# 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.

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**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)

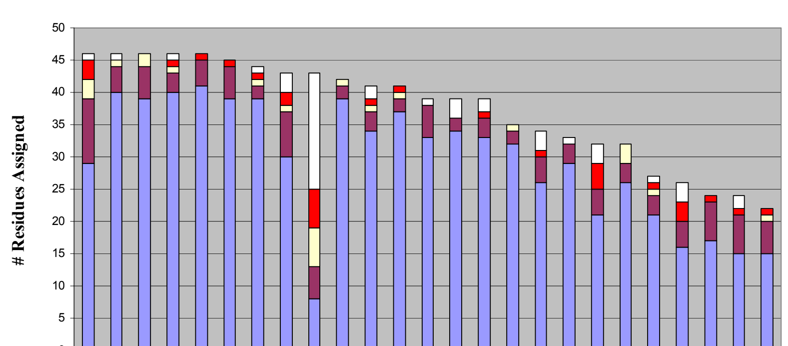
| Fac # | H 1 | M 2 | T 3 | T 4 | R 5 | L 6 | T 7 | R 8 | W 9 | L 10 | T 11 | A 12 | L 13 | D 14 | N 15 | F 16 | E 17 | A 18 | K 19 | M 20 | A 21 | L 22 | L 23 | P 24 | A 25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | H | M | T | T | 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; rows 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.