

Rapid Protein Extraction and Trypsin 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.

Ruan K, Lange R, Bec N, Bally C. *Biochem Biophys Res Commun* 1997; 239: 150.
Chappell E, Ballester J, Hood L, Lopez-Fernandez R. *J Chromatogr* 2000; 73: 121.
Simpson CD, Robinson MS, Lawrence KR, Tai F, Searles CA, Schrockner RT. *J Biomol Tech* 2003; 14: 172.
Ringham H, Bell R, Smeyers-Bebe G, Birkett J, Williams FA. *Electrophoresis* 2007; 28: 1022.
Pevsner P, Vecchiarelli D, Stall B, Remsen T, Anand S, Stern A. 2007. *British Mass Spectrometry Society*, Edinburgh, Scotland.
Pevsner P, Vecchiarelli D, Stall B, Remsen T, Anand S, Stern A, Samuel H. *British Mass Spectrometry Society*, 2007. *Robinson College, Cambridge*.
Pevsner P, Vecchiarelli D, Stall B, Remsen T, Anand S, Stern A, Samuel H, Birkett J, Williams FA. *British Mass Spectrometry Society*, 2007. *Robinson College, Cambridge*.
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PCT Sample Assembly

Cartridge Stack (12-48)

Microtubes (150µl)

Microtube Cartridge (6 or 8 tubes)



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, Bally 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.

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

PCT and Trypsin Digest

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

Pevsner P, Vecchiarelli D, Stall B, Remsen T, Anand S, Stern A. 2007. *British Mass Spectrometry Society*, Edinburgh, Scotland.
Pevsner P, Vecchiarelli D, Stall B, Remsen T, Kessler P, Levens N, Yang P, Stern A, Samuel H. *British Mass Spectrometry Society*, 2007. *Robinson College, Cambridge*.
Pevsner P, Vecchiarelli D, Stall B, Remsen T, Kessler P, Mornani M, Duddempudi S, Francois F, Stern A, Anand S. 2007. *British Mass Spectrometry Society*, Robinson College, Cambridge, UK.
Lopez-Ferrer D, Petritis K, Hixson KK, Heibek TH, Moore RJ, Below ME, Camp DG, Smith RD. *J Proteome Res* 2008; 7: 3276.

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 (ng/mL)	extraction efficiency (%)
HRT Na	control	26	2.25	8.0
HRT Nb	control	27	1.56	5.8
HRT Nc	control	9	0.68	7.6
HRT 1-6a	1 Gy	23	1.86	7.2
HRT 1-6b	1 Gy	12	0.94	7.8
HRT 1-6c	1 Gy	16	1.54	9.6
HRT 2-6a	2 Gy	29	3.15	10.9
HRT 2-6b	2 Gy	12	1.86	15.5
HRT 2-6c	2 Gy	14	1.14	8.2
HRT 4-6a	4 Gy	10	0.82	8.2
HRT 4-6b	4 Gy	18	1.41	7.8
HRT 4-6c	4 Gy	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 lesions. *American Chemical Society Mid-Atlantic Meeting*, Queensborough College, Queens, NY, May 2008. *American Chemical Society Mid-Atlantic Meeting*, Queensborough College, Queens, NY.
- Remsen T, Nathoin F, Talebian S, Kessler P, Liccardi F, Grifo J, et al. (2009). LC-MS identification of competent embryo biomarkers. *British Mass Spectrometry Society*, York, UK, September, 2008.
- Lopez-Ferrer D, Petritis K, Hixson KK, Heibek TH, Moore RJ, Below ME, Camp DG, Smith RD. *J Proteome Res* 2008; 7: 3276.
- Pevsner P, 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.

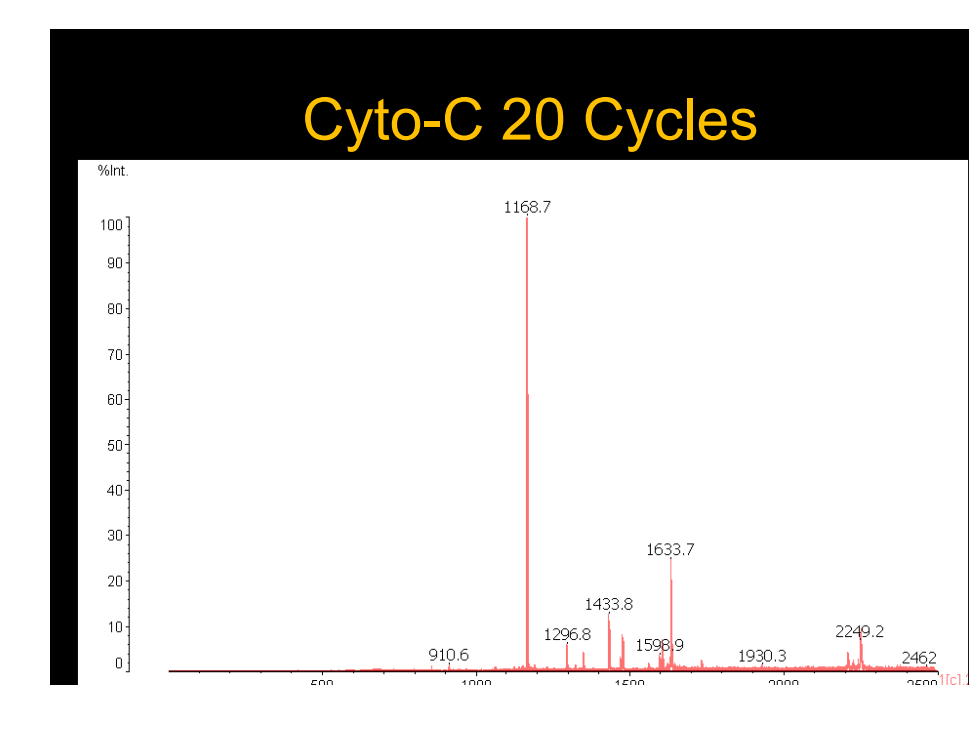
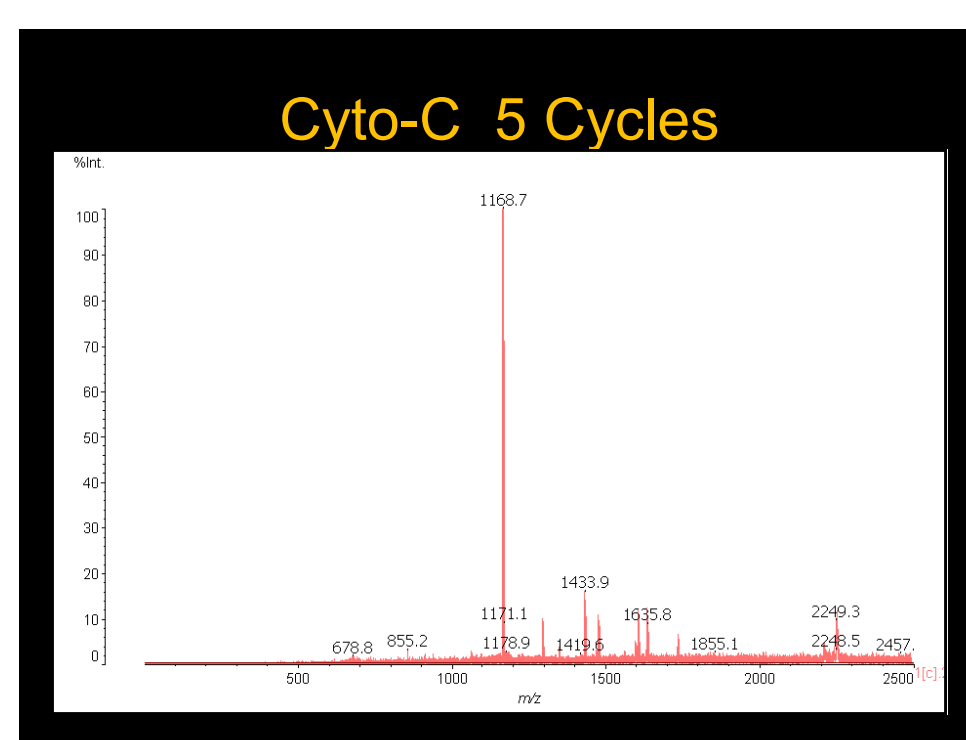
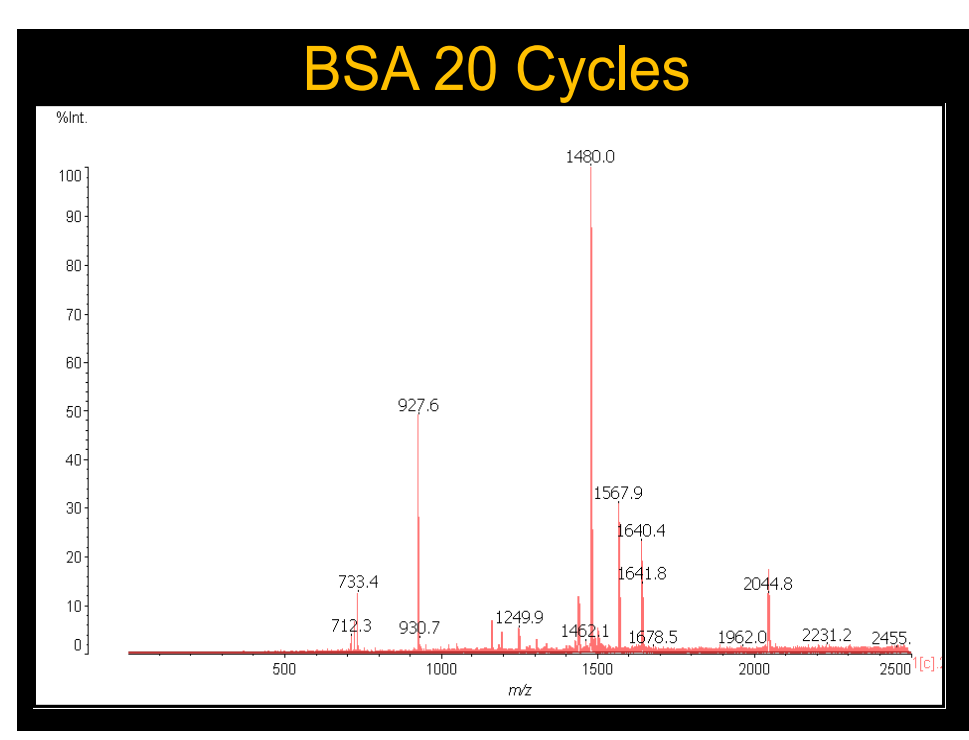
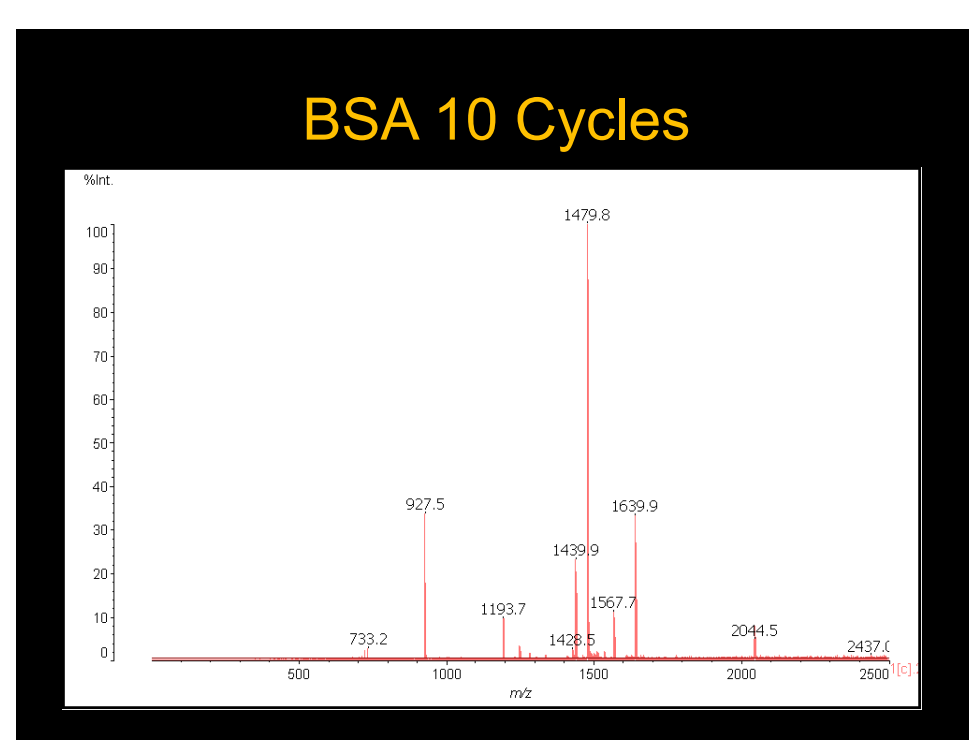
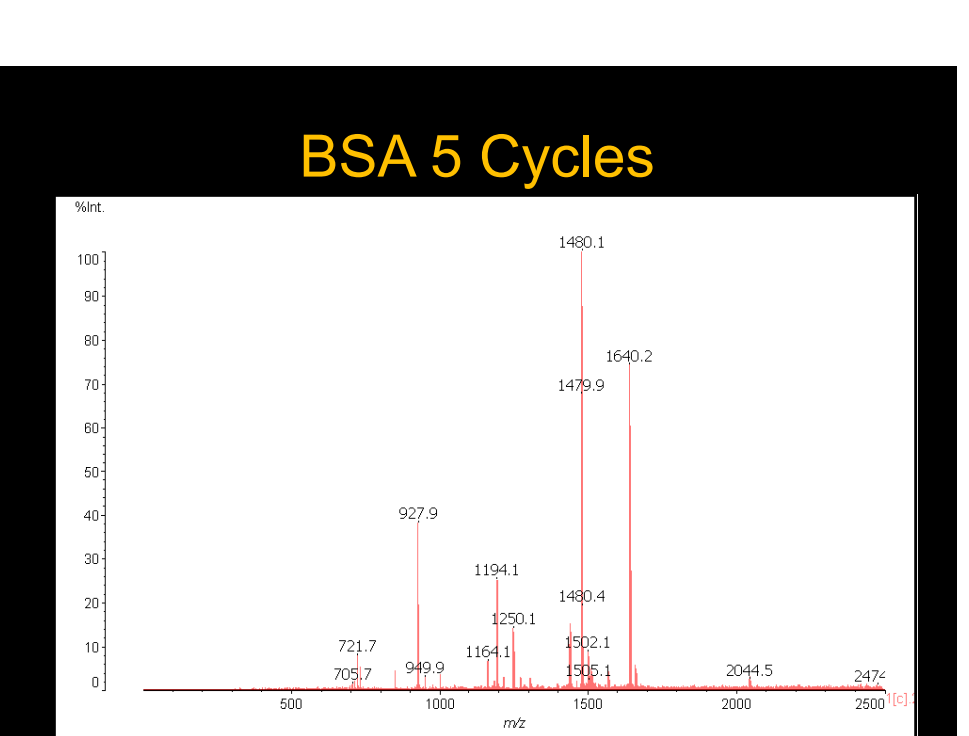
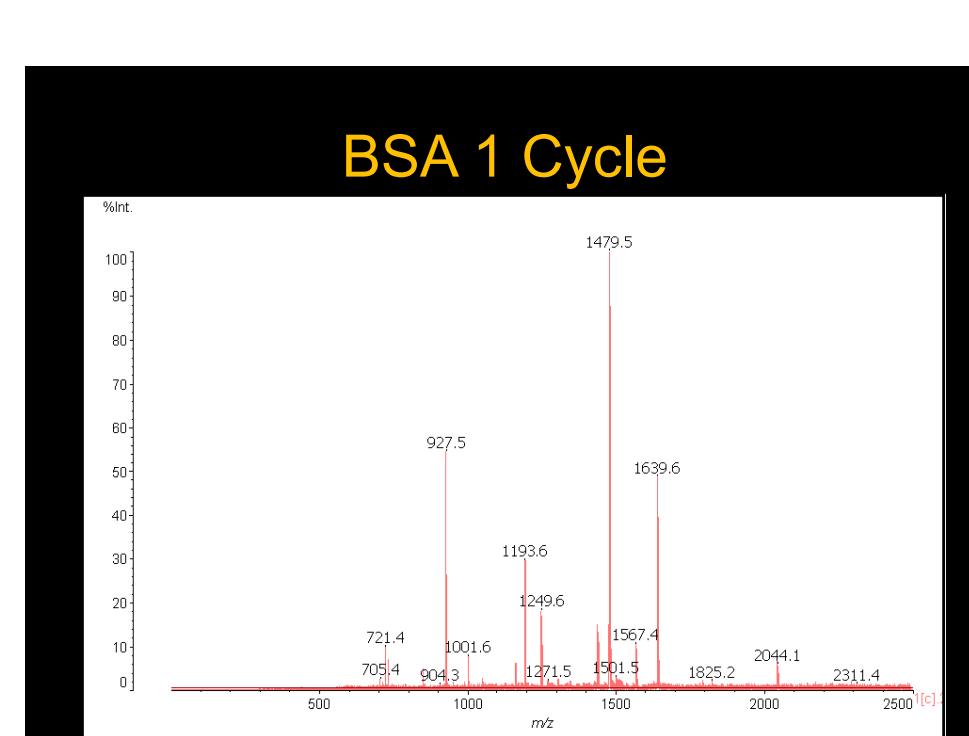
PCT Protocol

- 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)

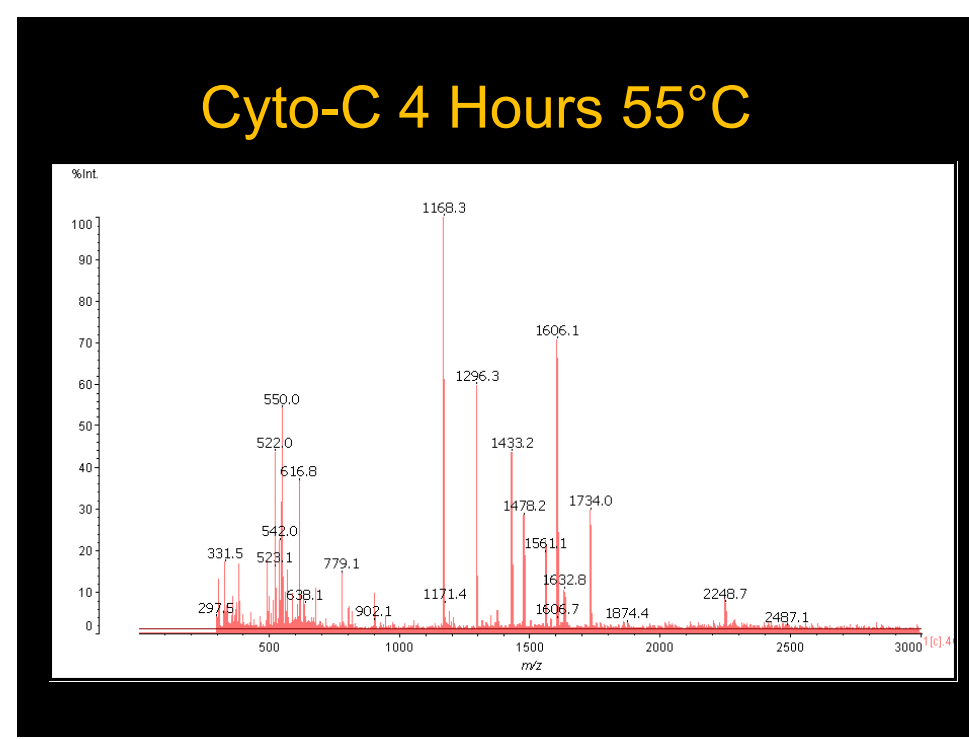
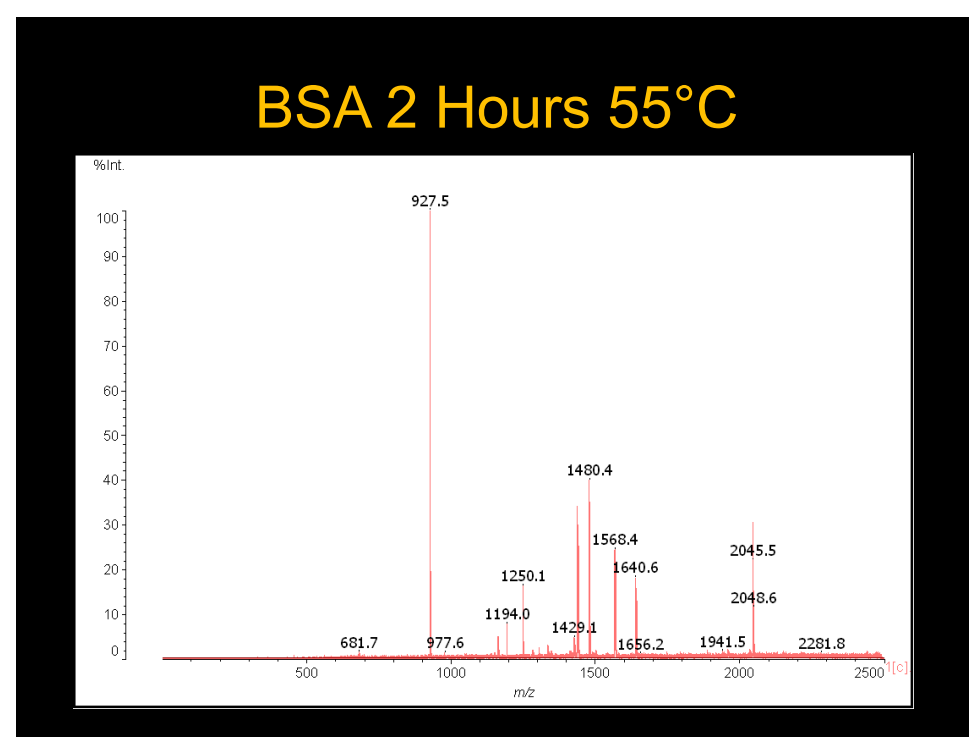
Reference Sample Protocol

- 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)

PCT Trypsin Digest Spectra



Reference Sample Spectra



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
10/200	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	No match	N/A
10/200	59	15
10/200	No match	N/A
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
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.

Conclusion

PCT tissue extraction is:

- 1) rapid
- 2) reproducible
- 3) high-yield

PCT trypsin digest is:

- 1) rapid with high % coverage
- 2) reproducible
- 3) Reduced analyte / trypsin ratio (~500:1)