Séquence expérimentale d’analyse MALDI

Digestion enzymatique « in gel »

Dépôt sur cible MALDI : PAC

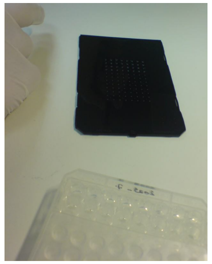
acquisition MS — Spectre de masse des peptides

Recherche Mascot — PMF

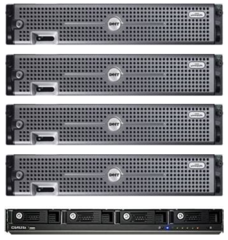
acquisition MSMS — Spectre de fragmentations de peptides

Recherche Mascot — PFF


Robot digester


Dépôt sur PAC


MALDI TOFTOF



Phenyx

Proteinscape

Prims

mascot

NAS 4.5To

Validation des résultats selon critères mascot

MS Validé selon critères mascot		X		X
MSMS Validé selon critères mascot		X	X	
	Signal insuffisant Quantité trop faible	Cas optimal	Peu de peptides (petite protéine) Pas de hit en MS Mais signal suffisant pour fragmenter et donc identification MSMS	Signal faible : peptides visibles suffisants pour une identification en MS mais pas assez de matériel pour fragmenter

Validation par le service
Contrôle mascot
Contrôle du spectre : les pics les plus intenses sont ils attribués ?

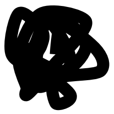
Rapport d’analyse

Plateforme Protéomique de l’Esplanade
IBMC
IBMP
GMGM

Digestion enzymatique « in gel »

Robot digester

Cycles de lavage des morceaux de gels
Acetonitryle / bicarbonate d'ammonium
+ réduction/alkylation des cystéines
DTT/iodoacetamide



Protéine

5heures

$-CH_2- SH + I-CH_2-CONH_2$ ---> $-CH_2-S-CH_2-CONH_2$

cysteine Iodoacetamide S-carbamidomethyl cysteine

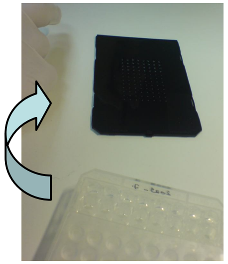
+ trypsine

12-15 heures

Mélange de peptides
PeptidesK
PeptidesR
Modif experimentale :
Cystéines modifiées par iodocacetamide :
carbamidomethylation

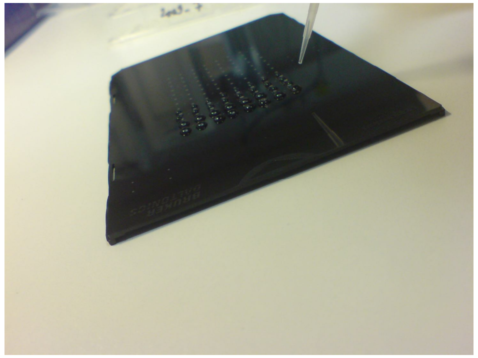
Extraction :
Diffusion passive
Ou en présence ACN/H2O/ac.formique

Dépôt sur cible MALDI : PAC



PAC : Préspotted AnchorChip
Cicle préspottée de matrice αcyano
Format 96
Calibrants prédéposés

Dépôt : 4 µl TFA 0.1%
+ 1µl échantillon



5' : les peptides co-cristallisent avec la matrice

Plateforme Protéomique de l'Esplanade
IBMC
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Séquence expérimentale d’analyse MALDI

Digestion enzymatique « in gel »	
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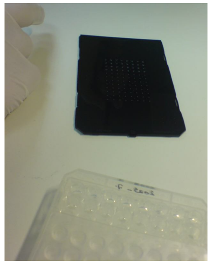
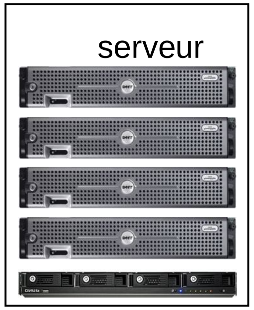

Dépôt sur PAC


MALDI TOFTOF



Phenyx
Proteinscape
Prims
mascot
NAS 4.5To

Plateforme Protéomique de l'Esplanade
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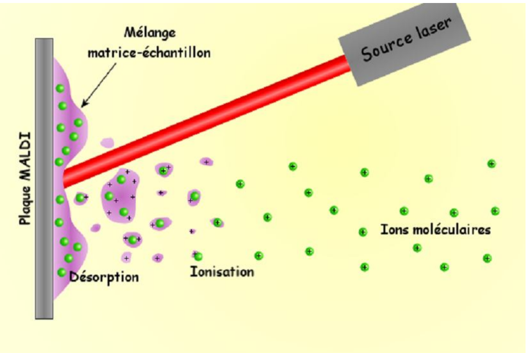
Acquisition MS

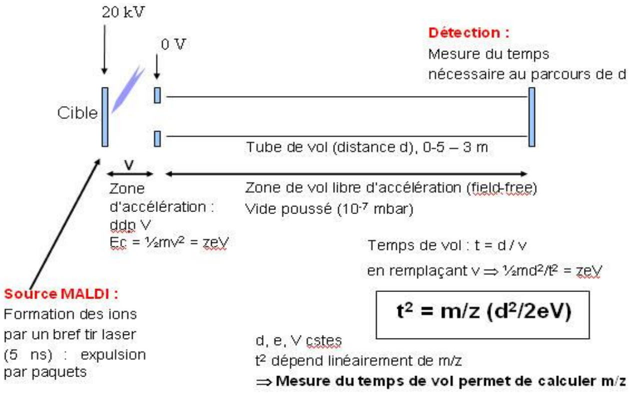
Ionisation MALDI



Ions MH+

Gamme de masse :
700-4000 da

TOF analysis



Détection :
Mesure du temps
nécessaire au parcours de d

Tube de vol (distance d), 0-5 – 3 m

Zone d'accélération :
ddp V
$Ec = ½mv^2 = zeV$

Zone de vol libre d'accélération (field-free)
Vide poussé (10^{-7} mbar)

Source MALDI :
Formation des ions par un bref tir laser (5 ns) : expulsion par paquets

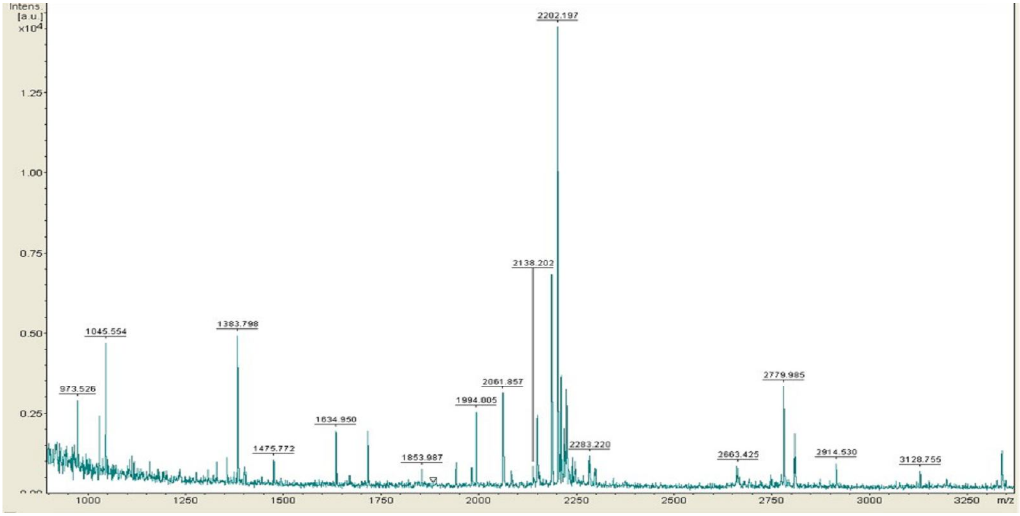
Temps de vol : $t = d / v$

en remplaçant v ⇒ $½md^2/t^2 = zeV$

$$\boxed{t^2 = m/z\ (d^2/2eV)}$$

d, e, V cstes
t^2 dépend linéairement de m/z
⇒ **Mesure du temps de vol permet de calculer m/z**

Spectre MS



Liste de pics :
masse de peptides MH+
700 – 4000 da
Précision < 50ppm

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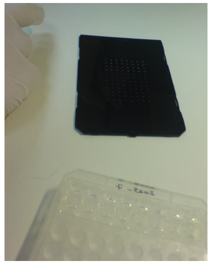
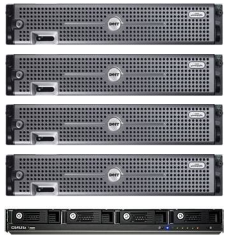


Dépôt sur PAC

MALDI TOFTOF

serveur



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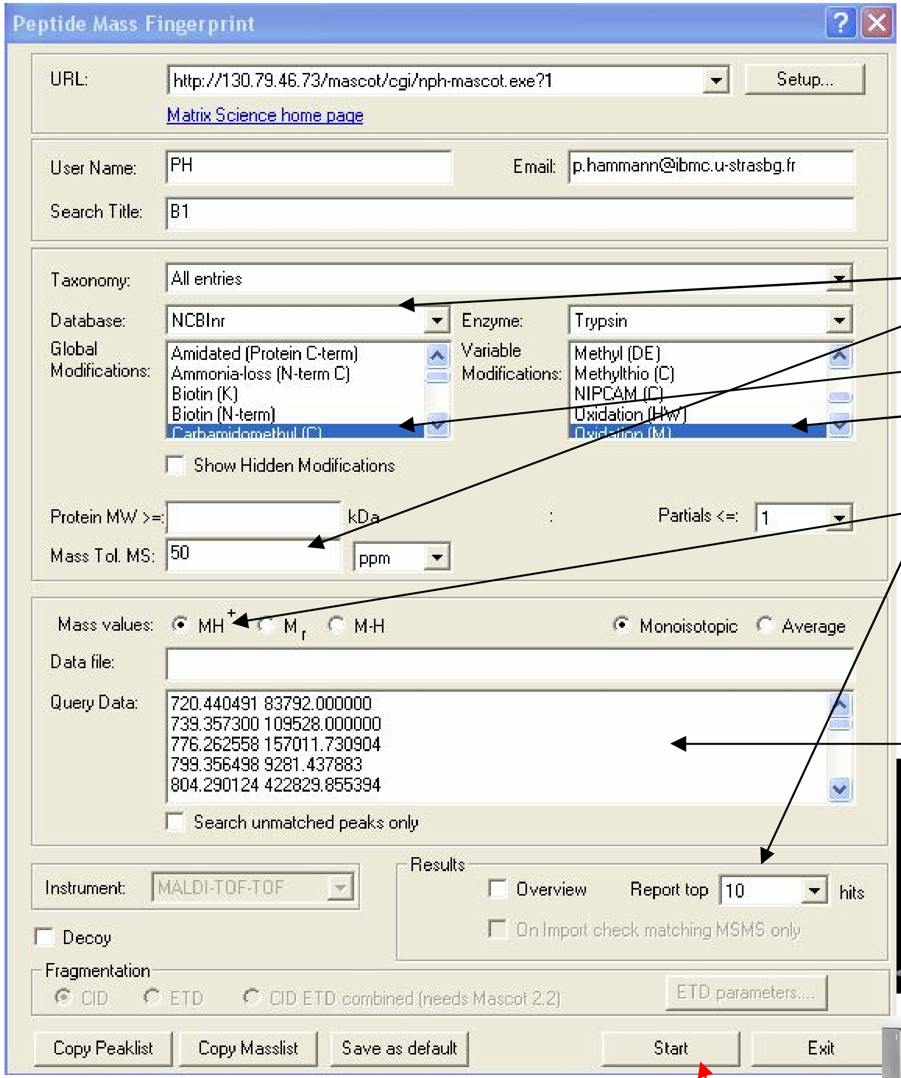


Peptide Mass Fingerprint

URL: http://130.79.46.73/mascot/cgi/nph-mascot.exe?1

Matrix Science home page

User Name: PH

Email: p.hammann@ibmc.u-strasbg.fr

Search Title: B1

Taxonomy: All entries

Database: NCBInr

Enzyme: Trypsin

Global Modifications:
- Amidated (Protein C-term)
- Ammonia-loss (N-term C)
- Biotin (K)
- Biotin (N-term)
- Carbamidomethyl (C)

Variable Modifications:
- Methyl (DE)
- Methylthio (C)
- NIPCAM (C)
- Oxidation (HW)
- Oxidation (M)

☐ Show Hidden Modifications

Protein MW >=: kDa

Partials <=: 1

Mass Tol. MS: 50 ppm

Mass values: ● MH^+ ○ M_r ○ M-H ● Monoisotopic ○ Average

Data file:

Query Data:

```
720.440491 83792.000000
739.357300 109528.000000
776.262558 157011.730904
799.356498 9281.437883
804.290124 422829.855394
```

☐ Search unmatched peaks only

Instrument: MALDI-TOF-TOF

☐ Decoy

Results

☐ Overview Report top 10 hits

☐ On Import check matching MSMS only

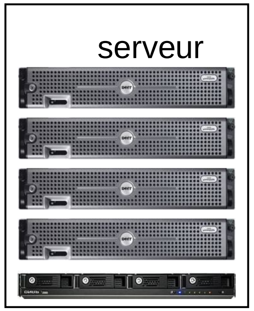
Fragmentation

● CID ○ ETD ○ CID ETD combined (needs Mascot 2.2)

Critères de recherche mascot en mode MS

Database NCBInr
Ms tolérance : 50 ppm
Modification
fixe : cyst.Carbamidométhylée
variable : Met oxydée
Rapport : 10 premières protéines
1 missed clivage
Ions MH+

Liste de masses



mascot

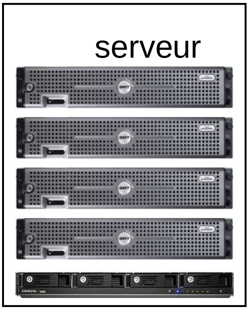
NAS 4.5To

Spectre MS
expérimental

Spectre MS
Théorique (NCBInr)

Match ?

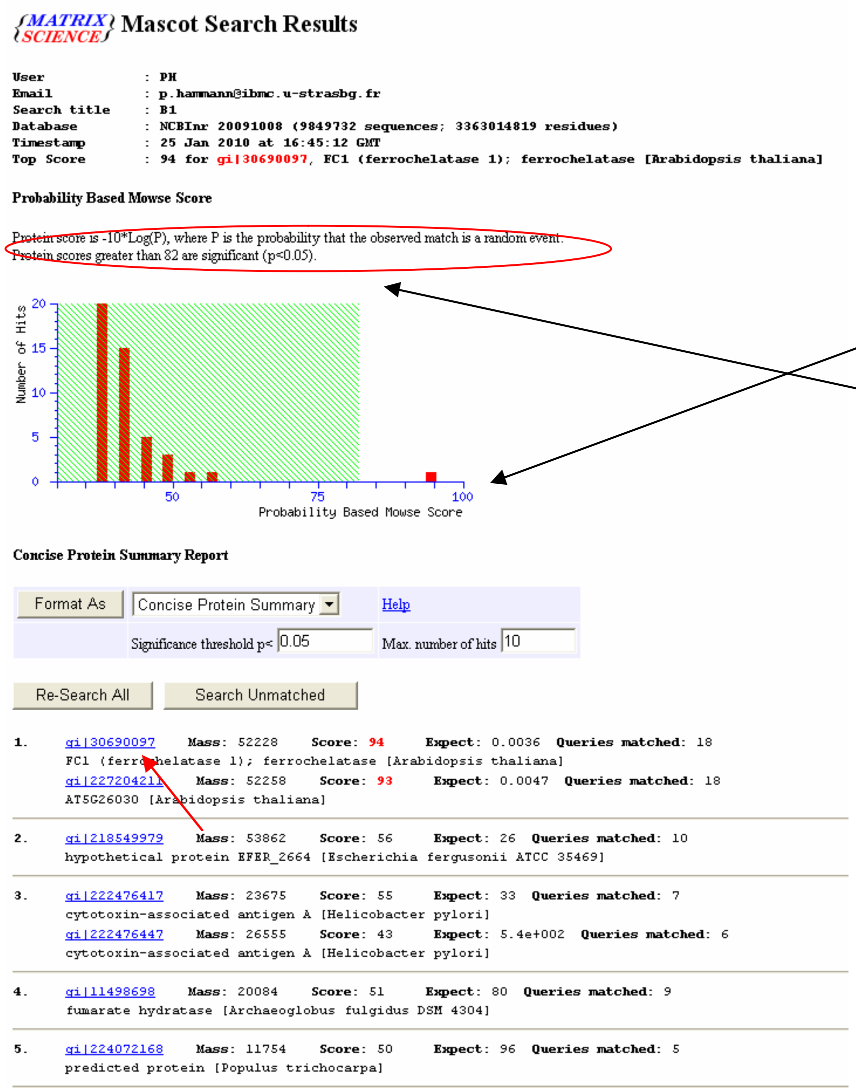
Résultat de recherche mascot
En mode MS



Résultats sous la forme de page web

mascot

NAS 4.5To



Mascot Search Results

```
User            : PH
Email           : p.hammann@ibmc.u-strasbg.fr
Search title    : B1
Database        : NCBInr 20091008 (9849732 sequences; 3363014819 residues)
Timestamp       : 25 Jan 2010 at 16:45:12 GMT
Top Score       : 94 for gi|30690097, FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana]
```

Probability Based Mowse Score

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 82 are significant (p<0.05).

Concise Protein Summary Report

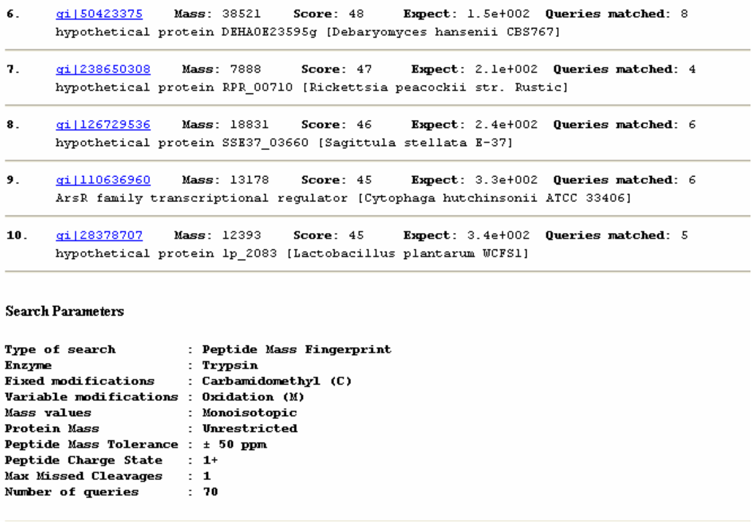
Significance threshold p< 0.05

Max. number of hits 10

```
1.    gi|30690097    Mass: 52228    Score: 94    Expect: 0.0036  Queries matched: 18
      FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana]
      gi|227204211    Mass: 52258    Score: 93    Expect: 0.0047  Queries matched: 18
      AT5G26030 [Arabidopsis thaliana]

2.    gi|218549979    Mass: 53862    Score: 56    Expect: 26  Queries matched: 10
      hypothetical protein EFER_2664 [Escherichia fergusonii ATCC 35469]

3.    gi|222476417    Mass: 23675    Score: 55    Expect: 33  Queries matched: 7
      cytotoxin-associated antigen A [Helicobacter pylori]
      gi|222476447    Mass: 26555    Score: 43    Expect: 5.4e+002  Queries matched: 6
      cytotoxin-associated antigen A [Helicobacter pylori]

4.    gi|11498698    Mass: 20084    Score: 51    Expect: 80  Queries matched: 9
      fumarate hydratase [Archaeoglobus fulgidus DSM 4304]

5.    gi|224072168    Mass: 11754    Score: 50    Expect: 96  Queries matched: 5
      predicted protein [Populus trichocarpa]
```

Mascot calcule une valeur seuil au delà de laquelle le hit est significatif à plus de 95% (dans NCBI nr)
Visualisation graphique : le hit doit sortir de la zone verte

Ici, le résultat est significatif dans NCBInr à plus de 95 % si le score est supérieur à 82



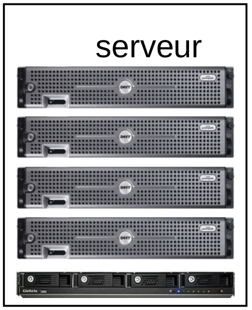
```
6.    gi|50423375    Mass: 38521    Score: 48    Expect: 1.5e+002  Queries matched: 8
      hypothetical protein DEHA0E23595g [Debaryomyces hansenii CBS767]

7.    gi|238650308    Mass: 7888    Score: 47    Expect: 2.1e+002  Queries matched: 4
      hypothetical protein RPR_00710 [Rickettsia peacockii str. Rustic]

8.    gi|126729536    Mass: 18831    Score: 46    Expect: 2.4e+002  Queries matched: 6
      hypothetical protein SSE37_03660 [Sagittula stellata E-37]

9.    gi|110636960    Mass: 13178    Score: 45    Expect: 3.3e+002  Queries matched: 6
      ArsR family transcriptional regulator [Cytophaga hutchinsonii ATCC 33406]

10.   gi|28378707    Mass: 12393    Score: 45    Expect: 3.4e+002  Queries matched: 5
      hypothetical protein lp_2083 [Lactobacillus plantarum WCFS1]
```

Search Parameters

```
Type of search          : Peptide Mass Fingerprint
Enzyme                  : Trypsin
Fixed modifications     : Carbamidomethyl (C)
Variable modifications  : Oxidation (M)
Mass values             : Monoisotopic
Protein Mass            : Unrestricted
Peptide Mass Tolerance  : ± 50 ppm
Peptide Charge State    : 1+
Max Missed Cleavages    : 1
Number of queries       : 70
```

✓ La protéine FC1 est validée en mode MS seul avec un score de 94

Résultats PMF mascot de la protéine



mascot

NAS 4.5To

Résultats sous la forme de page web

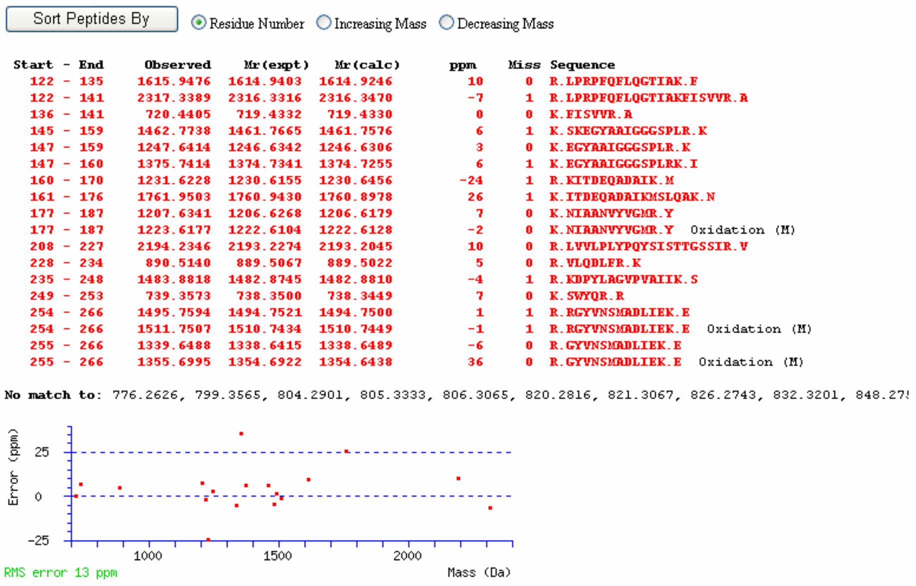
Mascot Search Results

Protein View

```
Match to: gi|30690097 Score: 94 Expect: 0.0036
FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana]

Nominal mass (Mr): 52228; Calculated pI value: 5.60
NCBI BLAST search of gi|30690097 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Arabidopsis thaliana
Links to retrieve other entries containing this sequence from NCBI Entrez:
gi|79328715 from Arabidopsis thaliana
gi|1170237 from Arabidopsis thaliana
gi|5107825 from Arabidopsis thaliana
gi|511081 from Arabidopsis thaliana
gi|2597828 from Arabidopsis thaliana
gi|110741028 from Arabidopsis thaliana

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 70
Number of mass values matched: 18
Sequence Coverage: 26%

Matched peptides shown in Bold Red

  1 MQATALSSGF NPLTKRKDHR FPRSCSQRNS LSLIQCDIKE RSFGESMTIT
 51 NRGLSFKTNV FEQARSVTGD CSYDETSAKA RSHVVAEDKI GVLLLNLGGP
101 ETLNDVQPFL YNLFADPDII RLPRPFQFLQ GTIAKFISVV RAPKSKEGYA
151 AIGGGSPLRK ITDEQADAIK MSLQAKNIAA NVYVGMRYWY PFTEEAVQQI
201 KKDKITRLVV LPLYPQYSIS TTGSSIRVLQ DLFRKDPYLA GVPVAIIKSW
251 YQRRGYVNSM ADLIEKELQT FSDPKEVMIF FSAHGVPVSY VENAGDPYQK
301 QMEECIDLIM EELKARGVLN DHKLAYQSRV GPVQWLKPYT DEVLVDLGKS
351 GVKSLLAVPV SFVSEHIETL EEIDMEYREL ALESGVENWG RVPALGLTPS
401 FITDLADAVI ESLPSAEAMS NPNAVVDSED SESSDAFSYI VKMFFGSILA
451 FVLLLSPKMF HAFRNL
```

En rouge gras : les peptides trouvés

% de recouvrement
Nombre de peptides trouvés



```
Start - End     Observed   Mr(expt)   Mr(calc)      ppm   Miss Sequence
  122 - 135    1615.9476  1614.9403  1614.9246       10     0  R.LPRPFQFLQGTIAK.F
  122 - 141    2317.3389  2316.3316  2316.3470       -7     1  R.LPRPFQFLQGTIAKFISVVR.A
  136 - 141     720.4405   719.4332   719.4330        0     0  K.FISVVR.A
  145 - 159    1462.7738  1461.7665  1461.7576        6     1  K.SKEGYAAIGGGSPLR.K
  147 - 159    1247.6414  1246.6342  1246.6306        3     0  K.EGYAAIGGGSPLR.K
  147 - 160    1375.7414  1374.7341  1374.7255        6     1  K.EGYAAIGGGSPLRK.I
  160 - 170    1231.6228  1230.6155  1230.6456      -24     1  R.KITDEQADAIK.M
  161 - 176    1761.9503  1760.9430  1760.8978       26     1  K.ITDEQADAIKMSLQAK.N
  177 - 187    1207.6341  1206.6268  1206.6179        7     0  K.NIAANVYVGMR.Y
  177 - 187    1223.6177  1222.6104  1222.6128       -2     0  K.NIAANVYVGMR.Y  Oxidation (M)
  208 - 227    2194.2346  2193.2274  2193.2045       10     0  R.LVVLPLYPQYSISTTGSSIR.V
  228 - 234     890.5140   889.5067   889.5022        5     0  R.VLQDLFR.K
  235 - 248    1483.8818  1482.8745  1482.8810       -4     1  R.KDPYLAGVPVAIIK.S
  249 - 253     739.3573   738.3500   738.3449        7     0  K.SWYQR.R
  254 - 266    1495.7594  1494.7521  1494.7500        1     1  R.RGYVNSMADLIEK.E
  254 - 266    1511.7507  1510.7434  1510.7449       -1     1  R.RGYVNSMADLIEK.E  Oxidation (M)
  255 - 266    1339.6488  1338.6415  1338.6489       -6     0  R.GYVNSMADLIEK.E
  255 - 266    1355.6995  1354.6922  1354.6438       36     0  R.GYVNSMADLIEK.E  Oxidation (M)

No match to: 776.2626, 799.3565, 804.2901, 805.3333, 806.3065, 820.2816, 821.3067, 826.2743, 832.3201, 848.27
```

RMS error 13 ppm

Représentation globale des précisions de masse pour chaque peptide.
L'erreur moyenne est bonne : 13 ppm

<50 ppm bon
<25 ppm excellent

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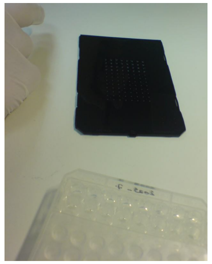
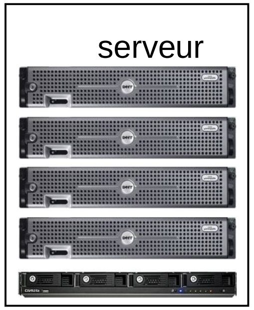


Dépôt sur PAC

MALDI TOFTOF



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→ Validation par le service
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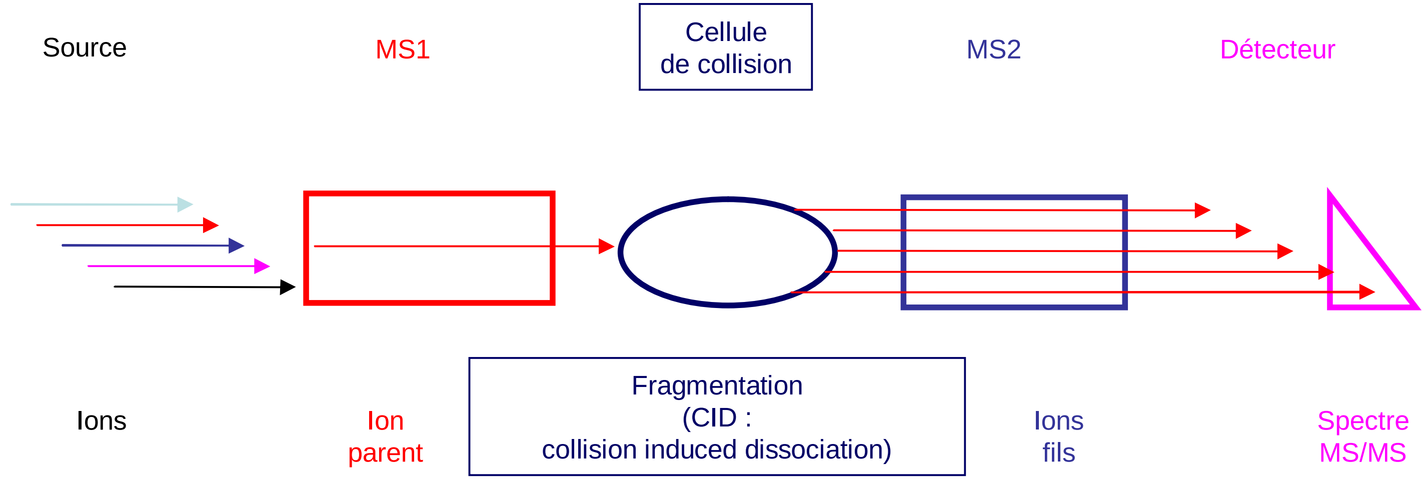
→ Rapport d'analyse

Mode MSMS
Principe de la spectrométrie de masse en tandem MS/MS



Source

MS1

Cellule de collision

MS2

Détecteur

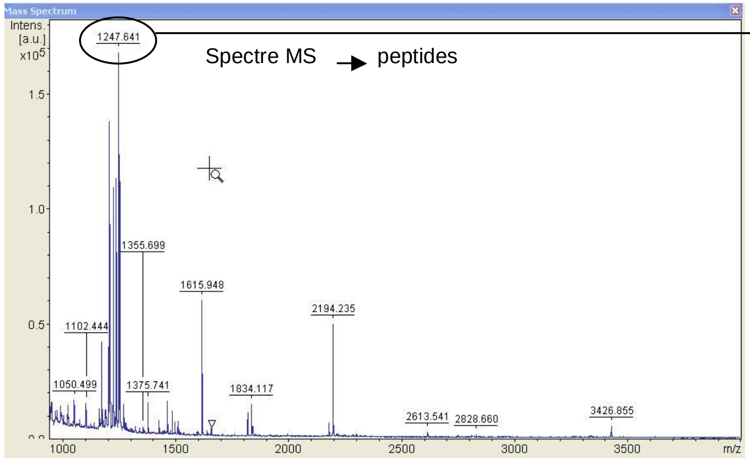
Ions

Ion parent

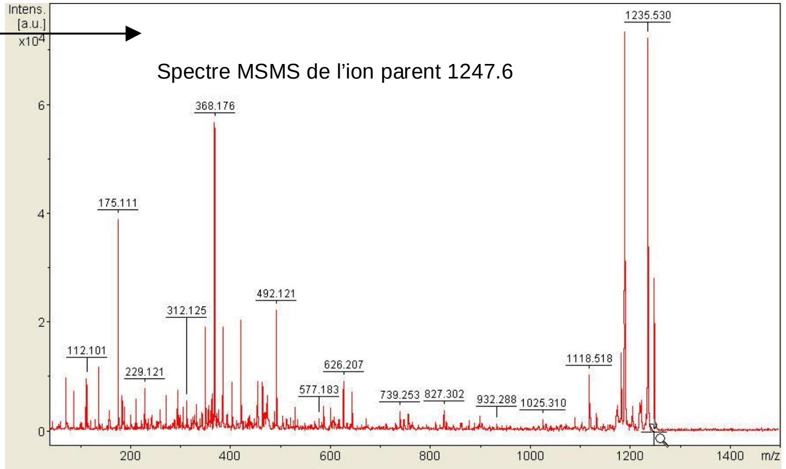
Fragmentation (CID : collision induced dissociation)

Ions fils

Spectre MS/MS



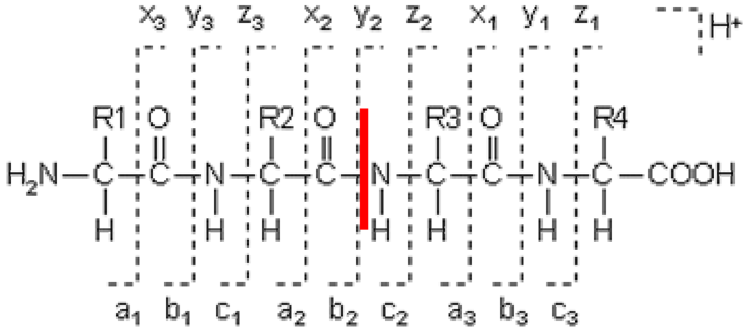
Spectre MS → peptides



Spectre MSMS de l'ion parent 1247.6

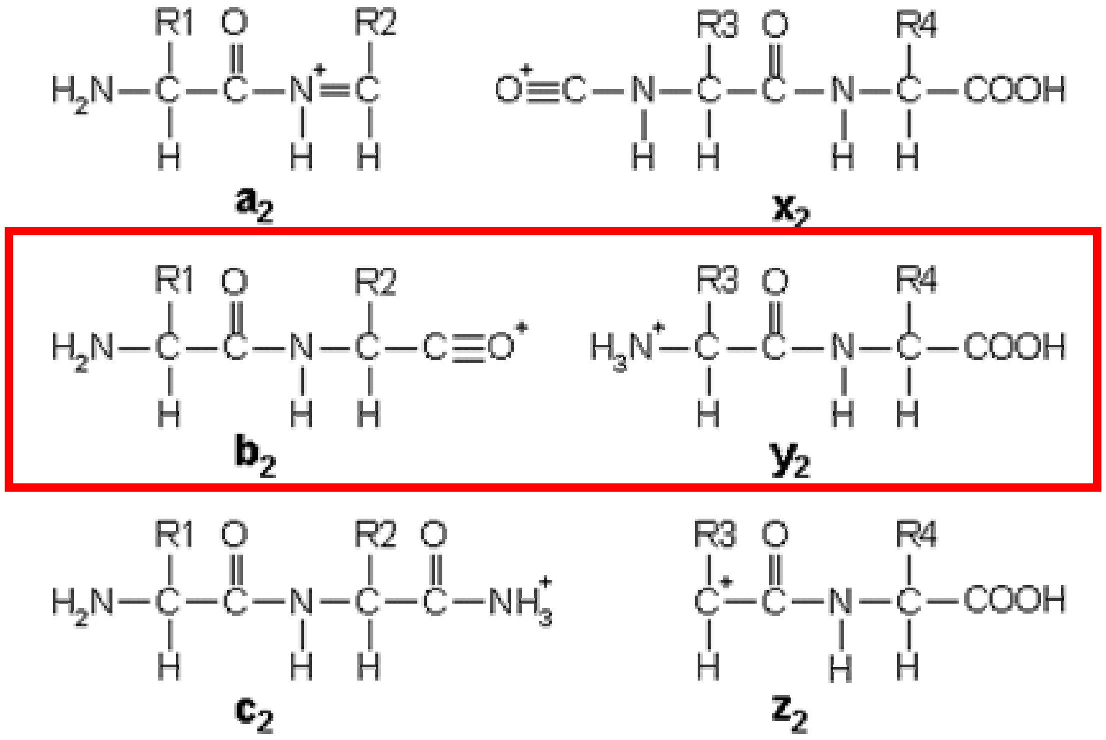
Interprétation d'un spectre de fragmentation

Fragments (ions) obtenus par MS/MS



Charge positive sur fragment Nter

Charge positive sur fragment Cter



Plateforme Protéomique de l'Esplanade
IBMC
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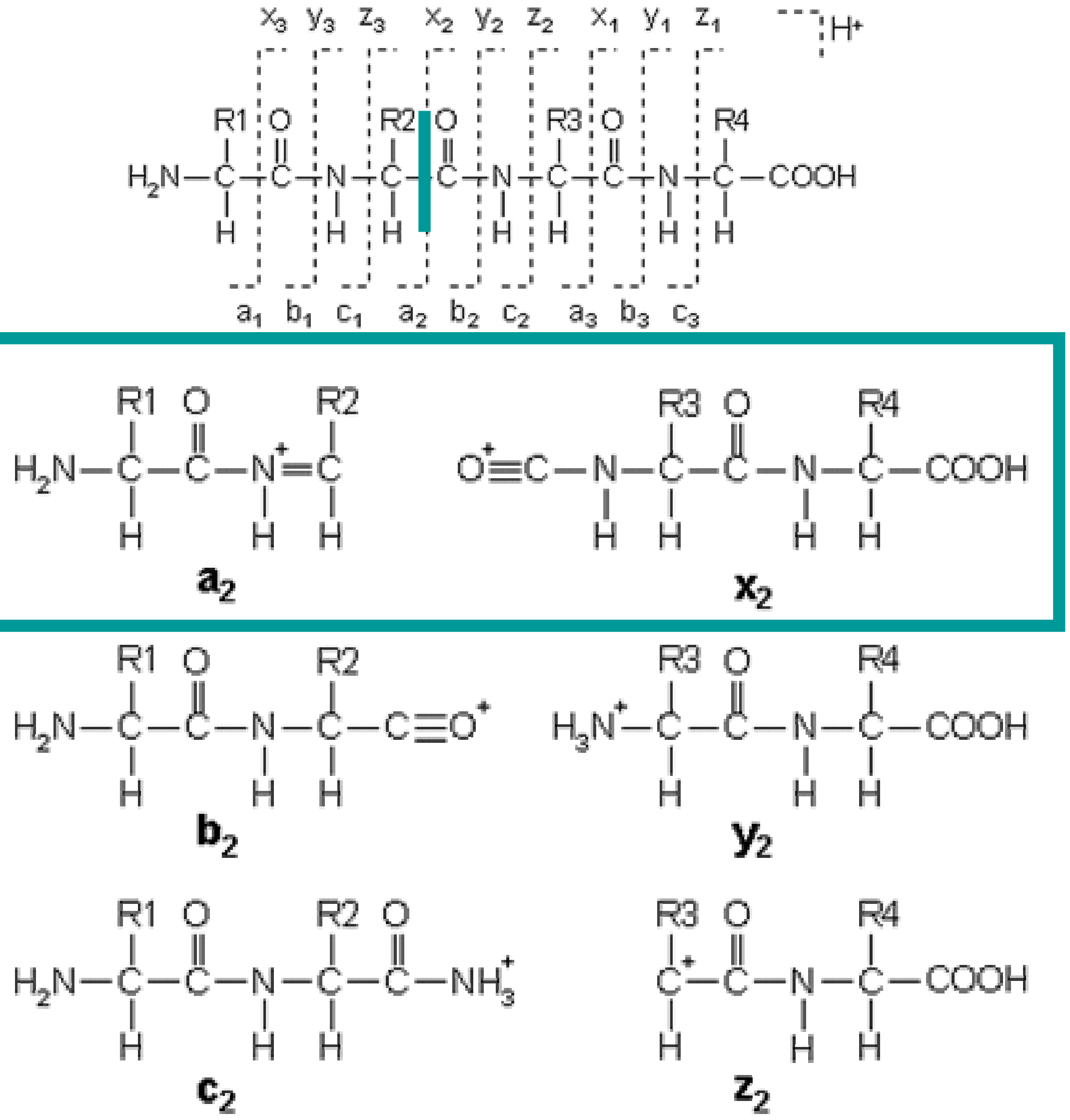
GMGM

http://www.matrixscience.com/help/fragmentation_help.html

Fragments (ions) obtenus par MS/MS

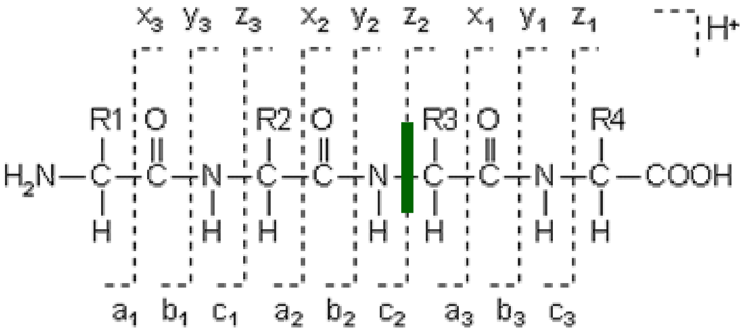


Charge positive
sur fragment Nter

Charge positive
sur fragment Cter

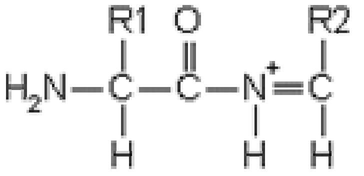
http://www.matrixscience.com/help/fragmentation_help.html

Fragments (ions) obtenus par MS/MS



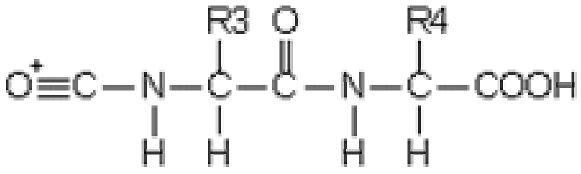
Charge positive sur fragment Nter

Charge positive sur fragment Cter



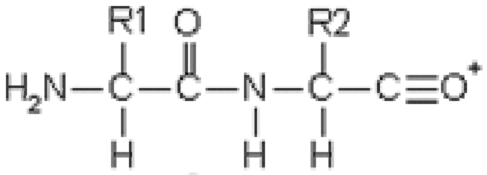
$H_2N-CH(R1)-C(=O)-N^{+}H=CH(R2)$

a_2



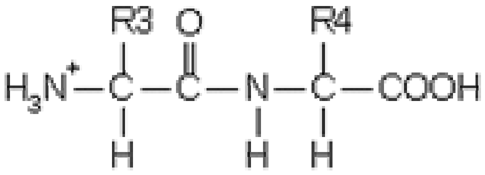
$O^{+}\equiv C-NH-CH(R3)-C(=O)-NH-CH(R4)-COOH$

x_2



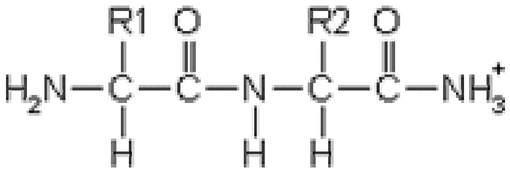
$H_2N-CH(R1)-C(=O)-NH-CH(R2)-C\equiv O^{+}$

b_2



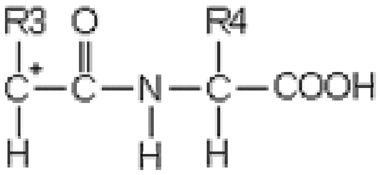
$H_3N^{+}-CH(R3)-C(=O)-NH-CH(R4)-COOH$

y_2



$H_2N-CH(R1)-C(=O)-NH-CH(R2)-C(=O)-NH_3^{+}$

c_2

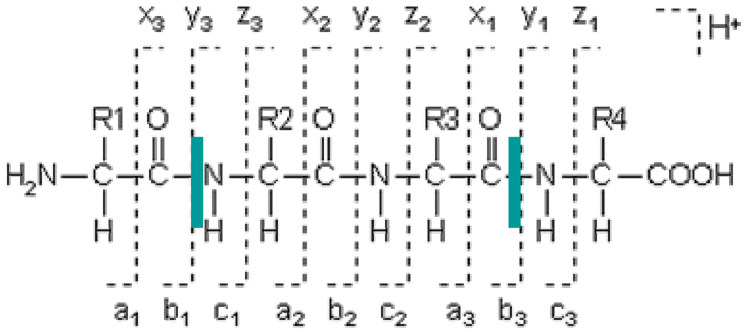


$C^{+}H(R3)-C(=O)-NH-CH(R4)-COOH$

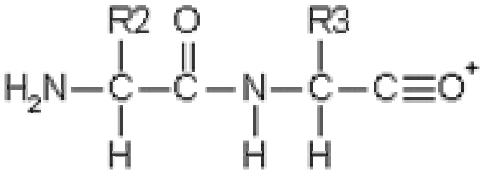
z_2

http://www.matrixscience.com/help/fragmentation_help.html

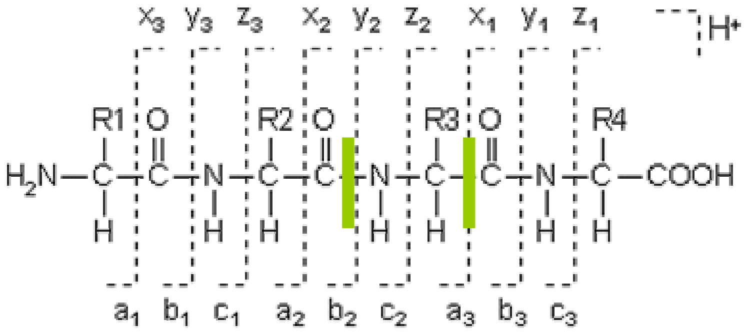
Fragments (ions) obtenus par MS/MS



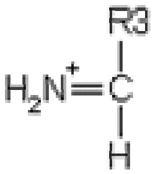
Fragments
internes


$$H_2N-\underset{H}{\overset{R2}{C}}-\overset{O}{\overset{\|}{C}}-\underset{H}{N}-\underset{H}{\overset{R3}{C}}-C\equiv O^+$$

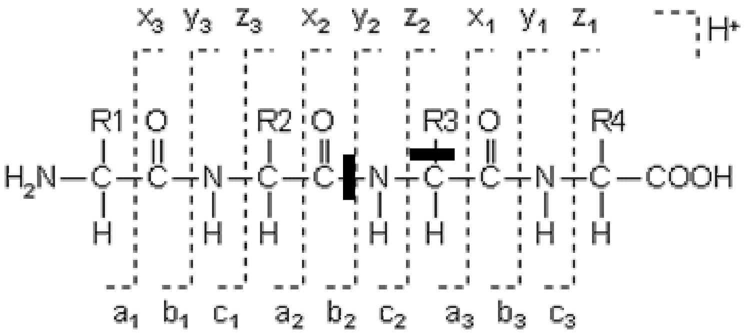
Fragments (ions) obtenus par MS/MS



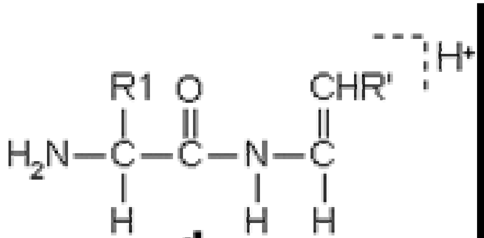
Ions immonium
(<200)



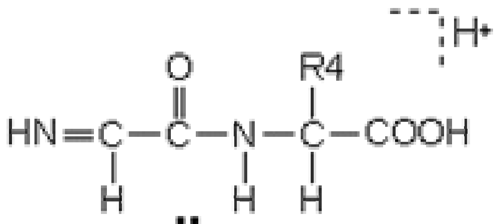
Fragmentation des peptides



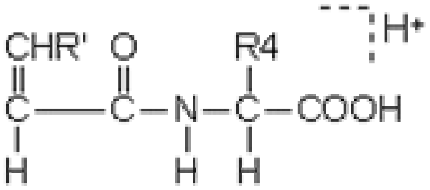
Ions satellites



d_2



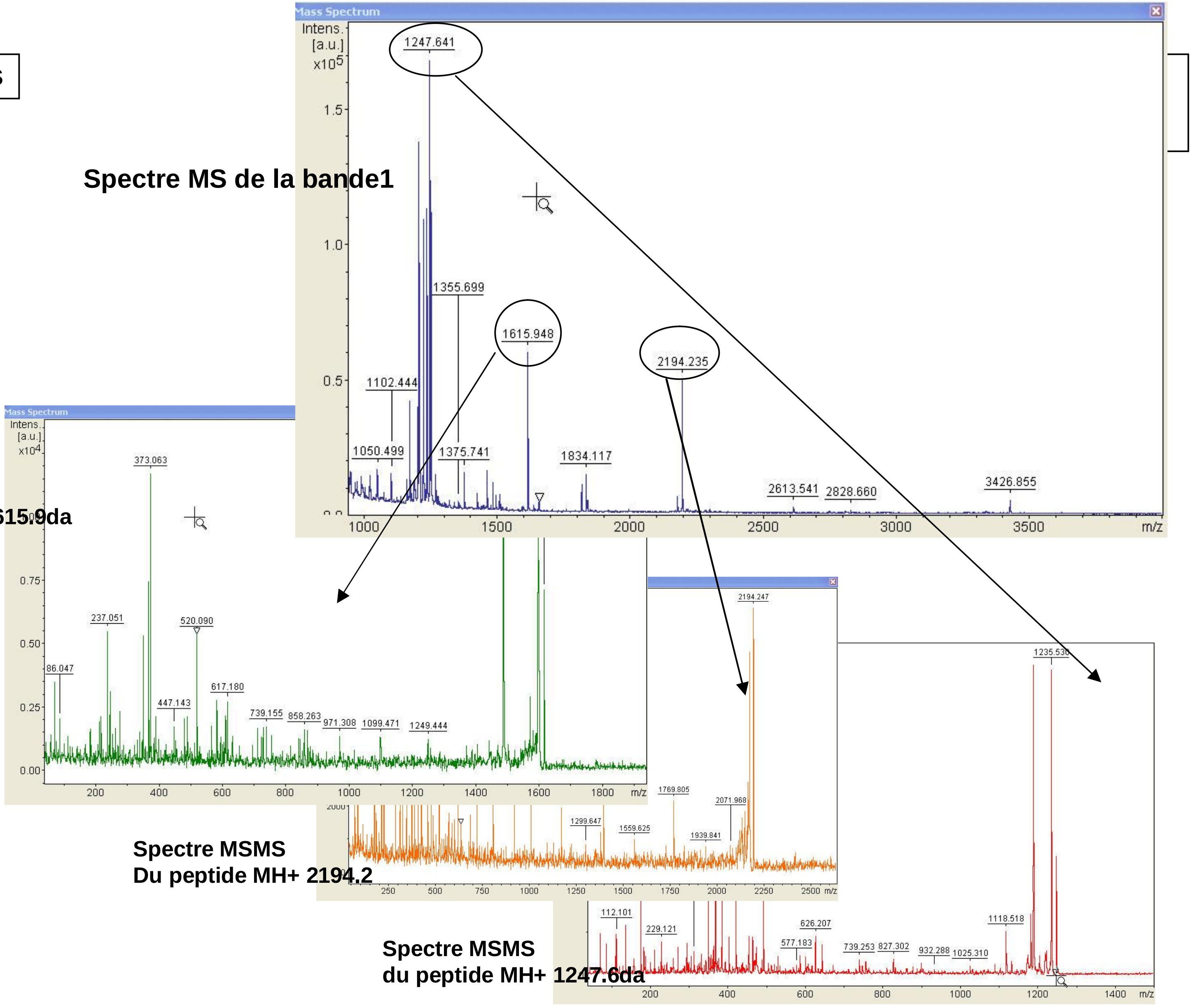
v_2



w_2

Au final, tous ces types d'ions sont susceptibles d'être vus en MALDI MSMS
Y,b,x,z,a,c,ions immonium, d, v, w, et fragments internes …
Y et b sont les plus abondants

Acquisition MALDI MSMS



Spectre MS de la bande1

Spectre MSMS du peptide 1615.9da

Spectre MSMS
Du peptide MH+ 2194.2

Spectre MSMS
du peptide MH+ 1247.6da

Plusieurs spectres MSMS sont réalisés à partir des ions parents intenses (peptides) détectés en mode MS
Le logiciel biotools compile les spectres MSMS et MS dans un seul fichier de résultats.

Biotools3.2

↓

Recherche MASCOT

Séquence expérimentale d'analyse MALDI

Digestion enzymatique « in gel »		
Dépôt sur cible MALDI : PAC		
acquisition	MS	Spectre de masse des peptides
Recherche Mascot		PMF
acquisition MSMS		Spectre de fragmentation de peptides
Recherche Mascot		**PFF or ions score results**

Validation des résultats selon critères mascot

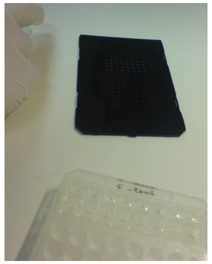
MS Validé selon critères mascot		X		X
MSMS Validé selon critères mascot		X	X	
	Signal insuffisant Quantité trop faible	Cas optimal	Peu de peptides (petite protéine) Pas de hit en MS Mais signal suffisant pour fragmenter et donc identification MSMS	Signal faible : peptides visibles suffisants pour une identification en MS mais pas assez de matériel pour fragmenter

Validation par le service
Contrôle mascot
Contrôle du spectre : les pics les plus intenses sont ils attribués ?

Rapport d'analyse

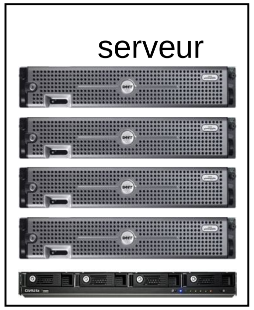
Plateforme Protéomique de l'Esplanade
IBMC
IBMP
GMGM

Robot digester

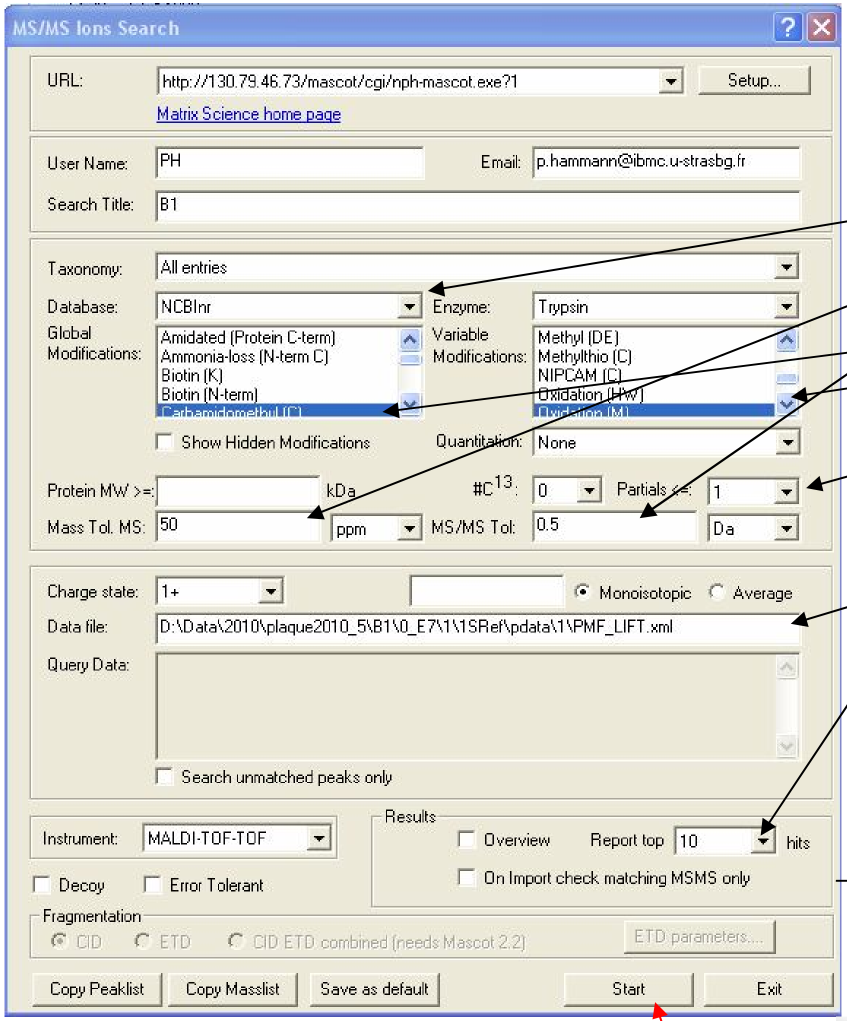


Dépôt sur PAC

MALDI TOFTOF



Phenyx
Proteinscape
Prims
mascot
NAS 4.5To



URL: http://130.79.46.73/mascot/cgi/nph-mascot.exe?1

User Name: PH
Email: p.hammann@ibmc.u-strasbg.fr
Search Title: B1

Taxonomy: All entries
Database: NCBInr
Enzyme: Trypsin
Global Modifications: Amidated (Protein C-term), Ammonia-loss (N-term C), Biotin (K), Biotin (N-term), Carbamidomethyl (C)
Variable Modifications: Methyl (DE), Methylthio (C), NIPCAM (C), Oxidation (HW), Oxidation (M)
☐ Show Hidden Modifications
Quantitation: None
Protein MW >=: kDa
#C13: 0
Partials <=: 1
Mass Tol. MS: 50 ppm
MS/MS Tol: 0.5 Da

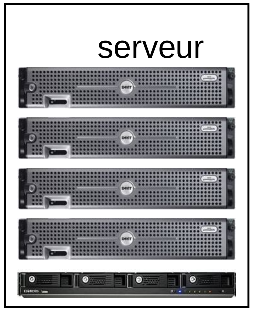
Charge state: 1+
● Monoisotopic ○ Average
Data file: D:\Data\2010\plaque2010_5\B1\0_E7\1\1SRef\pdata\1\PMF_LIFT.xml
Query Data:
☐ Search unmatched peaks only

Instrument: MALDI-TOF-TOF
Results
☐ Overview
Report top 10 hits
☐ On Import check matching MSMS only
☐ Decoy
☐ Error Tolerant
Fragmentation
● CID ○ ETD ○ CID ETD combined (needs Mascot 2.2)

Critères de recherche mascot en mode MSMS

Database NCBInr
Ms tolérance : 50 ppm
MSMS tolérance : 0.5 da
Modification
fixe : cyst.Carbamidométhylée
variable : Met oxydée
Rapport : 10 premières protéines
1 missed clivage

Fichier de résultat MS + nMSMS



mascot

NAS 4.5To

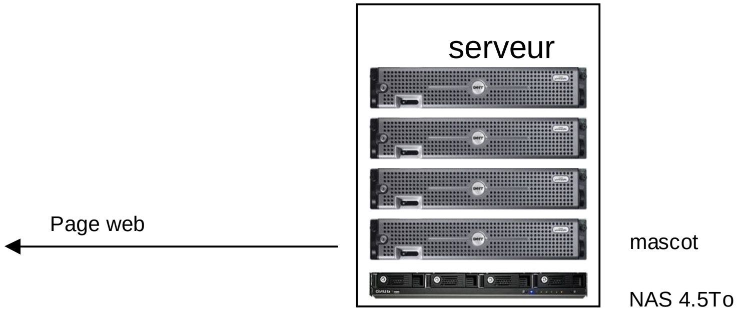
Spectre MSMS
Experimental
Masses de fragments
expérimentaux

Spectre MSMS
Théorique
Masses de fragments
théoriques

Match ?

Plateforme Protéomique
de l'Esplanade
IBMC
IBMP
GMGM

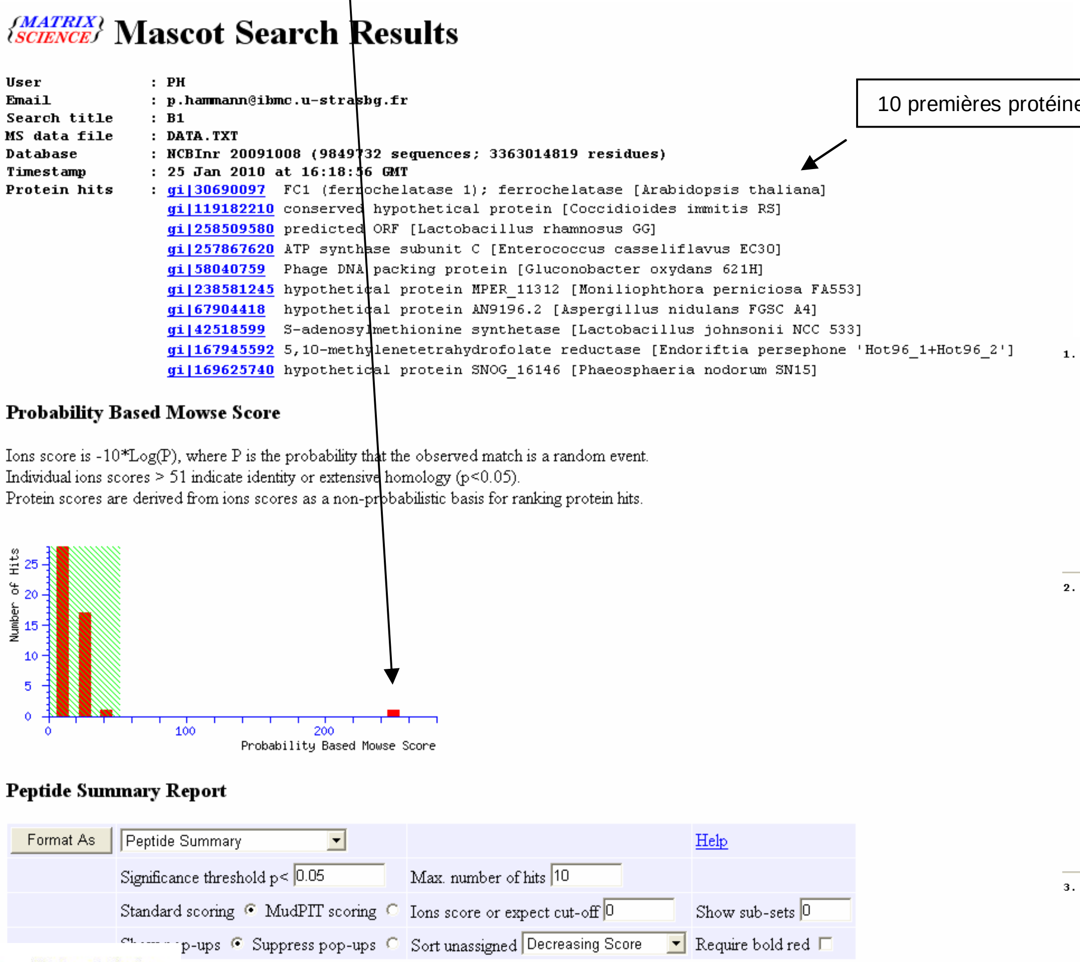
Résultat de recherche mascot MSMS : mode ion score



Pour chaque spectre MSMS, Mascot calcule une valeur seuil au delà de laquelle le hit est significatif à plus de 95%
Visualisation : le hit doit sortir de la zone verte

10 premières protéines

Détail des recherches par protéine



Mascot Search Results

```
User             : PH
Email            : p.hammann@ibmc.u-strasbg.fr
Search title     : B1
MS data file     : DATA.TXT
Database         : NCBInr 20091008 (9849732 sequences; 3363014819 residues)
Timestamp        : 25 Jan 2010 at 16:18:56 GMT
Protein hits     : gi|30690097  FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana]
                   gi|119182210 conserved hypothetical protein [Coccidioides immitis RS]
                   gi|258509580 predicted ORF [Lactobacillus rhamnosus GG]
                   gi|257867620 ATP synthase subunit C [Enterococcus casseliflavus EC30]
                   gi|58040759  Phage DNA packing protein [Gluconobacter oxydans 621H]
                   gi|238581245 hypothetical protein MPER_11312 [Moniliophthora perniciosa FA553]
                   gi|67904418  hypothetical protein AN9196.2 [Aspergillus nidulans FGSC A4]
                   gi|42518599  S-adenosylmethionine synthetase [Lactobacillus johnsonii NCC 533]
                   gi|167945592 5,10-methylenetetrahydrofolate reductase [Endoriftia persephone 'Hot96_1+Hot96_2']
                   gi|169625740 hypothetical protein SNOG_16146 [Phaeosphaeria nodorum SN15]
```

Probability Based Mowse Score

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 51 indicate identity or extensive homology (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

Peptide Summary Report

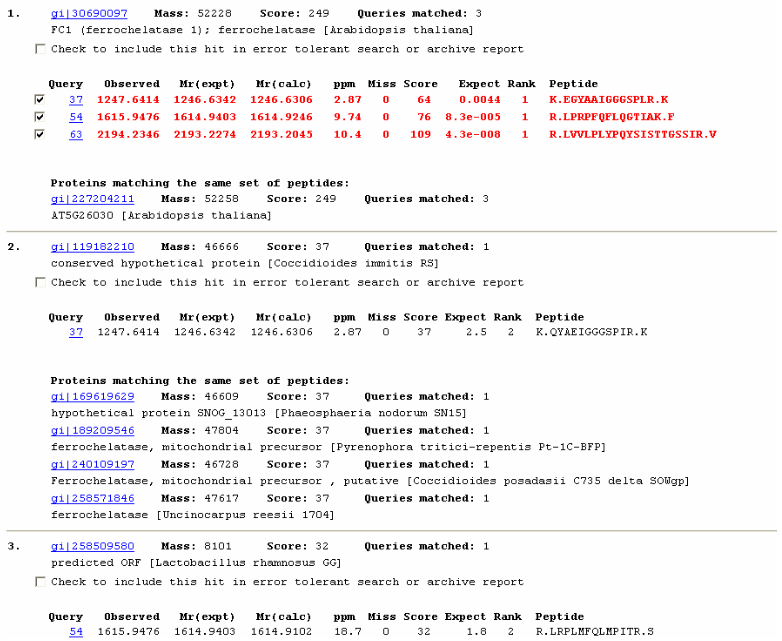
Format As | Peptide Summary ▾ | Help

Significance threshold p< 0.05 | Max. number of hits 10

Standard scoring ● MudPIT scoring ○ | Ions score or expect cut-off 0 | Show sub-sets 0

p-ups ● Suppress pop-ups ○ | Sort unassigned Decreasing Score ▾ | Require bold red ☐

Plateforme Protéomique
de l'Esplanade
IBMC
IBMP
GMGM



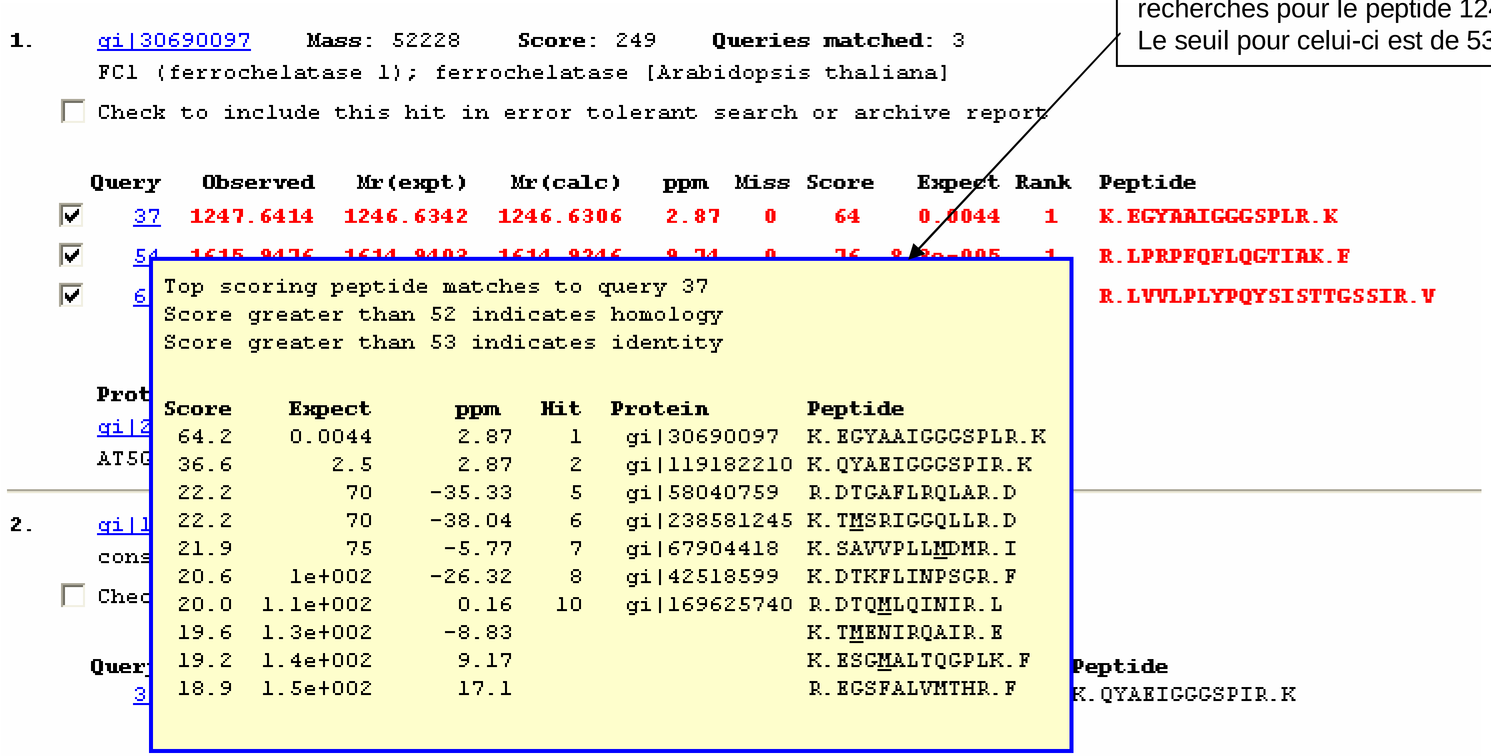
```
1.   gi|30690097     Mass: 52228     Score: 249     Queries matched: 3
     FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana]
   ☐ Check to include this hit in error tolerant search or archive report

     Query   Observed   Mr(expt)   Mr(calc)    ppm  Miss Score   Expect Rank  Peptide
   ☑    37  1247.6414  1246.6342  1246.6306   2.87    0    64   0.0044    1   K.EGYAAIGGGSPLR.K
   ☑    54  1615.9476  1614.9403  1614.9246   9.74    0    76  8.3e-005   1   R.LPRPFQFLQGTIAK.F
   ☑    63  2194.2346  2193.2274  2193.2045   10.4    0   109  4.3e-008   1   R.LVVLPLYPQYSISTTGSSIR.V

     Proteins matching the same set of peptides:
     gi|227204211     Mass: 52258     Score: 249     Queries matched: 3
     AT5G26030 [Arabidopsis thaliana]
-----------------------------------------------------------------------------------------------
2.   gi|119182210     Mass: 46666     Score: 37      Queries matched: 1
     conserved hypothetical protein [Coccidioides immitis RS]
   ☐ Check to include this hit in error tolerant search or archive report

     Query   Observed   Mr(expt)   Mr(calc)    ppm  Miss Score Expect Rank  Peptide
        37  1247.6414  1246.6342  1246.6306   2.87    0    37     2.5    2   K.QYAEIGGGSPIR.K

     Proteins matching the same set of peptides:
     gi|169619629     Mass: 46609     Score: 37      Queries matched: 1
     hypothetical protein SNOG_13013 [Phaeosphaeria nodorum SN15]
     gi|189209546     Mass: 47804     Score: 37      Queries matched: 1
     ferrochelatase, mitochondrial precursor [Pyrenophora tritici-repentis Pt-1C-BFP]
     gi|240109197     Mass: 46728     Score: 37      Queries matched: 1
     Ferrochelatase, mitochondrial precursor , putative [Coccidioides posadasii C735 delta SOWgp]
     gi|258571846     Mass: 47617     Score: 37      Queries matched: 1
     ferrochelatase [Uncinocarpus reesii 1704]
-----------------------------------------------------------------------------------------------
3.   gi|258509580     Mass: 8101      Score: 32      Queries matched: 1
     predicted ORF [Lactobacillus rhamnosus GG]
   ☐ Check to include this hit in error tolerant search or archive report

     Query   Observed   Mr(expt)   Mr(calc)    ppm  Miss Score Expect Rank  Peptide
        54  1615.9476  1614.9403  1614.9102   18.7    0    32     1.8    2   R.LRPLMFQLMPITR.S
```

Critères de validation d’un spectre MSMS

-Pour chaque spectre MSMS, Mascot calcule une valeur seuil au delà de laquelle le hit est significatif à plus de 95%
-Si plusieurs spectres de fragmentation matchent pour une même protéine, Mascot compile le résultat
-Code couleur pour visualiser les peptides fragmentées :

- -Rouge : score validé selon critère mascot/ **Noir** score
- -**gras** : peptide présent dans une seule protéines / non gras (peptide peut être trouvé dans plusieurs protéines différentes)

Pop-up de visualisation des recherches pour le peptide 1247da
Le seuil pour celui-ci est de 53

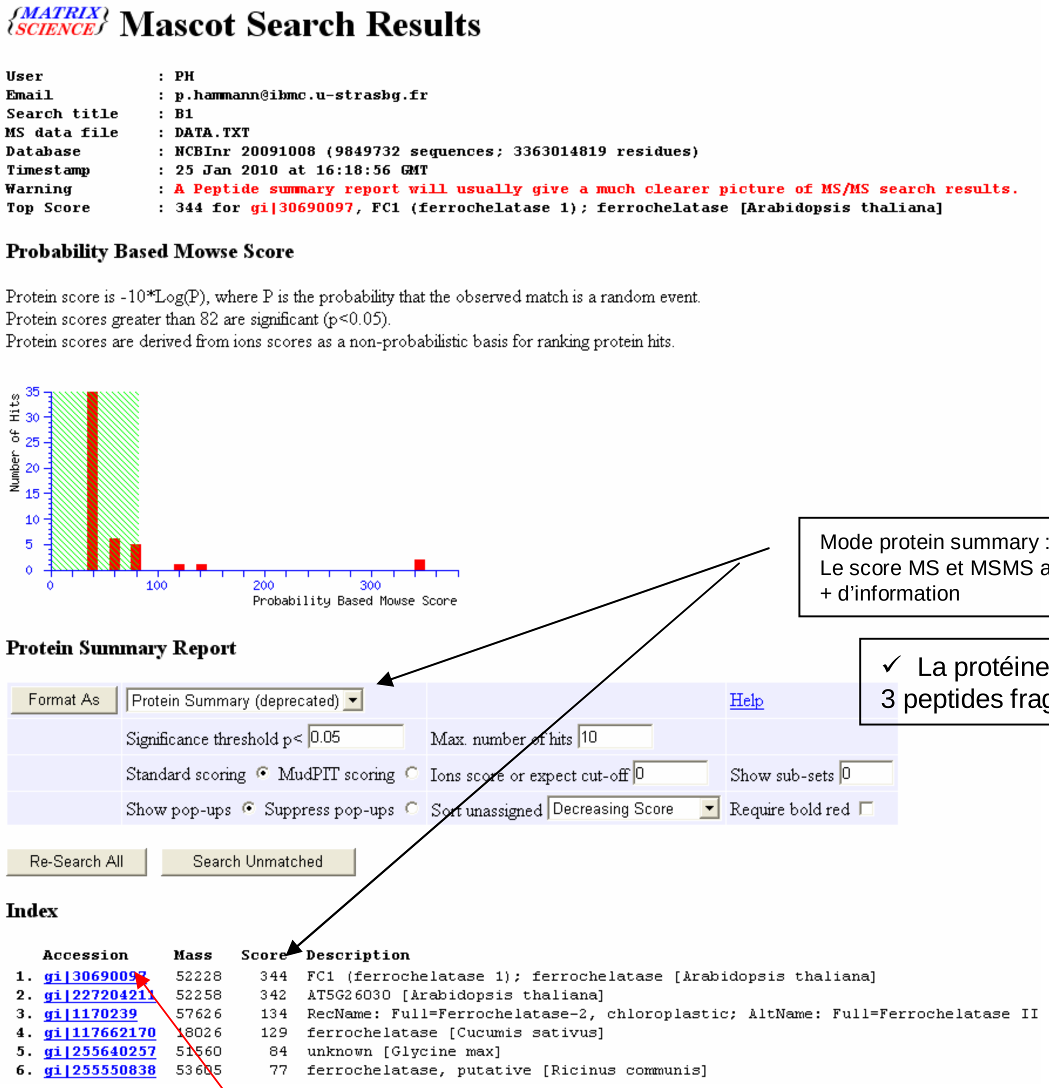


```
1.    gi|30690097     Mass: 52228     Score: 249     Queries matched: 3
      FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana]
   ☐ Check to include this hit in error tolerant search or archive report

     Query   Observed   Mr(expt)   Mr(calc)    ppm   Miss  Score    Expect   Rank  Peptide
   ☑   37   1247.6414  1246.6342  1246.6306   2.87    0     64     0.0044    1    K.EGYAAIGGGSPLR.K
   ☑   54   1615.9476  1614.9403  1614.9246   9.74    0     76                1    R.LPRPFQFLQGTIAK.F
   ☑   6                                                                           R.LVVLPLYPQYSISTTGSSIR.V

      Prot
      gi|2
      AT5G

2.    gi|1
      cons
   ☐ Chec

     Quer                                                                    Peptide
       3                                                                     K.QYAEIGGGSPIR.K
```

```
Top scoring peptide matches to query 37
Score greater than 52 indicates homology
Score greater than 53 indicates identity

Score     Expect       ppm   Hit   Protein         Peptide
 64.2     0.0044       2.87    1    gi|30690097   K.EGYAAIGGGSPLR.K
 36.6        2.5       2.87    2    gi|119182210  K.QYAEIGGGSPIR.K
 22.2         70     -35.33    5    gi|58040759   R.DTGAFLRQLAR.D
 22.2         70     -38.04    6    gi|238581245  K.TMSRIGGQLLR.D
 21.9         75      -5.77    7    gi|67904418   K.SAVVPLLMDMR.I
 20.6     1e+002     -26.32    8    gi|42518599   K.DTKFLINPSGR.F
 20.0   1.1e+002       0.16   10    gi|169625740  R.DTQMLQINIR.L
 19.6   1.3e+002      -8.83                       K.TMENIRQAIR.E
 19.2   1.4e+002       9.17                       K.ESGMALTQGPLK.F
 18.9   1.5e+002       17.1                       R.EGSFALVMTHR.F
```

✓ La protéine FC1 est validée en mode MSMS seul (ion score) avec 3 peptides fragmentés et un score total de 249

Résultat de recherche mascot combinant MS et MSMS : mode protein summary



Mascot Search Results

```
User              : PH
Email             : p.hammann@ibmc.u-strasbg.fr
Search title      : B1
MS data file      : DATA.TXT
Database          : NCBInr 20091008 (9849732 sequences; 3363014819 residues)
Timestamp         : 25 Jan 2010 at 16:18:56 GMT
Warning           : A Peptide summary report will usually give a much clearer picture of MS/MS search results.
Top Score         : 344 for gi|30690097, FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana]
```

Probability Based Mowse Score

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 82 are significant (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.

Protein Summary Report

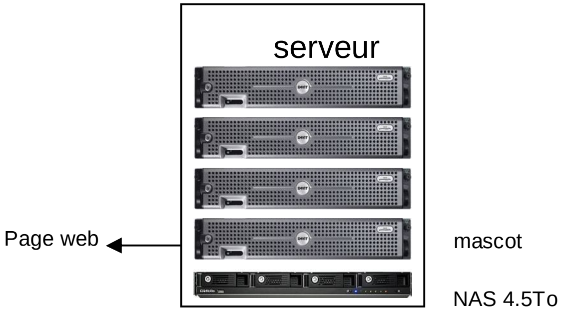
Format As: Protein Summary (deprecated)
Significance threshold p< 0.05
Max. number of hits 10
Standard scoring ● MudPIT scoring ○
Ions score or expect cut-off 0
Show sub-sets 0
Show pop-ups ● Suppress pop-ups ○
Sort unassigned Decreasing Score
Require bold red ☐

Index

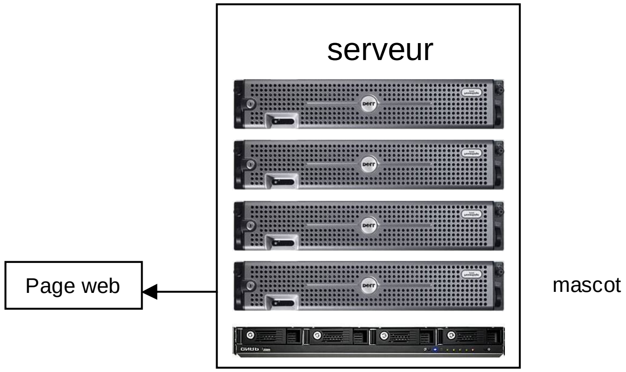
```
   Accession      Mass   Score  Description
1. gi|30690097    52228    344  FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana]
2. gi|227204211   52258    342  AT5G26030 [Arabidopsis thaliana]
3. gi|1170239     57626    134  RecName: Full=Ferrochelatase-2, chloroplastic; AltName: Full=Ferrochelatase II
4. gi|117662170   18026    129  ferrochelatase [Cucumis sativus]
5. gi|255640257   51560     84  unknown [Glycine max]
6. gi|255550838   53605     77  ferrochelatase, putative [Ricinus communis]
```

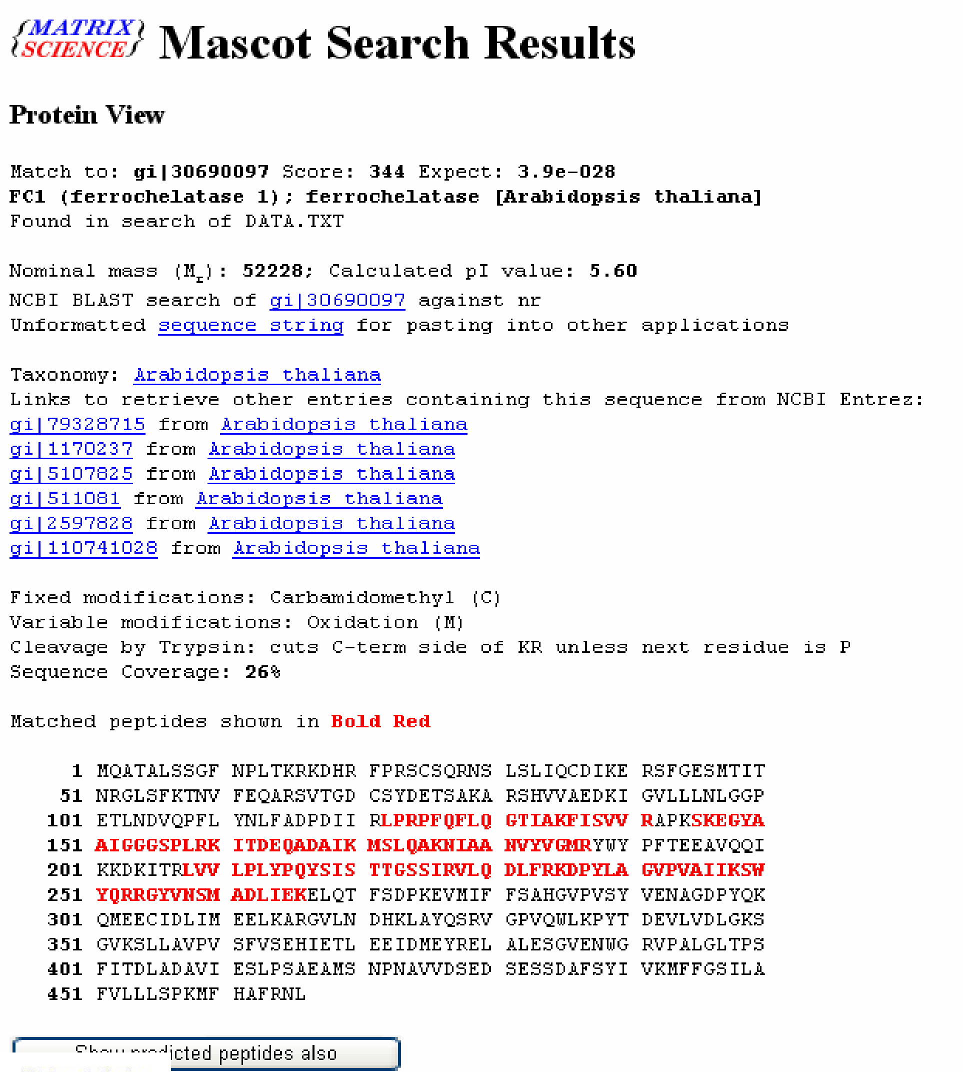
Mode protein summary :
Le score MS et MSMS augmente : 344
+ d'information

✓ La protéine FC1 est validée en mode MSMS et MS combiné avec 3 peptides fragmentés et un score total de 344



Résultat PFF mascot pour la protéine identifiée
Combinant MS et MSMS





Mascot Search Results

Protein View

Match to: **gi|30690097** Score: **344** Expect: **3.9e-028**
FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana]
Found in search of DATA.TXT

Nominal mass (M_r): **52228**; Calculated pI value: **5.60**
NCBI BLAST search of gi|30690097 against nr
Unformatted sequence string for pasting into other applications

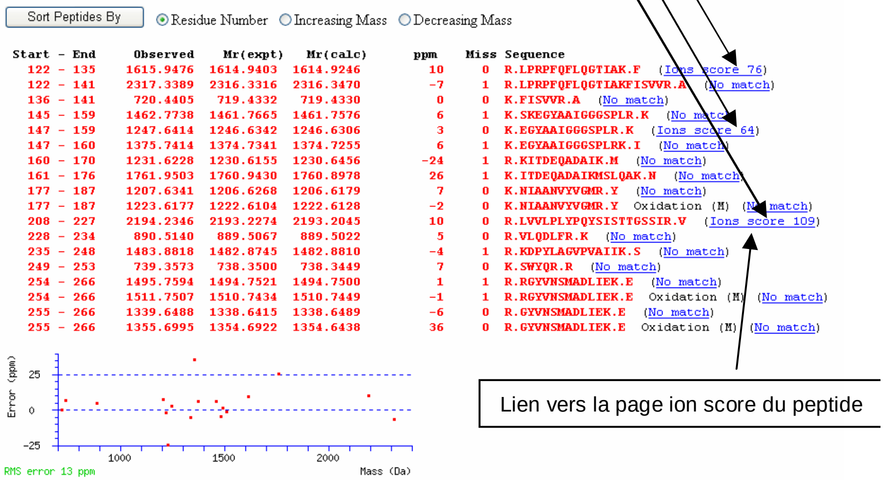
Taxonomy: Arabidopsis thaliana
Links to retrieve other entries containing this sequence from NCBI Entrez:
gi|79328715 from Arabidopsis thaliana
gi|1170237 from Arabidopsis thaliana
gi|5107825 from Arabidopsis thaliana
gi|511081 from Arabidopsis thaliana
gi|2597828 from Arabidopsis thaliana
gi|110741028 from Arabidopsis thaliana

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: **26%**

Matched peptides shown in **Bold Red**

1 MQATALSSGF NPLTKRKDHR FPRSCSQRNS LSLIQCDIKE RSFGESMTIT
51 NRGLSFKTNV FEQARSVTGD CSYDETSAKA RSHVVAEDKI GVLLLNLGGP
101 ETLNDVQPFL YNLFADPDII R**LPRPFQFLQ GTIAKFISVV** RAPK**SKEGYA**
151 **AIGGGSPLRK ITDEQADAIK MSLQAKNIAA NVYVGMR**YWY PFTEEAVQQI
201 KKDKITR**LVV LPLYPQYSIS TTGSSIRVLQ DLFRKDPYLA GVPVAIIKSW**
251 **YQRRGYVNSM ADLIEK**ELQT FSDPKEVMIF FSAHGVPVSY VENAGDPYQK
301 QMEECIDLIM EELKARGVLN DHKLAYQSRV GPVQWLKPYT DEVLVDLGKS
351 GVKSLLAVPV SFVSEHIETL EEIDMEYREL ALESGVENWG RVPALGLTPS
401 FITDLADAVI ESLPSAEAMS NPNAVVDSED SESSDAFSYI VKMFFGSILA
451 FVLLLSPKMF HAFRNL

3 peptides fragmentés
Et leurs ions score respectif

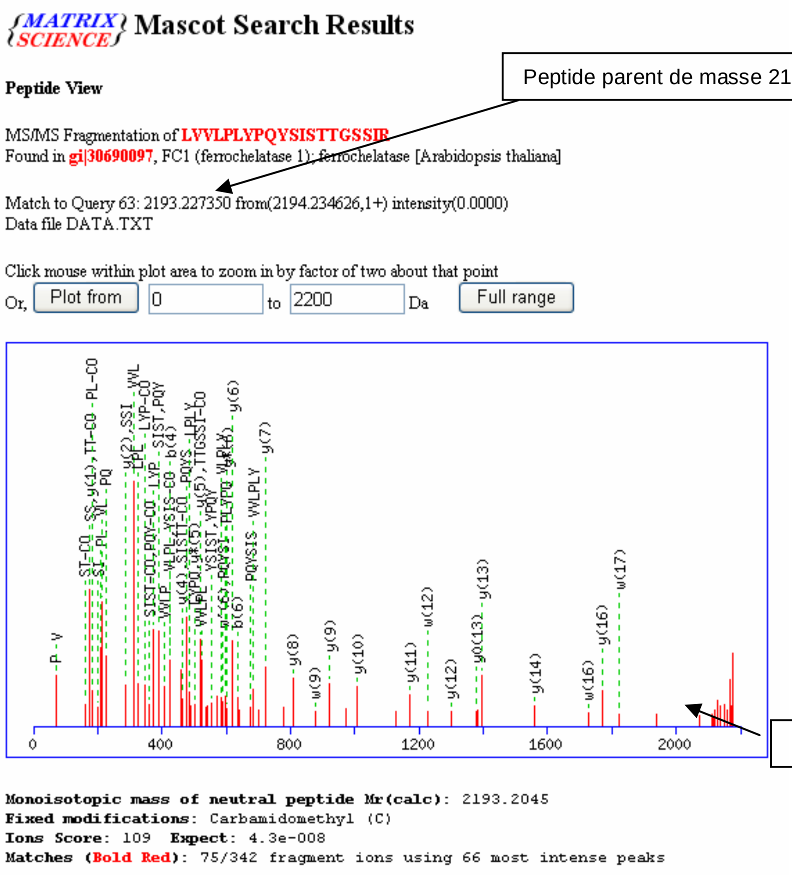


```
Start - End     Observed  Mr(expt)  Mr(calc)    ppm  Miss Sequence
  122 - 135    1615.9476 1614.9403 1614.9246     10     0 R.LPRPFQFLQGTIAK.F  (Ions score 76)
  122 - 141    2317.3389 2316.3316 2316.3470     -7     1 R.LPRPFQFLQGTIAKFISVVR.A  (No match)
  136 - 141     720.4405  719.4332  719.4330      0     0 K.FISVVR.A  (No match)
  145 - 159    1462.7738 1461.7665 1461.7576      6     1 K.SKEGYAAIGGGSPLR.K  (No match)
  147 - 159    1247.6414 1246.6342 1246.6306      3     0 K.EGYAAIGGGSPLR.K  (Ions score 64)
  147 - 160    1375.7414 1374.7341 1374.7255      6     1 K.EGYAAIGGGSPLRK.I  (No match)
  160 - 170    1231.6228 1230.6155 1230.6456    -24     1 R.KITDEQADAIK.M  (No match)
  161 - 176    1761.9503 1760.9430 1760.8978     26     1 K.ITDEQADAIKMSLQAK.N  (No match)
  177 - 187    1207.6341 1206.6268 1206.6179      7     0 K.NIAANVYVGMR.Y  (No match)
  177 - 187    1223.6177 1222.6104 1222.6128     -2     0 K.NIAANVYVGMR.Y  Oxidation (M)  (No match)
  208 - 227    2194.2346 2193.2274 2193.2045     10     0 R.LVVLPLYPQYSISTTGSSIR.V  (Ions score 109)
  228 - 234     890.5140  889.5067  889.5022      5     0 R.VLQDLFR.K  (No match)
  235 - 248    1483.8818 1482.8745 1482.8810     -4     1 R.KDPYLAGVPVAIIK.S  (No match)
  249 - 253     739.3573  738.3500  738.3449      7     0 K.SWYQR.R  (No match)
  254 - 266    1495.7594 1494.7521 1494.7500      1     1 R.RGYVNSMADLIEK.E  (No match)
  254 - 266    1511.7507 1510.7434 1510.7449     -1     1 R.RGYVNSMADLIEK.E  Oxidation (M)  (No match)
  255 - 266    1339.6488 1338.6415 1338.6489     -6     0 R.GYVNSMADLIEK.E  (No match)
  255 - 266    1355.6995 1354.6922 1354.6438     36     0 R.GYVNSMADLIEK.E  Oxidation (M)  (No match)
```

Lien vers la page ion score du peptide

Résultat MSMS MASCOT d’un peptide fragmenté :
Page mascot ion score du peptide 2193.2 da

Table de fragmentation :
Liste de tous les fragments théoriques
En rouge : fragments observés sur le spectre MSMS



Mascot Search Results

Peptide View

MS/MS Fragmentation of **LVVLPLYPQYSISTTGSSIR**
Found in **gi|30690097**, FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana]

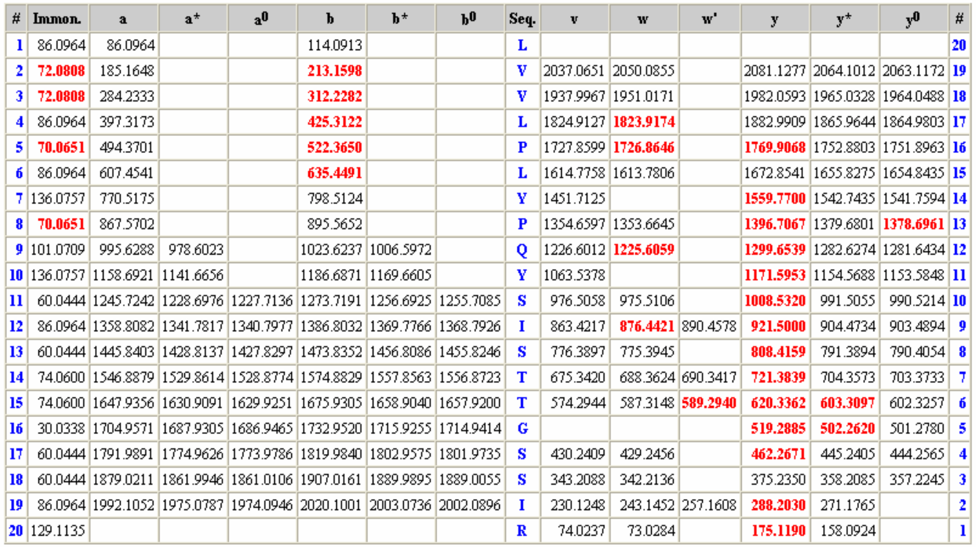
Match to Query 63: 2193.227350 from(2194.234626,1+) intensity(0.0000)
Data file DATA.TXT

Click mouse within plot area to zoom in by factor of two about that point
Or, Plot from 0 to 2200 Da Full range

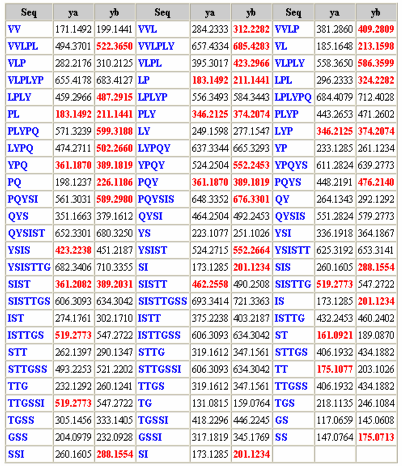
Monoisotopic mass of neutral peptide Mr(calc): 2193.2045
Fixed modifications: Carbamidomethyl (C)
Ions Score: 109 **Expect:** 4.3e-008
Matches (Bold Red): 75/342 fragment ions using 66 most intense peaks

Peptide parent de masse 2193.22 da

Spectre MSMS simplifié

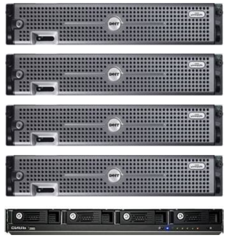


#	Immon.	a	a*	a^0	b	b*	b^0	Seq.	v	w	w'	y	y*	y^0	#
1	86.0964	86.0964			114.0913			L							20
2	**72.0808**	185.1648			**213.1598**			V	2037.0651	2050.0855		2081.1277	2064.1012	2063.1172	19
3	**72.0808**	284.2333			**312.2282**			V	1937.9967	1951.0171		1982.0593	1965.0328	1964.0488	18
4	86.0964	397.3173			**425.3122**			L	1824.9127	**1823.9174**		1882.9909	1865.9644	1864.9803	17
5	**70.0651**	494.3701			**522.3650**			P	1727.8599	**1726.8646**		**1769.9068**	1752.8803	1751.8963	16
6	86.0964	607.4541			**635.4491**			L	1614.7758	1613.7806		1672.8541	1655.8275	1654.8435	15
7	136.0757	770.5175			798.5124			Y	1451.7125			**1559.7700**	1542.7435	1541.7594	14
8	**70.0651**	867.5702			895.5652			P	1354.6597	1353.6645		**1396.7067**	1379.6801	**1378.6961**	13
9	101.0709	995.6288	978.6023		1023.6237	1006.5972		Q	1226.6012	**1225.6059**		**1299.6539**	1282.6274	1281.6434	12
10	136.0757	1158.6921	1141.6656		1186.6871	1169.6605		Y	1063.5378			**1171.5953**	1154.5688	1153.5848	11
11	60.0444	1245.7242	1228.6976	1227.7136	1273.7191	1256.6925	1255.7085	S	976.5058	975.5106		**1008.5320**	991.5055	990.5214	10
12	86.0964	1358.8082	1341.7817	1340.7977	1386.8032	1369.7766	1368.7926	I	863.4217	**876.4421**	890.4578	**921.5000**	904.4734	903.4894	9
13	60.0444	1445.8403	1428.8137	1427.8297	1473.8352	1456.8086	1455.8246	S	776.3897	775.3945		**808.4159**	791.3894	790.4054	8
14	74.0600	1546.8879	1529.8614	1528.8774	1574.8829	1557.8563	1556.8723	T	675.3420	688.3624	690.3417	**721.3839**	704.3573	703.3733	7
15	74.0600	1647.9356	1630.9091	1629.9251	1675.9305	1658.9040	1657.9200	T	574.2944	587.3148	**589.2940**	**620.3362**	**603.3097**	602.3257	6
16	30.0338	1704.9571	1687.9305	1686.9465	1732.9520	1715.9255	1714.9414	G				**519.2885**	**502.2620**	501.2780	5
17	60.0444	1791.9891	1774.9626	1773.9786	1819.9840	1802.9575	1801.9735	S	430.2409	429.2456		**462.2671**	445.2405	444.2565	4
18	60.0444	1879.0211	1861.9946	1861.0106	1907.0161	1889.9895	1889.0055	S	343.2088	342.2136		375.2350	358.2085	357.2245	3
19	86.0964	1992.1052	1975.0787	1974.0946	2020.1001	2003.0736	2002.0896	I	230.1248	243.1452	257.1608	**288.2030**	271.1765		2
20	129.1135							R	74.0237	73.0284		**175.1190**	158.0924		1



Seq	ya	yb	Seq	ya	yb	Seq	ya	yb
VV	171.1492	199.1441	VVL	284.2333	**312.2282**	VVLP	381.2860	**409.2809**
VVLPL	494.3701	**522.3650**	VVLPLY	657.4334	**685.4283**	VL	185.1648	**213.1598**
VLP	282.2176	310.2125	VLPL	395.3017	**423.2966**	VLPLY	558.3650	**586.3599**
VLPLYP	655.4178	683.4127	LP	**183.1492**	**211.1441**	LPL	296.2333	**324.2282**
LPLY	459.2966	**487.2915**	LPLYP	556.3493	584.3443	LPLYPQ	684.4079	712.4028
PL	**183.1492**	**211.1441**	PLY	**346.2125**	**374.2074**	PLYP	443.2653	471.2602
PLYPQ	571.3239	**599.3188**	LY	249.1598	277.1547	LYP	**346.2125**	**374.2074**
LYPQ	474.2711	**502.2660**	LYPQY	637.3344	665.3293	YP	233.1285	261.1234
YPQ	**361.1870**	**389.1819**	YPQY	524.2504	**552.2453**	YPQYS	611.2824	639.2773
PQ	198.1237	**226.1186**	PQY	**361.1870**	**389.1819**	PQYS	448.2191	**476.2140**
PQYSI	561.3031	**589.2980**	PQYSIS	648.3352	**676.3301**	QY	264.1343	292.1292
QYS	351.1663	379.1612	QYSI	464.2504	492.2453	QYSIS	551.2824	579.2773
QYSIST	652.3301	680.3250	YS	223.1077	251.1026	YSI	336.1918	364.1867
YSIS	**423.2238**	451.2187	YSIST	524.2715	**552.2664**	YSISTT	625.3192	653.3141
YSISTTG	682.3406	710.3355	SI	173.1285	**201.1234**	SIS	260.1605	**288.1554**
SIST	**361.2082**	**389.2031**	SISTT	**462.2558**	490.2508	SISTTG	**519.2773**	547.2722
SISTTGS	606.3093	634.3042	SISTTGSS	693.3414	721.3363	IS	173.1285	**201.1234**
IST	274.1761	302.1710	ISTT	375.2238	403.2187	ISTTG	432.2453	460.2402
ISTTGS	**519.2773**	547.2722	ISTTGSS	606.3093	634.3042	ST	**161.0921**	189.0870
STT	262.1397	290.1347	STTG	319.1612	347.1561	STTGS	406.1932	434.1882
STTGSS	493.2253	521.2202	STTGSSI	606.3093	634.3042	TT	**175.1077**	203.1026
TTG	232.1292	260.1241	TTGS	319.1612	347.1561	TTGSS	406.1932	434.1882
TTGSSI	**519.2773**	547.2722	TG	131.0815	159.0764	TGS	218.1135	246.1084
TGSS	305.1456	333.1405	TGSSI	418.2296	446.2245	GS	117.0659	145.0608
GSS	204.0979	232.0928	GSSI	317.1819	345.1769	SS	147.0764	**175.0713**
SSI	260.1605	**288.1554**	SI	173.1285	**201.1234**			

serveur



Page web

mascot

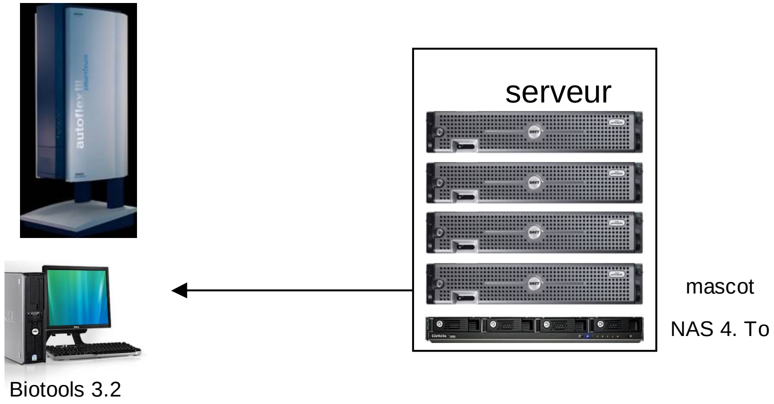
NAS 4.5To

Plateforme Protéomique
de l'Esplanade

IBMC
IBMP
GMGM

http://www.matrixscience.com/help/fragmentation_help.html

Visualisation du spectre fragmenté dans Biotools 3.2



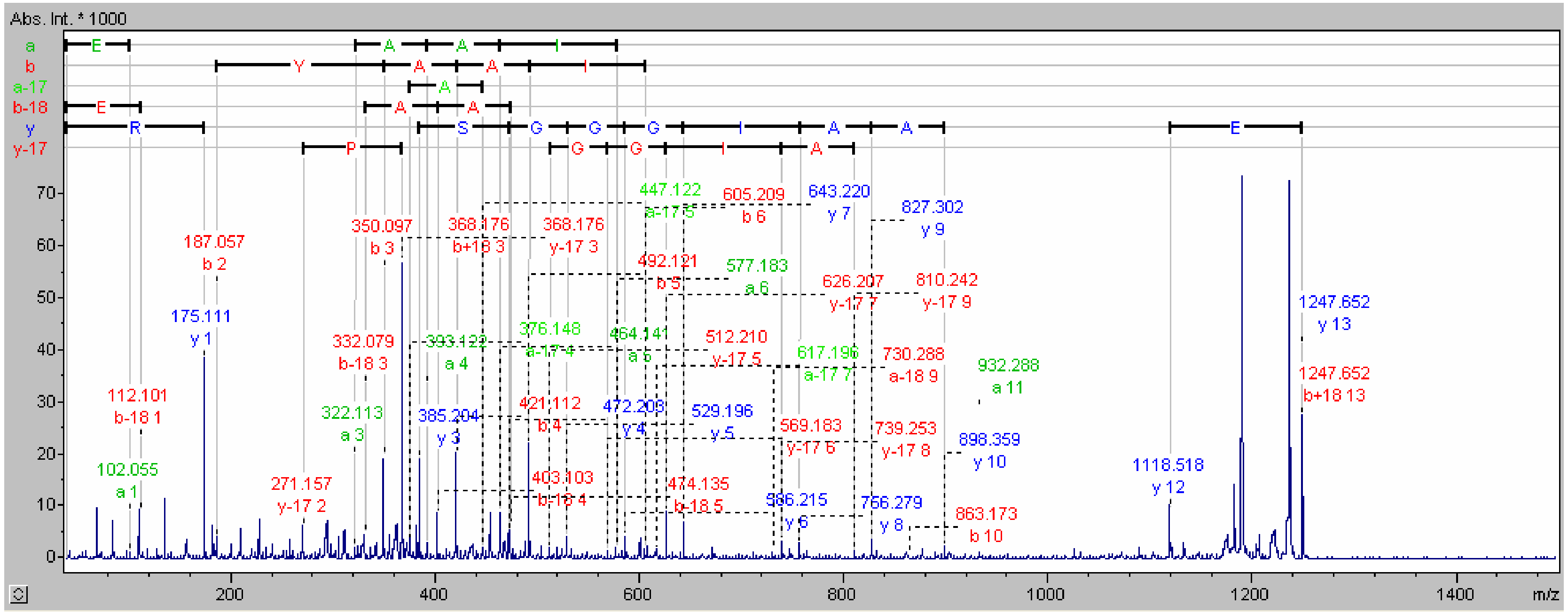
serveur

mascot

NAS 4. To

Biotools 3.2

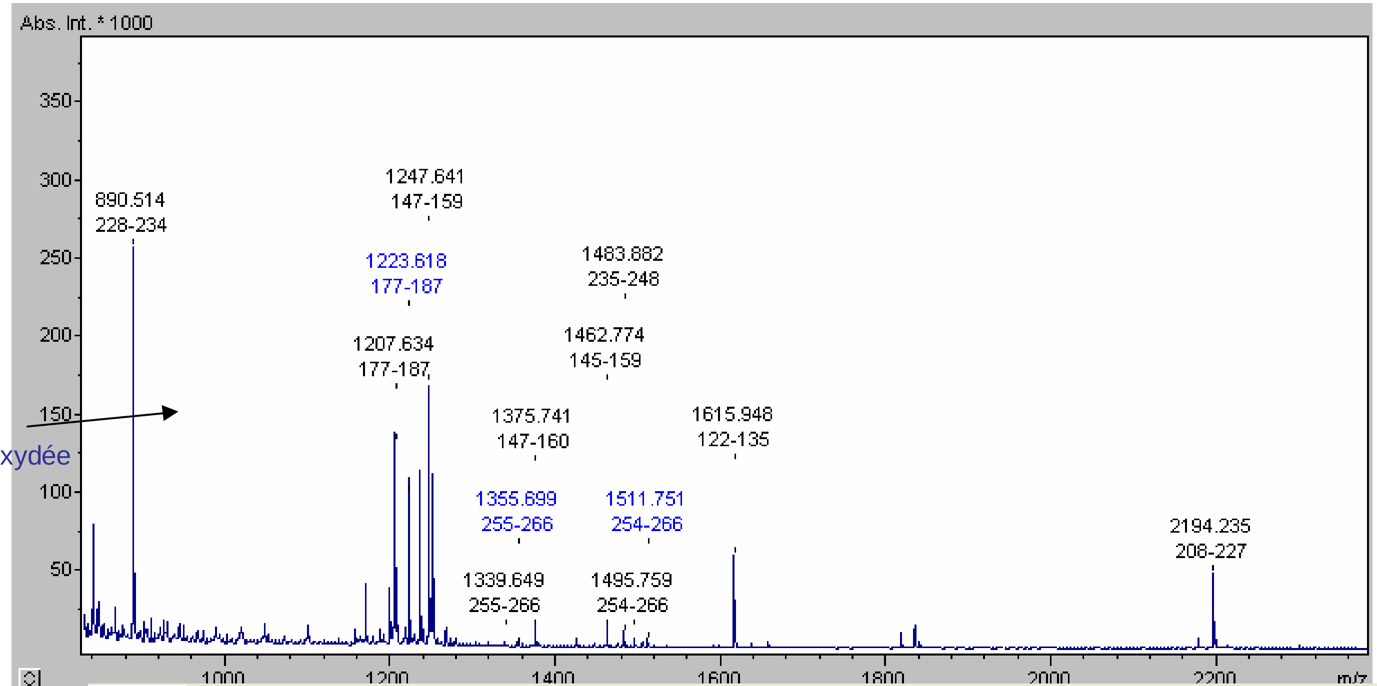
Spectre MSMS de l’ion parent 1293.2 da



Compilation des résultats d’analyse MS et MSMS dans biotools 3.2

Biotools 3.2

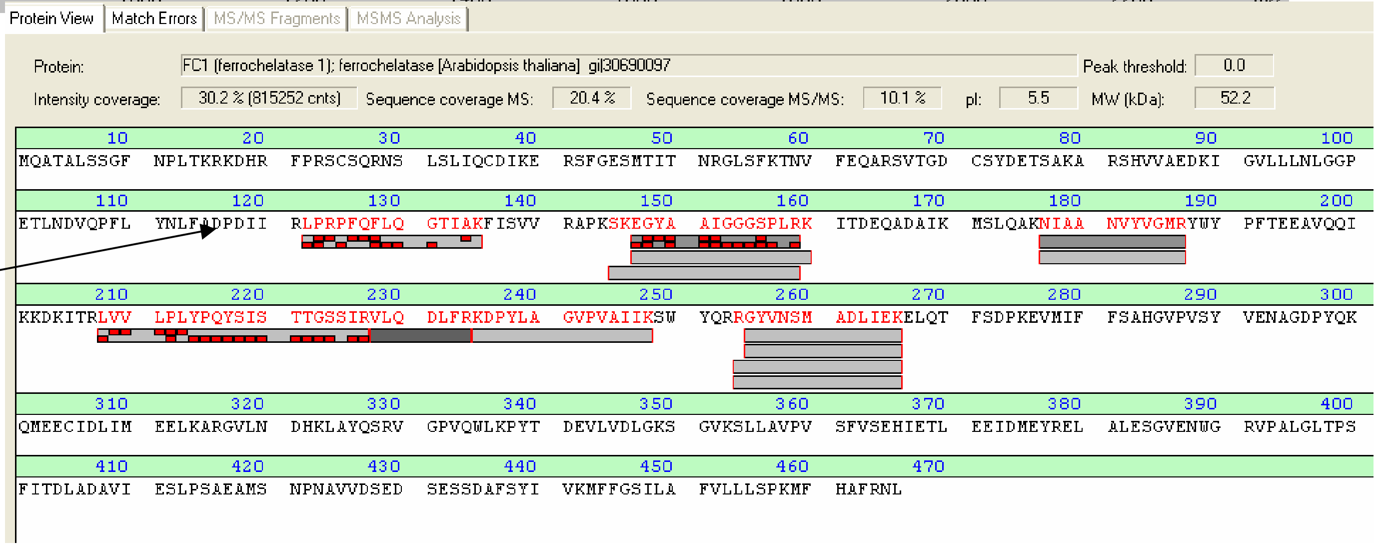
En noir peptides MH+
En bleu peptides Met oxydée



- ✓ Validé
- ✓En MS
- ✓En MSMS

3 peptides

Petits carrés rouges :
Peptide fragmenté
Gris peptide MS

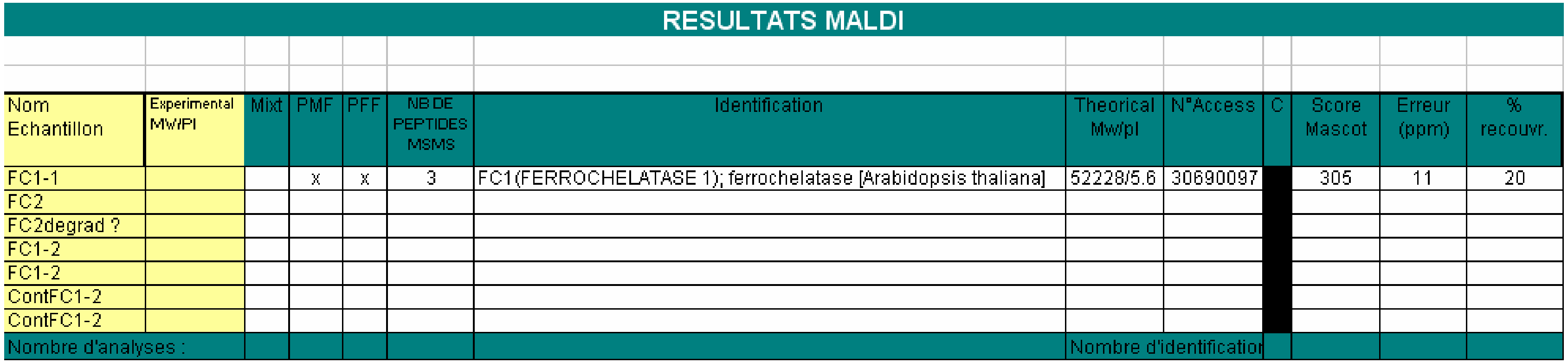


Protein: FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana] gi|30690097
Peak threshold: 0.0
Intensity coverage: 30.2 % (815252 cnts)
Sequence coverage MS: 20.4 %
Sequence coverage MS/MS: 10.1 %
pI: 5.5
MW (kDa): 52.2

```
        10         20         30         40         50         60         70         80         90        100
MQATALSSGF NPLTKRKDHR FPRSCSQRNS LSLIQCDIKE RSFGESMTIT NRGLSFKTNV FEQARSVTGD CSYDETSAKA RSHVVAEDKI GVLLLNLGGP

       110        120        130        140        150        160        170        180        190        200
ETLNDVQPFL YNLFADPDII RLPRPFQFLQ GTIAKFISVV RAPKSKEGYA AIGGGSPLRK ITDEQADAIK MSLQAKNIAA NVYVGMRYWY PFTEEAVQQI

       210        220        230        240        250        260        270        280        290        300
KKDKITRLVV LPLYPQYSIS TTGSSIRVLQ DLFRKDPYLA GVPVAIIKSW YQRRGYVNSM ADLIEKELQT FSDPKEVMIF FSAHGVPVSY VENAGDPYQK

       310        320        330        340        350        360        370        380        390        400
QMEECIDLIM EELKARGVLN DHKLAYQSRV GPVQWLKPYT DEVLVDLGKS GVKSLLAVPV SFVSEHIETL EEIDMEYREL ALESGVENWG RVPALGLTPS

       410        420        430        440        450        460        470
FITDLADAVI ESLPSAEAMS NPNAVVDSED SESSDAFSYI VKMFFGSILA FVLLLSPKMF HAFRNL
```

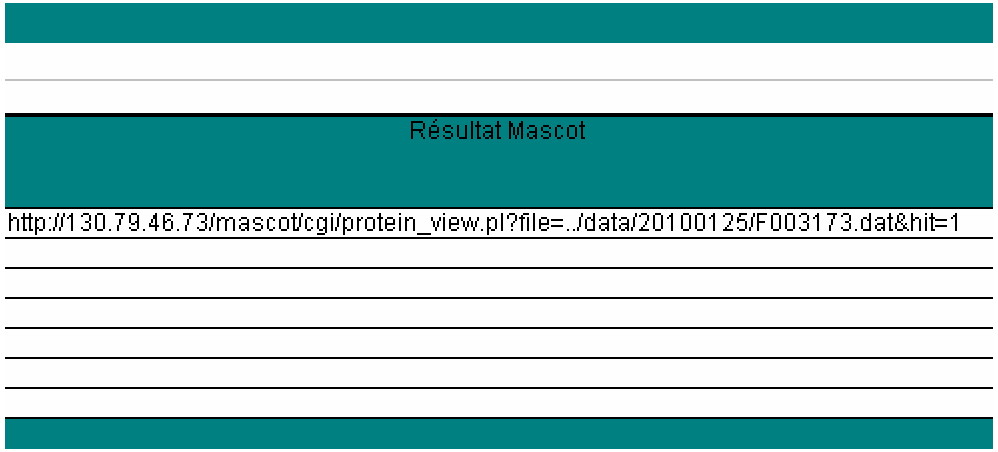
Plateforme Protéomique de l'Esplanade
IBMC
IBMP
GMGM

→ **Tableau synthétique de résultats d’analyse**



RESULTATS MALDI

Nom Echantillon	Experimental MW/PI	Mixt	PMF	PFF	NB DE PEPTIDES MSMS	Identification	Theorical Mw/pI	N°Access	C	Score Mascot	Erreur (ppm)	% recouvr.
FC1-1			x	x	3	FC1(FERROCHELATASE 1); ferrochelatase [Arabidopsis thaliana]	52228/5.6	30690097		305	11	20
FC2												
FC2degrad ?												
FC1-2												
FC1-2												
ContFC1-2												
ContFC1-2												
Nombre d'analyses :							Nombre d'identificatior					

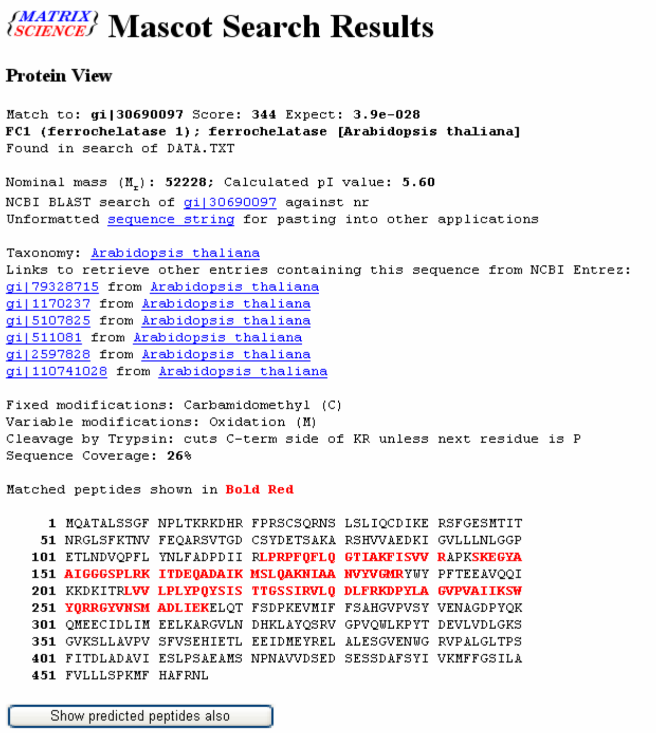


Résultat Mascot

http://130.79.46.73/mascot/cgi/protein_view.pl?file=../data/20100125/F003173.dat&hit=1

↓

Mail
G.Bonnard



Mascot Search Results

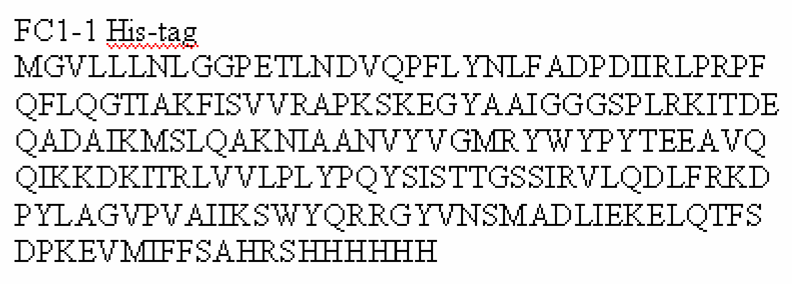
Protein View

```
Match to: gi|30690097 Score: 344 Expect: 3.9e-028
FC1 (ferrochelatase 1); ferrochelatase [Arabidopsis thaliana]
Found in search of DATA.TXT

Nominal mass (Mr): 52228; Calculated pI value: 5.60
NCBI BLAST search of gi|30690097 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Arabidopsis thaliana
Links to retrieve other entries containing this sequence from NCBI Entrez:
gi|79328715 from Arabidopsis thaliana
gi|1170237 from Arabidopsis thaliana
gi|5107825 from Arabidopsis thaliana
gi|511081 from Arabidopsis thaliana
gi|2597828 from Arabidopsis thaliana
gi|110741028 from Arabidopsis thaliana

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Sequence Coverage: 26%

Matched peptides shown in Bold Red

    1 MQATALSSGF NPLTKRKDHR FPRSCSQRNS LSLIQCDIKE RSFGESMTIT
   51 NRGLSFKTNV FEQARSVTGD CSYDETSAKA RSHVVAEDKI GVLLLNLGGP
  101 ETLNDVQPFL YNLFADPDII RLPRPFQFLQ GTIAKFISVV RAPKSKEGYA
  151 AIGGGSPLRK ITDEQADAIK MSLQAKNIAA NVYVGMRYWY PFTEEAVQQI
  201 KKDKITRLVV LPLYPQYSIS TTGSSIRVLQ DLFRKDPYLA GVPVAIIKSW
  251 YQRRGYVNSM ADLIEKELQT FSDPKEVMIF FSAHGVPVSY VENAGDPYQK
  301 QMEECIDLIM EELKARGVLN DHKLAYQSRV GPVQWLKPYT DEVLVDLGKS
  351 GVKSLLAVPV SFVSEHIETL EEIDMEYREL ALESGVENWG RVPALGLTPS
  401 FITDLADAVI ESLPSAEAMS NPNAVVDSED SESSDAFSYI VKMFFGSILA
  451 FVLLLSPKMF HAFRNL
```

Analyse de protéines recombinantes

But : obtenir le % de recouvrement maximal sur le spectre MS

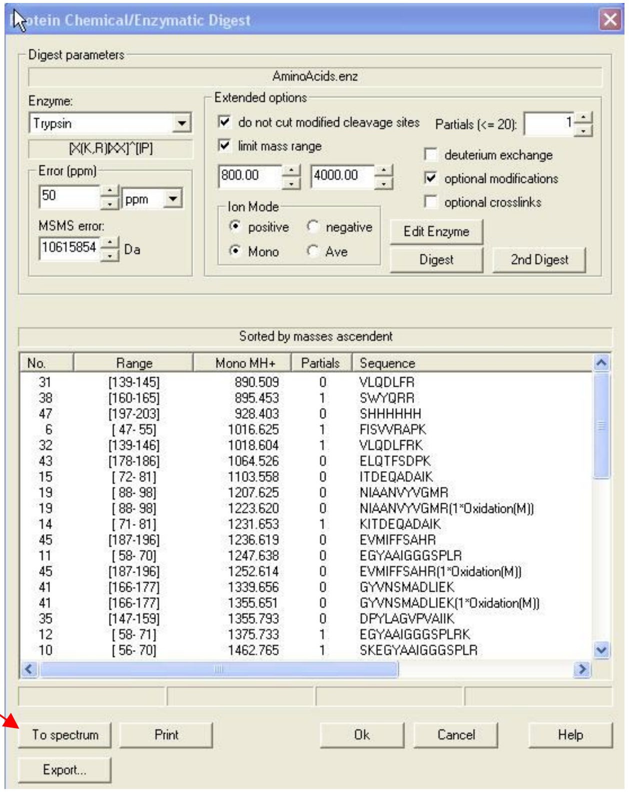
Biotools 3.2
Sequence editor



FC1-1 His-tag
MGVLLLNLGGPETLNDVQPFLYNLFADPDIIRLPRPF
QFLQGTIAKFISVVRAPKSKEGYAAIGGGSPLRKITDE
QADAIKMSLQAKNIAANVYVGMRYWYPYTEEAVQ
QIKKDKITRLVVLPLYPQYSISTTGSSIRVLQDLFRKD
PYLAGVPVAIIKSWYQRRGYVNSMADLIEKELQTFS
DPKEVMIFFSAHRSHHHHHH

Digestion théorique de votre séquence + tag
paramètres de coupure :
- Ions MH+
- Cysteines modifiées
- 1 missed clivage
- Méthionines oxydées

Appliquée au spectre expérimental :
Recherche de peptides (petits) non labellés en mode automatique
Augmentation de la couverture de séquence



otein Chemical/Enzymatic Digest

Digest parameters
AminoAcids.enz
Enzyme: Trypsin
[X(K,R)][XX]^[IP]
Error (ppm): 50 ppm
MSMS error: 10615854 Da

Extended options
☑ do not cut modified cleavage sites
☑ limit mass range
800.00 4000.00
Partials (<= 20): 1
☐ deuterium exchange
☑ optional modifications
☐ optional crosslinks

Ion Mode
● positive ○ negative
● Mono ○ Ave

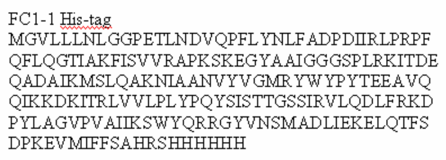
Sorted by masses ascendent

No.	Range	Mono MH+	Partials	Sequence
31	[139-145]	890.509	0	VLQDLFR
38	[160-165]	895.453	1	SWYQRR
47	[197-203]	928.403	0	SHHHHHH
6	[47- 55]	1016.625	1	FISVVRAPK
32	[139-146]	1018.604	1	VLQDLFRK
43	[178-186]	1064.526	0	ELQTFSDPK
15	[72- 81]	1103.558	0	ITDEQADAIK
19	[88- 98]	1207.625	0	NIAANVYVGMR
19	[88- 98]	1223.620	0	NIAANVYVGMR(1*Oxidation(M))
14	[71- 81]	1231.653	1	KITDEQADAIK
45	[187-196]	1236.619	0	EVMIFFSAHR
11	[58- 70]	1247.638	0	EGYAAIGGGSPLR
45	[187-196]	1252.614	0	EVMIFFSAHR(1*Oxidation(M))
41	[166-177]	1339.656	0	GYVNSMADLIEK
41	[166-177]	1355.651	0	GYVNSMADLIEK(1*Oxidation(M))
35	[147-159]	1355.793	0	DPYLAGVPVAIIK
12	[58- 71]	1375.733	1	EGYAAIGGGSPLRK
10	[56- 70]	1462.765	1	SKEGYAAIGGGSPLR

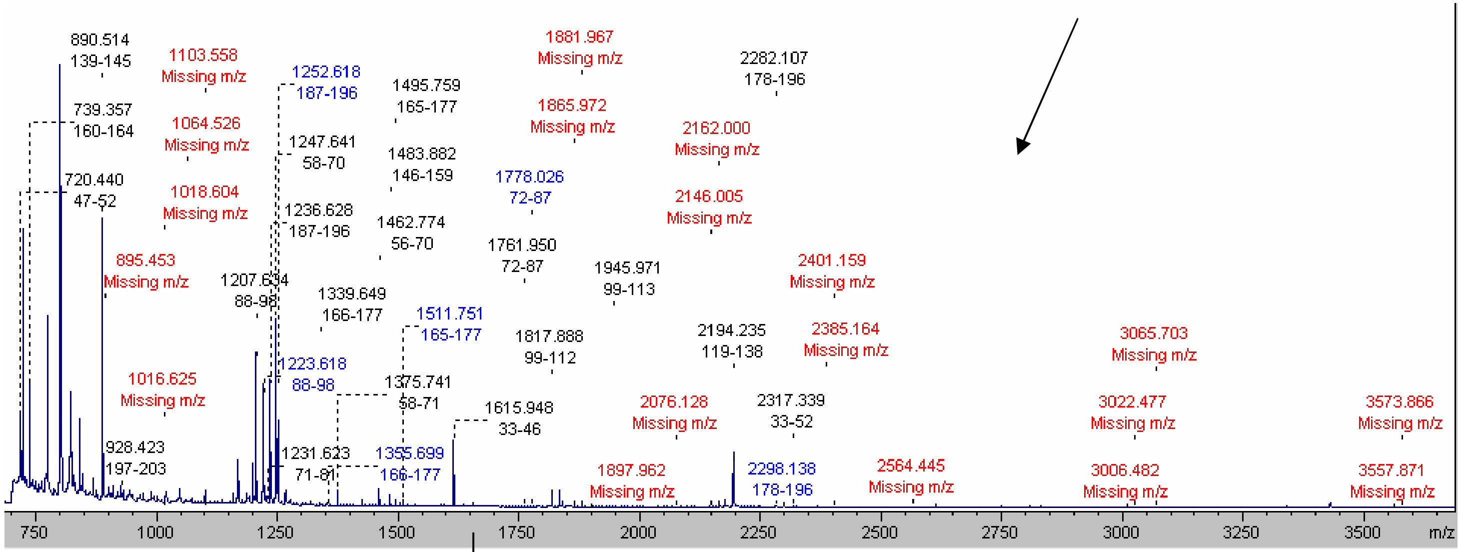
Plateforme Protéomique de l'Esplanade
IBMC
IBMP
GMGM

Biotools 3.2
Sequence editor

Protéine recombinante :
Recherche des peptides manquants sur le spectre

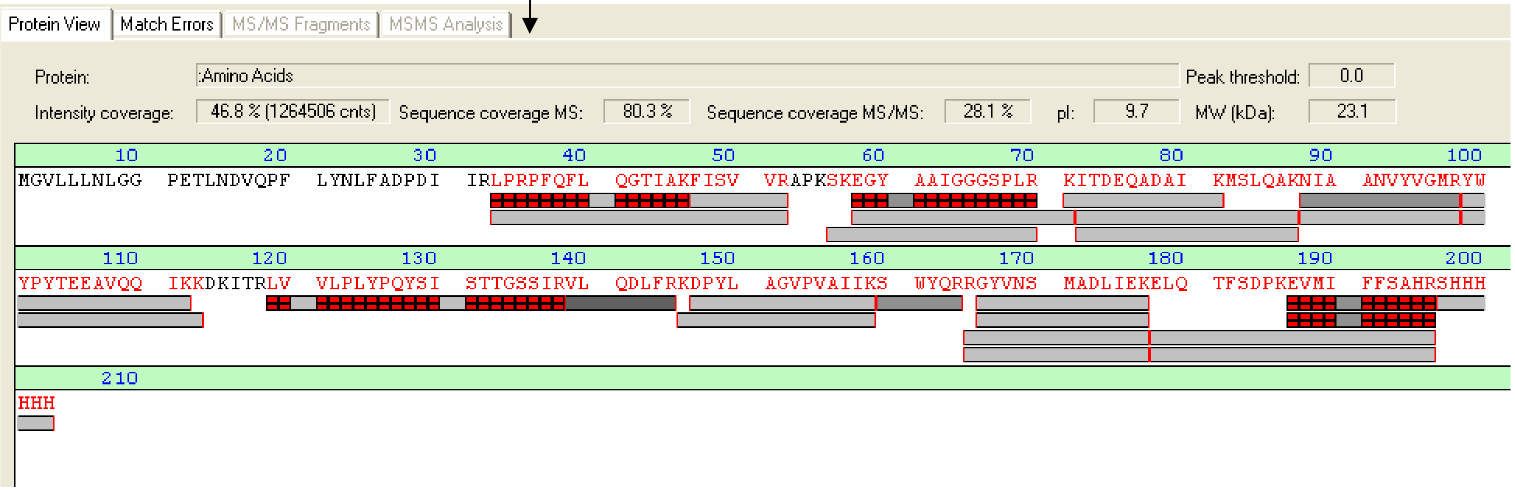


FC1-1 His-tag
MGVLLLNLGGPETLNDVQPFLYNLFADPDIIRLPRPF
QFLQGTIAKFISVVRAPKSKEGYAAIGGGSPLRKITDE
QADAIKMSLQAKNIAANVYVGMRYWYPYTEEAVQ
QIKKDKITRLVVLPLYPQYSISTTGSSIRVLQDLFRKD
PYLAGVPVAIIKSWYQRRGYVNSMADLIEKELQTFS
DPKEVMIFFSAHRSHHHHHH



Séquence appliquée au spectre expérimental : Recherche de peptides (petits) non labellés (missing) en mode automatique.

Augmentation de la couverture de séquence

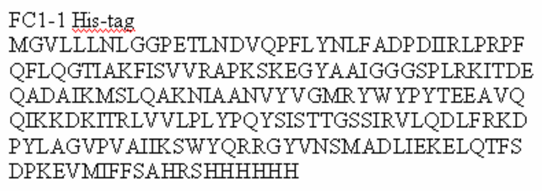


Protein: :Amino Acids
Peak threshold: 0.0
Intensity coverage: 46.8 % (1264506 cnts)
Sequence coverage MS: 80.3 %
Sequence coverage MS/MS: 28.1 %
pI: 9.7
MW (kDa): 23.1

```
         10         20         30         40         50         60         70         80         90        100
MGVLLLNLGG PETLNDVQPF LYNLFADPDI IRLPRPFQFL QGTIAKFISV VRAPKSKEGY AAIGGGSPLR KITDEQADAI KMSLQAKNIA ANVYVGMRYW
        110        120        130        140        150        160        170        180        190        200
YPYTEEAVQQ IKKDKITRLV VLPLYPQYSI STTGSSIRVL QDLFRKDPYL AGVPVAIIKS WYQRRGYVNS MADLIEKELQ TFSDPKEVMI FFSAHRSHHH
        210
HHH
```

Plateforme Protéomique
de l'Esplanade
IBMC
IBMP
GMGM

Contrôle qualité de protéines recombinantes : tableau de digestion théorique / expérimentale

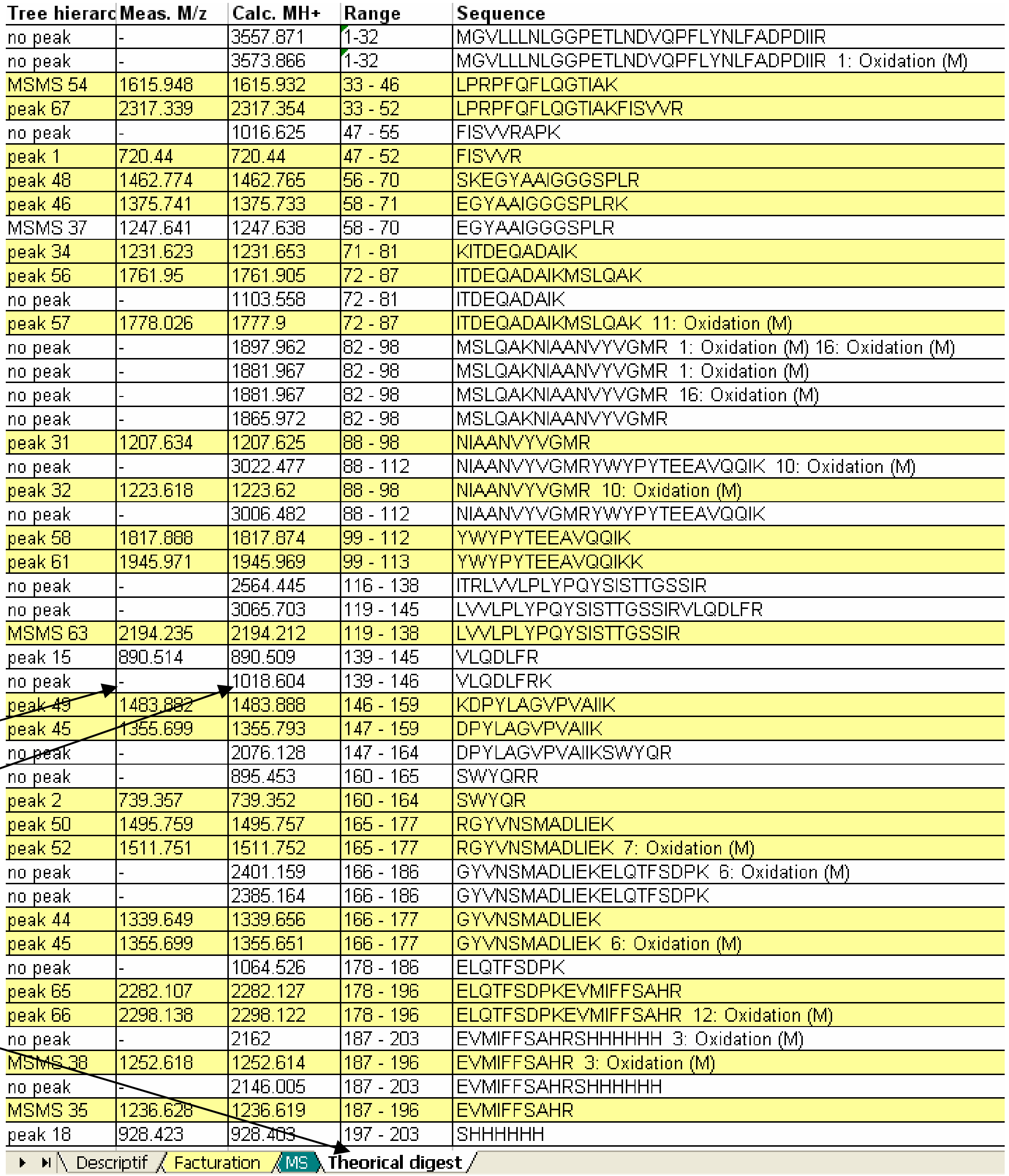


FC1-1 His-tag
MGVLLLNLGGPETLNDVQPFLYNLFADPDIIRLPRPF
QFLQGTIAKFISVVRAPKSKEGYAAIGGGSPLRKITDE
QADAIKMSLQAKNIAANVYVGMRYWYPYTEEAVQ
QIKKDKITRLVVLPLYPQYSISTTGSSIRVLQDLFRKD
PYLAGVPVAIIKSWYQRRGYVNSMADLIEKELQTFS
DPKEVMIFFSAHRSHHHHHH

Digestion théorique de votre séquence + tag
Paramètres :
Ions MH+
Cysteines modifiées
1 missed clivage
Methionines oxydées

Colonne peptides Experimentaux / Colonne peptides prédits

Tableau des résultats collé dans l'onglet theorical digest du formulaire d'analyse 2010



Tree hierarc	Meas. M/z	Calc. MH+	Range	Sequence
no peak	-	3557.871	1-32	MGVLLLNLGGPETLNDVQPFLYNLFADPDIIR
no peak	-	3573.866	1-32	MGVLLLNLGGPETLNDVQPFLYNLFADPDIIR 1: Oxidation (M)
MSMS 54	1615.948	1615.932	33 - 46	LPRPFQFLQGTIAK
peak 67	2317.339	2317.354	33 - 52	LPRPFQFLQGTIAKFISVVR
no peak	-	1016.625	47 - 55	FISVVRAPK
peak 1	720.44	720.44	47 - 52	FISVVR
peak 48	1462.774	1462.765	56 - 70	SKEGYAAIGGGSPLR
peak 46	1375.741	1375.733	58 - 71	EGYAAIGGGSPLRK
MSMS 37	1247.641	1247.638	58 - 70	EGYAAIGGGSPLR
peak 34	1231.623	1231.653	71 - 81	KITDEQADAIK
peak 56	1761.95	1761.905	72 - 87	ITDEQADAIKMSLQAK
no peak	-	1103.558	72 - 81	ITDEQADAIK
peak 57	1778.026	1777.9	72 - 87	ITDEQADAIKMSLQAK 11: Oxidation (M)
no peak	-	1897.962	82 - 98	MSLQAKNIAANVYVGMR 1: Oxidation (M) 16: Oxidation (M)
no peak	-	1881.967	82 - 98	MSLQAKNIAANVYVGMR 1: Oxidation (M)
no peak	-	1881.967	82 - 98	MSLQAKNIAANVYVGMR 16: Oxidation (M)
no peak	-	1865.972	82 - 98	MSLQAKNIAANVYVGMR
peak 31	1207.634	1207.625	88 - 98	NIAANVYVGMR
no peak	-	3022.477	88 - 112	NIAANVYVGMRYWYPYTEEAVQQIK 10: Oxidation (M)
peak 32	1223.618	1223.62	88 - 98	NIAANVYVGMR 10: Oxidation (M)
no peak	-	3006.482	88 - 112	NIAANVYVGMRYWYPYTEEAVQQIK
peak 58	1817.888	1817.874	99 - 112	YWYPYTEEAVQQIK
peak 61	1945.971	1945.969	99 - 113	YWYPYTEEAVQQIKK
no peak	-	2564.445	116 - 138	ITRLVVLPLYPQYSISTTGSSIR
no peak	-	3065.703	119 - 145	LVVLPLYPQYSISTTGSSIRVLQDLFR
MSMS 63	2194.235	2194.212	119 - 138	LVVLPLYPQYSISTTGSSIR
peak 15	890.514	890.509	139 - 145	VLQDLFR
no peak	-	1018.604	139 - 146	VLQDLFRK
peak 49	1483.882	1483.888	146 - 159	KDPYLAGVPVAIIK
peak 45	1355.699	1355.793	147 - 159	DPYLAGVPVAIIK
no peak	-	2076.128	147 - 164	DPYLAGVPVAIIKSWYQR
no peak	-	895.453	160 - 165	SWYQRR
peak 2	739.357	739.352	160 - 164	SWYQR
peak 50	1495.759	1495.757	165 - 177	RGYVNSMADLIEK
peak 52	1511.751	1511.752	165 - 177	RGYVNSMADLIEK 7: Oxidation (M)
no peak	-	2401.159	166 - 186	GYVNSMADLIEKELQTFSDPK 6: Oxidation (M)
no peak	-	2385.164	166 - 186	GYVNSMADLIEKELQTFSDPK
peak 44	1339.649	1339.656	166 - 177	GYVNSMADLIEK
peak 45	1355.699	1355.651	166 - 177	GYVNSMADLIEK 6: Oxidation (M)
no peak	-	1064.526	178 - 186	ELQTFSDPK
peak 65	2282.107	2282.127	178 - 196	ELQTFSDPKEVMIFFSAHR
peak 66	2298.138	2298.122	178 - 196	ELQTFSDPKEVMIFFSAHR 12: Oxidation (M)
no peak	-	2162	187 - 203	EVMIFFSAHRSHHHHHH 3: Oxidation (M)
MSMS 38	1252.618	1252.614	187 - 196	EVMIFFSAHR 3: Oxidation (M)
no peak	-	2146.005	187 - 203	EVMIFFSAHRSHHHHHH
MSMS 35	1236.628	1236.619	187 - 196	EVMIFFSAHR
peak 18	928.423	928.403	197 - 203	SHHHHHH

Plateforme Protéomique
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IBMC
IBMP
GMGM

Plus loin : analyse bas débit

Site de clivage et modifications

Stratégie d'analyse :

- **% de recouvrement maximal (idem protéine recombinante)**

But : dégrossir au maximum pour cerner les zones « noires », susceptibles de porter la (les) modifications.

Moyens : Quantité abondante de matériel (bande visible au bleu) pour MS et MSMS de qualité
Enzymes : trypsine, Asp N, gluC, ArgC, LysC

- **Bio-informatique :**

Prédiction de motifs de PTM

Prédiction d'adduits de masse par sequence editor (biotools 3.2) et recherche sur spectre expérimental. →

- **Stratégie d'enrichissement**

Exemple : phosphopeptides sur colonne imac.

- **Stratégie différentielle** : traitement enzymatique ciblant la PTM

Exemple : Avec ou sans phosphatase

↓

différentiel visualisé sur spectres MS.