Digestion with Pressure Cycling Technology (PCT)

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University of Missouri

What is PCT?

PCT is the application of cycling high and low hydrostatic pressures to a solid (tissue) or liquid (trypsin digest) sample.

Suzuki C, Suzuki K. *J Biochem* 1962; **52**: 67.

Ruan K, Lange R, Bec N, Balny C. *Biochem Biophys Res Commun* 1997; **239**: 150.

Chicon R, Belloque J, Recio I, Lopez-Fandino R. *J Dairy Res* 2006; **73**: 121.

Smejkal GB, Robinson MH, Lawrence NP, Tao F, Saravis CA, Schumacher RT. *J Biomol Tech* 2006;**17**: 173.

Ringham H, Bell RL, Smejkal GB, Behnke J, Witzmann FA. *Electrophoresis* 2007; **28**: 1022.

Pevsner P, Vecchione D, Stall B, Remsen T, Anand S, Stern A. 2007. British Mass Spectrometry Society, Edinburgh, Scotland,

Pevsner P, Vecchione D, Remsen T, Kessler P, Levers N, Yang P, Stern A, Samuels H. British Mass Spectrometry Society, 2007 Robinson College,Cambridge .

Pevsner P, Vecchione D, 1 Remsen T, Kessler P, Momeni M, Duddempudi S, Francois F, Stern A, Anand S. 2007 British Mass Spectrometry LCMS Meeting, Robinson College, Cambridge, UK,

PCT Sample Assembly

Microtubes (150µl)

Microtube Cartridge
(6 or 8 tubes)

Cartridge
Stack
(12-48)

PCT Barocycler

How does PCT Work?

- Pressures between 29k-145k PSI (2-10 kbar) denature protein
- Water-water and protein-water hydrogen bonds increase.
- Protein-protein hydrogen bonds decrease.

Silva JL, Weber G. *Annu Rev Phys Chem* 1993; **44**: 89.

How does PCT Work?

- Hydrophobic groups exposed to water.
- Water penetrates protein, increasing internal pressure.
- Hydrogen bonds responsible for secondary and tertiary structure of protein destabilize.

Ruan K, Lange R, Bec N, Balny C. *Biochem Biophys Res Commun* 1997; **239**: 150.

How does PCT Work?

- Non-native salt bridges, hydrophobic, and electrostatic interactions disrupted
  - –Electrostatic replusions increase.
  - –Repulsions favor unfolding.

Balny C. *Journal of Physics: Condensed Matter* 2004; **16**: S1245.

PCT and Trypsin Digest

- Pressures >35k PSI(2.4 kbar) will denature trypsin.

Pevsner P, Vecchione D, Stall B, Remsen T, Anand S, Stern A. 2007. British Mass Spectrometry Society, Edinburgh, Scotland

Pevsner P, Vecchione D, Remsen T, Kessler P, Levers N, Yang P, Stern A, Samuels H. British Mass Spectrometry Society, 2007 Robinson College, Cambridge .

Pevsner P, Vecchione D, 1 Remsen T, Kessler P, Momeni M, Duddempudi S, Francois F, Stern A, Anand S. 2007. British Mass Spectrometry LCMS Meeting, Robinson College, Cambridge, UK

Lopez-Ferrer D, Petritis K, Hixson KK, Heibeck TH, Moore RJ, Belov ME, Camp DG, Smith RD. *J Proteome Res* 2008; **7**: 3276.

PCT Applications

- Direct protein extraction from tissue
- Trypsin digest

Example of PCT Protein Extraction from Tissue

Extraction of Protein from Irradiated Mouse Heart

- Control (Normal Mouse Heart)
- Experimental [Mouse Heart 6 days post-total body irradiation (TBI)]
  -1 Gy, 2 Gy, and 4 Gy samples
  - All samples run in triplicate
  - Bradford Protein Assay

PCT Mouse Heart Protein Extraction

tube	sample	tissue mass mg	protein assay mg/mL	extraction efficiency (%)
HRT Na	control	28	2.25	8.0
HRT Nb		27	1.56	5.8
HRT Nc		**9**	0.68	7.6
HRT 1-6a	1 Gy	23	1.66	7.2
HRT 1-6b		**12**	0.94	7.8
HRT 1-6c		16	1.54	9.6
HRT 2-6a	2 Gy	29	3.15	10.9
HRT 2-6b		12	1.86	15.5
HRT 2-6c		**14**	1.14	8.2
HRT 4-6a	4 Gy	**10**	0.82	8.2
HRT 4-6b		18	1.41	7.8
HRT 4-6c		13	1.58	12.2
				9.1 ± 2.6

Why PCT Extraction?

- Reproducibility
- Speed
- Frozen samples can be processed
- Virtually adiabatic
- Ambient environment
- Instrument reliability
  - 30k cycles before our first repair

PCT Trypsin Digest

PCT Trypsin Digest (2007-2010)

- Remsen, T, Kessler, P, Francois, F, Stern, A, Anand, S, & Pevsner, P (**2008**). Imaging MALDI of colorectal carcinoma - field defects in satellite tissue. American Chemical Society Mid-Atlantic meeting, Queensborough College, Queens, NY, May, **2008**. *American Chemical Society Mid-Atlantic Meeting,* Queensborough College, Queens, NY.
- Remsen, T, Naftolin, F, Talebian, S, Kessler, P, Licciardi, F, Grifo, J, et al. (**2008**). LCMS identification of competent embryo biomarkers. British Mass Spectrometry Society, York, UK, September, **2008**.
- Lopez-Ferrer D, Petritis K, Hixson KK, Heibeck TH, Moore RJ, Belov ME, Camp DG,2nd, Smith RD. *J Proteome Res* **2008**; **7**: 3276.
- Pevsner PH, Melamed J, Remsen T, Kogus A, Francois F, Kessler P, Stern A, Anand S. *Biomarkers Med* **2009**; **3**: 55.

Trypsin/ Calibrant Prep

- **Trypsin**
  - –Trypsin was dissolved in 100 mM ammonium bicarbonate
- **Calibrant**
  - –Calibrants were dissolved in 0.1% TFA/Acetonitrile (ACN), 30/70.

BSA/Cyto-C Prep

- BSA*
  - –1158 µg BSA / 2 µg trypsin in 160 µL of 100 mM ammonium bicarbonate
- Cytochrome C*
  - –1158 µg Cyto-C / 2 µg trypsin in 160 µL of 100 mM ammonium bicarbonate

*No alkylation or reduction was performed on any samples.

PCT Protocol

- Experimental Protocol
  - 35 kPSI (2.4 kbar)
  - 40 seconds/cycle (with compressor)
  - Cycle= 20 sec 35k PSI / 20 sec 1 atm (5 seconds with continuous N_2 gas supply)
- BSA (16) and Cyto-C (16)
  - 1 cycle (n=4)
  - 5 cycles (n=4)
  - 10 cycles (n=4)
  - 20 cycles (n=4)

Reference Sample Protocol

- BSA (4) & Cyto-C (4)
  - 30 Minutes, 55˚C (n=1)
  - 1 Hour, 55˚C (n=1)
  - 2 Hours, 55˚C (n=1)
  - 4 Hours, 55˚C (n=1)

Matrix

- 70% ACN/ 30% CHCA

5 mg/mL

MALDI Instrument

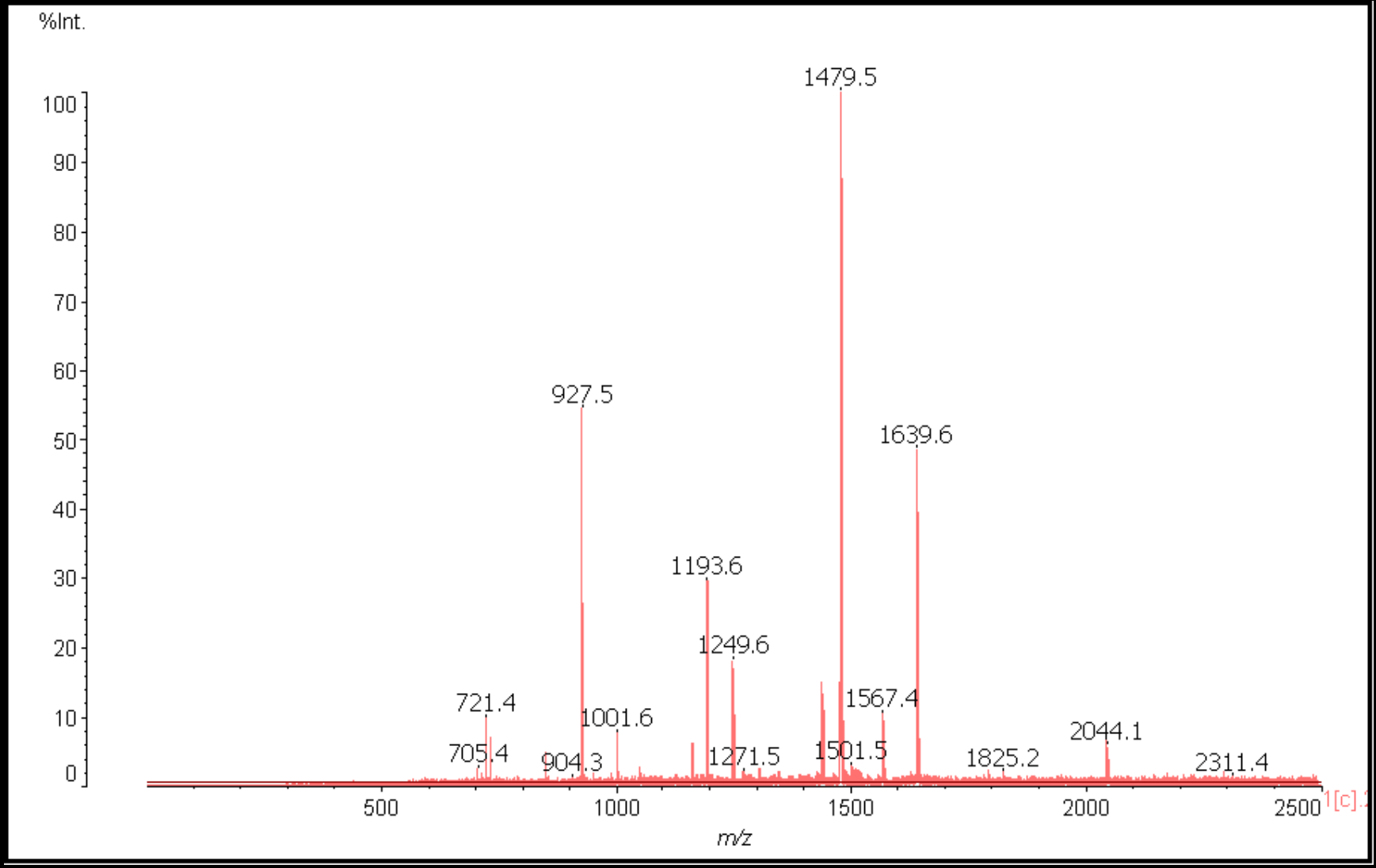
- Axima TOF^2 (MALDI TOF-TOF Shimadzu, Kyoto, Japan)
- Reflectron mode

Results

- MASCOT Identification of Cytochrome C & BSA
  - Numbered scores represent exact matches.
  - Coverage maps indicate % coverage and matching peptide sequences.

PCT Trypsin Digest Spectra

BSA 1 Cycle


%Int.
100
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80
70
60
50
40
30
20
10
0
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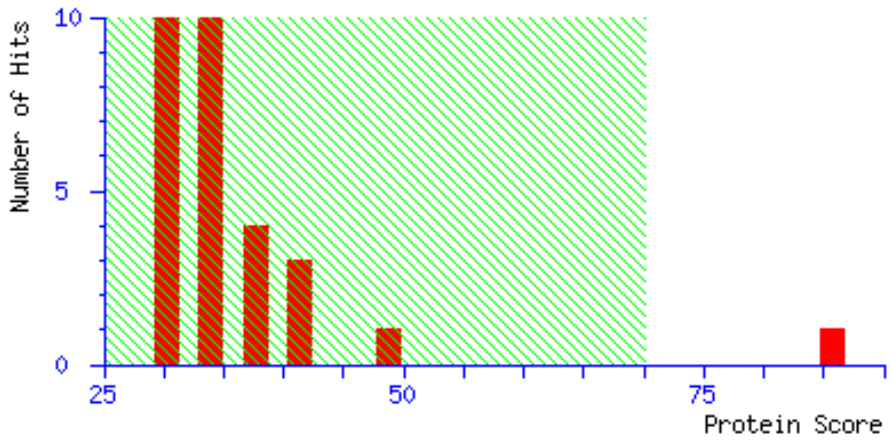
927.5
1639.6
1193.6
1249.6
721.4
1001.6
1567.4
2044.1
705.4
904.3
1271.5
1501.5
1825.2
2311.4
500
1000
1500
2000
2500
m/z

BSA 1 Cycle Score

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 70 are significant (p<0.05).



Concise Protein Summary Report

Format As	Concise Protein Summary	Help
	Significance threshold p< 0.05	Max. number of hits AUTO

Re-Search All | Search Unmatched

1. ALBU_BOVIN **Mass:** 69248 **Score:** 86 **Expect:** 0.0014 **Matches:** 13
Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4

BSA 1 Cycle Coverage Map

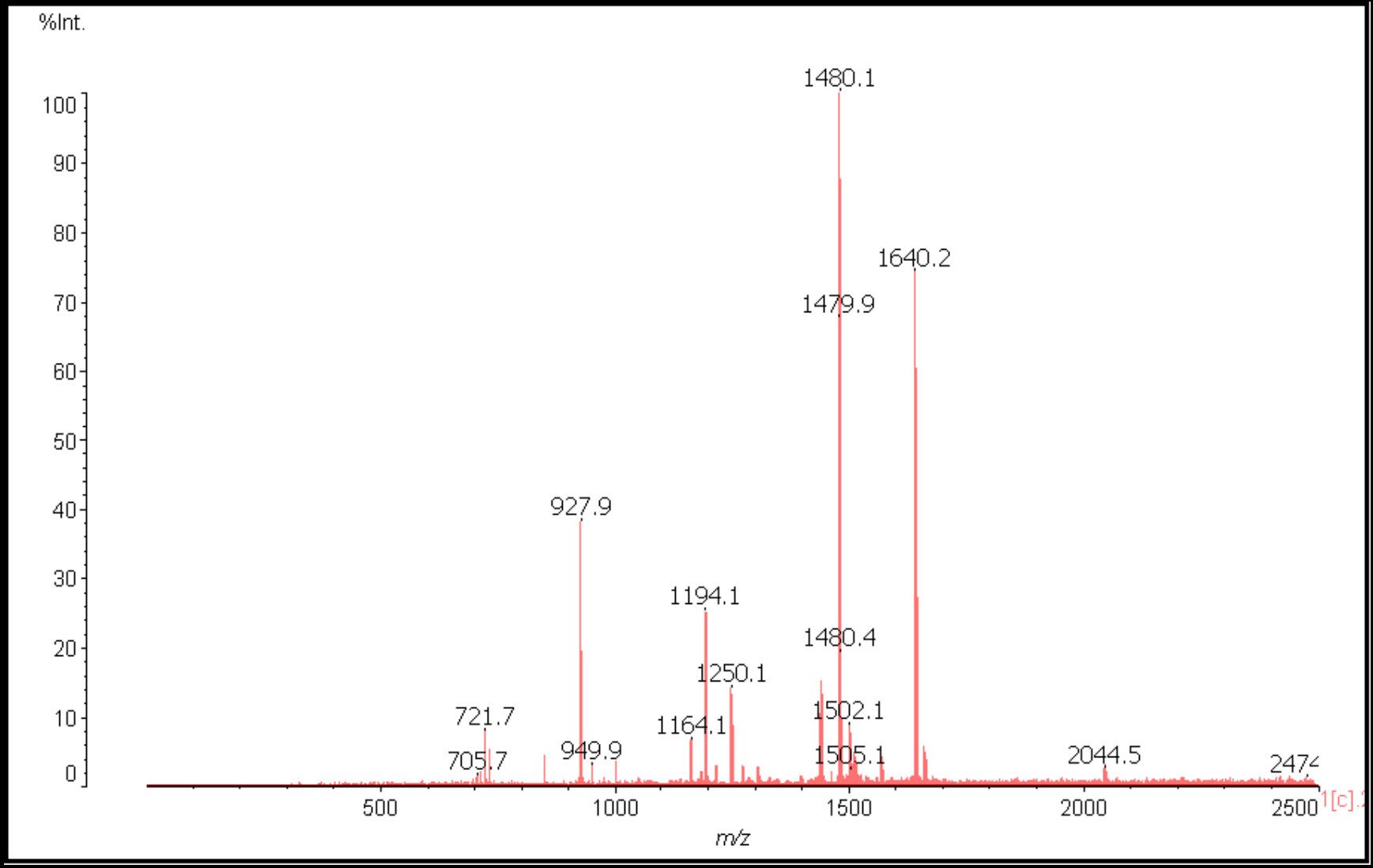
Taxonomy: Bos taurus

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **20**
Number of mass values matched: **13**
Sequence Coverage: **20%**

Matched peptides shown in **Bold Red**

```
     1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
    51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
   101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
   151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
   201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
   251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
   301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
   351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
   401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
   451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
   501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
   551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
   601 STQTALA
```

BSA 5 Cycles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90
80
70
60
50
40
30
20
10
0
1480.1
1640.2
1479.9
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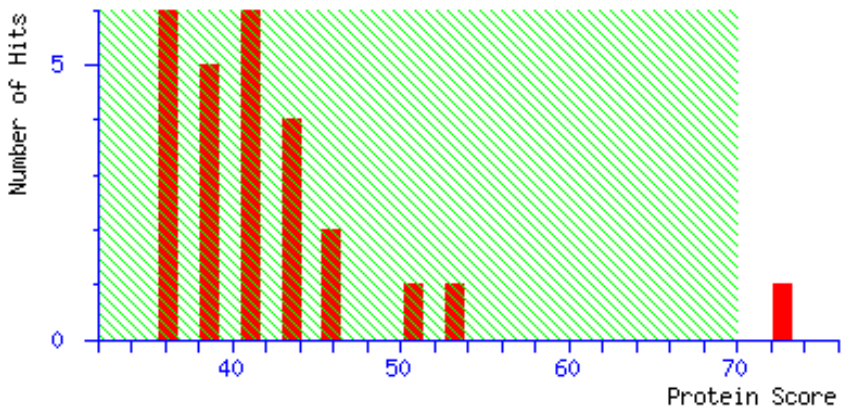
1480.4
1250.1
721.7
1164.1
1502.1
705.7
949.9
1505.1
2044.5
500
1000
1500
2000
2500
m/z

BSA 5 Cycles Score

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 70 are significant (p<0.05).



Concise Protein Summary Report

Format As	Concise Protein Summary	Help
	Significance threshold p< 0.05	Max. number of hits AUTO

Re-Search All | Search Unmatched

1. ALBU_BOVIN **Mass:** 69248 **Score:** 73 **Expect:** 0.028 **Matches:** 13
Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4

BSA 5 Cycles – Coverage Map

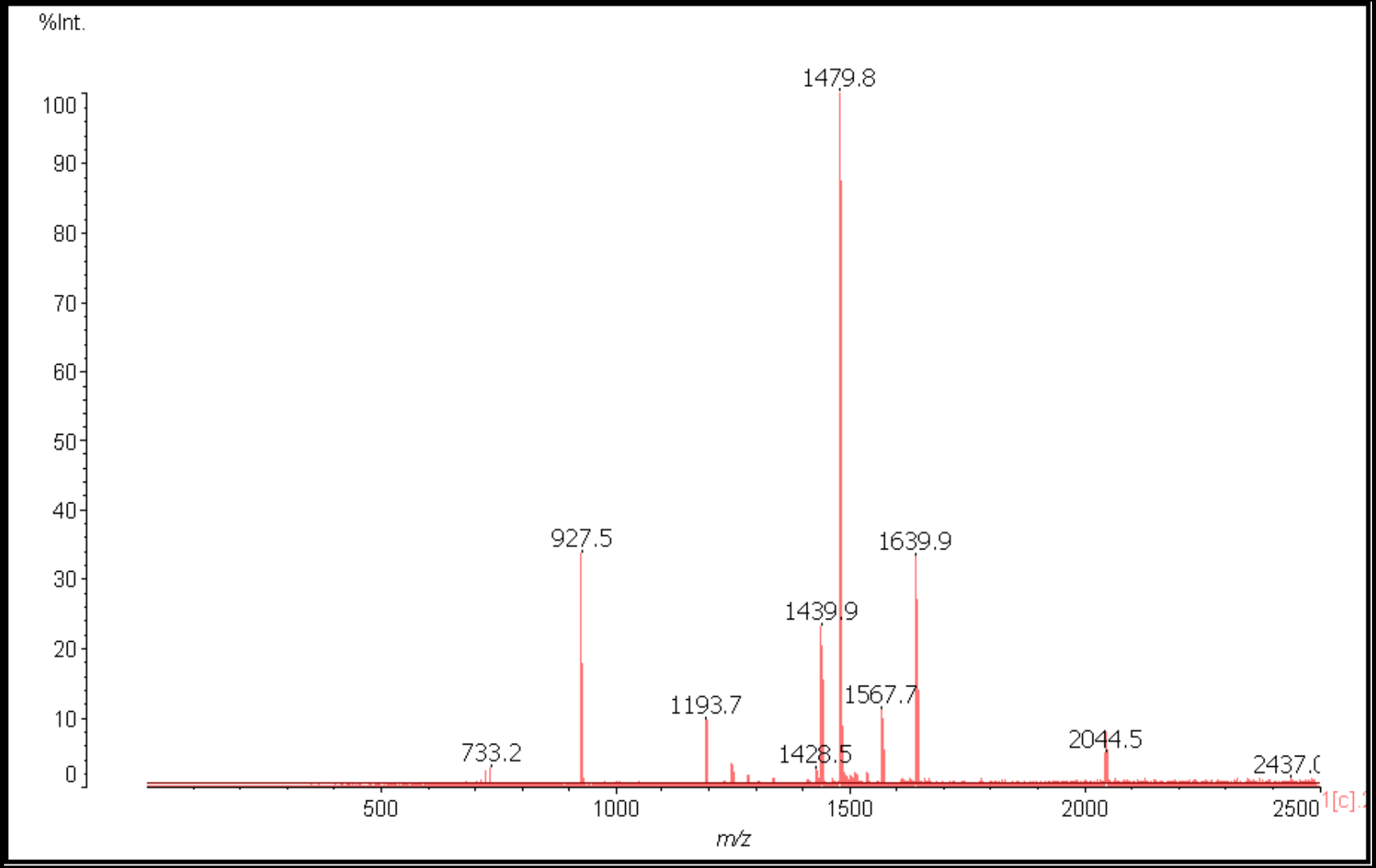
Taxonomy: Bos taurus

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **20**
Number of mass values matched: **13**
Sequence Coverage: **18%**

Matched peptides shown in **Bold Red**

```
     1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
    51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
   101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
   151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
   201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
   251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
   301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
   351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
   401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
   451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
   501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
   551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
   601 STQTALA
```

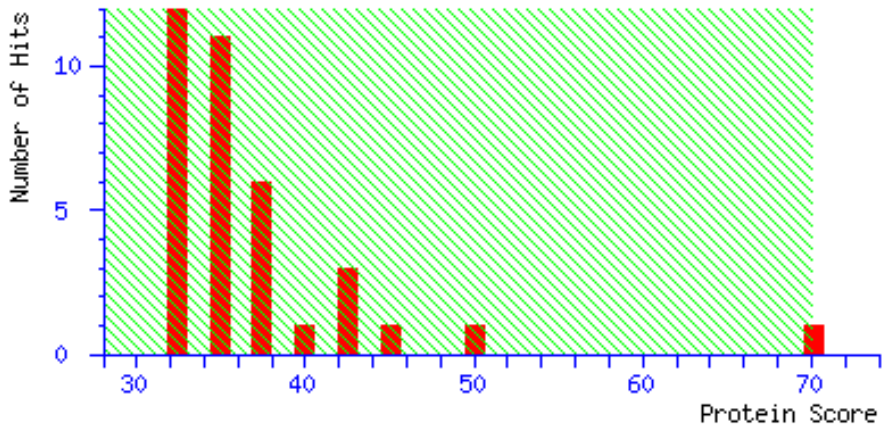
BSA 10 Cycles



BSA 10 Cycles Score

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 70 are significant (p<0.05).



Concise Protein Summary Report

Format As	Concise Protein Summary	Help
	Significance threshold p< 0.05	Max. number of hits AUTO

Re-Search All | Search Unmatched

1. ALBU_BOVIN **Mass:** 69248 **Score:** 70 **Expect:** 0.05 **Matches:** 11
Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4

BSA 10 Cycles - Coverage Map

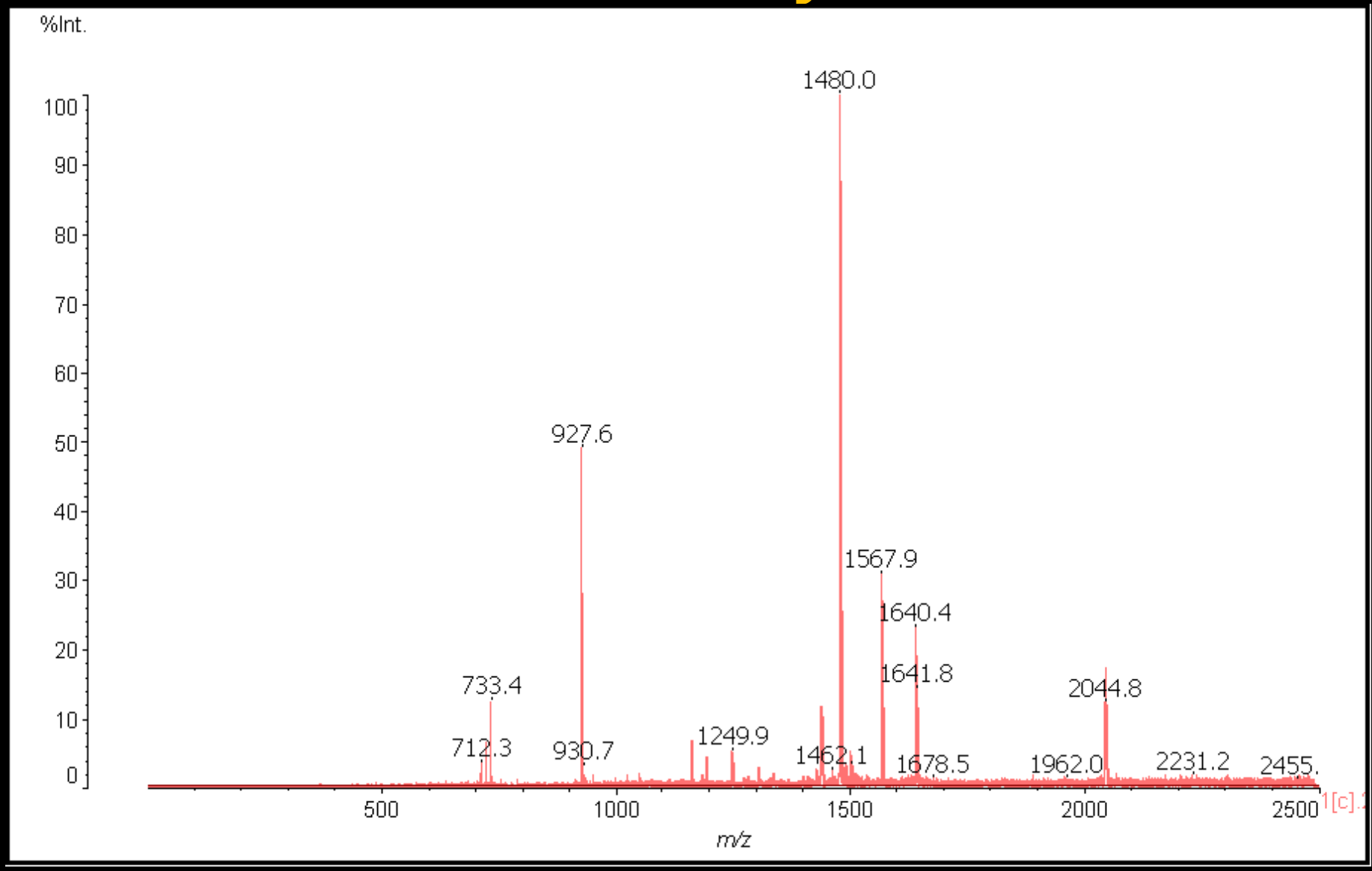
Taxonomy: Bos taurus

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **20**
Number of mass values matched: **11**
Sequence Coverage: **17%**

Matched peptides shown in **Bold Red**

```
     1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
    51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
   101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
   151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
   201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
   251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
   301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
   351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
   401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
   451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
   501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
   551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
   601 STQTALA
```

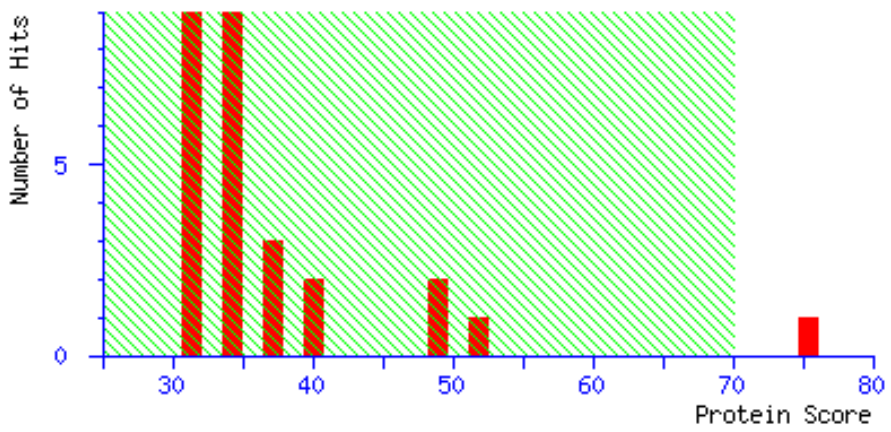
BSA 20 Cycles



BSA 20 Cycles Score

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 70 are significant (p<0.05).



Concise Protein Summary Report

Format As | Concise Protein Summary | Help

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

Re-Search All | Search Unmatched

1. CYC_HORSE **Mass:** 11825 **Score:** 75 **Expect:** 0.015 **Matches:** 7

Cytochrome c OS=Equus caballus GN=CYCS PE=1 SV=2

BSA 20 Cycles - Coverage Map

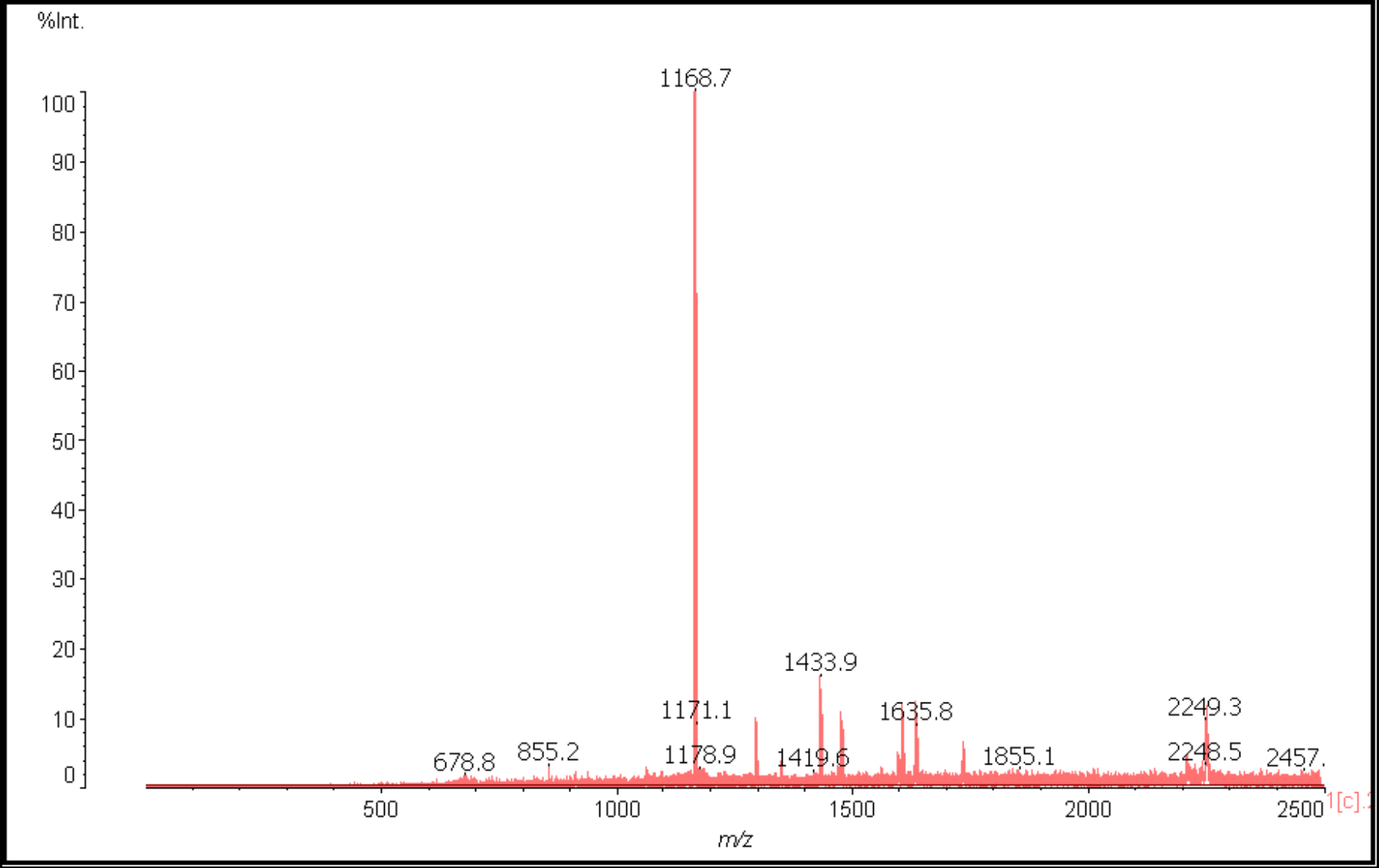
Taxonomy: Bos taurus

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **20**
Number of mass values matched: **11**
Sequence Coverage: **19%**

Matched peptides shown in **Bold Red**

```
    1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
   51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
  101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
  151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
  201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
  251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
  301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
  351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
  401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
  451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
  501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
  551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
  601 STQTALA
```

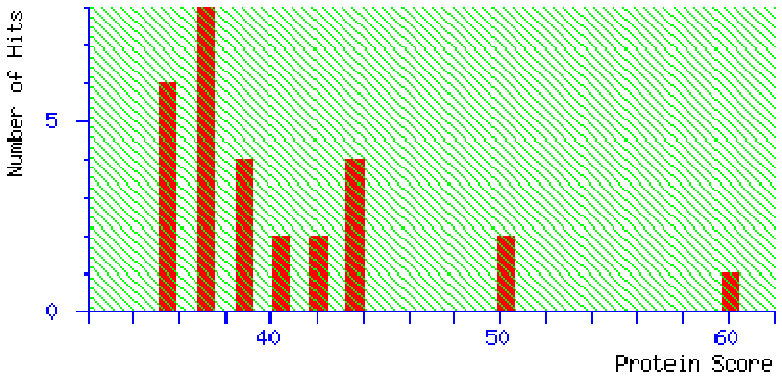
Cyto-C 5 Cycles



Cyto-C 5 Cycles Score

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 70 are significant (p<0.05).



Concise Protein Summary Report

Format As | Concise Protein Summary | Help

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

Re-Search All | Search Unmatched

1. CYC_HORSE **Mass:** 11825 **Score:** 60 **Expect:** 0.51 **Matches:** 6
Cytochrome c OS=Equus caballus GN=CYCS PE=1 SV=2
CYC_EQUAS **Mass:** 11811 **Score:** 45 **Expect:** 17 **Matches:** 5
Cytochrome c OS=Equus asinus GN=CYCS PE=1 SV=2
CYC_EQUBU **Mass:** 11811 **Score:** 45 **Expect:** 17 **Matches:** 5
Cytochrome c OS=Equus burchelli GN=CYCS PE=1 SV=2

Cyto-C 5 Cycles -Coverage Map

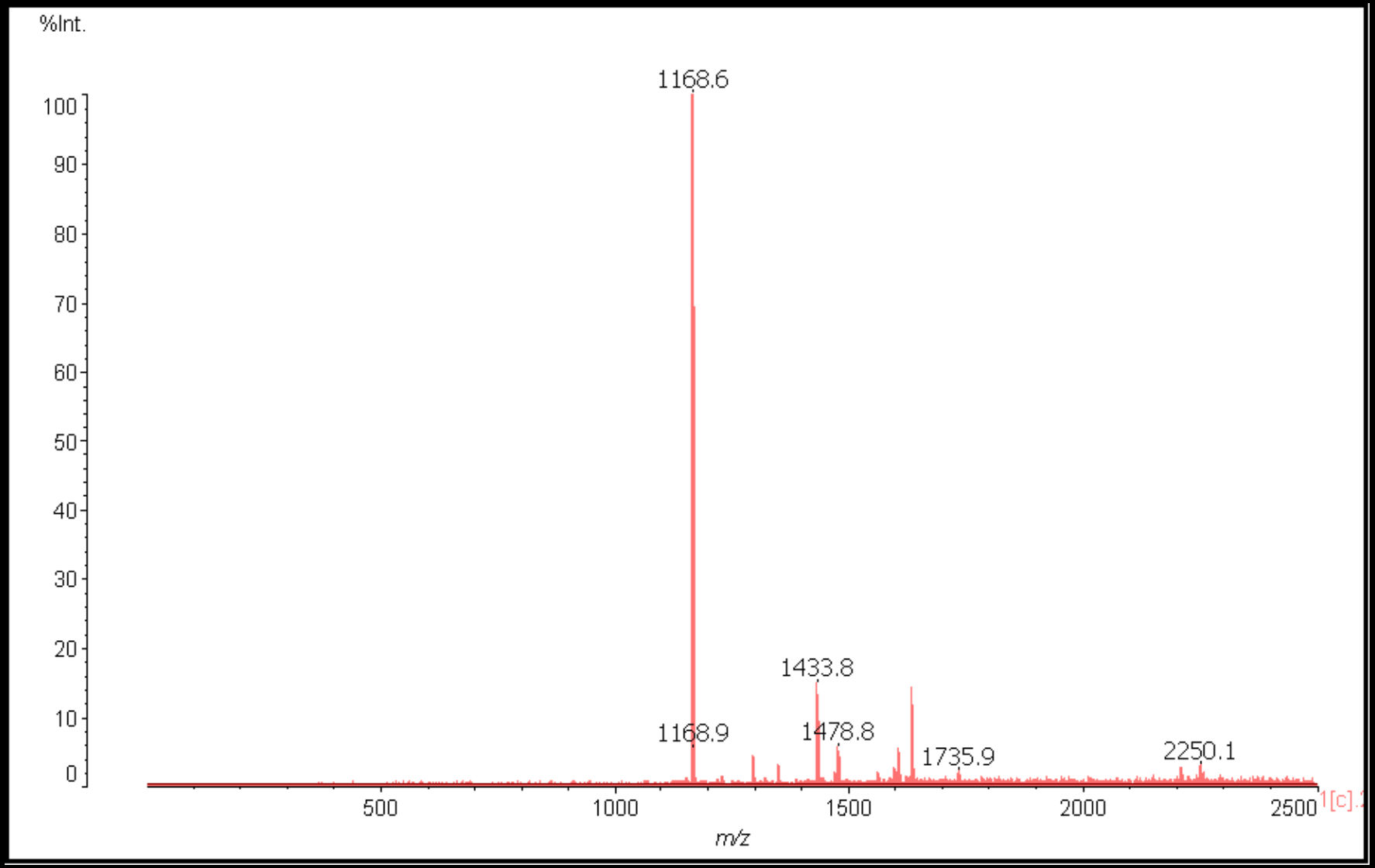
Taxonomy: Equus caballus

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **20**
Number of mass values matched: **6**
Sequence Coverage: **50%**

Matched peptides shown in **Bold Red**

```
     1 MGDVEKGKKI FVQKCAQCHT VEKGGKHKTG PNLHGLFGRK TGQAPGFTYT
    51 DANKNKGITW KEETLMEYLE NPKKYIPGTK MIFAGIKKKT EREDLIAYLK
   101 KATNE
```

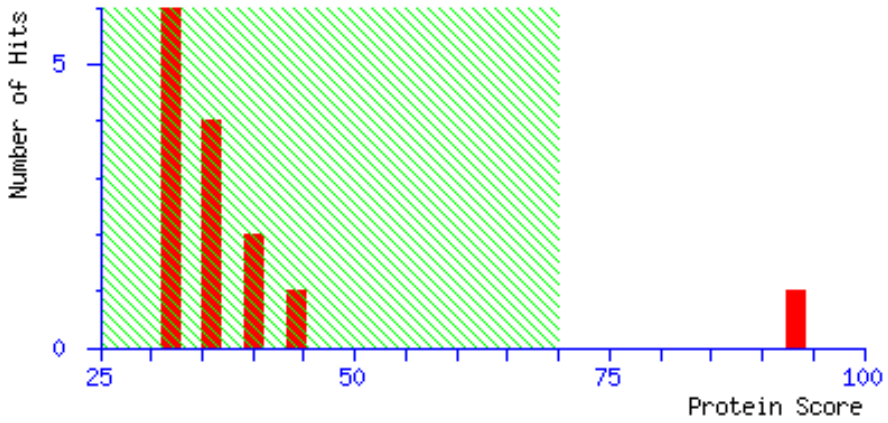
Cyto-C 10 Cycles



Cyto-C 10 Cycles

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 70 are significant (p<0.05).



Concise Protein Summary Report

Format As | Concise Protein Summary | Help

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

Re-Search All | Search Unmatched

1. CYC_HORSE **Mass:** 11825 **Score:** 93 **Expect:** 0.00024 **Matches:** 9
Cytochrome c OS=Equus caballus GN=CYCS PE=1 SV=2

Cyto-C 10 Cycles – Coverage Map

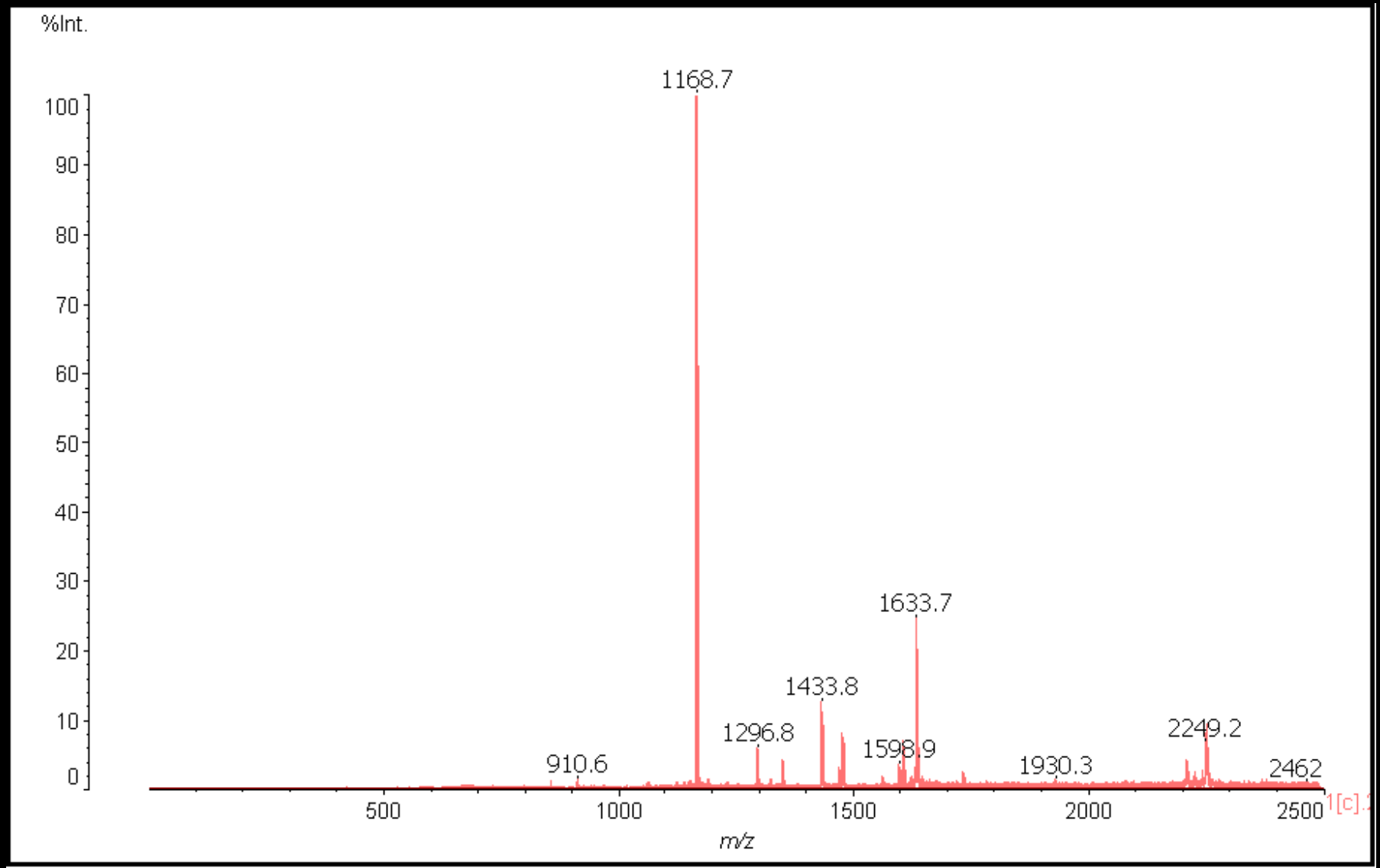
Taxonomy: Equus caballus

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **20**
Number of mass values matched: **9**
Sequence Coverage: **62%**

Matched peptides shown in **Bold Red**

```
     1 MGDVEKGKKI FVQKCAQCHT VEKGGKHKTG PNLHGLFGRK TGQAPGFTYT
    51 DANKNKGITW KEETLMEYLE NPKKYIPGTK MIFAGIKKKT EREDLIAYLK
   101 KATNE
```

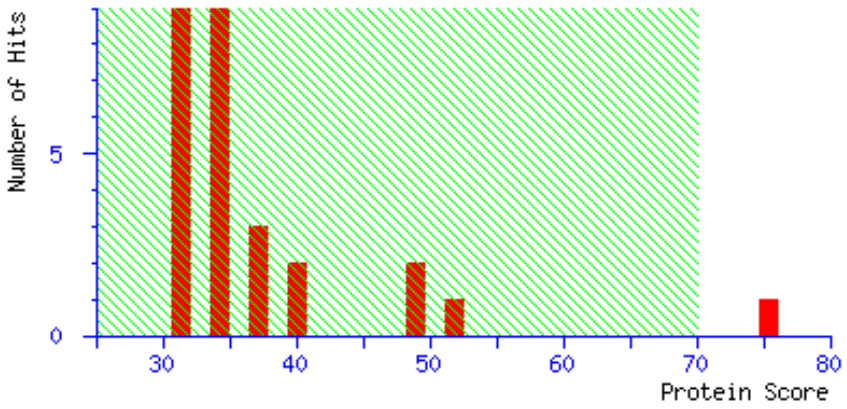
Cyto-C 20 Cycles



Cyto-C 20 Cycles Score

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 70 are significant (p<0.05).



Concise Protein Summary Report

Format As | Concise Protein Summary | Help

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

Re-Search All | Search Unmatched

1. CYC_HORSE **Mass:** 11825 **Score:** 75 **Expect:** 0.015 **Matches:** 7
Cytochrome c OS=Equus caballus GN=CYCS PE=1 SV=2

Cyto-C 20 Cycles – Coverage Map

Taxonomy: Equus caballus

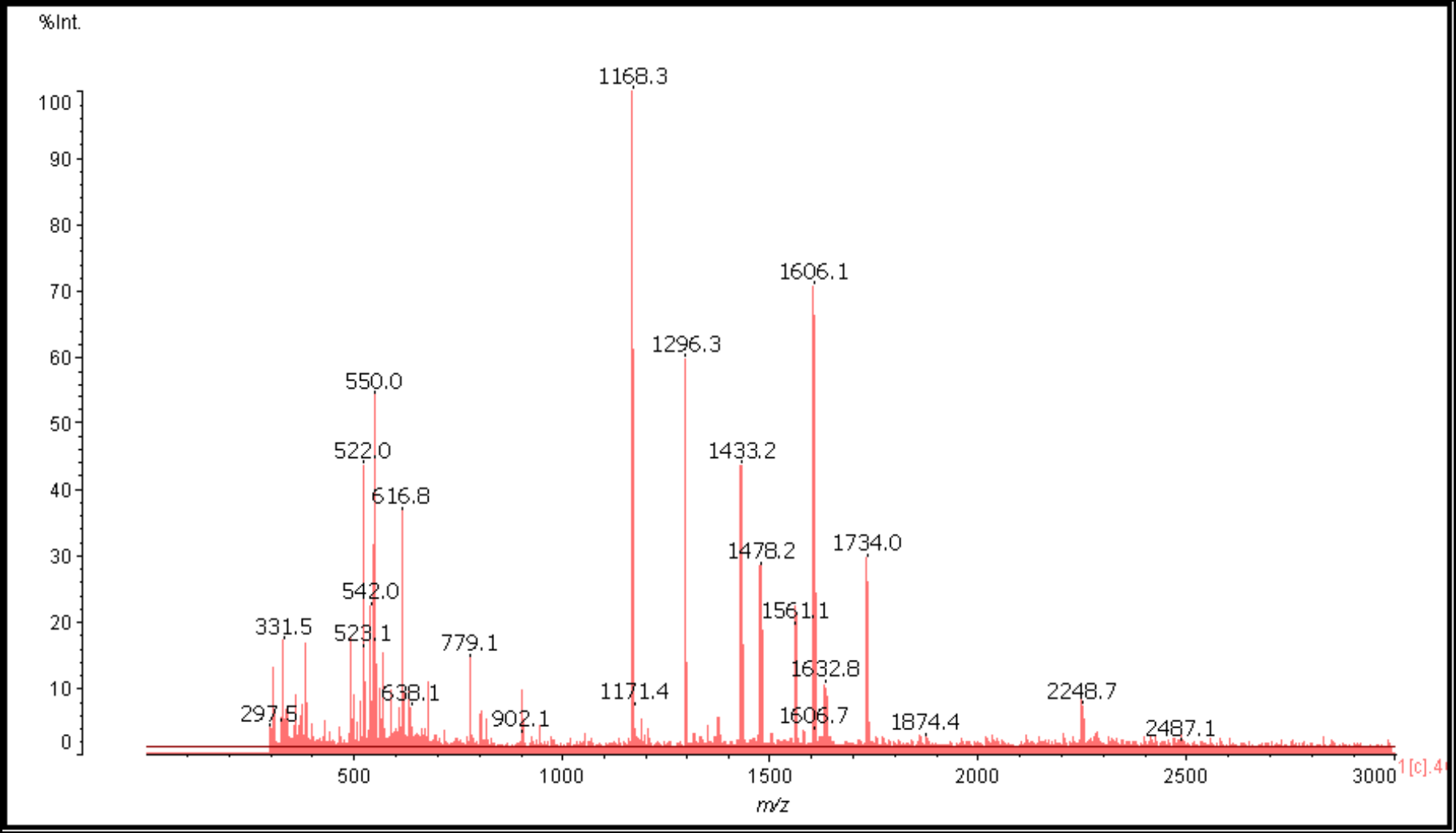
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **20**
Number of mass values matched: **7**
Sequence Coverage: **50%**

Matched peptides shown in **Bold Red**

```
     1 MGDVEKGKKI FVQKCAQCHT VEKGGKHKTG PNLHGLFGRK TGQAPGFTYT
    51 DANKNKGITW KEETLMEYLE NPKKYIPGTK MIFAGIKKKT EREDLIAYLK
   101 KATNE
```

Reference Sample Spectra

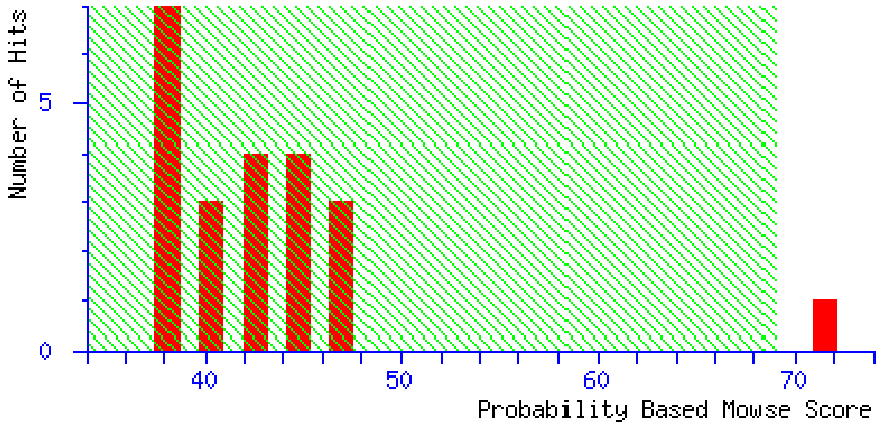
Cyto-C 4 Hours 55℃



Cyto-C 4 Hours 55℃ Score

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 69 are significant (p<0.05).



Concise Protein Summary Report

Format As	Concise Protein Summary	Help
	Significance threshold p< 0.05	Max. number of hits 5

Re-Search All | Search Unmatched

1. CYC_HORSE **Mass:** 11825 **Score:** 72 **Expect:** 0.033 **Queries matched:** 11
Cytochrome c OS=Equus caballus GN=CYCS PE=1 SV=2

Cyto-C 4 Hours 55℃ - Coverage Map

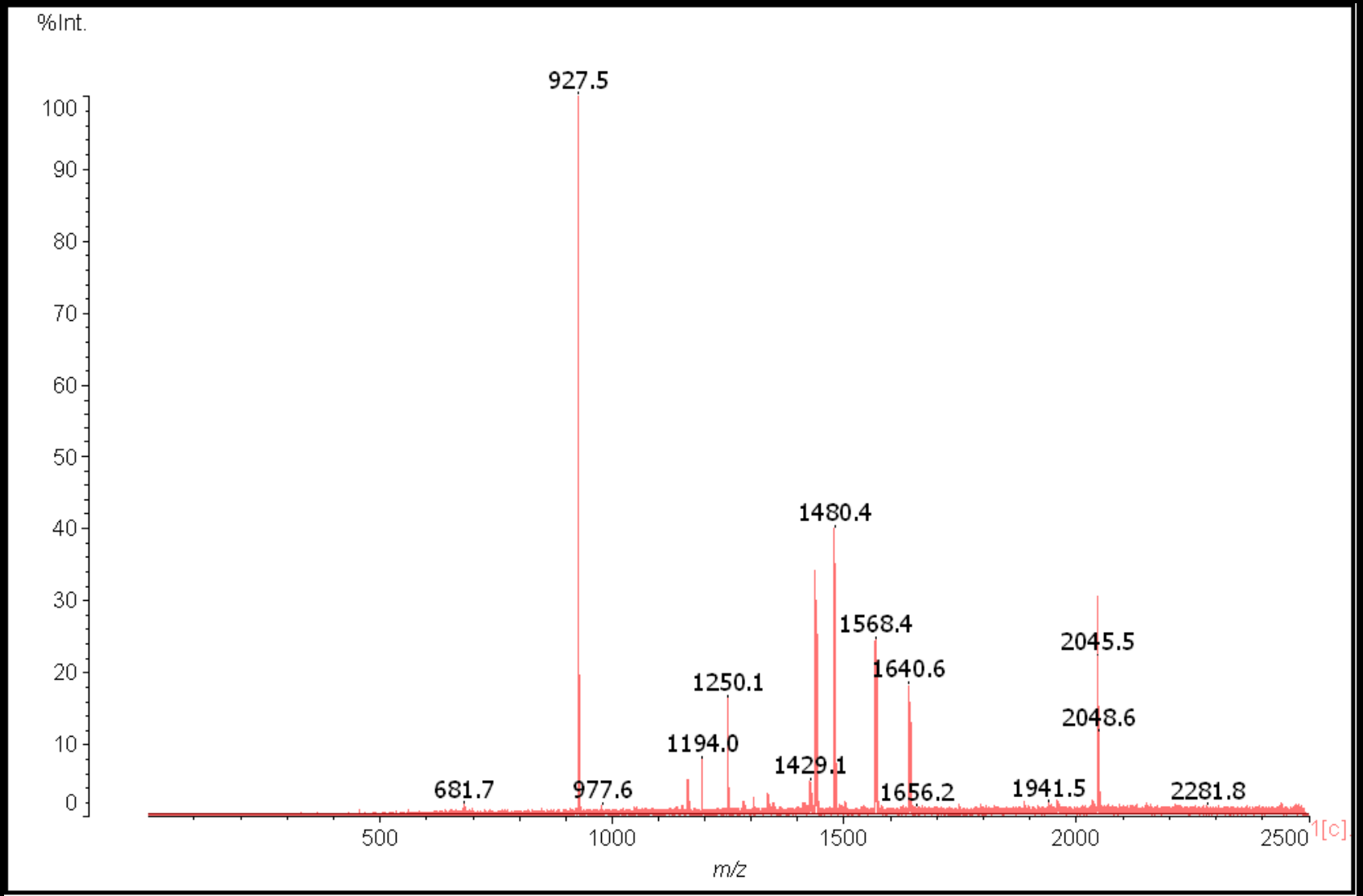
Taxonomy: Equus caballus

Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **20**
Number of mass values matched: **11**
Sequence Coverage: **68%**

Matched peptides shown in **Bold Red**

```
     1 MGDVEKGKKI FVQKCAQCHT VEKGGKHKTG PNLHGLFGRK TGQAPGFTYT
    51 DANKNKGITW KEETLMEYLE NPKKYIPGTK MIFAGIKKKT EREDLIAYLK
   101 KATNE
```

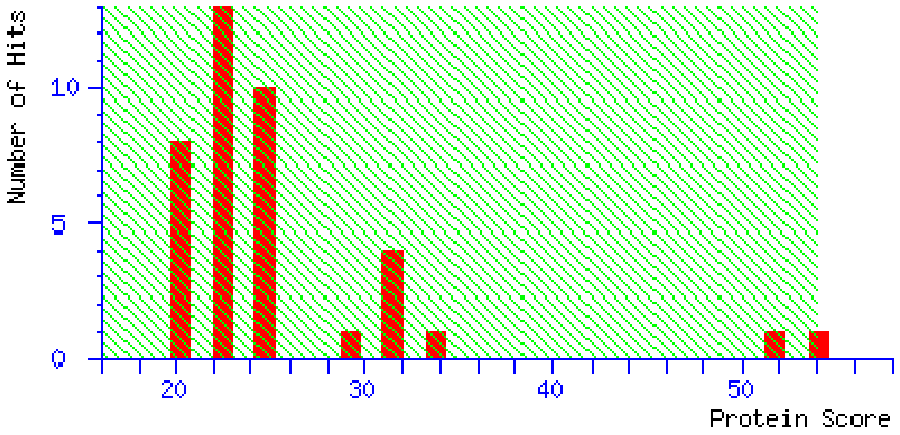
BSA 2 Hours 55℃



BSA 2 Hours 55℃ Score

Mascot Score Histogram

Protein score is -10*Log(P), where P is the probability that the observed match is a random event.
Protein scores greater than 54 are significant (p<0.05).



Concise Protein Summary Report

Format As	Concise Protein Summary	Help
	Significance threshold p< 0.05	Max. number of hits AUTO

Re-Search All Search Unmatched

1. ALBU_BOVIN **Mass:** 69248 **Score:** 54 **Expect:** 0.049 **Matches:** 12
Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4

BSA 2 Hours 55℃ – Coverage Map

Taxonomy: Bos taurus

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **20**
Number of mass values matched: **12**
Sequence Coverage: **20%**

Matched peptides shown in **Bold Red**

```
  1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
 51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
601 STQTALA
```

BSA (Bovine Serum Albumin) + Trypsin + PCT (Samples 1-8)		
158 µL BSA (1158 µg) + 2 µL trypsin (2 µg); BSA: Trypsin, 579:1		
Runs (Cycles/Seconds)	**MASCOT Score**	**Sequence Coverage (%)**
1/20	86	20
1/20	No match	N/A
1 /20	No match	N/A
1/20	No match	N/A
5/100	73	18
5/100	No match	N/A
5/100	No match	N/A
5/100	No match	N/A

BSA (Bovine Serum Albumin) + Trypsin + PCT (Samples 8-16)		
158 µL BSA (1158 µg) + 2 µL trypsin (2 µg); BSA: Trypsin, 579:1		
Runs (Cycles/Seconds)	**MASCOT Score**	**Sequence Coverage (%)**
10/200	70	17
10/200	59	15
10/200	No match	N/A
10/200	No match	N/A
20/400	54	17
20/400	69	19
20/400	65	20
20/400	No match	N/A

Cyto-C (Horse Heart Cytochrome-C) + Trypsin + PCT (Samples 1-8)		
158 µL Cyto-C (1158 µg) + 2 µL trypsin (2 µg); Cyto-C: Trypsin, 579:1		
Runs (Cycles/Seconds)	**MASCOT Score**	**Sequence Coverage (%)**
1/20	No match	N/A
1/20	No match	N/A
1/20	No match	N/A
1/20	No match	N/A
5/100	52	44
5/100	60	50
5/100	52	44
5/100	No match	N/A

Cyto-C (Horse Heart Cytochrome-C) + Trypsin + PCT (Samples 8-16)		
158 µL Cyto-C (1158 µg) + 2 µL trypsin (2 µg); Cyto-C: Trypsin, 579:1		
Runs (Cycles/Seconds)	**MASCOT Score**	**Sequence Coverage (%)**
10/200	77	50
10/200	54	44
10/200	93	62
10/200	60	50
20/400	59	50
20/400	65	50
20/400	63	50
20/400	75	50

BSA Reference Samples @ 55 °C		
Time	Mascot Score	Sequence Coverage (%)
30 Minutes	No match	N/A
1 Hour	41	16
2 Hours	54	20
4 Hours	No match	N/A

Cytochrome C Reference Samples @ 55 °C		
Time	Mascot Score	Sequence Coverage (%)
30 Minutes	No match	N/A
1 Hour	No match	N/A
2 Hours	No match	N/A
4 Hours	99	78

Data Summary

- Protein identification is cycle-dependent.
- PCT digest in minutes achieves equal coverage compared to water bath digest (55˚C) at 2 and 4 hours, respectively, for BSA and Cytochrome C.

Tertiary Structure Disulfide Bond Effects on Trypsin Digest

- BSA
  - 34 cysteine residues
  - 17 disulfide bonds
- Cytochrome C
  - 2 cysteine residues
  - (CAQC- no disulfide bond)

P. Restani, C. Ballabio, A. Cattaneo, et. al. *Allergy* 2004; 59: 21.

Why PCT Trypsin Digest?

- Reduced digest time (hrs to mins)
- Ambient temperature digest
- Reduced analyte / trypsin ratio (~500:1)
- No tissue maceration

Conclusion

- PCT tissue extraction is:
  1) rapid
  2) reproducible
  3) high-yield

- PCT trypsin digest is:
  1) rapid with high % coverage
  2) reproducible

Pevsner Laboratory

Back Row (L to R): Alvis Yuen, Nick Sobol, Daniel Weissman,Jennifer-Leigh Oprihory, Ariel Gross

Middle Row: Tiffany Remsen, Arko Mukherjee, Robert Chouake

Front Row: Michael Yee & Paul Pevsner