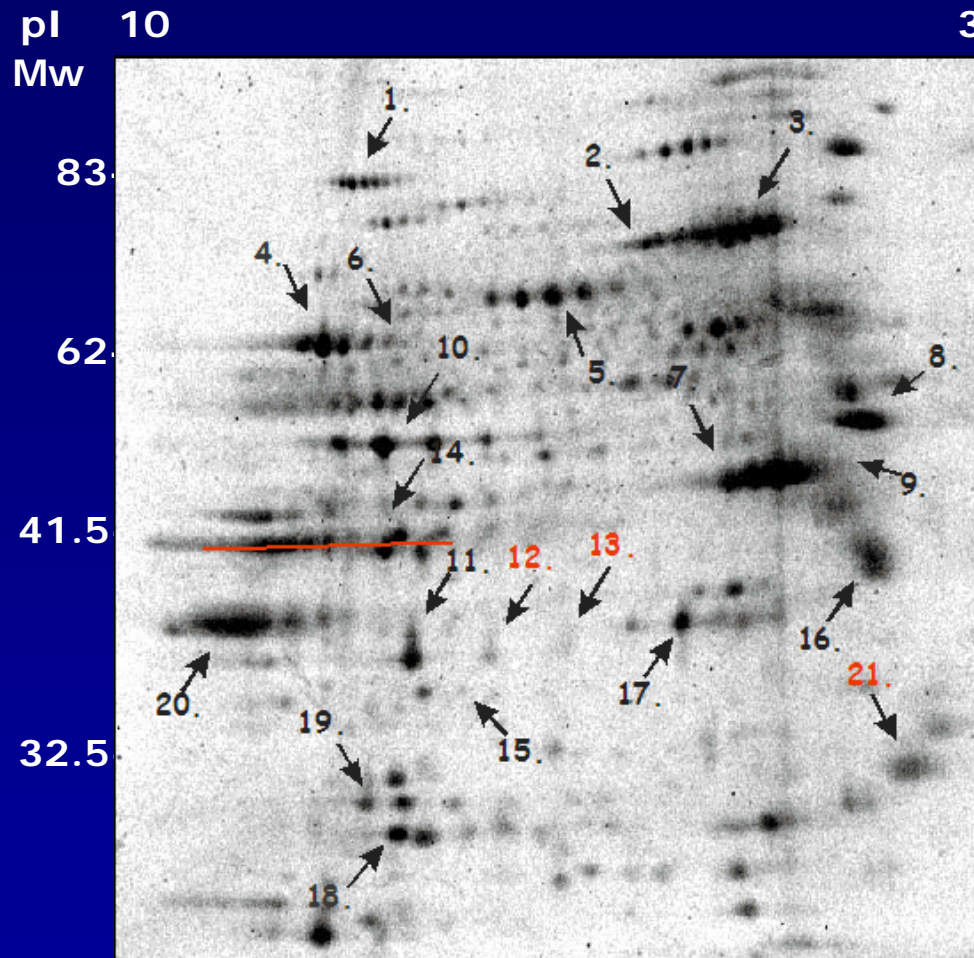
Match to: [gi|55957359](#) Score: 86 Expect: 0.0076
glutamate dehydrogenase 1 [Homo sapiens]

Nominal mass (M_r): 61359; Calculated pI value: 7.66

NCBI BLAST search of [gi|55957359](#) against nr

Unformatted [sequence string](#) for pasting into other applications

2DE protein databases



1. Q99798 Aconitase, mitochondrial
2. P02768 human serum albumin
3. P11142 heat shock cognate 71 kDa protein
4. P30613 human pyruvate kinase
5. Q16555 human dihydropyrimidinase related protein-2 (DRP-2)
6. P00367 Glutamate dehydrogenase
7. P0251 gamma-actin
8. P06576 ATP synthase beta chain, mitochondrial (precursor)
9. NOHUG human gamma-enolase
10. P06733 human alpha-enolase
11. and 12. P40925 Malate dehydrogenase
14. P04075 human fructose-bisphosphate aldolase A and C
15. gi14757780 Formylgluthathione hydrolase
16. P14136 glial fibrillary acidic protein, astrocyte (GFAP)
17. P07195 human L-lactate dehydrogenase B
18. Triosephosphate isomerase
19. P00915 Carbonic anhydrase Ii
20. P04406 human 3-phosphate dehydrogenase
21. 14-3-3 zeta/delta

<http://www.expasy.org>