ABRF-2003 ESRG: Analysis of a PVDF-Bound Known Protein with a Single Amino-Terminus.

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ABSTRACT:

The ABRF-2003ESRG sample is the 15th in a series of studies designed to allow participating members to evaluate their laboratory's abilities to analyze n-terminal proteins or peptide samples. This years study was done on a single protein that had been electroblotted to PVDF membrane. The original sample was submitted as part of a protein complex for analysis to an ESRG member's laboratory. One of the bands observed after SDS-PAGE analysis (another of which has already been used for the ABRF-2002ESRG study), running at an apparent molecular weight of 49kDa, was selected for this year's study. This sample demonstrated a single n-terminal sequence with initial yields around 2 pmol. Participants will be asked to assign sequence, identify the protein if possible, and return the tabular yield data to the ESRG for analysis. Comparison of initial yields, repetitive yields and overall performance of the instruments will be made, as well as run conditions and other factors that may affect the final results.

Materials and Methods

Sample Preparation:

This sample was prepared at the same time as the sample for the 2002 study. The protein complex* was present in several milliliters of solution. This was mixed with an equal volume of 2X Laemmli resolubilization buffer plus B-mercaptoethanol and boiled for 3 minutes. The protein was then run onto ten 15 lane precast 10-20% tris glycine 1.0 mm gels (NOVEX). After electrophoresis, the proteins were electrophoretically transferred to PVDF (NOVEX, 0.22 um) at 250 mAmps for 2 hours in a full immersion transfer tank system. PVDF membranes were then stained with 0.05% Coomassie blue G-250 in 50% methanol/10% acetic acid for five minutes, followed by destaining with 40% methanol/10% acetic acid and finally washing with water overnight. Bands used in this study were excised and placed into 0.5 ml microcentrifuge tubes and stored at room temperature for 1 year prior to being selected for this study. Individual bands (14 total) were sequenced for 25 amino acids by ESRG members on various models of sequencers to determine the expected performance of the sample. Amino acid analyses of three bands detected 9.4 pmole, 13.4 pmole, and 18.2 pmole of protein, with the average being 13.8 pmole. Assuming 15% sequencing yield from the PVDF, the expected average sequencing yield was approximately 2.0 pmole.

*Our thanks to Dr. Robert Macnab (Yale University, New Haven, CT, USA) and Dr. Tohru Minamino (Protonic Nanomachine ERATO, Kyoto, Japan) for the use of their protein complex in this study.

TABLE 1: ABRF-2003 ESRG Sequence Assignments from Facilities

Fac #	Instrument	PC	TC	PW	TW	-	Length of Call	Residues Assigned	Positive Accuracy	Total Accuracy
1	ABD 492-HT	21	0	0	3	0	24	24	100%	88%
2	ABD 494-HT	14	9	0	1	1	25	24	100%	96%
3	ABD 494-HT	23	1	0	0	1	25	24	100%	100%
4	ABD 492-HT	25	1	1	1	1	29	28	96%	93%
5	ABD 494-HT	9	1	0	0	0	10	10	100%	100%
6	ABD 494-HT	13	6	0	3	3	25	22	100%	86%
7	ABD 494-HT	29	2	0	0	2	33	31	100%	100%
8	ABD 494-HT	24	1	0	0	1	26	25	100%	100%
9	ABD 494-cLC	19	0	0	0	1	20	19	100%	100%
10	ABD 494-HT	4	1	0	1	1	7	6	100%	83%
11	ABD 494-HT	5	13	2	0	0	20	20	71%	90%
12	ABD 494-HT	13	3	1	1	2	20	18	93%	89%
13	ABD 477	10	3	1	0	2	16	14	91%	93%
14	ABD 492-HT	18	1	0	1	0	20	20	100%	95%
15	ABD 494-HT	17	2	2	4	0	25	25	89%	76%
16	ABD 494-cLC	20	1	0	0	0	21	21	100%	100%
17	HP G-1005A	48	0	1	0	1	50	49	98%	98%
18	ABD 494-HT	22	3	0	0	0	25	25	100%	100%
19	ABD 494-HT	5	0	0	0	0	5	5	100%	100%
20	ABD 492-cLC	26	4	0	1	3	34	31	100%	97%
21	ABD 494-cLC	21	1	0	1	1	24	23	100%	96%
22	ABD 494-HT	13	2	0	0	0	15	15	100%	100%
23	ABD 494-HT	24	0	0	0	1	25	24	100%	100%
24	SHIM PPSQ	5	0	5	1	1	12	11	50%	45%
25	ABD 494-HT	14	3	0	0	1	18	17	100%	100%
26	ABD 494-HT	22	1	0	1	1	25	24	100%	96%
27	ABD 477	0	0	2	6	1	9	8	0%	0%
28	ABD 494-cLC	18	5	1	2	0	26	26	95%	88%
29	ABD 492-cLC	25	0	0	0	0	25	25	100%	100%
30	ABD 494-HT	18	5	0	0	2	25	23	100%	100%
31	HP G-1005A	24	0	0	0	1	25	24	100%	100%
32	ABD 494-HT	25	0	0	0	0	25	25	100%	100%
33	ABD 492-HT	13	2	0	1	1	17	16	100%	94%
34	ABD 494-HT	12	10	0	0	0	22	22	100%	100%
35	ABD 494-HT	11	1	0	0	3	15	12	100%	100%
36	ABD 494-HT	15	7	0	1	2	25	23	100%	96%
37	HP G-1005A	10	1	0	1	3	15	12	100%	92%
38	ABD 494-HT	26	1	0	0	0	27	27	100%	100%
39	ABD 49HT	20	1	0	0	1	22	21	100%	100%
40	ABD 494-cLC	10	1	6	0	8	25	17	63%	65%
41	HP G-1005A	25	0	0	0	0	25	25	100%	100%
42	ABD 494-HT	15	7	0	1	2	25	23	100%	96%
43	ABD 494-HT	15	2	1	0	2	20	18	94%	94%
44	ABD 477	6	3	1	1	1	12	11	86%	82%
45	ABD 494-cLC	5	0	1	0	0	6	6	83%	83%
46	ABDS492-HT	12	3	0	0	0	15	15	100%	100%
Totals or Averages		769	108	25	32	51	985	934	96.9%	93.9%

Expected Sequence (residues 1–25)

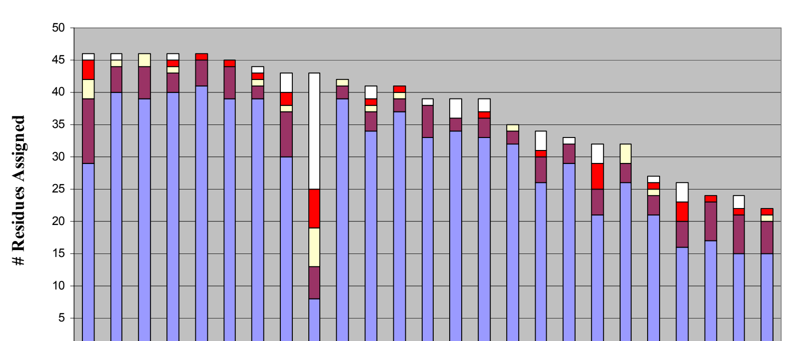
Fac #	H 1	M 2	T 3	T 4	R 5	L 6	T 7	R 8	W 9	L 10	T 11	A 12	L 13	D 14	N 15	F 16	E 17	A 18	K 19	M 20	A 21	L 22	L 23	P 24	A 25
1	H	M	T	T	R	L	T	R	l	L	T	A	L	D	N	F	E	A	s	M	A	v	L	P	
2	s	M	Tl	Tl	rl	La	T	rav	-	L	Ta	Ad	Lg	D	N	F	E	A	K	M	A	L	l	P	A
3	H	M	T	T	R	L	T	R	-	L	T	A	L	D	N	F	E	A	K	M	A	L	L	p	A
4	H	M	T	T	R	L	T	R	N	L	-	A	L	D	N	F	E	A	K	M	A	L	L	P	a
5	H	M	T	T	R	L	T	R	w	L															
6	s	M	T	T	R	L	T	R	c	L	T	A	L	d	N	F	-	v	-	q	a	vfli	qkr	-	gaik
7	H	M	T	T	R	L	T	R	w	L	T	A	L	D	N	F	E	A	K	M	A	L	L	P	A
8	hk	M	T	T	R	L	T	R	-	L	T	A	L	D	N	F	E	A	K	M	A	L	L	P	A
9	H	M	T	T	R	L	T	R	-	L	T	A	L	D	N	F	E	A	K	M					
10	H	M	T	-	R	l	q																		
11	HS	MPI	TLA	TIA	K	E	E	AQ	Q	LD	T	M	LA	D	QN	F	ER	A	RK	M					
12	H	M	T	T	R	L	T	-	-	L	l	A	L	LD	N	F	e	A	kq	V					
13	h	M	t	T	R	L	T	R	A	L	-	A	-	D	N	f									
14	h	M	T	T	R	L	T	R	r	L	T	A	L	D	N	F	E	A	K	M					
15	H	M	T	T	R	L	T	rag	lpv	L	T	A	L	D	N	F	el	A	K	ND	E	da	L	gadi	v
16	H	M	T	T	R	L	T	R	w	L	T	A	L	D	N	F	E	A	K	M	A				
17	H	M	T	T	R	L	T	R	W	L	T	A	L	D	N	F	E	A	K	M	A	L	L	P	A
18	H	M	T	T	R	L	T	R	W	L	T	A	L	D	N	F	ea	A	K	M	A	la	la	P	A
19	H	M	T	T	R																				
20	H	M	T	T	R	L	T	R	-	L	T	A	L	D	N	F	E	A	K	M	A	L	L	P	A
21	H	M	T	T	R	L	T	R	W	L	T	A	L	D	N	F	E	A	a	M	A	-	L	p	
22	H	M	T	T	R	L	T	Ra	w	L	T	A	L	D	N										
23	H	M	T	T	R	L	T	R	-	L	T	A	L	D	N	F	E	A	K	M	A	L	L	P	A
24	P	M	T	T	R	L	K	-	Y	P	P	l													
25	H	M	T	T	R	L	T	R	-	L	t	A	L	D	n	F	E	a							
26	h	M	T	T	R	L	T	R	-	L	T	A	L	D	N	F	E	A	a	M	A	L	L	P	A
27	r	P	P	n	v	qi	-	n	p																
28	H	M	T	T	R	L	T	R	P	L	T	A	L	D	N	F	E	A	r	M	a	g	l	p	a
29	H	M	T	T	R	L	T	R	W	L	T	A	L	D	N	F	E	A	K	M	A	L	L	P	A
30	H	M	T	T	R	L	T	R	-	L	T	A	L	D	N	F	E	A	k	m	A	-	l	p	a
31	H	M	T	T	R	L	T	R	-	L	T	A	L	D	N	F	E	A	K	M	A	L	L	P	A
32	H	M	T	T	R	L	T	R	W	L	T	A	L	D	N	F	E	A	K	M	A	L	L	P	A
33	Hgs	M	T	T	R	L	T	R	-	L	T	A	L	D	N	f	l								
34	hsg	M	TLQ	TAI	RL	LA	T	RA	w	LGVA	T	A	L	D	N	F	E	A	K	V	dqgeal	L			
35	-	mgyl	T	T	R	L	T	-	-	L	T	A	L	D	N										
36	hdgsa	M	T	T	R	L	T	R	-	L	T	A	l	D	N	F	-	a	K	M	r	l	lg	pgt	apl
37	H	-	T	T	R	L	T	R	-	L	T	A	l	-	d										
38	H	M	T	T	R	L	T	R	W	L	T	A	L	D	N	F	E	A	k	M	A	L	L	P	A
39	H	M	T	T	R	L	T	R	-	L	T	A	L	D	N	F	E	A	K	M	A	l			
40	Hwn	M	A	T	R	L	T	R	Dh	L	T	H	L	-	-	Q	-	-	-	S	-	-	L	-	G
41	H	M	T	T	R	L	T	R	W	L	T	A	L	D	N	F	E	A	K	M	A	L	L	P	A
42	H	Mti	Tl	T	R	La	T	r	pg	L	T	A	Ldi	D	-	F	E	A	-	M	A	L	l	p	A
43	M	M	T	T	r	L	T	r	-	L	T	A	L	-	N	F	E	A	K	M					
44	h	M	T	N	R	L	t	m	-	L	t	A													
45	Q	M	T	T	R	L																			
46	H	MT	T	T	R	L	T	RW	W	L	T	A	L	D	NG										

Expected Sequence (residues 26–50; only facilities with assignments past residue 25)

Fac #	V 26	R 27	R 28	Y 29	G 30	R 31	L 32	T 33	R 34	A 35	T 36	G 37	L 38	V 39	L 40	E 41	A 42	T 43	G 44	L 45	Q 46	L 47	P 48	L 49	G 50
4	V	s	R	Y																					
7	V	-	R	Y	g	-	L	T																	
8	V																								
17	V	R	R	Y	G	R	L	T	R	A	T	G	L	V	L	E	A	T	G	L	Q	-	P	V	G
20	V	-	r	Y	g	-	l	t	I																
28	v																								
38	V	R																							

Legend: Facility number (Fac#), Positive Correct (PC), Tentative Correct (TC), Positive Wrong (PW), Tentative Wrong (TW), No call (-). Upper case letter represent positive calls and lower case letters represent tentative assignments. Assignments where m ultiple positive calls were made with the correct amino acid were counted as a tentative correct. Assignments where multiple positive calls were made without the correct assignment were counted as a positive wrong. Positive Accuracy = PC/(PC+PW), total accuracy = (PC+TC)/(PC+TC+PW+TW). Company abbreviations are AppliedBiosystems (ABD), Hewlett Packard/Agilent (HP), Shimadzu (SHIM). It should be noted that facility #41 reported only the first 25 amino acids on the supplied data report sheet but did mak e assignments past 25 on the AA/pmol table supplied for repetitive yield calculations. Facility #41 data from cycle #26 on was: VRRYGRLTRATglVlE-TglQ-p.

Sequence Calls For First 25 Residues for ABRF-2003 ESRG



	H	M	T	T	R	L	T	R	W	L	T	A	L	D	N	F	E	A	K	M	A	L	L	P	A
NC	1	1	0	1	0	0	1	3	18	0	2	0	1	3	2	0	3	1	3	0	1	3	0	2	0
TW	3	0	0	1	1	1	1	2	6	0	1	1	0	0	1	0	1	0	4	0	1	3	1	1	1
PW	3	1	2	1	0	0	1	1	6	1	1	1	0	0	0	1	0	0	0	3	1	0	0	0	1
TC	10	4	5	3	4	5	2	7	5	2	3	2	5	2	3	2	4	3	4	3	3	4	6	6	5
PC	29	40	39	40	41	39	39	30	8	39	34	37	33	34	33	32	26	29	21	26	21	16	17	15	15

Amino Acid

Figure 1: Bar graph representation of the first 25 residues of the data shown in Table 1. NC represents a "-" from Table 1. Note very poor results for Tryptophan #9, which is consistent with past ABRF Edman sample results.

TABLE 2: Summary of Sequence Assignments for ABRF-2003 ESRG Compared With Other ABRF Edman Sequence Studies of Proteins

Description	Equation	ABRF-2003 ESRG	ABRF-2002 ESRG	ABRF-99SEQ (protein)	ABRF-95SEQ	ABRF-94SEQ
Total # cycles	PC+TC+PW+TW	934	837	560	1801	1637
# responses	R	46	31	45	71	78
Total # correct	PC+TC	877	630	518	1361	1505
Total # wrong	PW+TW	57	207	42	440	132
Total # positive	PC+PW	794	596	474	1647	1492
Total # tentative	TC+TW	140	241	86	154	145
Avg. # correct	(PC+TC)/R	19.1	20.3	11.5	19.1	19.3
Avg. # positive	(PC+PW)/R	17.3	19.2	10.5	23.2	19.1
Avg. # incorrect	(PW+TW)/R	1.2	6.7	0.9	6.2	1.7
Accuracy of PC calls	PC/(PC+PW)	96.9%	76.3%	98.7%	78.0%	95.5%
Accuracy of TC calls	TC/(TC+TW)	77.1%	72.6%	58.1%	45.0%	55.0%

Calls were designated as positive correct (PC), positive wrong (PW), tentative correct (TC), tentative wrong (TW), and number of responses (R).

TABLE 3: Summary of Initial and Repetitive Yields For ABRF-2003 ESRG

Facility	IY [1] MET(2) (pmol)	RY [2] MET (2,20)	RY [2] LEU (6,10,13,22)	RY [2] ALA (12,18,21,25)	# PC All	# PC First 25	# Correct First 25	%PC First 25
1	3.91	93.60%	89.90%	92.60%	21	21	21	100%
2	2.97	95.20%	93.30%	94.40%	14	14	23	100%
3	1.92	89.50%	89.60%	93.40%	23	23	24	100%
4	1.22	93.10%	94.90%	80.00%	25	22	23	96%
5	1.73		87.50%		9	9	10	100%
6	0.97	89.80%	91.20%	96.50%	13	13	19	100%
7	2.71	90.40%	90.40%	92.00%	29	24	25	100%
8	2.59	91.80%	92.40%	95.60%	24	23	24	100%
9	2.26	88.30%	85.50%	90.70%	19	19	19	100%
10	1.17				4	4	5	100%
11	1.51	90.90%	92.50%	85.70%	5	5	18	71%
12	0.83	87.80%	81.00%	116.90%	13	13	16	93%
13	0				10	10	13	91%
14	2.06		88.80%		18	18	19	100%
15	1.73		81.50%	95.40%	17	17	19	89%
16	29.9 [1]	94.50%	96.60%	67.90%	20	20	21	100%
17	0				48	25	25	100%
18	1.22		92.10%	90.60%	22	22	25	100%
19	2.01				5	5	5	100%
20	2.25		95.20%	87.90%	26	24	24	100%
21	1.96	89.60%	89.80%	84.40%	21	21	22	100%
22	0				13	13	15	100%
23	1.42	90.20%	89.80%	91.80%	24	24	24	100%
24	1.71		87.60%		5	5	5	50%
25	0				14	14	17	100%
26	0.24	96.20%	95.30%	94.50%	22	22	23	100%
27	0				0	0	0	0%
28	0				18	18	22	95%
29	2.3	91.70%	88.50%	90.70%	25	25	25	100%
30	1.96	85.20%	78.90%	91.20%	18	18	23	100%
31	2.6	95.30%	92.70%	90.10%	24	24	24	100%
32	2.94				25	25	25	100%
33	1.44		102.00%		13	13	15	100%
34	1.98	90.20%	87.80%	89.70%	12	12	22	100%
35	0.52		71.80%		11	11	12	100%
36	1.83	90.80%	87.70%	94.30%	15	15	22	100%
37	0		92.60%		10	10	11	100%
38	1.3	91.40%	91.80%	93.50%	26	24	25	100%
39	2.54	91.80%	93.90%	92.20%	20	20	21	100%
40	1.67				10	10	11	63%
41	3.58	94.10%	95.40%	92.70%	25	25	25	100%
42	0		94.70%		15	15	22	100%
43	0		88.30%	89.20%	15	15	17	94%
44	2.64		81.80%		6	6	9	86%
45	0				5	5	5	83%
46	3				12	12	15	100%
Averages:	1.96	91.43%	89.79%	91.30%	17	16	18	94%

1. Initial yields (IY) of MET 2 are reported as background corrected (pmol yield cycle 2 minus pmol yield cycle 1) pmol values. Facility #16's MET values are questionable, as shown by comparison to the pmol yield of their HIS 1 (3.1 pmol). Therefore, the IY from facility #16 was not used in the average calculation.

2. Repetitive yields (RY) were calculated using background corrected pmol values (cycle yield minus previous cycle yield) only if the amino acids were positively or tentatively assigned. The formula was as follows, using MET 2 and 20 as an example: (M20/M2) raised to the 1/(n20-n2) power where M20 and M2 represents the background correct pmol value of MET at cycle 20 and 2 and n20-n2 represents the difference in the two cycles. Repetitive yields for the 4 LEU and the 4 ALA were averaged. Facilities #32 and #46 only provided pmol data for assigned amino acids, therefore no RY calculation was made as the pmol values could not be background corrected.

TABLE 4: Best Responses for the ABRF-2003 ESRG

Best Responses for First 25 Residues [1]

Facility #	# PC	# TC	% PC	Protein ID	IY (M2)	RY	Sequencer
41	25	0	100%	PC	3.7	94%	HP G-1005A
29	25	0	100%	PC	2.3	89.80%	ABD Procise-cLC
32	25	0	100%	PC	2.9		ABD Procise-HT
17	25	0	100%	PC			HP G-1005A
38	24	1	100%	PC	1.3	92.50%	ABD Procise-HT
7	24	1	100%	TC	2.7	91.10%	ABD Procise-HT
31	24	0	100%	-	2.6	91.70%	HP G-1005A
20	24	0	100%	-	2.2	91.60%	ABD Procise-cLC
23	24	0	100%	-	1.4	90.80%	ABD Procise-HT
8	23	1	100%	-	2.6	93.80%	ABD Procise
3	23	1	100%	-	1.9	91.30%	ABD Procise-HT
18	22	3	100%	PC	1.2	91.30%	ABD Procise-HT

[1] Determined from data presented in Table 3. Only responses with a %PC of 100% for the first 25 residues were considered. Responses were sorted according to #PC, # total correct (PC+TC), RY and finally IY.

Responses that went significantly beyond 25 residues

Facility #	#PC	#TC	%PC	#PW	#TW	Sequencer
41	41	5	100%	0	0	HP G-1005A
17	48	0	98%	1	0	HP G-1005A
7	29	2	100%	0	0	ABD Procise-HT
20	26	4	100%	0	1	ABD Procise cLC
4	25	1	96%	1	1	ABD Procise

TABLE 5: Performance of Edman Sequencers Used to Analyze ABRF-2003 ESRG

Manufacturer	Model	R [1]	# PC	# TC	# PW	# TW	# -	% PC [2]	% TC [2]	# correct/R [2]	RY [3]	IY (pm) [3]
ABD	Procise-HT	30	497	89	7	19	28	98.6%	82.4%	19.5	90.8%	1.8
ABD	Procise-cLC	8	144	12	8	4	13	94.7%	75.0%	19.5	88.1%	2.1
ABD	477	3	16	6	4	7	4	80.0%	46.0%	7.3	81.8%	2.6
Shimadzu	PPSQ-23A	1	5	0	5	0	0	50.0%	NA	5	87.6%	2.3
HP	G-1005A	4	107	1	1	1	5	99.1%	50.0%	27	93.1%	3.1

[1] Number of instruments that analyzed ABRF-2003 ESRG.
[2] Calculated as described in TABLE 2.
[3] Repetitive (RY) and initial (IY) yields from each instrument were included in the averages only if a complete pmol/cycle table was provided.

TABLE 6: Database Search Results for ABRF-2003 ESRG

Fac #	Positive ID (Y/N)	Correct ID (Y/N)	Search Engine	Database(s)
1	y	y	NCBI BLAST	nr
2	y	y	GCG Wisc, FINDPATTERNS	SP
3	y	y	FASTA	GPTR
4	y	y	BLAST	GB. Nr
5	y	y	MS Edman (PP)	SP
7	y	y	FASTA	nr
8	y	y	MS Edman (PP)	NCBI
9	y[1]			
12	n	n	BLAST	
13	y	y	BLASTP	SP
14	y	y	BLASTP	nr
15	n	y	BLAST	nr
16	y	y	BLASTP	nr
17	n	y	BLAST	
20	y	y	FASTA3	SP swall
22	y	y	FASTA	SP/PIR
23	y	y	FASTA	PIR
24	n	n	BLAST, FASTA	SP
25	y	y	BLAST	
26	y	y	EMBI	nr
27	n	n	BLAST	nr
30	y	y	FASTA3	SWALL
31	y	y	FASTA	nr
32	y	y	MPSearch EMBL	SP
33	y	y	BLAST	nr SP
34	y	y	Protein Prospector	SP
35	y	y	FASTA3 EBI	SWALL
36	n	n	FASTA GCG	SP
37			FASTA EMBL	SWALL
38	y	y	BLAST	nr
40	n	n	BLAST	BLAST
41	y	y	BLASTP, TBLASTN	nr
42	y	y	FASTA EMBL-EBI	SWALL
43	y	y	BLAST	nr
44	n	n	Protein Info	nr
45	n	n	FASTA 3	
46	y	y	GCG FASTA	5P-nr

1. Facility #9 performed a search and reported an unambiguous identification, however, no protein name was given on the data sheet.

Table 7: Results from 14 bands tested by ESRG members

		average
IY (M2) in pmole	1.3 to 3.6	2.23
RY (Leu)	81.1% to 101.0%	93.9%
Length of call	5* to 25	20
Residues assigned	4* to 23	17
# PC	213	15
# TC	26	2
Unassigned	42	3
# Trp assignments	2 (of 14)	

* Power failure shut down instrument.

Note: Reproducibility of data was very good within member labs that tested more than one band.

Sequencer models used: ABD Procise HT, ABD Procise cLC

Conclusions:

The participating facilities had very good success with this PVDF sample of a protein with a single amino-terminus. 48% were able to assign 25 or more amino acids, 72% made no wrong assignments (100% PA), 96.9% of the positive assignments were correct, and 65% of the facilities were able to correctly identify the protein. The accuracy of the assignments compares favorably to previous protein studies that distributed solution samples. Given the variability in the amount of protein in each band (a 2-fold difference detected in three analyzed bands), the initial yield (IY) is not a good indicator of the performance of a sequencer. Repetitive yield (RY), length of call, and positive accuracy (PA) are better indicators, but are not definitive as the ability of the person reading the data will affect the results. As shown in Table 7, the ESRG members produced results similar to the study's participants.