Outils bio-informatiques pour la protéomique- Comparaison TheGPM/Xcalibur Sequest- Gelprint: génération de gels 2D in-silico

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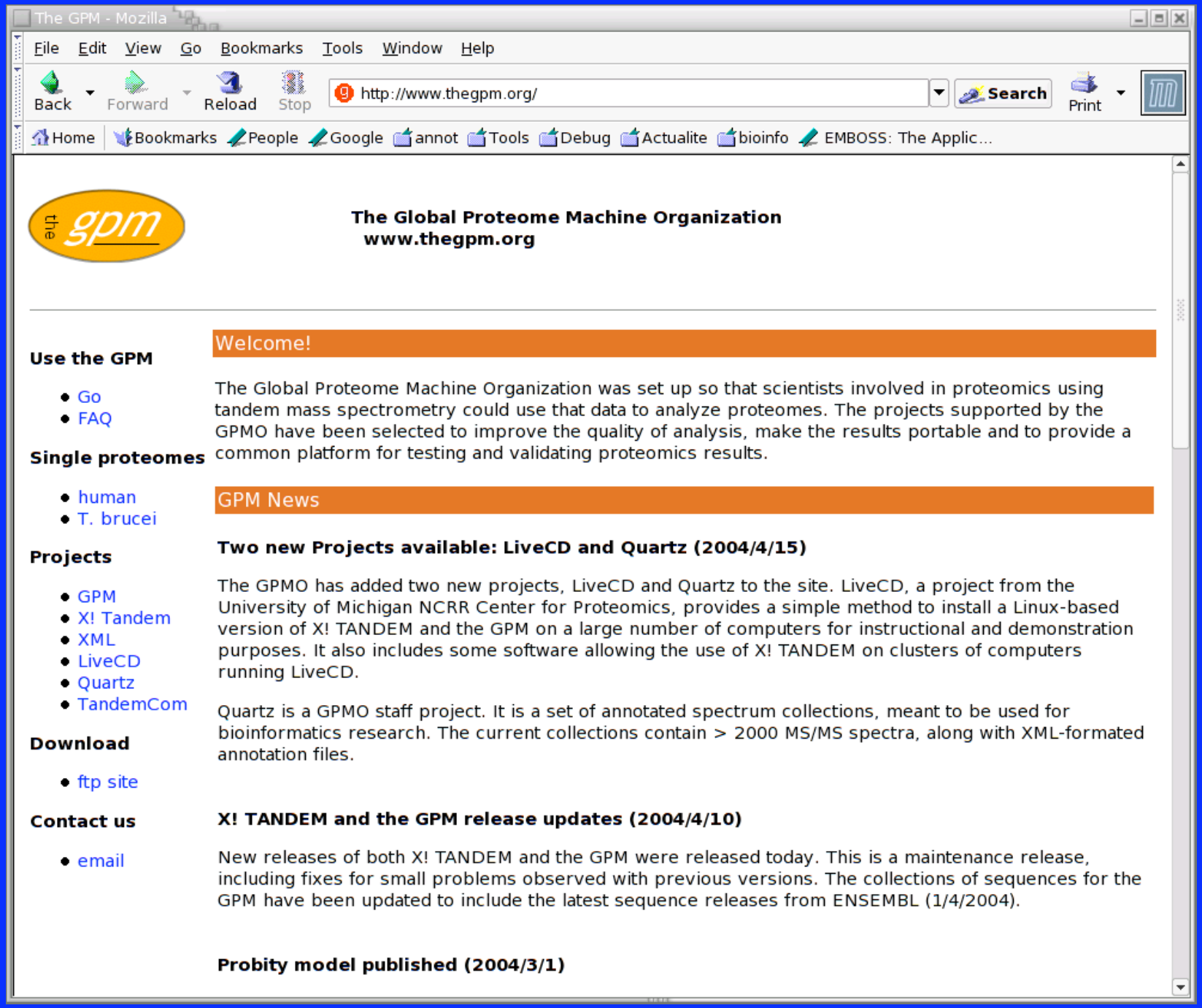
13402 Marseille cedex 20

De quoi parlerons nous?

- Présentation de TheGPM
- Mimivirus: un virus géant vivant dans les amibes
- Comparaison TheGPM/Sequest pour l'identification des protéines par spectrométrie de masse MS/MS


The GPM - Mozilla
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The Global Proteome Machine Organization
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Projects
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X! Tandem
XML
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Quartz
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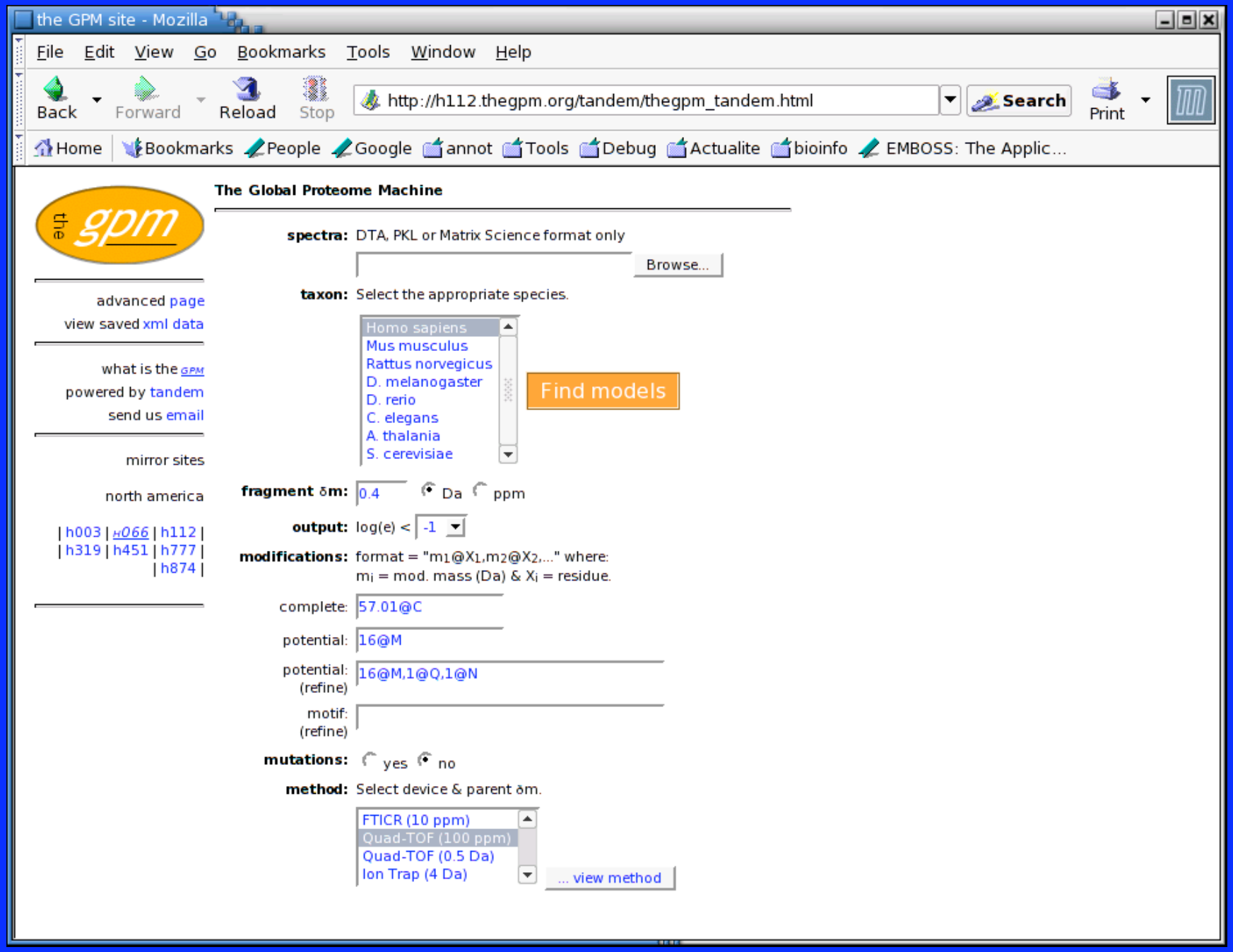
Welcome!
The Global Proteome Machine Organization was set up so that scientists involved in proteomics using tandem mass spectrometry could use that data to analyze proteomes. The projects supported by the GPMO have been selected to improve the quality of analysis, make the results portable and to provide a common platform for testing and validating proteomics results.
GPM News
Two new Projects available: LiveCD and Quartz (2004/4/15)
The GPMO has added two new projects, LiveCD and Quartz to the site. LiveCD, a project from the University of Michigan NCRR Center for Proteomics, provides a simple method to install a Linux-based version of X! TANDEM and the GPM on a large number of computers for instructional and demonstration purposes. It also includes some software allowing the use of X! TANDEM on clusters of computers running LiveCD.
Quartz is a GPMO staff project. It is a set of annotated spectrum collections, meant to be used for bioinformatics research. The current collections contain > 2000 MS/MS spectra, along with XML-formated annotation files.
X! TANDEM and the GPM release updates (2004/4/10)
New releases of both X! TANDEM and the GPM were released today. This is a maintenance release, including fixes for small problems observed with previous versions. The collections of sequences for the GPM have been updated to include the latest sequence releases from ENSEMBL (1/4/2004).
Probity model published (2004/3/1)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| h003 | h066 | h112 |
| h319 | h451 | h777 |
| h874 |
The Global Proteome Machine
spectra: DTA, PKL or Matrix Science format only
Browse...
taxon: Select the appropriate species.
Homo sapiens
Mus musculus
Rattus norvegicus
D. melanogaster
D. rerio
C. elegans
A. thalania
S. cerevisiae
Find models
fragment δm: 0.4 Da ppm
output: log(e) < -1
modifications: format = "m1@X1,m2@X2,..." where:
mi = mod. mass (Da) & Xi = residue.
complete: 57.01@C
potential: 16@M
potential: (refine) 16@M,1@Q,1@N
motif: (refine)
mutations: yes no
method: Select device & parent δm.
FTICR (10 ppm)
Quad-TOF (100 ppm)
Quad-TOF (0.5 Da)
Ion Trap (4 Da)
... view method

Par défaut GPM est configuré pour utiliser les protéomes suivants:

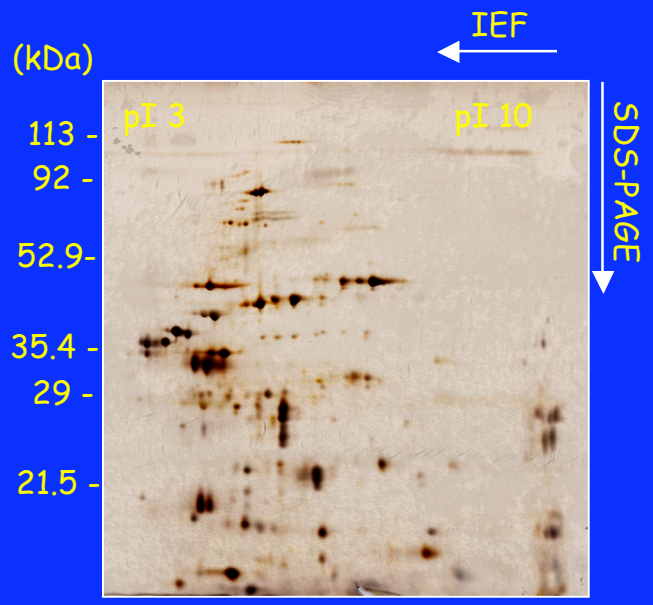
1. Human (34d NCBI 34, 10 May 2004)
2. Mouse (32b NCBI m32, 1 Apr. 2004)
3. Rat (RGSC 3.1b, 9 Feb. 2004)
4. Zebra fish (3b WTSI Zv3, 1 Apr. 2004)
5. Fruit fly (BGDP 3.1, 2 Jul. 2003)
6. C. elegans (116a WS 116, 1 Apr. 2004)
7. Yeast (SCD, 15 Nov 2003)
8. A. thalania (ATH1, v. 5.0, Jan 29, 2004)

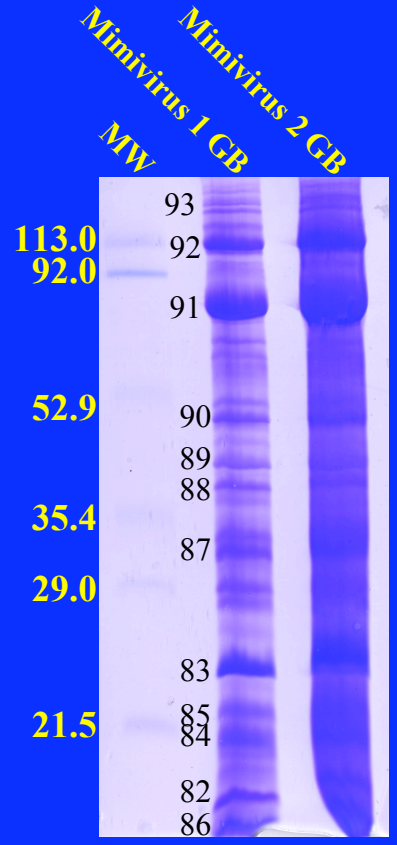
Et fonctionne sur une machine standard, sous Windows ou Linux: (nous avons teste linux)

1. 2.4 GHz Pentium IV processors;
2. 256 MB memory
3. 40 GB, 7200 rpm EIDE hard drive
4. Apache HTTP server, v. 2
5. ActiveState Perl

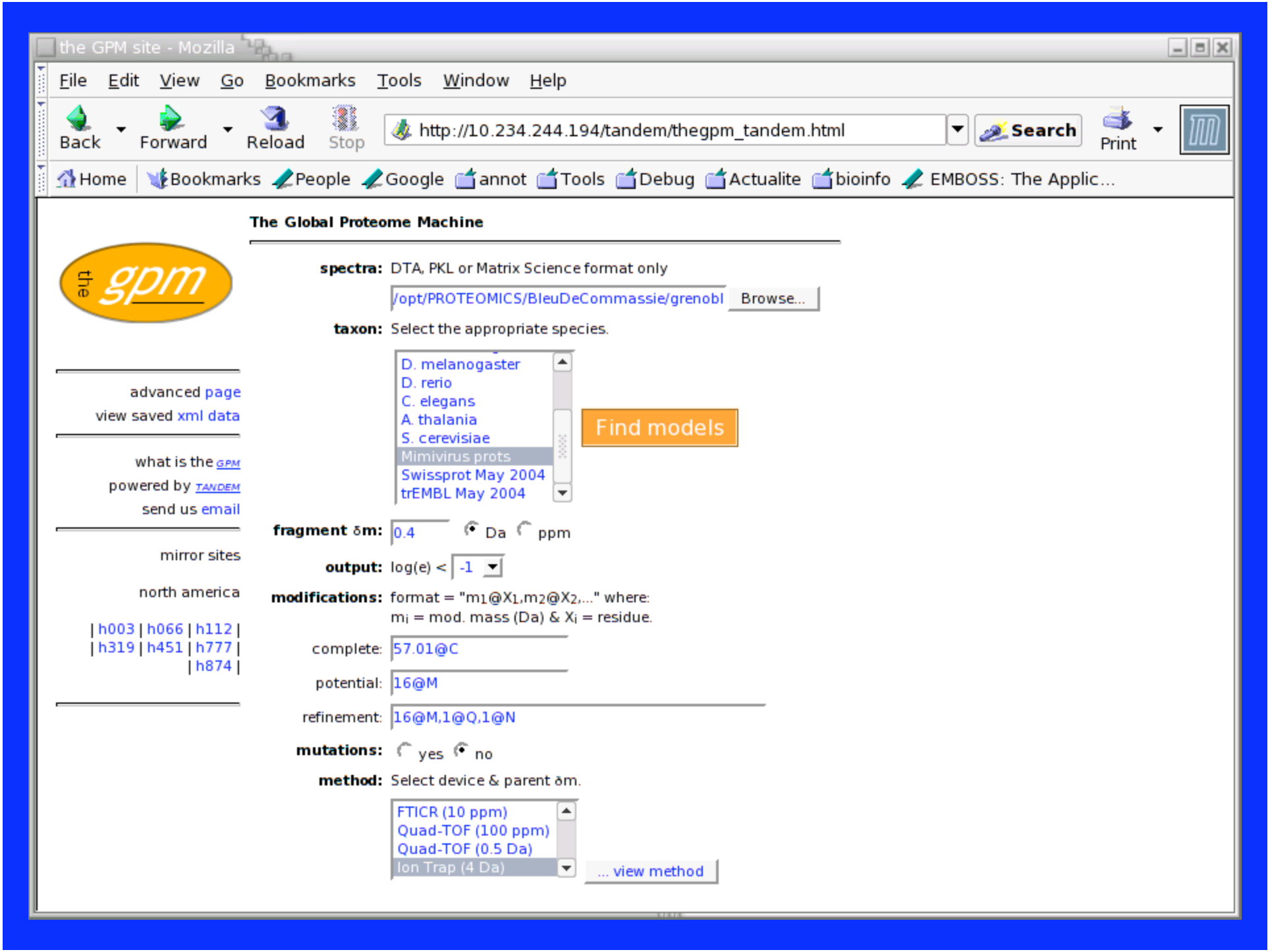
Notre expérience est basée sur des spots issu de gels:

- 2D coloré au nitrate d'argent
- 1 D coloré au bleu de Coomassie





Spots analysés sur une trappe à ions LCQ Deca XP plus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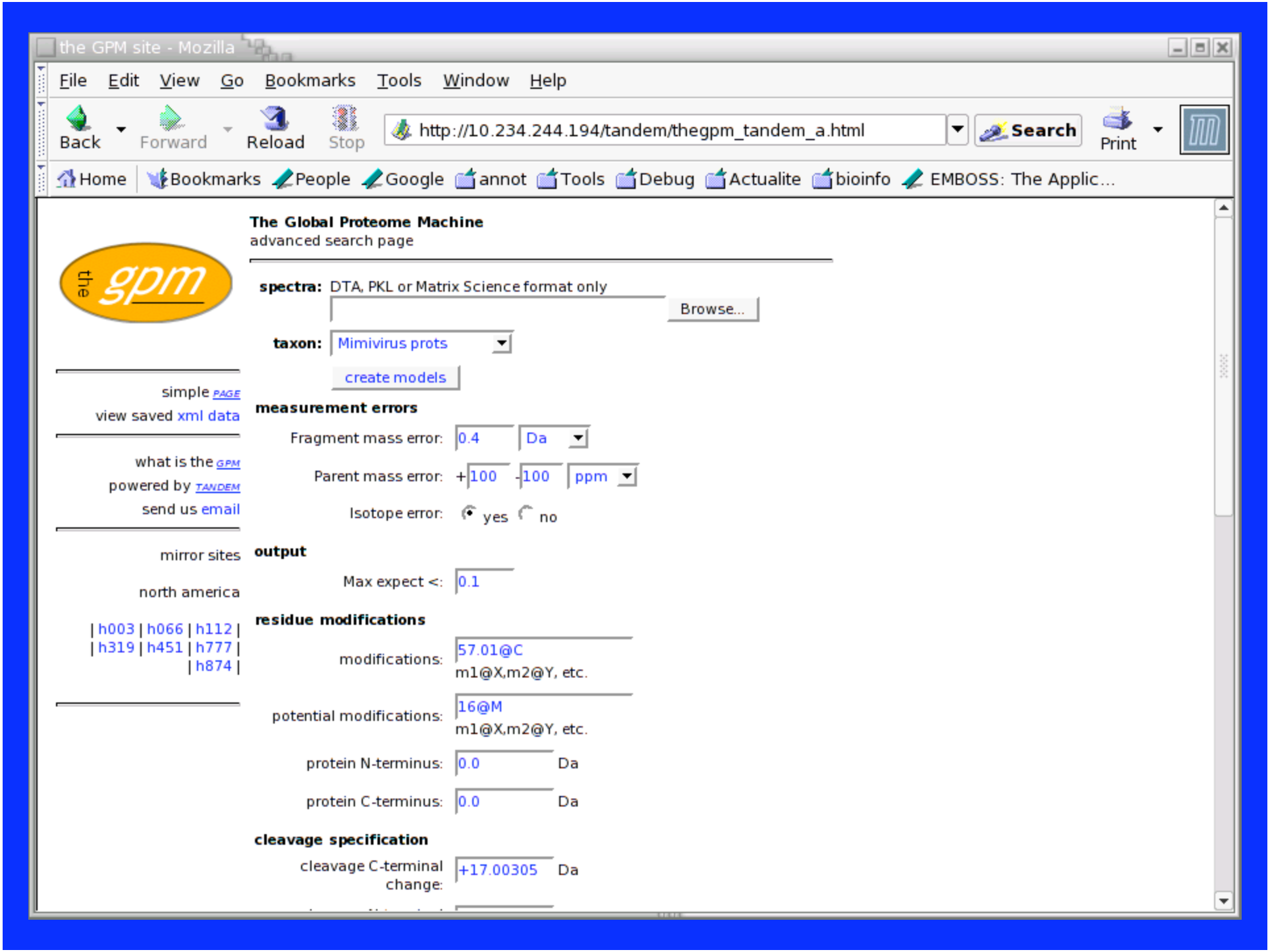
mirror sites
north america
| h003 | h066 | h112 |
| h319 | h451 | h777 |
| h874 |
The Global Proteome Machine
spectra: DTA, PKL or Matrix Science format only
/opt/PROTEOMICS/BleuDeCommassie/grenobl
Browse...
taxon: Select the appropriate species.
D. melanogaster
D. rerio
C. elegans
A. thalania
S. cerevisiae
Mimivirus prots
Swissprot May 2004
trEMBL May 2004
Find models
fragment δm: 0.4 Da ppm
output: log(e) < -1
modifications: format = "m1@X1,m2@X2,..." where:
mi = mod. mass (Da) & Xi = residue.
complete: 57.01@C
potential: 16@M
refinement: 16@M,1@Q,1@N
mutations: yes no
method: Select device & parent δm.
FTICR (10 ppm)
Quad-TOF (100 ppm)
Quad-TOF (0.5 Da)
Ion Trap (4 Da)
... view method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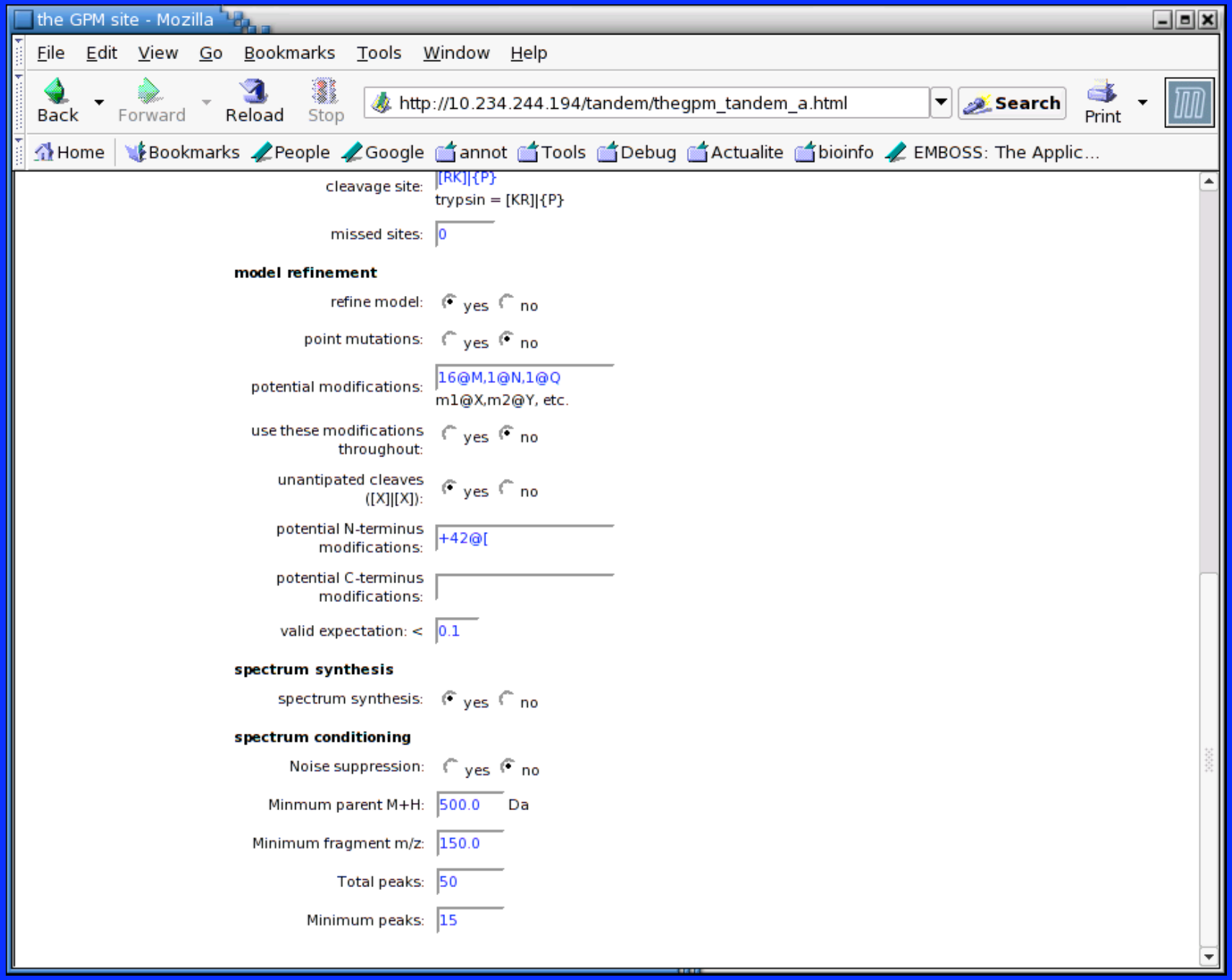
spectra: DTA, PKL or Matrix Science format only
Browse...
taxon: Mimivirus prots
create models
measurement errors
Fragment mass error: 0.4 Da
Parent mass error: +100 -100 ppm
Isotope error: yes no
output
Max expect <: 0.1
residue modifications
modifications: 57.01@C
m1@X,m2@Y, etc.
potential modifications: 16@M
m1@X,m2@Y, etc.
protein N-terminus: 0.0 Da
protein C-terminus: 0.0 Da
cleavage specification
cleavage C-terminal change: +17.00305 Da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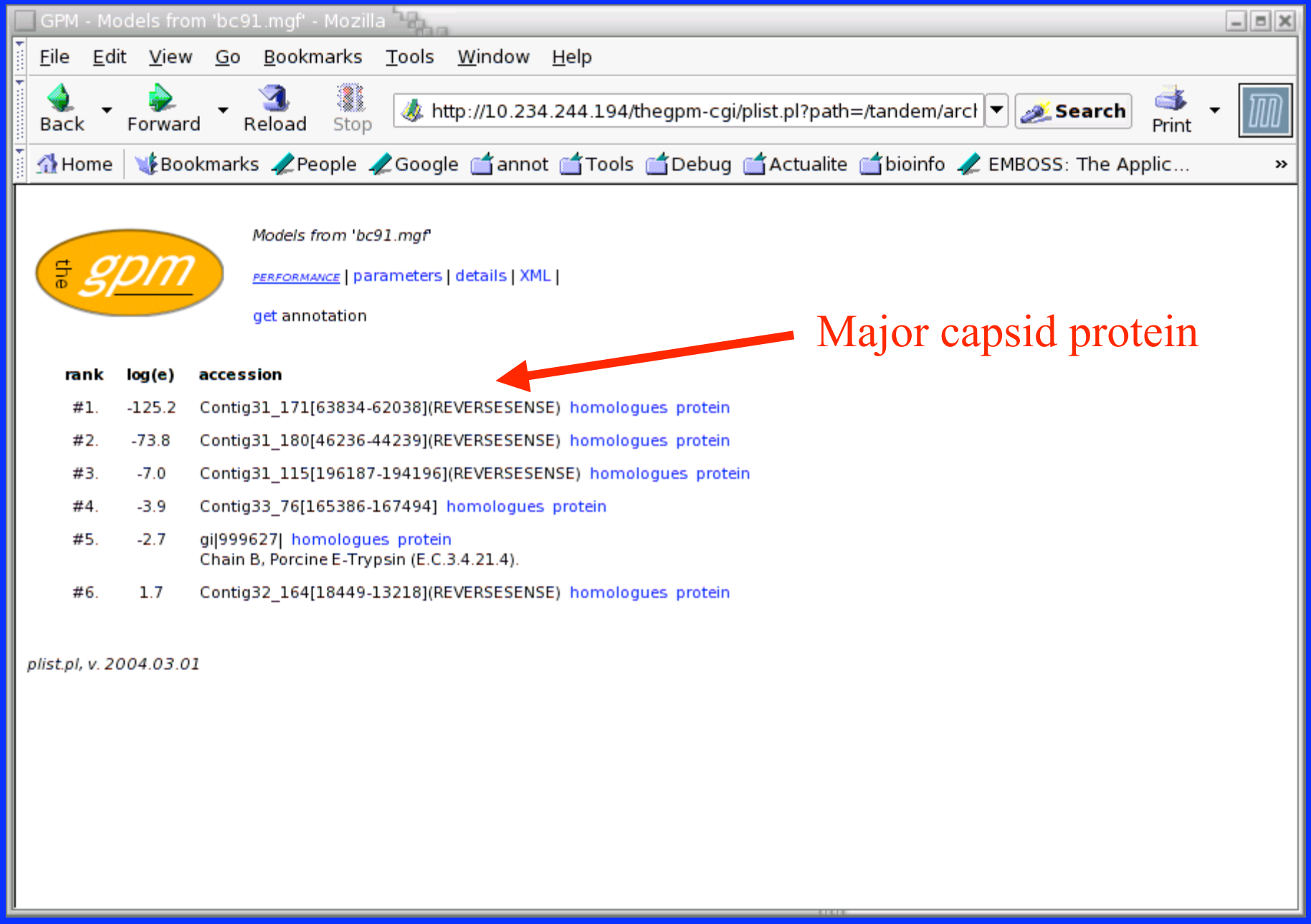
cleavage site: [RK]|{P}
trypsin = [KR]|{P}
missed sites: 0
model refinement
refine model: yes no
point mutations: yes no
potential modifications: 16@M,1@N,1@Q
m1@X,m2@Y, etc.
use these modifications throughout: yes no
unantipated cleaves ([X][X]): yes no
potential N-terminus modifications: +42@[
potential C-terminus modifications:
valid expectation: < 0.1
spectrum synthesis
spectrum synthesis: yes no
spectrum conditioning
Noise suppression: yes no
Minmum parent M+H: 500.0 Da
Minimum fragment m/z: 150.0
Total peaks: 50
Minimum peaks: 15


GPM - Models from 'bc91.mgf' - Mozilla
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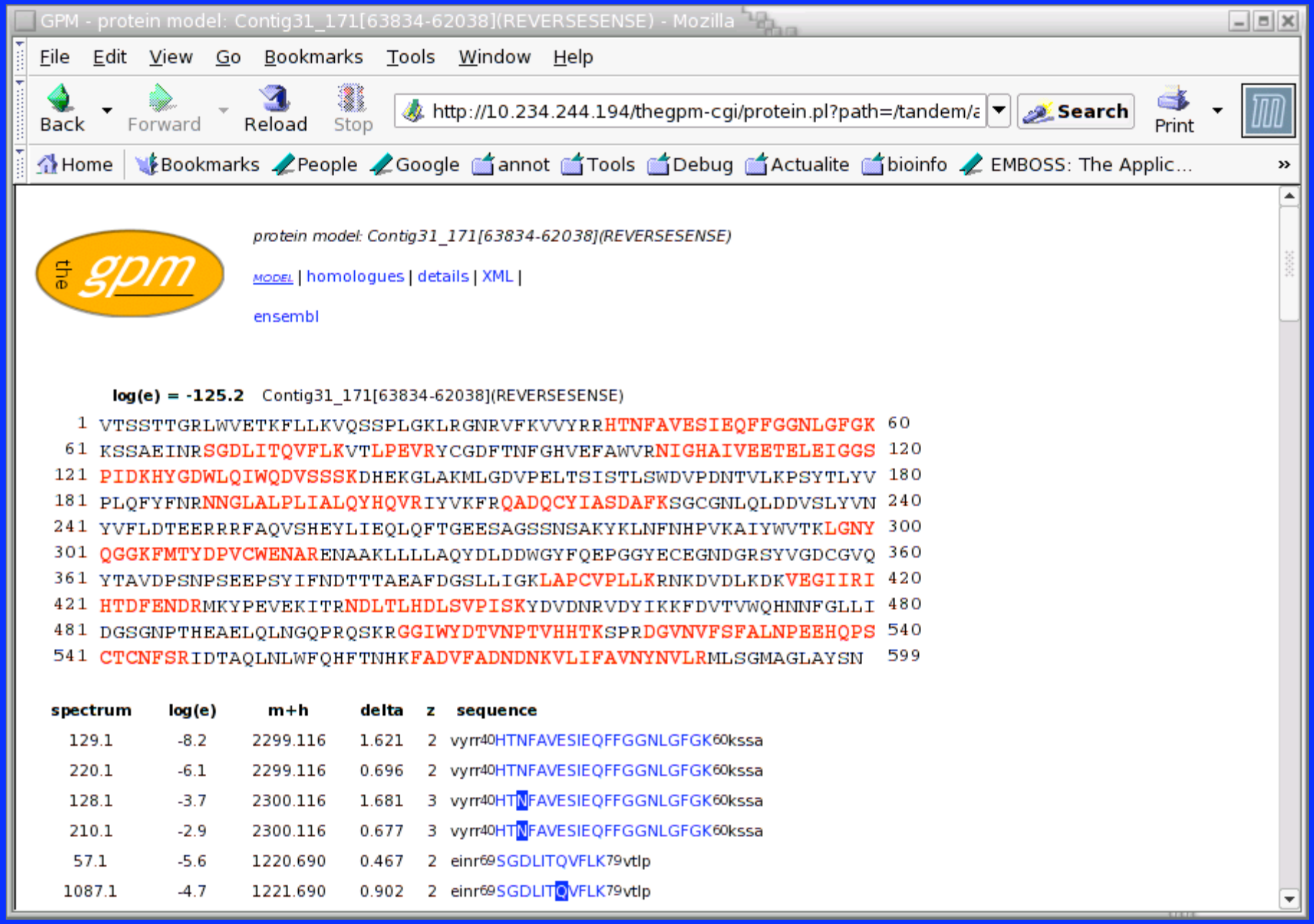
Models from 'bc91.mgf'
PERFORMANCE | parameters | details | XML |
get annotation
Major capsid protein
rank log(e) accession
#1. -125.2 Contig31_171[63834-62038](REVERSESENSE) homologues protein
#2. -73.8 Contig31_180[46236-44239](REVERSESENSE) homologues protein
#3. -7.0 Contig31_115[196187-194196](REVERSESENSE) homologues protein
#4. -3.9 Contig33_76[165386-167494] homologues protein
#5. -2.7 gi|999627| homologues protein
Chain B, Porcine E-Trypsin (E.C.3.4.21.4).
#6. 1.7 Contig32_164[18449-13218](REVERSESENSE) homologues protein
plist.pl, v. 2004.03.01


GPM - protein model: Contig31_171[63834-62038](REVERSESENSE) - Mozilla
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the gpm
protein model: Contig31_171[63834-62038](REVERSESENSE)
MODEL | homologues | details | XML |
ensembl
log(e) = -125.2 Contig31_171[63834-62038](REVERSESENSE)
1 VTSSTTGRLWVETKFLLKVQSSPLGKLRGNRVFKVVYRRHTNFAVESIEQFFGGNLGFGK 60
61 KSSAEINRSGDLITQVFLKVTLPEVRYCGDFTNFGHVEFAWVRNIGHAIVEETELEIGGS 120
121 PIDKHYGDWLQIWQDVSSSKDHEKGLAKMLGDVPELTSISTLSWDVPDNTVLKPSYTLYV 180
181 PLQFYFNRNNGLALPLIALQYHQVRIYVKFRQADQCYIASDAFKSGCGNLQLDDVSLYVN 240
241 YVFLDTEERRRFAQVSHEYLIEQLQFTGEESAGSSNSAKYKLNFNHPVKAIYWVTKLGNY 300
301 QGGKFMTYDPVCWENARENAAKLLLLAQYDLDDWGYFQEPGGYECEGNDGRSYVGDCGVQ 360
361 YTAVDPSNPSEEPSYIFNDTTTAEAFDGSLLIGKLAPCVPLLKRNKDVDLKDKVEGIIRI 420
421 HTDFENDRMKYPEVEKITRNDLTLHDLSVPISKYDVDNRVDYIKKFDVTVWQHNNFGLLI 480
481 DGSGNPTHEAELQLNGQPRQSKRGGIWYDTVNPTVHHTKSPRDGVNVFSFALNPEEHQPS 540
541 CTCNFSRIDTAQLNLWFQHFTNHKFADVFADNDNKVLIFAVNYNVLRMLSGMAGLAYSN 599
spectrum log(e) m+h delta z sequence
129.1 -8.2 2299.116 1.621 2 vyrr40HTNFAVESIEQFFGGNLGFGK60kssa
220.1 -6.1 2299.116 0.696 2 vyrr40HTNFAVESIEQFFGGNLGFGK60kssa
128.1 -3.7 2300.116 1.681 3 vyrr40HTNFAVESIEQFFGGNLGFGK60kssa
210.1 -2.9 2300.116 0.677 3 vyrr40HTNFAVESIEQFFGGNLGFGK60kssa
57.1 -5.6 1220.690 0.467 2 einr69SGDLITQVFLK79vtlp
1087.1 -4.7 1221.690 0.902 2 einr69SGDLITQVFLK79vtlp

GPM - peptide model: 129.1.1 of Contig31_171[63834-62038](REVERSESENSE) - Mozilla

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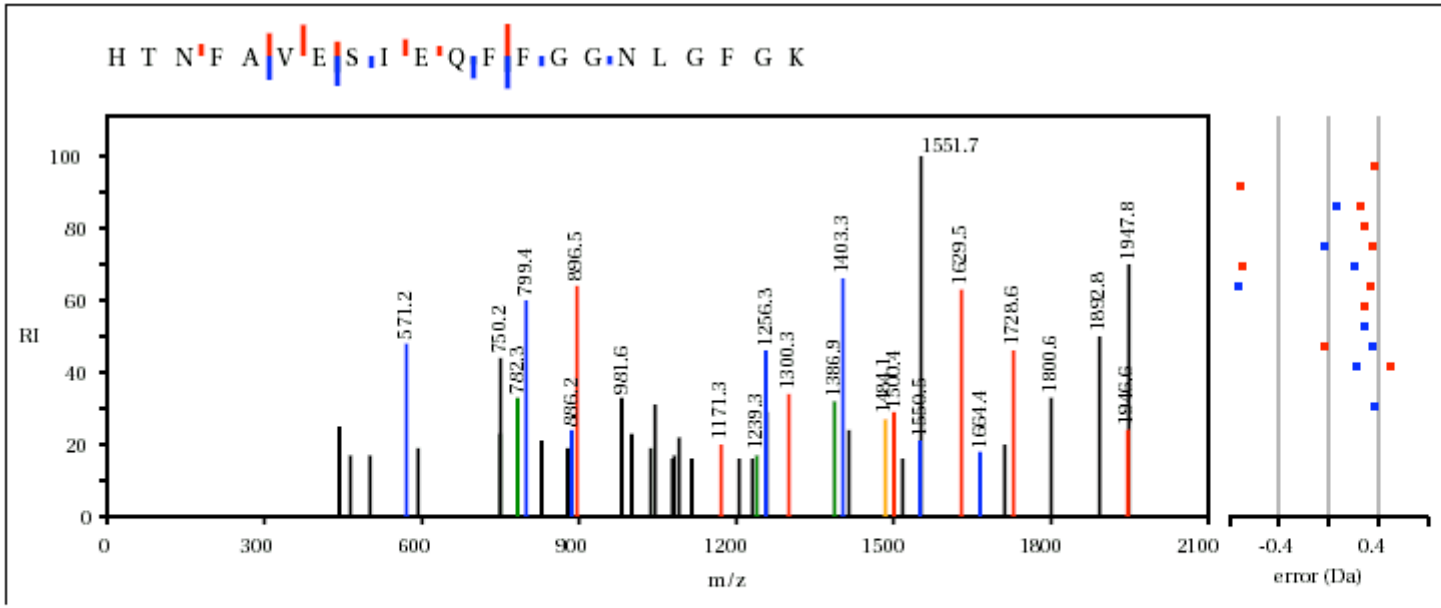
peptide model: 129.1.1 of Contig31_171[63834-62038](REVERSESENSE)

MODEL | PROTEIN | HOMOLOGUES | XML |

ADOBE SVG PLUGIN required to view spectrum

#	log(e)	m+h	delta	z	sequence
129	-8.2	2299.116	1.621	2	40HTNFAVESIEQFFGGNLGFGK60

BC91_01_010.1248.1250.2.dta



bond	+1y	+1y*	+1b	+1b*
H1	2162.055	2145.039	138.062	121.046
T2	2061.008	2043.992	239.110	222.094
N3	**1946.965**	1929.949	353.153	336.137
F4	1799.896	1782.880	500.221	483.205

GPM - peptide model: 129.1.1 of Contig31_171[63834-62038](REVERSESENSE) - Mozilla

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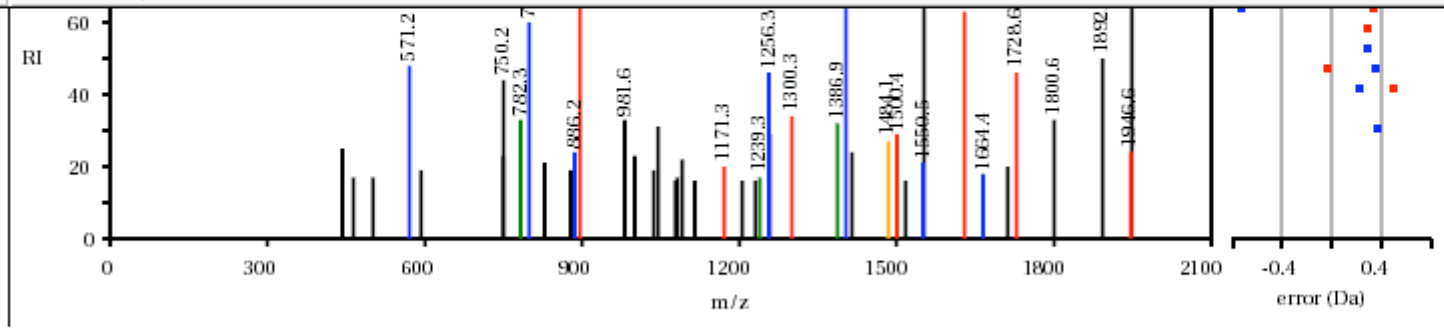
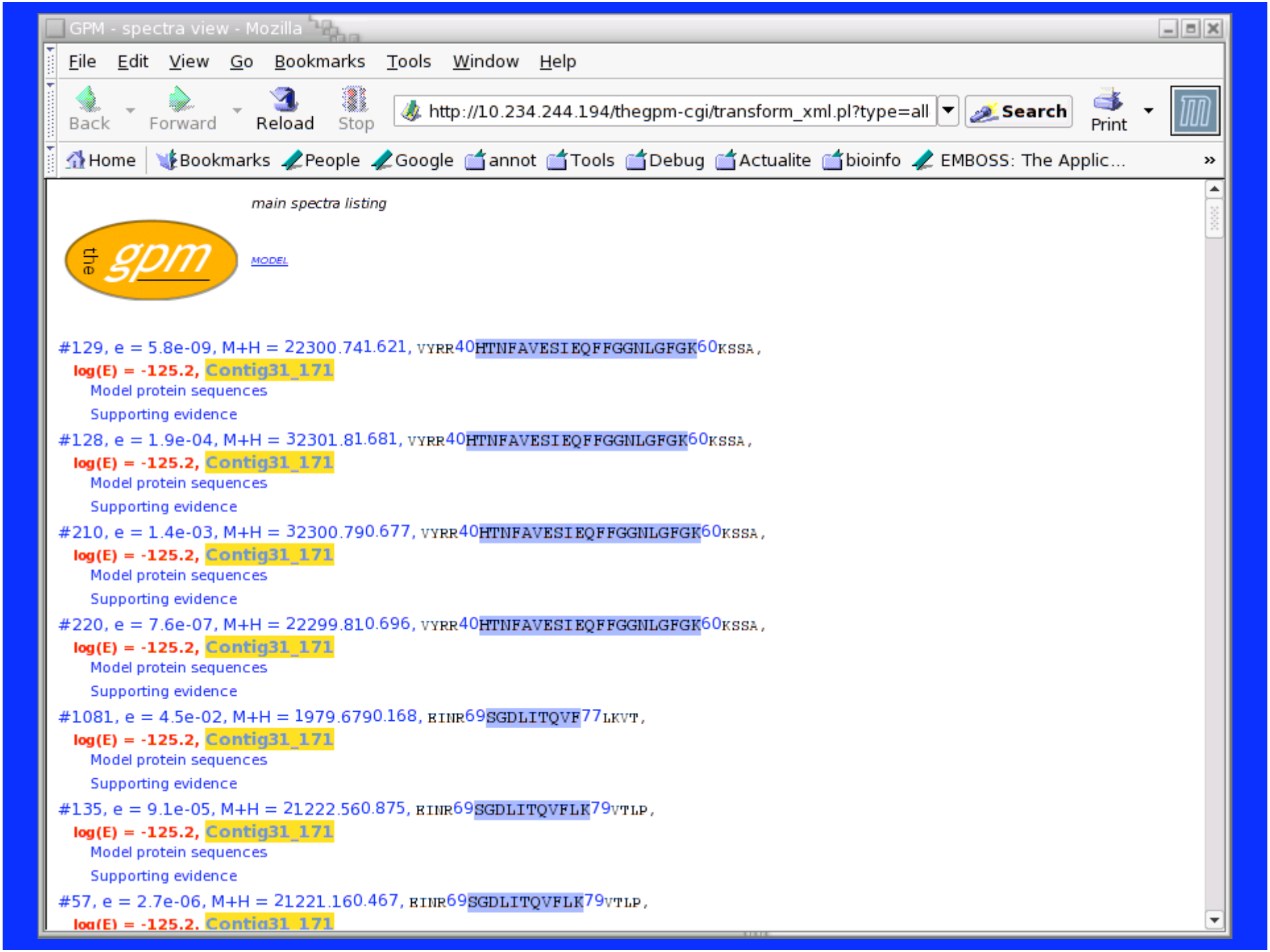


bond	+1y	+1y*	+1b	+1b*
H1	2162.055	2145.039	138.062	121.046
T2	2061.008	2043.992	239.110	222.094
N3	**1946.965**	1929.949	353.153	336.137
F4	1799.896	1782.880	500.221	483.205
A5	**1728.859**	1711.843	**571.258**	554.242
V6	**1629.791**	1612.775	670.327	653.311
E7	**1500.748**	**1483.732**	**799.369**	**782.353**
S8	1413.716	1396.700	**886.401**	869.385
I9	**1300.632**	1283.616	999.485	982.469
E10	**1171.590**	1154.574	1128.528	1111.512
Q11	1043.531	1026.515	**1256.586**	**1239.570**
F12	**896.463**	879.447	**1403.655**	**1386.639**
F13	749.394	732.378	**1550.723**	1533.707
G14	692.373	675.357	1607.745	1590.729
G15	635.351	618.335	**1664.766**	1647.750
N16	521.308	504.292	1778.809	1761.793
L17	408.224	391.208	1891.893	1874.877
G18	351.203	334.187	1948.915	1931.899
F19	204.134	187.118	2095.983	2078.967
G20	147.113	130.097	2153.005	2135.989

peptide.pl, v. 2004.03.01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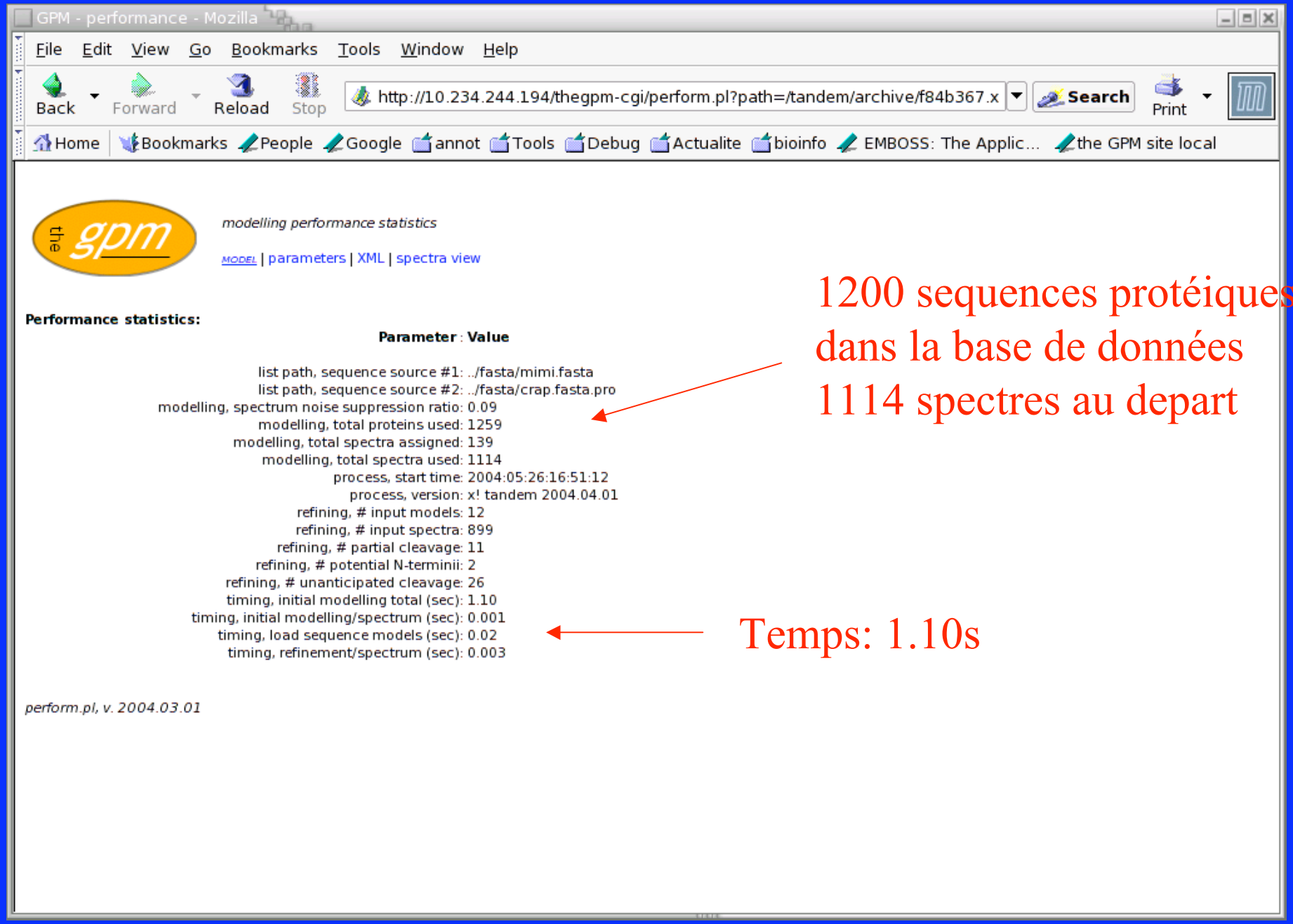
main spectra listing
the gpm
MODEL
#129, e = 5.8e-09, M+H = 22300.741.621, VYRR40HTNFAVESIEQFFGGNLGFGK60KSSA,
log(E) = -125.2, Contig31_171
Model protein sequences
Supporting evidence
#128, e = 1.9e-04, M+H = 32301.81.681, VYRR40HTNFAVESIEQFFGGNLGFGK60KSSA,
log(E) = -125.2, Contig31_171
Model protein sequences
Supporting evidence
#210, e = 1.4e-03, M+H = 32300.790.677, VYRR40HTNFAVESIEQFFGGNLGFGK60KSSA,
log(E) = -125.2, Contig31_171
Model protein sequences
Supporting evidence
#220, e = 7.6e-07, M+H = 22299.810.696, VYRR40HTNFAVESIEQFFGGNLGFGK60KSSA,
log(E) = -125.2, Contig31_171
Model protein sequences
Supporting evidence
#1081, e = 4.5e-02, M+H = 1979.6790.168, EINR69SGDLITQVF77LKVT,
log(E) = -125.2, Contig31_171
Model protein sequences
Supporting evidence
#135, e = 9.1e-05, M+H = 21222.560.875, EINR69SGDLITQVFLK79VTLP,
log(E) = -125.2, Contig31_171
Model protein sequences
Supporting evidence
#57, e = 2.7e-06, M+H = 21221.160.467, EINR69SGDLITQVFLK79VTLP,
log(E) = -125.2, Contig31_171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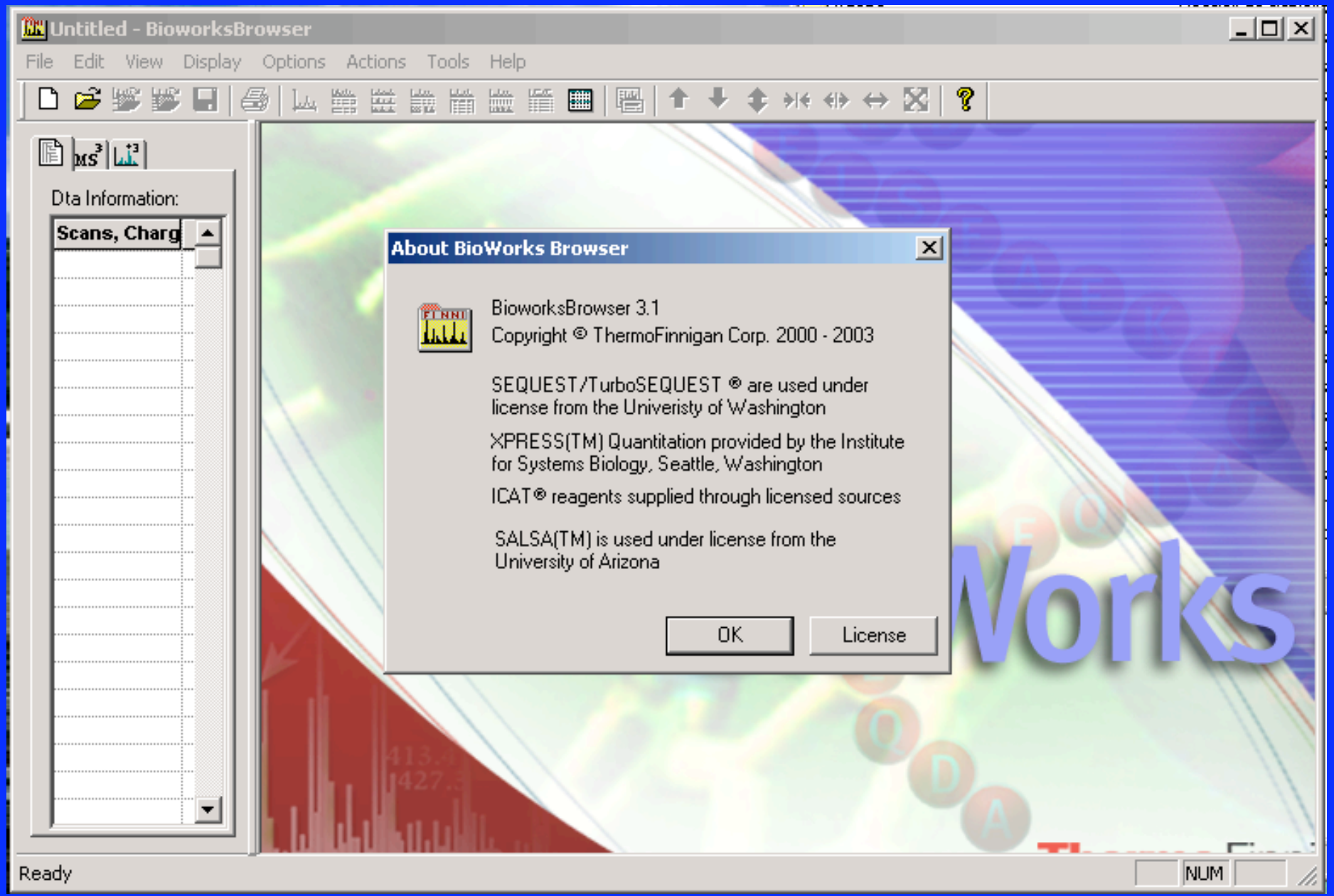
the gpm
modelling performance statistics
MODEL | parameters | XML | spectra view
Performance statistics:
Parameter : Value
list path, sequence source #1: ../fasta/mimi.fasta
list path, sequence source #2: ../fasta/crap.fasta.pro
modelling, spectrum noise suppression ratio: 0.09
modelling, total proteins used: 1259
modelling, total spectra assigned: 139
modelling, total spectra used: 1114
process, start time: 2004:05:26:16:51:12
process, version: x! tandem 2004.04.01
refining, # input models: 12
refining, # input spectra: 899
refining, # partial cleavage: 11
refining, # potential N-terminii: 2
refining, # unanticipated cleavage: 26
timing, initial modelling total (sec): 1.10
timing, initial modelling/spectrum (sec): 0.001
timing, load sequence models (sec): 0.02
timing, refinement/spectrum (sec): 0.003
perform.pl, v. 2004.03.01
1200 sequences protéiques
dans la base de données
1114 spectres au depart
Temps: 1.10s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Scans, Charg
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Dta Information: 1114

Scans, Charg
2, +1
4 - 6, +1
12, +1
14, +1
16, +1
24 - 26, +1
28, +2
28, +3
40, +2
40, +3
74, +1
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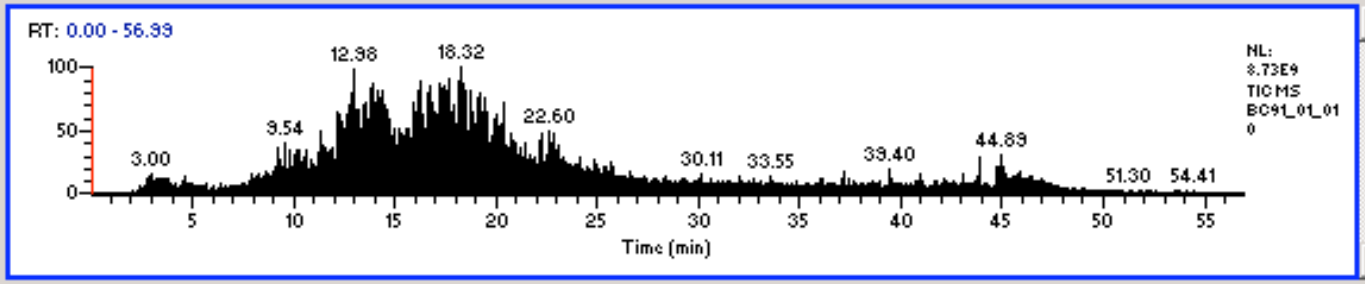
82, +1
86, +1
88, +2
88, +3
90, +1
92, +1
142 - 144, +2

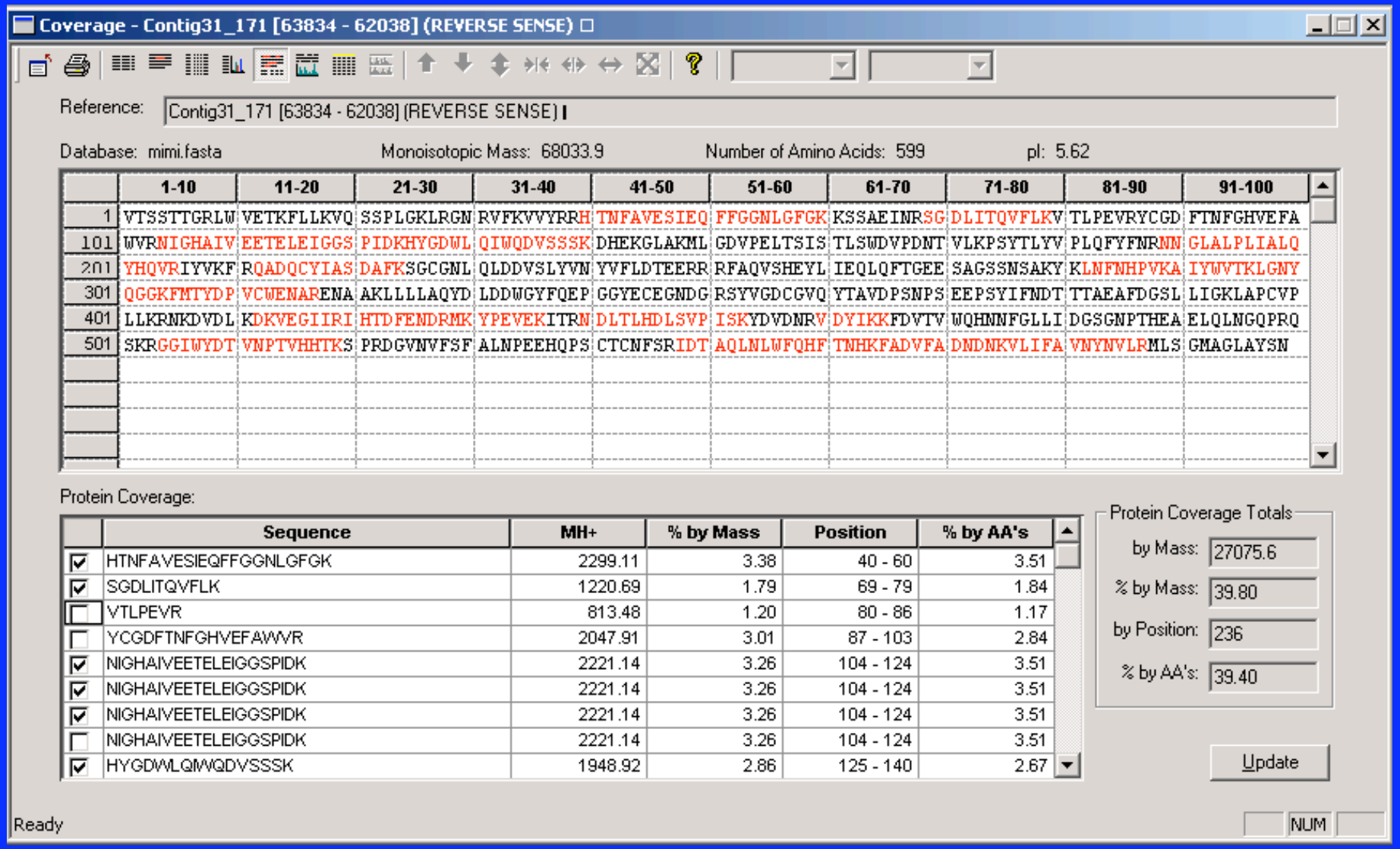


Number of traces: 1

Filter: single, xc (+/- 1,2,3)=1.50,2.00,2.50

	Reference					Score	Accessior	
	Scan(s)	Sequence	MH+	Charge	XC	Delta Cn	Sp	
#1	**Contig31_171 [63834 - 62038] (REVERSE SENSE)**					**1168.3**		
	142 - 144	-.LGNYQGGK.	836.43	2	2.474	0.560	511.8	
	150 - 152	-.LGNYQGGK.	836.43	1	2.131	0.375	273.9	
	316	-.VDYIKK.-	765.45	1	1.921	0.339	428.4	
	322	-.LGNYQGGK.	836.43	1	1.665	0.108	195.8	
	374 - 376	-.YPEVEK.-	764.38	1	1.897	0.252	290.7	
	446 - 448	-.IHTDFENDR.-	1146.52	2	2.627	0.663	360.4	
	452	-.IHTDFENDR.-	1146.52	1	1.524	0.287	202.3	
	458	-.IHTDFENDR.-	1146.52	1	2.326	0.622	458.5	
	462 - 464	-.VEGIIR.-	686.42	1	1.657	0.374	297.9	
	466	-.VEGIIR.-	686.42	2	2.139	0.455	507.8	
	486	-.MKYPEVEK.-	1023.52	3	2.584	0.278	904.1	

Ready NUM


Coverage - Contig31_171 [63834 - 62038] (REVERSE SENSE)
Reference:
Contig31_171 [63834 - 62038] (REVERSE SENSE)
Database: mimi.fasta
Monoisotopic Mass: 68033.9
Number of Amino Acids: 599
pI: 5.62
1-10 11-20 21-30 31-40 41-50 51-60 61-70 71-80 81-90 91-100
1 VTSSTTGRLW VETKFLLKVQ SSPLGKLRGN RVFKVVYRRH TNFAVESIEQ FFGGNLGFGK KSSAEINRSG DLITQVFLKV TLPEVRYCGD FTNFGHVEFA
101 WVRNIGHAIV EETELEIGGS PIDKHYGDWL QIWQDVSSSK DHEKGLAKML GDVPELTSIS TLSWDVPDNT VLKPSYTLYV PLQFYFNRNN GLALPLIALQ
201 YHQVRIYVKF RQADQCYIAS DAFKSGCGNL QLDDVSLYVN YVFLDTEERR RFAQVSHEYL IEQLQFTGEE SAGSSNSAKY KLNFNHPVKA IYWVTKLGNY
301 QGGKFMTYDP VCWENARENA AKLLLLAQYD LDDWGYFQEP GGYECEGNDG RSYVGDCGVQ YTAVDPSNPS EEPSYIFNDT TTAEAFDGSL LIGKLAPCVP
401 LLKRNKDVDL KDKVEGIIRI HTDFENDRMK YPEVEKITRN DLTLHDLSVP ISKYDVDNRV DYIKKFDVTV WQHNNFGLLI DGSGNPTHEA ELQLNGQPRQ
501 SKRGGIWYDT VNPTVHHTKS PRDGVNVFSF ALNPEEHQPS CTCNFSRIDT AQLNLWFQHF TNHKFADVFA DNDNKVLIFA VNYNVLRMLS GMAGLAYSN
Protein Coverage:
Sequence MH+ % by Mass Position % by AA's
HTNFAVESIEQFFGGNLGFGK 2299.11 3.38 40 - 60 3.51
SGDLITQVFLK 1220.69 1.79 69 - 79 1.84
VTLPEVR 813.48 1.20 80 - 86 1.17
YCGDFTNFGHVEFAWVR 2047.91 3.01 87 - 103 2.84
NIGHAIVEETELEIGGSPIDK 2221.14 3.26 104 - 124 3.51
NIGHAIVEETELEIGGSPIDK 2221.14 3.26 104 - 124 3.51
NIGHAIVEETELEIGGSPIDK 2221.14 3.26 104 - 124 3.51
NIGHAIVEETELEIGGSPIDK 2221.14 3.26 104 - 124 3.51
HYGDWLQIWQDVSSSK 1948.92 2.86 125 - 140 2.67
Protein Coverage Totals
by Mass: 27075.6
% by Mass: 39.80
by Position: 236
% by AA's: 39.40
Update
Ready
NUM

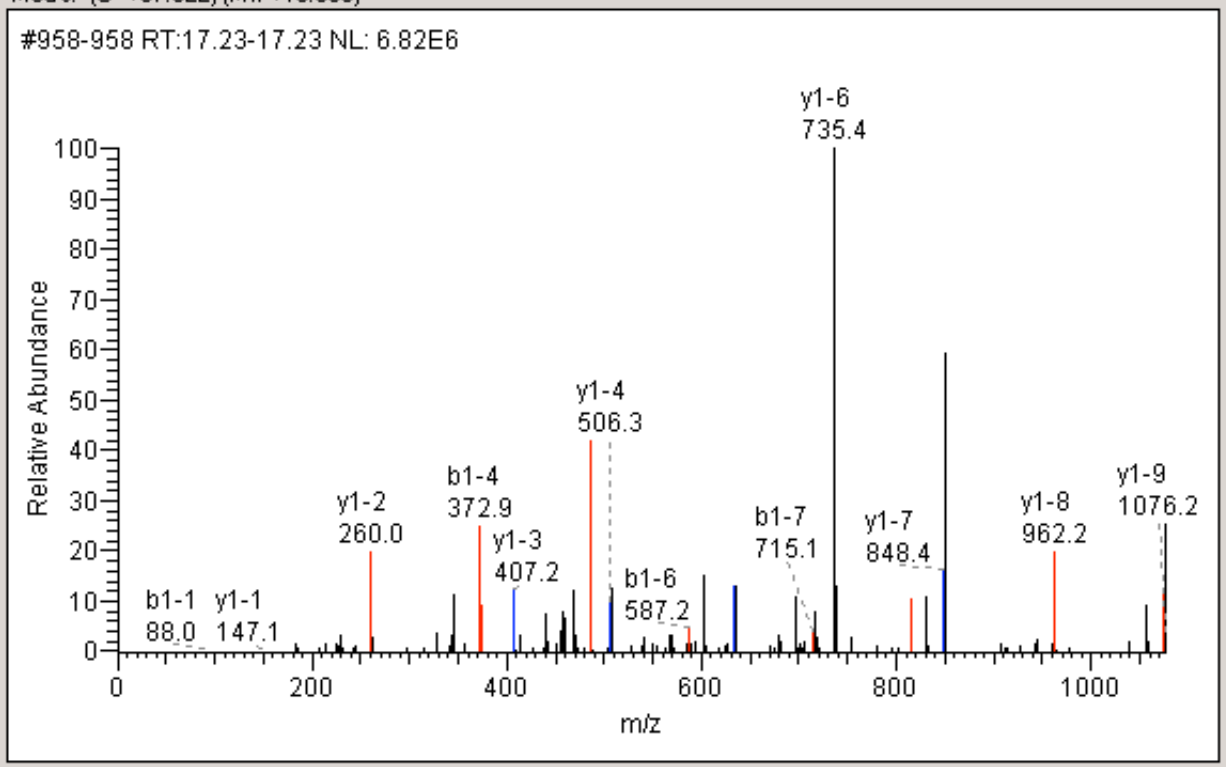
DisplayIons - SGDLITQVFLK

Display the ion series for charge state: +1

Dta: BC91_01_010.958.958.2 Precursor mass: 611.80 Mass type: Monoisotopic

Mod's: (C* +57.022) (M# +15.999)

	AA	A Ions	B Ions	Y Ions	
1	S		88.04	-	11
2	G		145.06	1133.64	10
3	D		260.09	1076.62	9
4	L		373.17	961.59	8
5	I		486.26	848.51	7
6	T		587.30	735.42	6
7	Q		715.36	634.37	5
8	V		814.43	506.32	4
9	F		961.50	407.25	3
10	L		1074.58	260.18	2
11	K		-	147.09	1



Ready

NUM

Sur 10 échantillons, 8 identifications coherentes

Echantillon	Id match	Score gpm	Score sequest
82	-	-	-
83	29_13 34_197 34_273	- - -22.9	30 20 -
84	34_129 34_156	-19.9 -16.1	190 40
85	34_69 34_129	-14.7 -3.5	100 20
86	29_35	-13.9	30.2
87	31_171 31_180 31_7 31_161	-56.1 -26.6 -16.1 -13.1	300 110 70 20
88	31_161 34_103 34_288	-49 -23.7 -13.8	348 72 30
89	31_161 34_152	-50 -5.7	210 30
90	31_161	-111	1140
91	31_171 31_180 31_115	-125.2 -73.8 -7	1168 290 30

Conclusion:

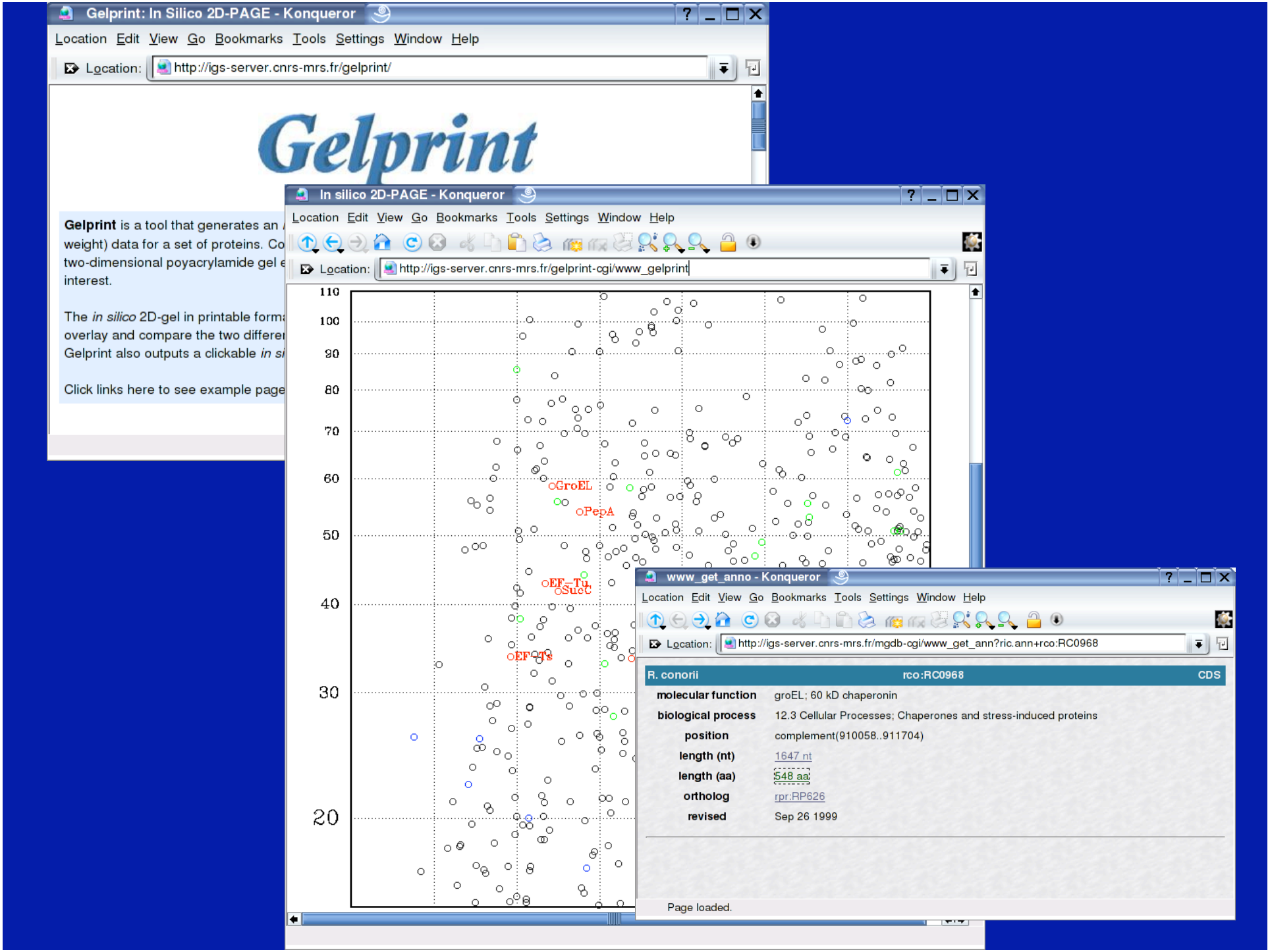
Une différence considérable en temps de calcul:

theGPM: 1 s
Sequest : 10 mn

Pour des résultats similaires

2D-gel of *Rickettsia conorii*

2D-gel provided by P. Renest et al.


Gelprint: In Silico 2D-PAGE - Konqueror
Location Edit View Go Bookmarks Tools Settings Window Help
Location: http://igs-server.cnrs-mrs.fr/gelprint/
Gelprint
Gelprint is a tool that generates an
weight) data for a set of proteins. Co
two-dimensional poyacrylamide gel e
interest.
The in silico 2D-gel in printable form
overlay and compare the two differe
Gelprint also outputs a clickable in si
Click links here to see example page
In silico 2D-PAGE - Konqueror
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100
90
80
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60
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GroEL
PepA
EF-Tu
SucC
EF-Ts
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R. conorii rco:RC0968 CDS
molecular function groEL; 60 kD chaperonin
biological process 12.3 Cellular Processes; Chaperones and stress-induced proteins
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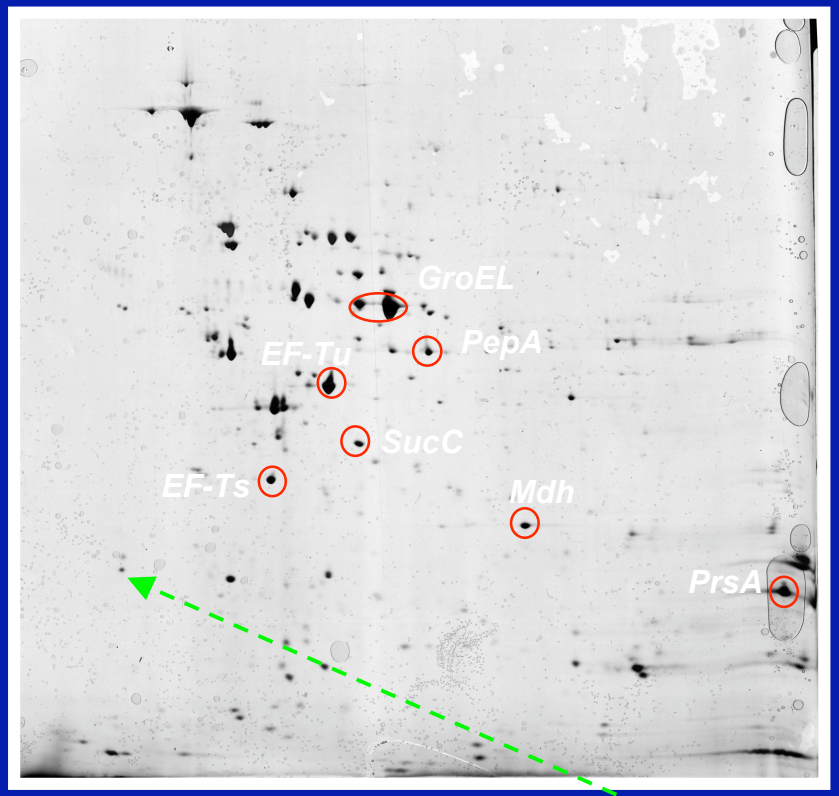
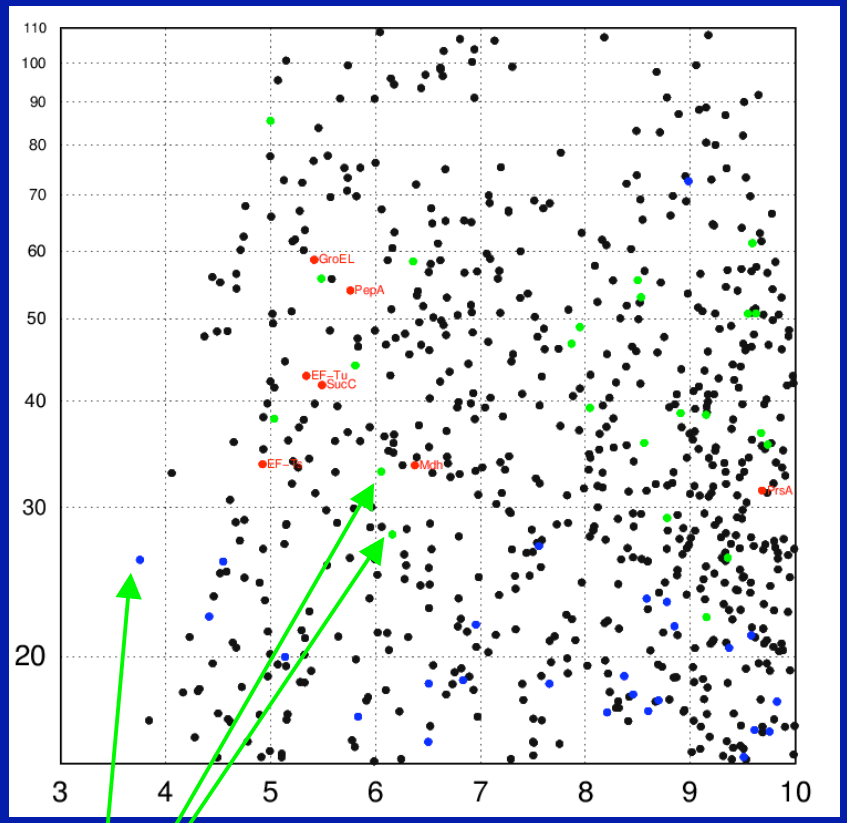
length (aa) 548 aa
ortholog rpr:RP626
revised Sep 26 1999
Page loaded.

2D gel (experimental)



2D gel (*in silico*)



?

Proteins of interest !

"Split genes and Repeat-containing proteins"

Input data for Gelprint

- List of theoretical (pre-computed) pI and Mw for a set of proteins
- Ranges of pI/Mw to show in the in silico 2D-gel
- Database address to make links from in silico 2D-gel to the database

- Gelprint generates in silico 2D-gels in Postscript/PDF that fit to your “real” gel
  - Size of your 2D-gel (in centimeter)
  - Some references points for fitting (i.e. positions of some identified spots on the real 2D-gel (in centimeter)

Gelprint

http://igs-server.cnrs-mrs.fr/gelprint/

Acknowledgements

Patricia Renesto
Didier Raoult
(Unite des Rickettsies, CNRS UMR6020, Marseille)