

Digestion with Pressure Cycling Technology (PCT)

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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 (150 μ l)



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 repulsions 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, 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 N₂ 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

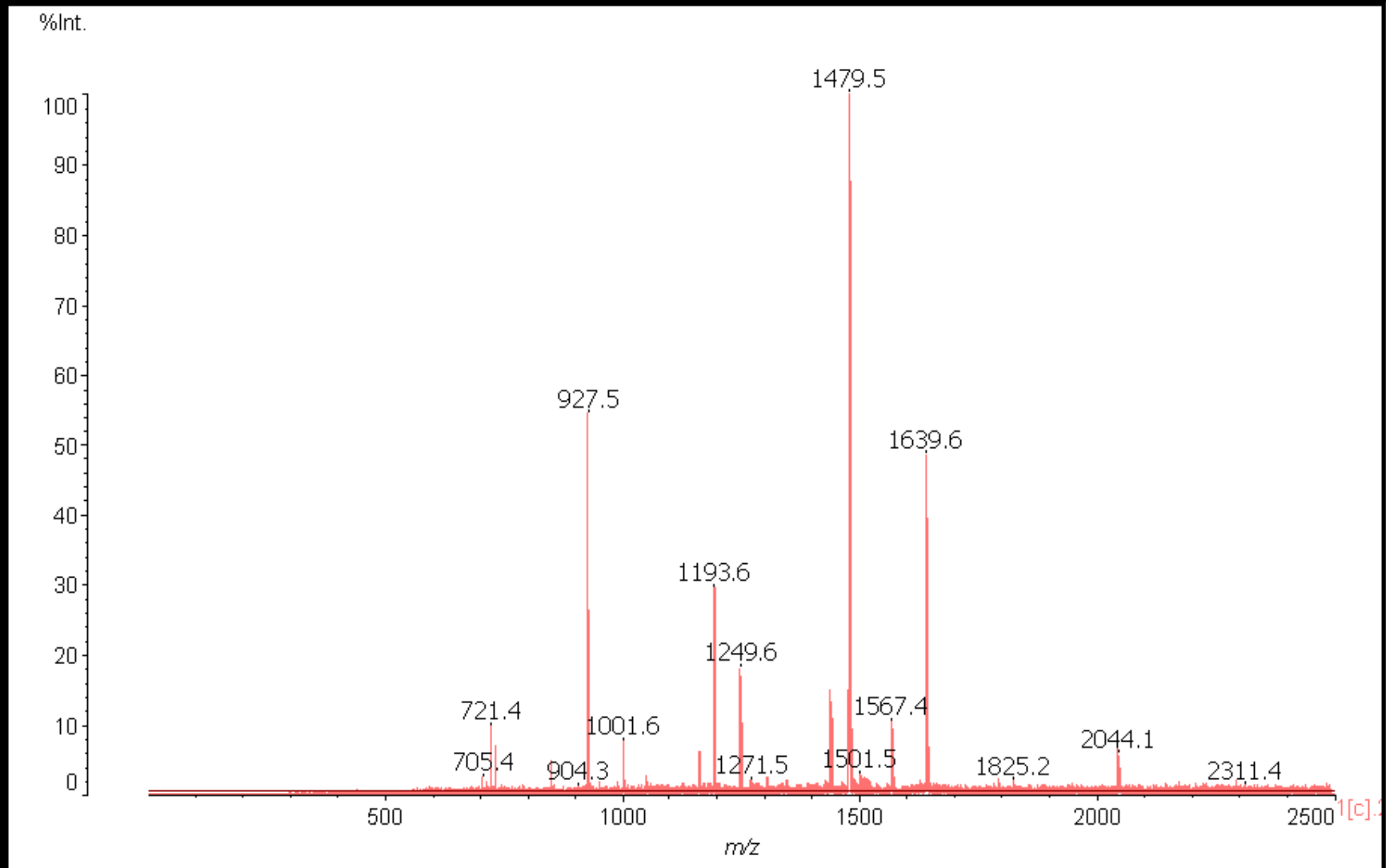
- Axima TOF² (MALDI TOF-TOF Shimadzu, Kyoto, Japan)
- Reflectron mode

Results

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

PCT Trypsin Digest Spectra

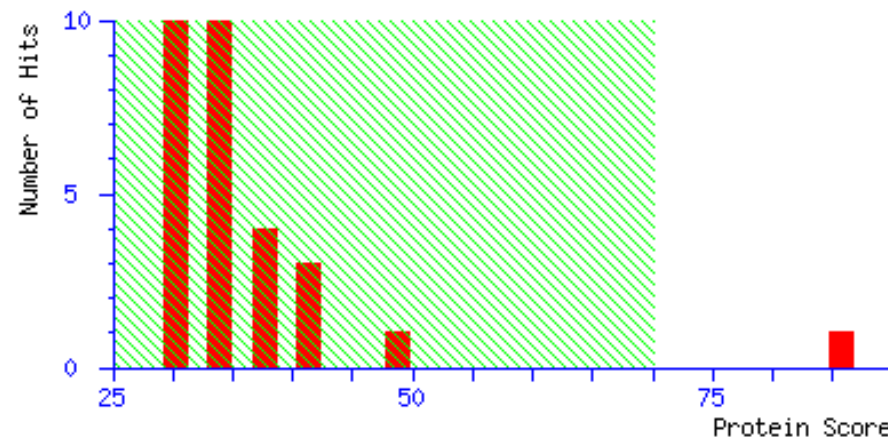
BSA 1 Cycle



BSA 1 Cycle Score

Mascot Score Histogram

Protein score is $-10 \cdot \log(P)$, 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

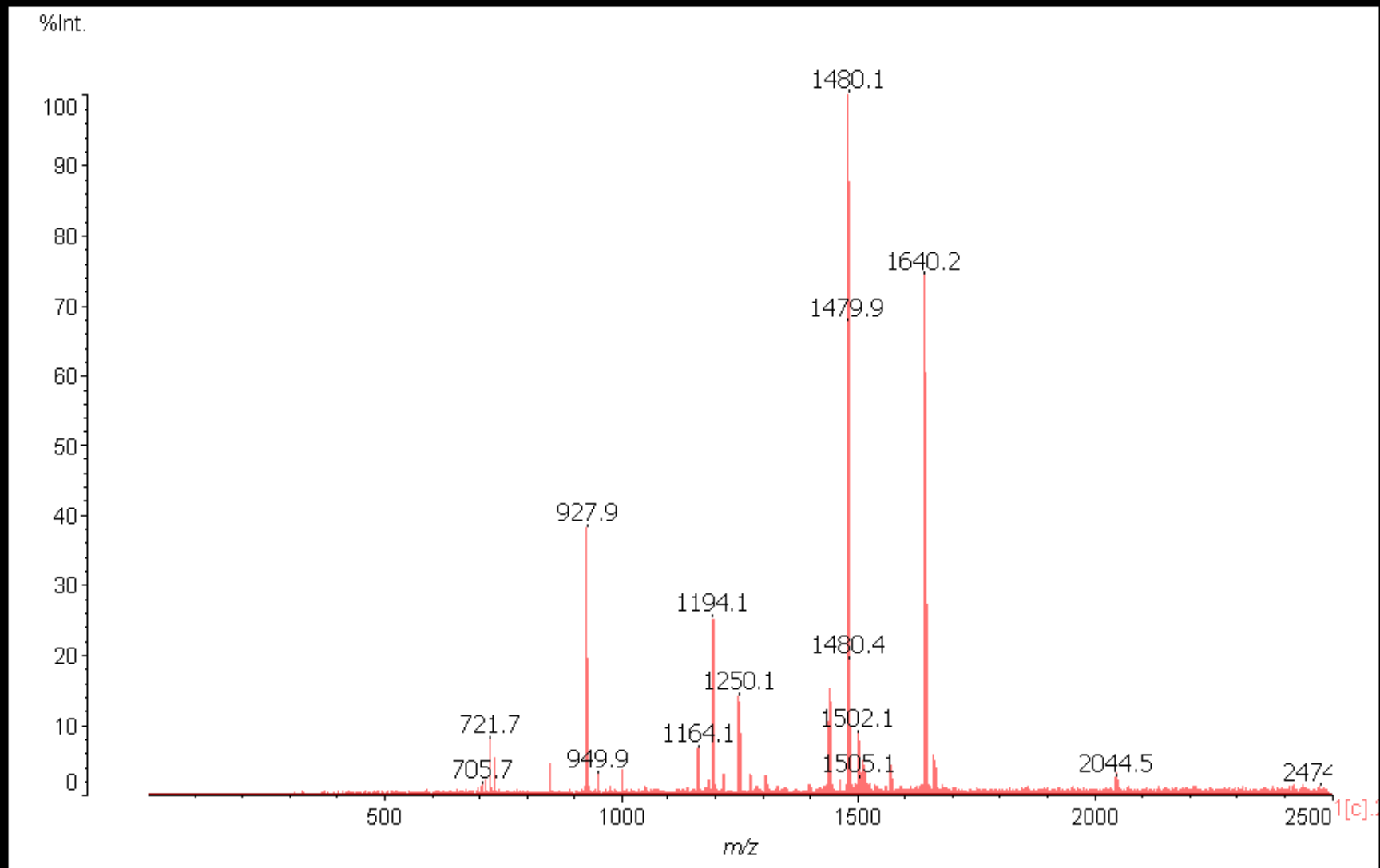
Number of mass values matched: 13

Sequence Coverage: 20%

Matched peptides shown in **Bold Red**

1	MKWVTFISLL	LLFSSAYSRG	VFRR DT HKSE	LAH RFKDLGE	EH FKGLVLIA
51	FSQYLQQCPF	DEHV KLV NEL	TE FAKTCVAD	ESHAGCEKSL	HTLFGDELCK
101	VASLRETYGD	MADCCEKQEP	ERNECFLSHK	DDSPDLPKLK	PDPNTLCDEF
151	KADEKKFUGK	YLYE LARRHP	YFYAPELLYY	ANKYNGVFQE	CCQAEDKGAC
201	LLPKIETMRE	KVLASSARQR	LRCASIQKFG	ER AL KAWSVA	RL SQKFPKAE
251	FVEVTKLVTD	LTKVHKECCH	GDLLECADDR	ADLAKYICDN	QDTISSKLKE
301	CCDKPLLEKS	HCIAEVEKDA	IPENLPPLTA	DFAEDKDVCK	NYQEAK DA FL
351	GS FLYEYSRR	HPE YAVSVLL	RL AKEYEATL	EECCAKDDPH	ACYSTVFDKL
401	KHL VDEPQNL	IK QNCDQFEK	LGE YGFQNAL	IV RYTRKVPQ	VST PTLVEVS
451	R SLGKVGTRC	CTKPESERMP	CTEDYLSLIL	NRLCVLHEKT	PVSEKVTKCC
501	TESLVNRRPC	FSALTPDETY	VPKAFDEKLF	TFHADICTLP	DTEKQIKKQT
551	ALVELLKHKP	KATEEQLKTV	MENFVAFVDK	CCAADD K EAC	FA VEGPKLVV
601	STQTALA				

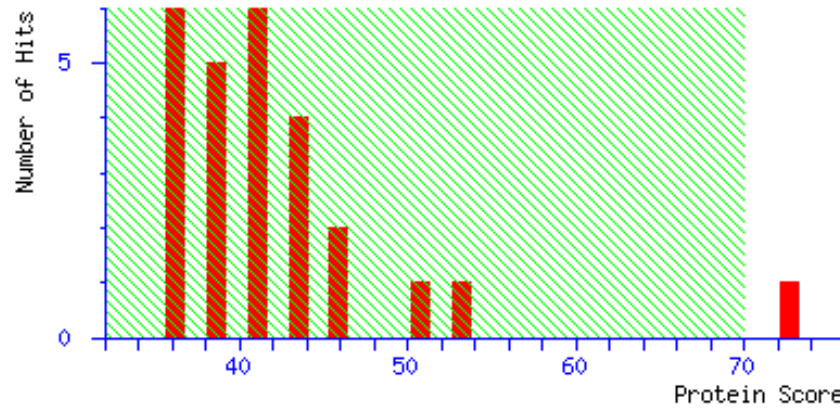
BSA 5 Cycles



BSA 5 Cycles Score

Mascot Score Histogram

Protein score is $-10 \cdot \log(P)$, 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 [Help](#)

Significance threshold $p <$ Max. number of hits

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

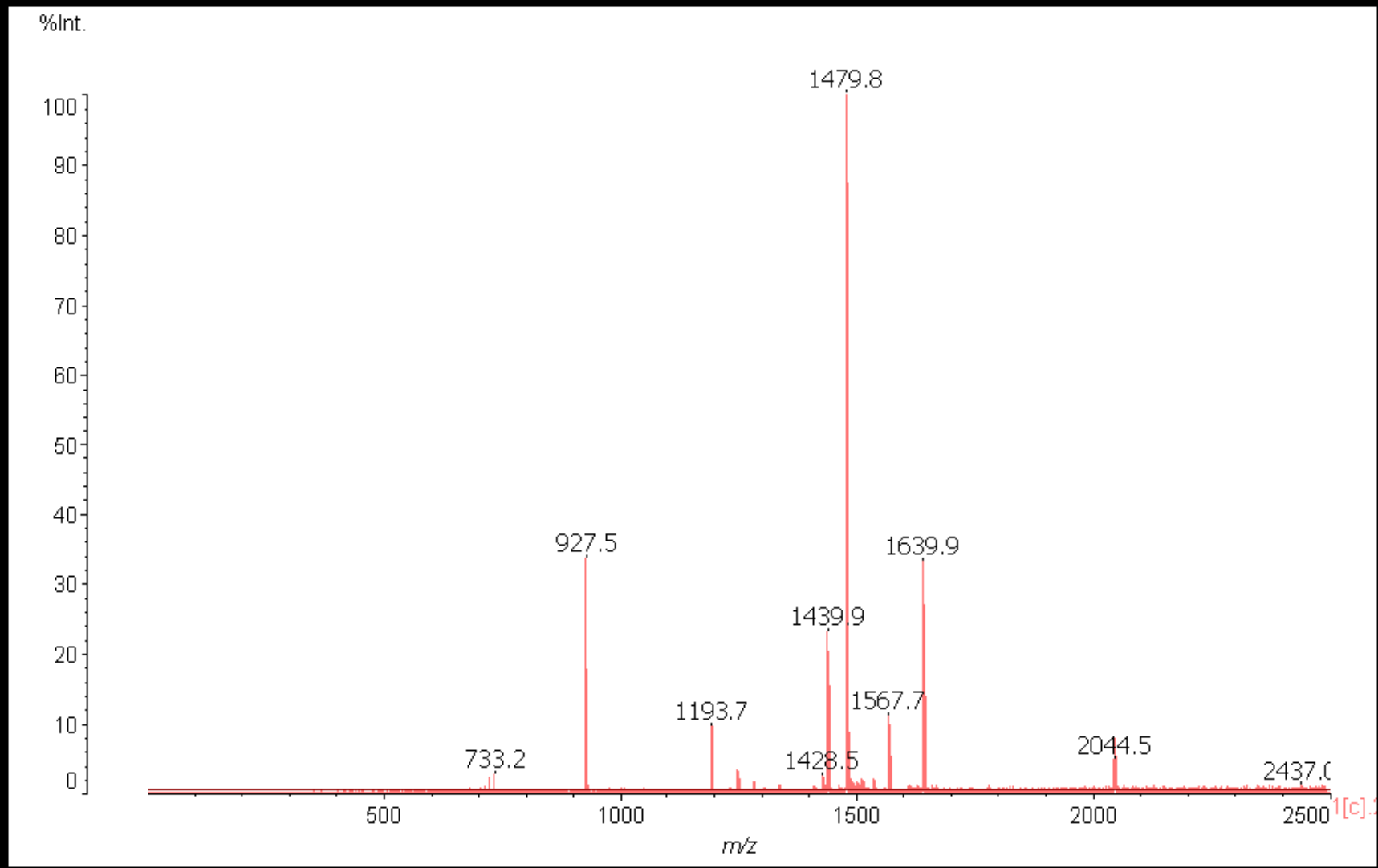
Number of mass values matched: 13

Sequence Coverage: 18%

Matched peptides shown in **Bold Red**

1	MKWVTFISLL	LLFSSAYSERG	VFRR DT HKSE	IA HRFKDLGE	EH FKGLVLIA
51	FSQYLQCCPF	DEHV KLV NEL	TE FAKTCVAD	ESHAGCEKSL	HTLFGDELCK
101	VASLRETYGD	MADCCEKQEP	ERNECFLSHK	DDSPDLPKLK	PDPNTLCDEF
151	KADEKKFUGK	YL YE IA RRHP	YFYAPELLYY	ANKYNGVFOE	CCQAEDKGAC
201	LLPKIETMRE	KVLASSARQR	LRCASIQKFG	ER AL KAW SVA	RL SQKFPKAE
251	FVEVTKLVTD	LTKVHKECCH	GDLLECADDR	ADLAKYICDN	QDTISSKLKE
301	CCDKPLLEKS	HCIAEVEKDA	IPENLPPLTA	DFAEDKDVCK	NYQEAK DA FL
351	GS FLYEYSRR	HP EYAVSVLL	RL AKEYEATL	EECCA KDD PH	ACYSTVFDKL
401	KHLVDEPQNL	IKQNCDOFEK	LGE YGFQ NA L	IV RYTRK VP Q	VST PTLVEVS
451	R SLGKVGTRC	CTKPESERMP	CTEDYLSLIL	NRL C VL HE KT	PV SEK V TKCC
501	TESLVMRRPC	FSALTPDETY	VPKAFDEKLF	TFHADICTLP	DTEKQIKKQT
551	ALVELLKHKP	KATEEQLKTV	MENFVAFVVK	CCAADDKEAC	FAVEGPKLVV
601	STQTALA				

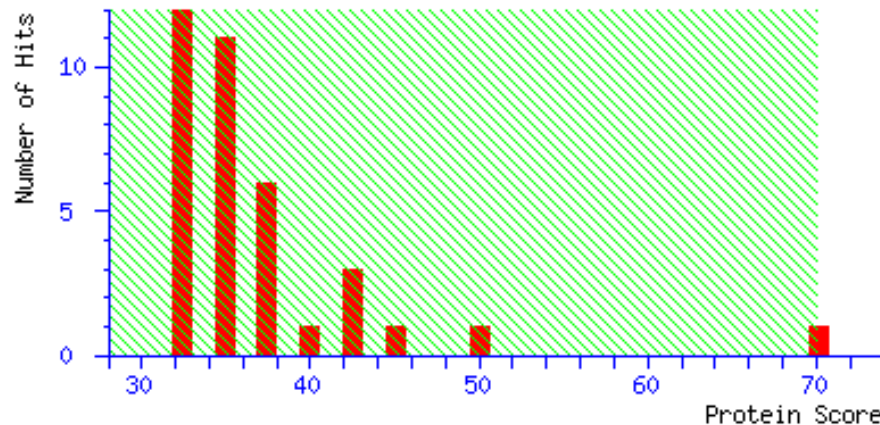
BSA 10 Cycles



BSA 10 Cycles Score

Mascot Score Histogram

Protein score is $-10 \cdot \log(P)$, 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 <input type="button" value="v"/>	Help
Significance threshold $p <$	<input type="text" value="0.05"/>	Max. number of hits <input type="text" value="AUTO"/>
<input type="button" value="Re-Search All"/>	<input type="button" value="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

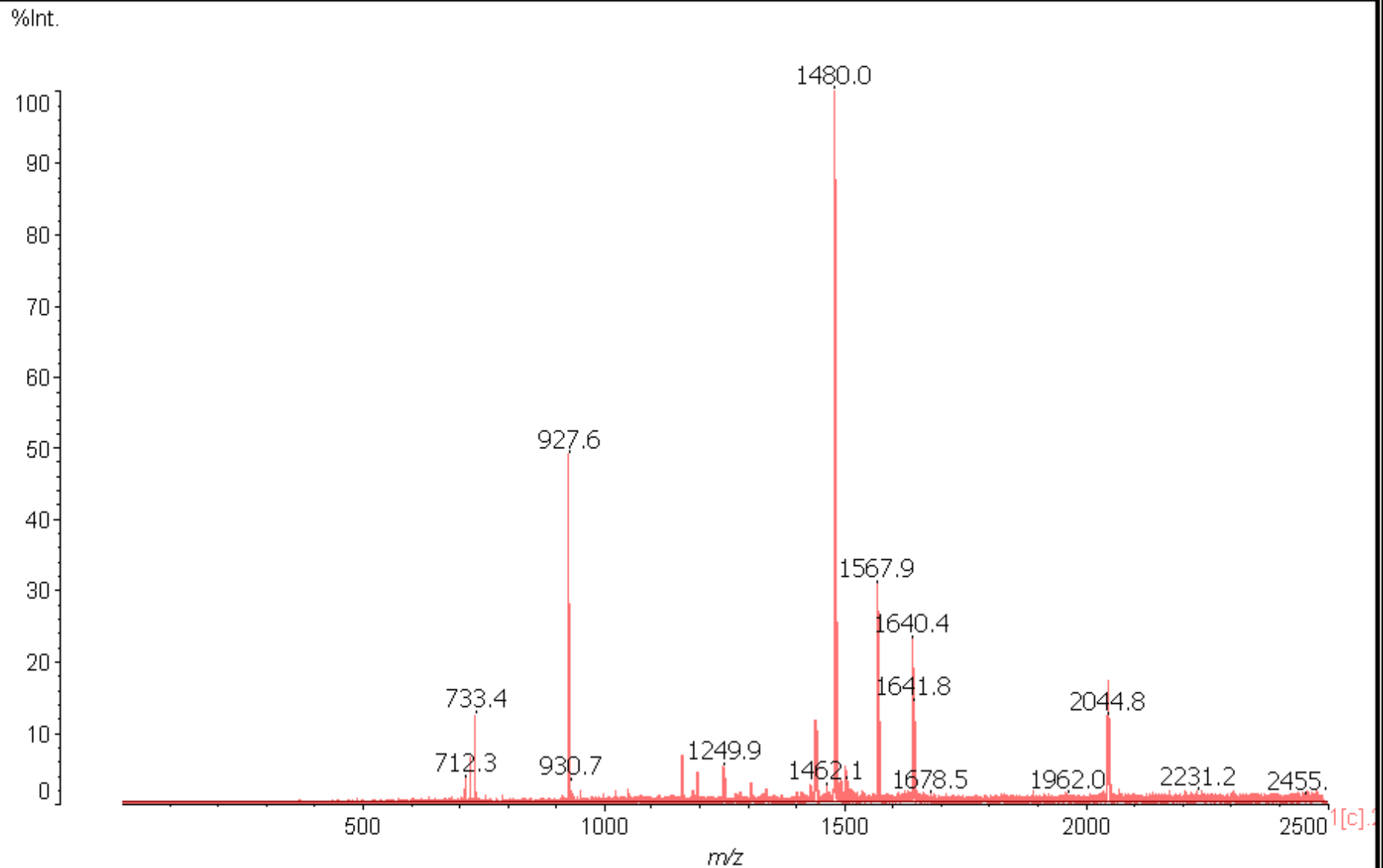
Number of mass values matched: 11

Sequence Coverage: 17%

Matched peptides shown in **Bold Red**

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

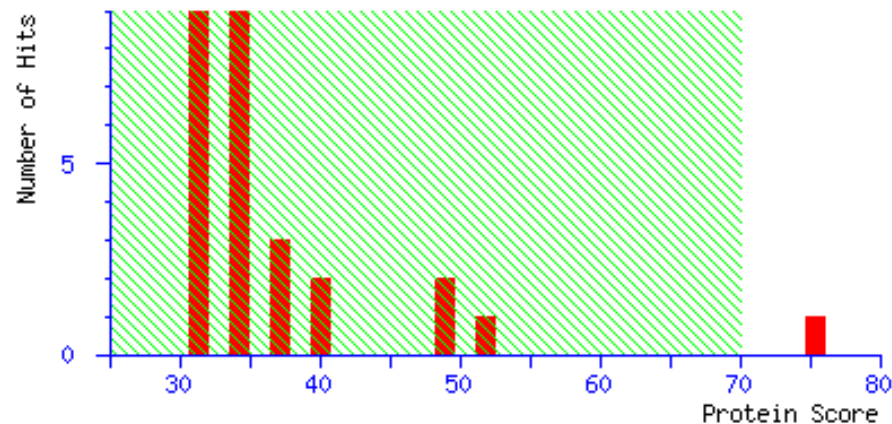
BSA 20 Cycles



BSA 20 Cycles Score

Mascot Score Histogram

Protein score is $-10 \cdot \log(P)$, 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	

- [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

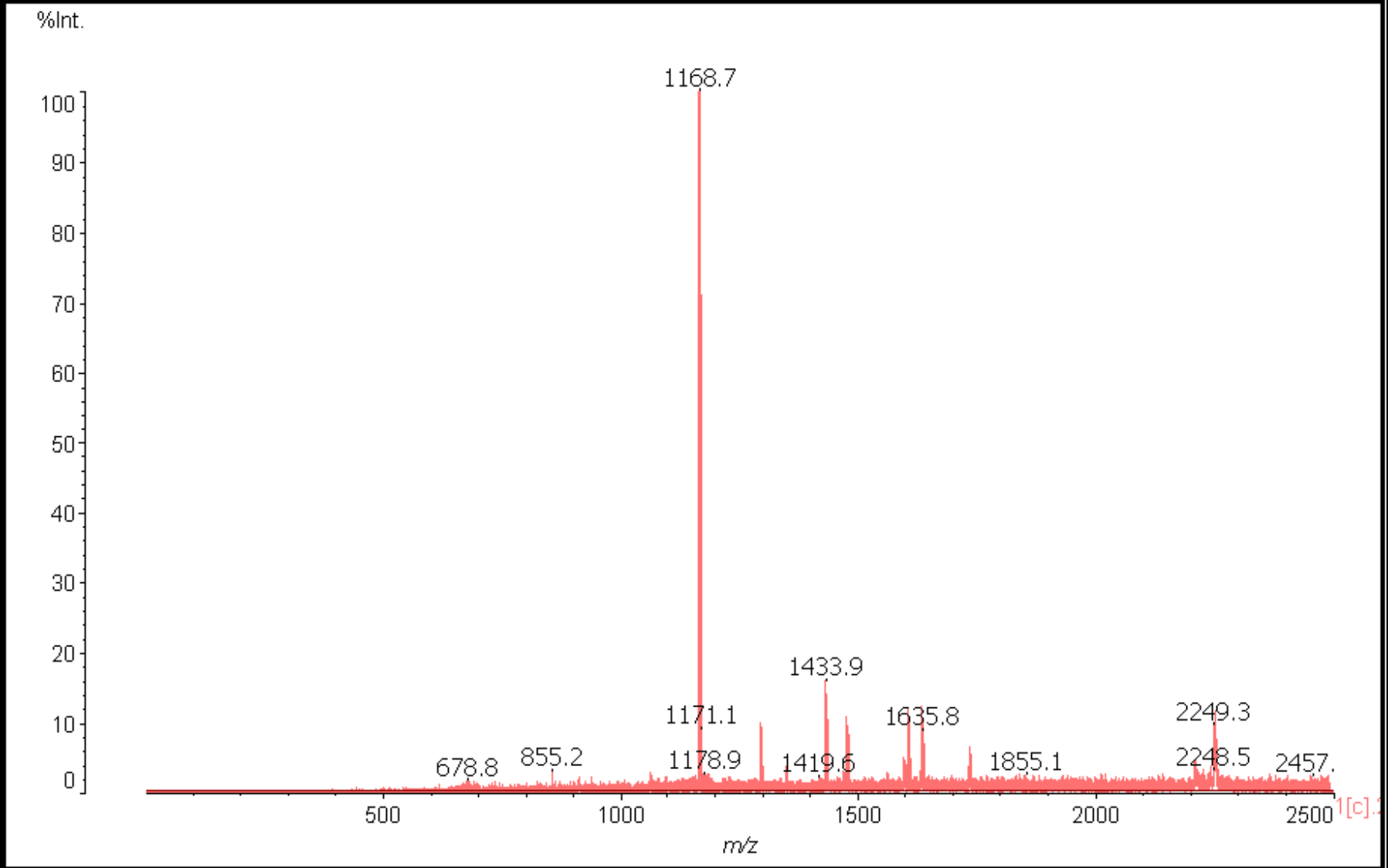
Number of mass values matched: 11

Sequence Coverage: 19%

Matched peptides shown in **Bold Red**

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

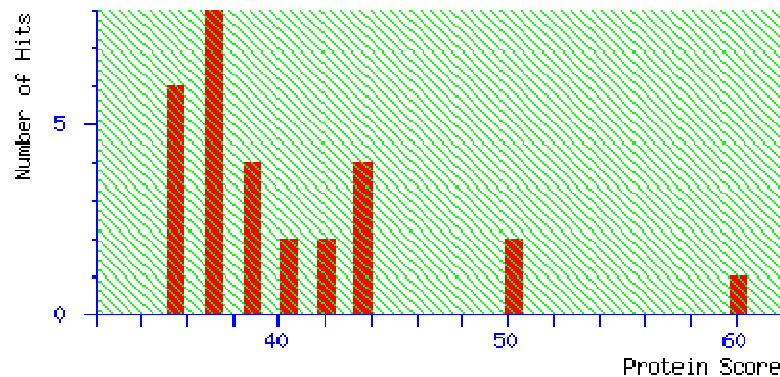
Cyto-C 5 Cycles



Cyto-C 5 Cycles Score

Mascot Score Histogram

Protein score is $-10 \cdot \log(P)$, 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 $p <$

Max. number of hits

- [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

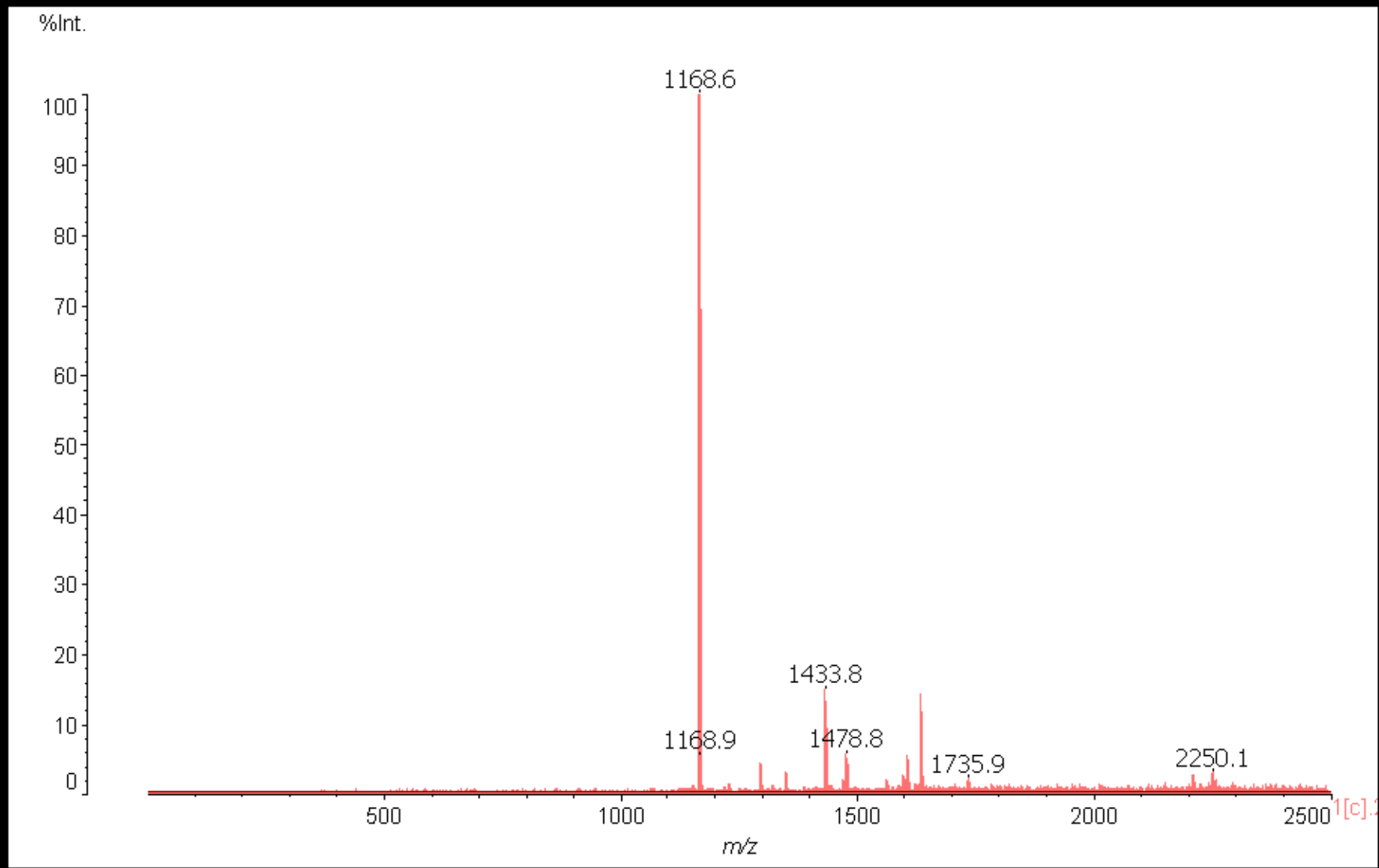
Number of mass values matched: 6

Sequence Coverage: 50%

Matched peptides shown in **Bold Red**

```
1 MGDVEKGKKI FVQKCAQCHT VEGGKHKTG PNLHGLEGRK TGQAPGFYI  
51 DANKNKGITW KEETLMEYLE NPKKYIPGTK MIFAGIKKKT EREDLIAYLK  
101 KATNE
```

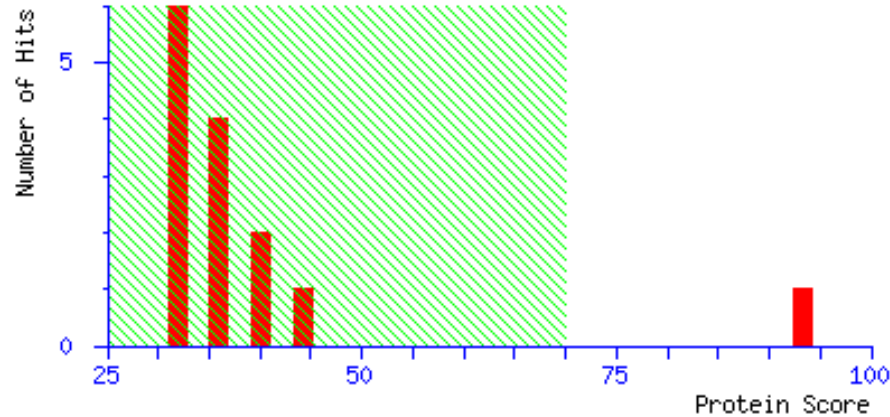
Cyto-C 10 Cycles



Cyto-C 10 Cycles

Mascot Score Histogram

Protein score is $-10 \cdot \log(P)$, 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 [Help](#)

Significance threshold $p <$ Max. number of hits

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

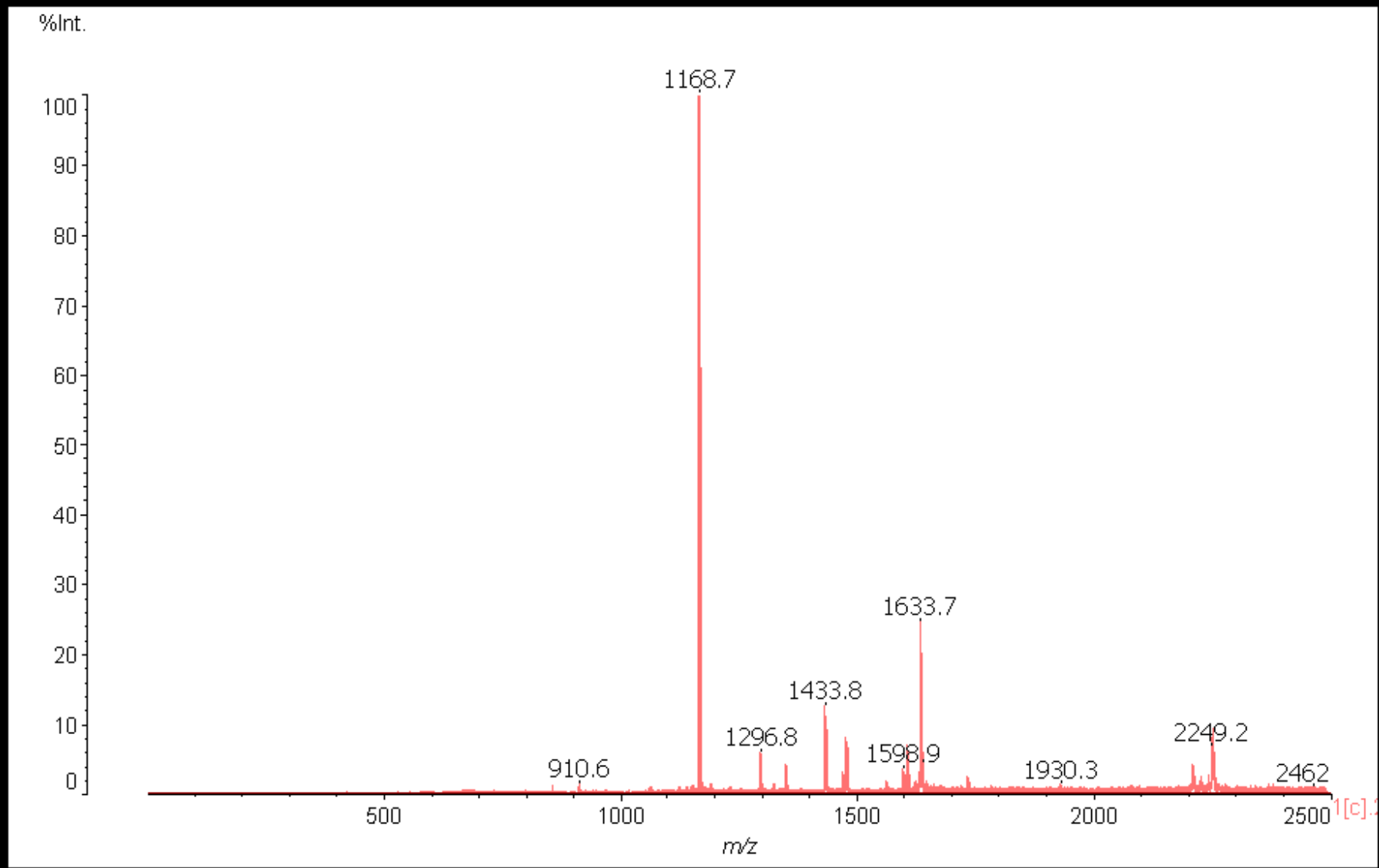
Number of mass values matched: 9

Sequence Coverage: 62%

Matched peptides shown in **Bold Red**

1 MGDVEKGKKI **FVQKCAQCHT VEKGGKHKTG PNLHGLFGRK TGQAPGFYIT**
51 **DANKNKGITW KEETLMEYLE NPKKYIPGTK MIFAGIKKKT EREDLIAYLK**
101 KATNE

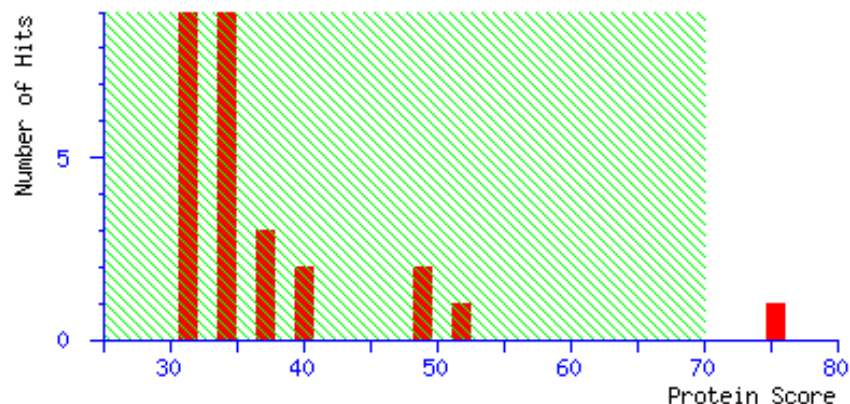
Cyto-C 20 Cycles



Cyto-C 20 Cycles Score

Mascot Score Histogram

Protein score is $-10 \cdot \log(P)$, 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	

- [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

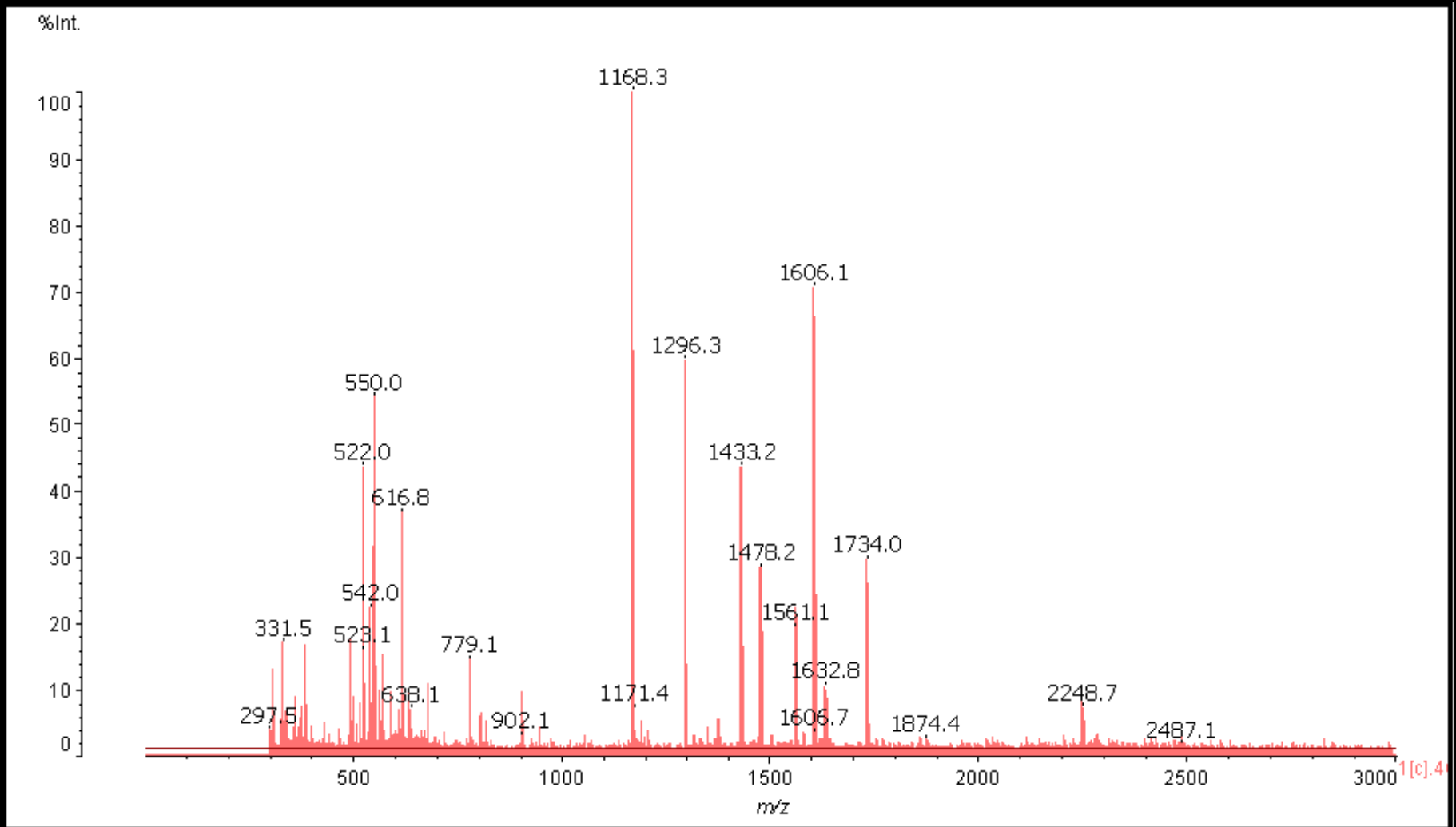
Sequence Coverage: 50%

Matched peptides shown in **Bold Red**

```
1 MGDVEKGKKI FVQKCAQCHT VEGGGKHKTG PNLHGLEGRK TGQAPGFYIT  
51 DANKNKGITW KEETLMEYLE NPKKYIPGTK MIFAGIKKKT EREDLIAYLK  
101 KATNE
```

Reference Sample Spectra

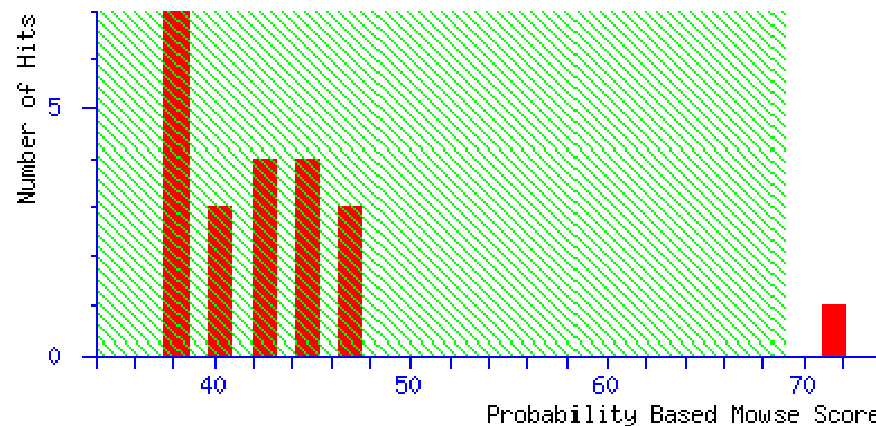
Cyto-C 4 Hours 55°C



Cyto-C 4 Hours 55°C Score

Probability Based Mowse Score

Protein score is $-10 \cdot \log(P)$, where P is the probability that the observed match is a random event. Protein scores greater than 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°C - 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

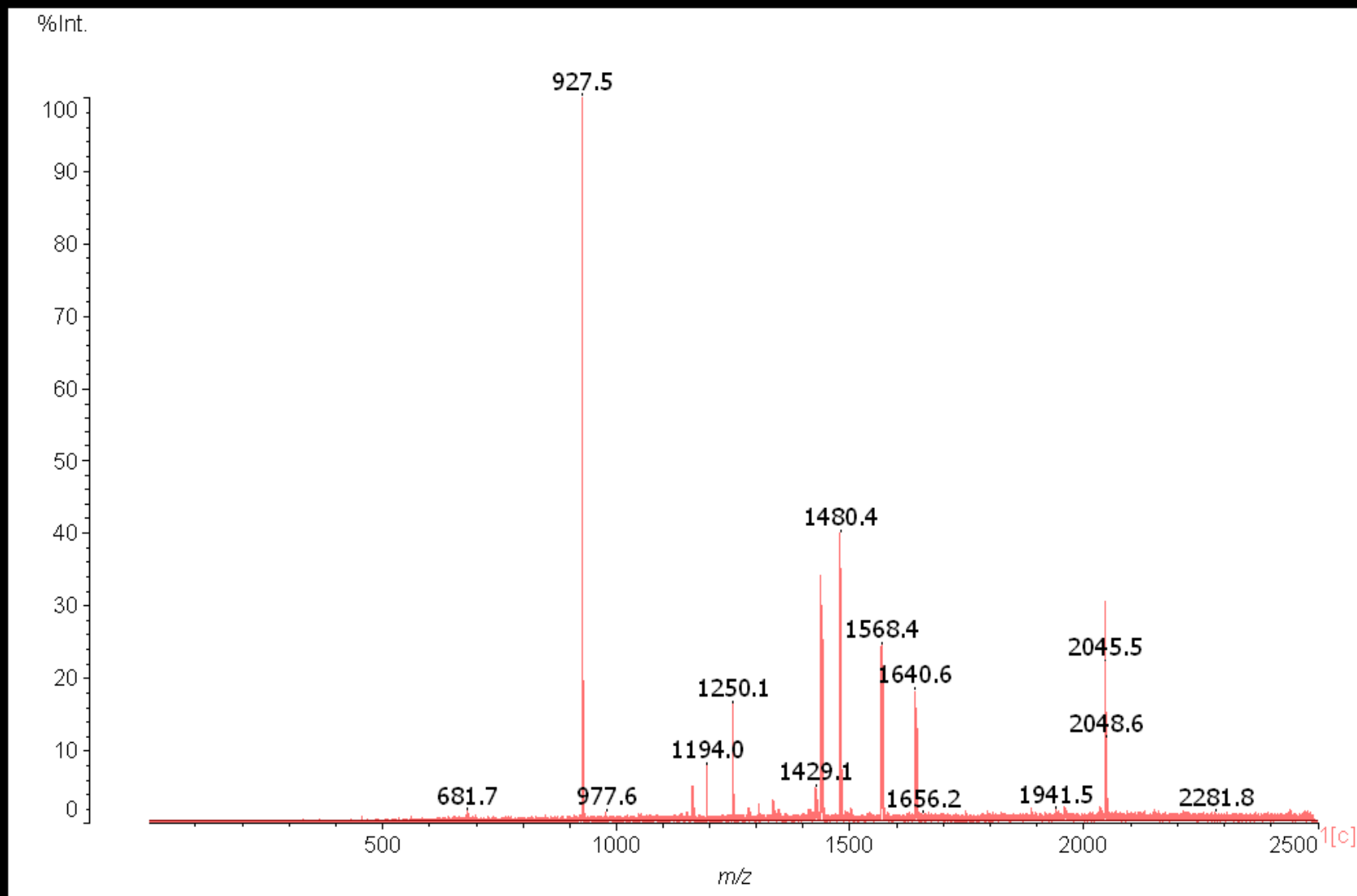
Number of mass values matched: 11

Sequence Coverage: 68%

Matched peptides shown in **Bold Red**

```
1 MGDVEKGGKI FVQKCAQCHT VEKGKHKTG PNLHGLFGRK TGQAPGFYYT  
51 DANKNKGITW KEETLMEYLE NPKKYIPGTK MIFAGIKKKT EREDLIAYLK  
101 KATNE
```

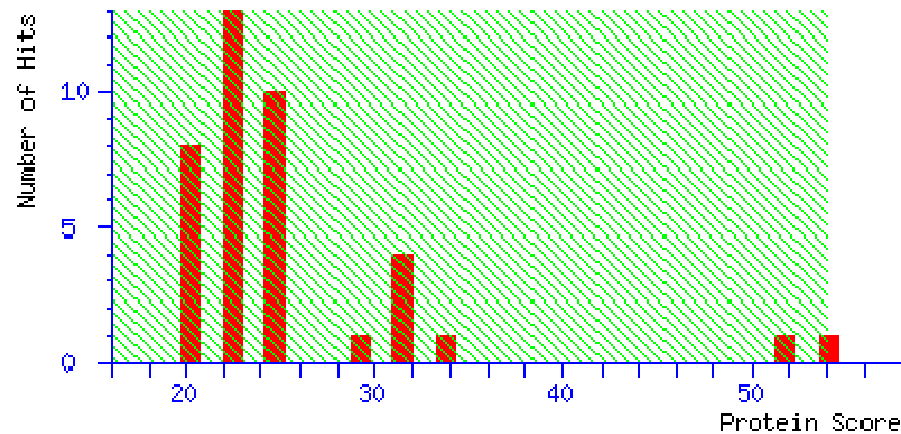
BSA 2 Hours 55°C



BSA 2 Hours 55°C Score

Mascot Score Histogram

Protein score is $-10 \cdot \log(P)$, 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 $p <$ <input type="text" value="0.05"/>		Max. number of hits <input type="text" value="AUTO"/>
<input type="button" value="Re-Search All"/>	<input type="button" value="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°C – 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 IAHREKDLGE EHFKGLVLIA
51 FSQYLQOCPF 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 MENFVAFVVK CCAADDKEAC FAVEGPKLVV
601 STQTALA
```

BSA (Bovine Serum Albumin) + Trypsin + PCT (Samples 1-8)

158 μ L BSA (1158 μ g) + 2 μ L trypsin (2 μ g); 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 μ L BSA (1158 μ g) + 2 μ L trypsin (2 μ g); 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 μ L Cyto-C (1158 μ g) + 2 μ L trypsin (2 μ g); 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 μ L Cyto-C (1158 μ g) + 2 μ L trypsin (2 μ g); 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

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Daniel Weissman, Jennifer-Leigh Oprihory, Ariel Gross

Middle Row: Tiffany Remsen, Arko Mukherjee,
Robert Chouake

Front Row: Michael Yee & Paul Pevsner