

1. Your laboratory is located on which continent?			
		Response Percent	Response Total
Africa		0%	0
Asia		4%	2
Australia		2%	1
Europe		12%	6
North America		80%	40
South America		2%	1
Total Respondents			50
(skipped this question)			1

2. What best describes your laboratory setting?			
		Response Percent	Response Total
University /Academic Laboratory		58.8%	30
Pharmaceutical / Biotech Company		23.5%	12
For profit Hospital / Medical Center		0%	0
Non profit Hospital / Medical Center		5.9%	3
For profit Research Institute		0%	0
Non profit Research Institute		3.9%	2
Contract Laboratory		2%	1
Government Laboratory		3.9%	2
Other (please specify)		2%	1
Total Respondents			51
(skipped this question)			0

3. What best describes your laboratory function?			
		Response Percent	Response Total
Core Facility / Research Resource		78.4%	40
Research Laboratory		15.7%	8
Quality Control Laboratory		0%	0
Other (please specify)		5.9%	3
Total Respondents			51
(skipped this question)			0

4. How long has your lab been doing Edman degradation sequencing?			
		Response Percent	Response Total
Less than 5 years		3.9%	2
Between 5 and 10 years		15.7%	8
Between 10 and 15 years		25.5%	13
More than 15 years		54.9%	28
Total Respondents			51
(skipped this question)			0

5. What other services are offered or techniques performed in your laboratory? Check all that apply			
		Response Percent	Response Total
Peptide Synthesis		46%	23
Amino Acid Analysis		56%	28
DNA Sequencing		28%	14
Oligonucleotide synthesis		18%	9
HPLC of proteins or peptides		90%	45
FPLC of proteins or peptides		20%	10
SDS-PAGE (1D gels)		58%	29
Electroblotting to PVDF membranes		54%	27
2D gel electrophoresis		48%	24
DIGE gels		6%	3
Gel image analysis		34%	17
Automated spot picking		18%	9
Manual spot picking		40%	20
Automated enzymatic digestion		16%	8
Manual enzymatic digestion		84%	42
Biacore analysis		14%	7
Carbohydrate analysis		16%	8
MALDI-TOF		68%	34
ESI-MS		62%	31
LC-MS/MS		64%	32
Other (please specify)		12%	6
Total Respondents			50
(skipped this question)			1

6. How many of the following Edman sequencers (functioning or nonfunctioning) do you have?						
	0	1	2	3	4 or more	Response Total
Applied Biosystems 470A	83% (10)	0% (0)	17% (2)	0% (0)	0% (0)	12
Applied Biosystems 477	77% (10)	15% (2)	8% (1)	0% (0)	0% (0)	13
Applied Biosystems 473/475/476	71% (10)	29% (4)	0% (0)	0% (0)	0% (0)	14
Applied Biosystems Procise 49X HT	6% (2)	69% (22)	19% (6)	3% (1)	3% (1)	32
Applied Biosystems Procise 49X cLC	31% (8)	62% (16)	8% (2)	0% (0)	0% (0)	26
Applied Biosystems ProciseC (C-terminal)	92% (11)	8% (1)	0% (0)	0% (0)	0% (0)	12
Agilent /Hewlett Packard G1000A	77% (10)	23% (3)	0% (0)	0% (0)	0% (0)	13
Agilent /Hewlett Packard G1005	85% (11)	15% (2)	0% (0)	0% (0)	0% (0)	13
Agilent /Hewlett Packard 421-NC	100% (10)	0% (0)	0% (0)	0% (0)	0% (0)	10
Porton 2090E	83% (10)	17% (2)	0% (0)	0% (0)	0% (0)	12
Beckman LF 3000	75% (9)	25% (3)	0% (0)	0% (0)	0% (0)	12
Shimadzu PPSQ-23	100% (10)	0% (0)	0% (0)	0% (0)	0% (0)	10
Other	91% (10)	9% (1)	0% (0)	0% (0)	0% (0)	11
Total Respondents						49
(skipped this question)						2

7. Indicate the number of your functioning sequencers in each age range? (Functioning is defined as sequencers that are still being used)						
	0	1	2	3	4 or more	Response Total
Less than 5 years old	27% (6)	59% (13)	9% (2)	0% (0)	5% (1)	22
Between 5 and 10 years old	9% (3)	71% (24)	18% (6)	3% (1)	0% (0)	34
Between 10 and 15 years old	31% (5)	62% (10)	6% (1)	0% (0)	0% (0)	16
More than 15 years old	100% (6)	0% (0)	0% (0)	0% (0)	0% (0)	6
Total Respondents						49
(skipped this question)						2

8. Do you have any non-functioning sequencers that are less than 5 years old? (Non-functioning sequencers have had chemicals removed and are no longer used.)			
		Response Percent	Response Total
yes		4.3%	2
no		95.7%	44
Total Respondents			46
(skipped this question)			5

9. If you have permanently shut down and no longer use any sequencers in your lab within the last 3 years, indicate the reasons below. Check all that apply.			
		Response Percent	Response Total
Have not shutdown any sequencers		66.7%	24
Sequencer too old to operate reliably		11.1%	4
Needs repair but lack replacement parts		2.8%	1
Lack of skilled personnel to operate		2.8%	1
Lack of sample requests		11.1%	4
Too expensive to operate		5.6%	2
Other (please specify)		11.1%	4
Total Respondents			36
(skipped this question)			15

10. Would you anticipate purchasing a new Edman chemical protein sequencer with in the next 3-5 years?			
		Response Percent	Response Total
yes		36.7%	18
no		63.3%	31
Total Respondents			49
(skipped this question)			2

11. If you answered yes what is the most likely reason? Check all that apply			
		Response Percent	Response Total
Handle increased sample demands		38.9%	7
Replace existing sequencer		72.2%	13
Other (please specify)		11.1%	2
Total Respondents			18
(skipped this question)			33

12. Which describes your sequencer chemical and supply purchases. Select all that apply.			
		Response Percent	Response Total
Purchase all chemicals from sequencer manufacturer		59.2%	29
Purchase all chemicals from another sequencer manufacturer		0%	0
Purchase some chemicals from any sequencer manufacturer and some from other sources		34.7%	17
Purchase and prepare all chemicals from sources other than a sequencer manufacturer		6.1%	3
Purchase HPLC columns from sequencer manufacturer		51%	25
Purchase HPLC columns from other sources		22.4%	11
Other (please specify)		8.2%	4
Total Respondents			49
(skipped this question)			2

13. If you purchase reagents from other sources indicate all reasons below:			
		Response Percent	Response Total
Chemicals no longer available from instrument manufacturer		19%	4
Less expensive from other sources		71.4%	15
Better or more consistent quality from other sources		23.8%	5
Other (please specify)		9.5%	2
Total Respondents			21
(skipped this question)			30

14. Which describes your sequencer maintenance and repair habits? Select all that apply.			
		Response Percent	Response Total
Purchase service contracts for total coverage		36.7%	18
Partial coverage contracts		18.4%	9
No service contract, call service when needed		18.4%	9
Use tech support or service engineer to help troubleshoot and make repairs on your own		32.7%	16
Comfortable enough to troubleshoot and make most repairs without company assistance		38.8%	19
Other (please specify)		8.2%	4
Total Respondents			49
(skipped this question)			2

15. How many individuals in your lab perform any aspect of Edman sequencing; sequencer operation, data analysis, maintenance, etc...			
		Response Percent	Response Total
1		61.2%	30
2		30.6%	15
3		2%	1
4		6.1%	3
more than 4		0%	0
Total Respondents			49
(skipped this question)			2

16. Has the number of individuals performing Edman sequencing in your group increased, decreased, or stayed the same over the last 3 years			
		Response Percent	Response Total
increased		14.3%	7
decreased		8.2%	4
stay the same		77.6%	38
Total Respondents			49
(skipped this question)			2

17. For each individual in your lab that devotes any effort to doing Edman sequencing, specify their years of experience.					
	0-5 years	5-10 years	10-15 years	15 or more years	Response Total
Individual #1	14% (7)	24% (12)	18% (9)	43% (21)	49
Individual #2	53% (10)	21% (4)	5% (1)	21% (4)	19
Individual #3	33% (1)	33% (1)	33% (1)	0% (0)	3
Individual #4	33% (1)	0% (0)	33% (1)	33% (1)	3
more than 4 individuals	0% (0)	0% (0)	0% (0)	0% (0)	0
Total Respondents					49
(skipped this question)					2

18. What percent effort of the individuals total responsibility in question 17 is dedicated to Edman sequencing					
	0-25%	25-50%	50-75%	75-100%	Response Total
Individual #1	53% (26)	20% (10)	14% (7)	12% (6)	49
Individual #2	74% (14)	11% (2)	16% (3)	0% (0)	19
Individual #3	33% (1)	33% (1)	33% (1)	0% (0)	3
Individual #4	33% (1)	67% (2)	0% (0)	0% (0)	3
Total Respondents					49
(skipped this question)					2

19. Has the responsibility for Edman sequencing of these individuals increased, decreased, or stayed the same over the past 3 years				
	increased	decreased	stay the same	Response Total
Individual #1	12% (6)	24% (12)	63% (31)	49
Individual #2	37% (7)	16% (3)	47% (9)	19
Individual #3	0% (0)	33% (1)	67% (2)	3
Individual #4	0% (0)	33% (1)	67% (2)	3
Total Respondents				49
(skipped this question)				2

20. On average, how many samples does your lab sequence per month?			
		Response Percent	Response Total
1-5		15.9%	7
5-10		27.3%	12
10-15		20.5%	9
15-20		9.1%	4
20-30		6.8%	3
more than 30		20.5%	9
Total Respondents			44
(skipped this question)			7

21. Over the past 3 years, has the number of samples increased, decreased, or stayed the same?			
		Response Percent	Response Total
increased		22.7%	10
decreased		43.2%	19
stayed the same		34.1%	15
Total Respondents			44
(skipped this question)			7

22. Over the next 3 years do you anticipate the number of samples increasing, decreasing, or staying the same			
		Response Percent	Response Total
increasing		20%	9
decreasing		11.1%	5
staying the same		51.1%	23
cannot predict		17.8%	8
Total Respondents			45
(skipped this question)			6

23. Indicate what percentage of your samples have been run for the following number of cycles over the past year.							
	0%	1-20%	21-40%	41-60%	61-80%	81-100%	Response Total
1 to 10 cycles	3% (1)	11% (4)	13% (5)	26% (10)	32% (12)	16% (6)	38
10-20 cycles	0% (0)	48% (21)	23% (10)	11% (5)	11% (5)	7% (3)	44
20-30 cycles	12% (4)	79% (26)	6% (2)	0% (0)	3% (1)	0% (0)	33
30-40 cycles	39% (11)	57% (16)	4% (1)	0% (0)	0% (0)	0% (0)	28
40-50 cycles	67% (16)	33% (8)	0% (0)	0% (0)	0% (0)	0% (0)	24
more than 50	90% (18)	10% (2)	0% (0)	0% (0)	0% (0)	0% (0)	20
Total Respondents							44
(skipped this question)							7

24. Indicate the approximate percentage of the types of samples on which your lab performs Edman sequencing.							
	0%	1-20%	21-40%	41-60%	61-80%	81-100%	Response Total
Determine N-terminus from a cleavage or processing event	2% (1)	50% (21)	26% (11)	14% (6)	7% (3)	0% (0)	42
Determine N-terminus for quality control or conformation of recombinant proteins	2% (1)	55% (22)	20% (8)	20% (8)	2% (1)	0% (0)	40
Quality control of synthetic peptides	26% (9)	71% (24)	3% (1)	0% (0)	0% (0)	0% (0)	34
Denovo sequencing of proteins with undefined genomes	14% (5)	70% (26)	11% (4)	3% (1)	3% (1)	0% (0)	37
Protein identification of gel bands or spots	5% (2)	50% (19)	32% (12)	13% (5)	0% (0)	0% (0)	38
Need entire protein/peptide sequence	58% (19)	36% (12)	3% (1)	0% (0)	3% (1)	0% (0)	33
Radioisotope labeled sequencing; eg P32-labeled phosphorylation site determination	73% (24)	27% (9)	0% (0)	0% (0)	0% (0)	0% (0)	33
Core users prefer Edman sequence data	46% (13)	36% (10)	11% (3)	4% (1)	0% (0)	4% (1)	28
Other	62% (8)	31% (4)	0% (0)	8% (1)	0% (0)	0% (0)	13
Total Respondents							44
(skipped this question)							7

25. In the past three years would you say that the demand for these types of requests has increased, decreased, or stayed the same.				
	Increased	Decreased	Stayed the same	Response Total
Determine N-terminus from a cleavage or processing event	34% (15)	14% (6)	52% (23)	44
Determine N-terminus for quality control or conformation of recombinant proteins	22% (9)	22% (9)	56% (23)	41
Quality control of synthetic peptides	6% (2)	31% (11)	63% (22)	35
Denovo sequencing of proteins with undefined genomes	11% (4)	43% (16)	46% (17)	37
Protein identification of gel bands or spots	24% (9)	37% (14)	39% (15)	38
Need entire protein/peptide sequence	14% (5)	31% (11)	54% (19)	35
Radioisotope labeled sequencing; eg P32-labeled phosphorylation site determination	6% (2)	44% (15)	50% (17)	34
Core users prefer Edman sequence data	4% (1)	21% (6)	75% (21)	28
Other	0% (0)	0% (0)	100% (7)	7
Total Respondents				45
(skipped this question)				6

26. If you use Edman sequencing for identification of 1D gel bands or 2D spots, what percent of the identifications turn out to be proteins with known sequences found in a database.			
		Response Percent	Response Total
0-25%		4.5%	2
25-50%		13.6%	6
50-75%		13.6%	6
75-100%		54.5%	24
Do not use Edman sequencing for protein identifications of gel bands or spots		13.6%	6
Total Respondents			44
(skipped this question)			7

27. Does your lab also perform mass spectrometry based protein analysis?			
		Response Percent	Response Total
Yes		80%	36
No		20%	9
Total Respondents			45
(skipped this question)			6

28. Sometime within the next 3 years, are you planning on purchasing a mass spectrometer to perform some of the protein analysis that is currently being done with Edman sequencing?			
		Response Percent	Response Total
Yes		31.1%	14
No		68.9%	31
Total Respondents			45
(skipped this question)			6

29. If you are planning on purchasing a mass spectrometer for protein work, will this eliminate the need for Edman sequencing in your lab?			
		Response Percent	Response Total
Yes		0%	0
No		54.8%	23
Not purchasing a mass spectrometer		45.2%	19
Total Respondents			42
(skipped this question)			8

30. Are there other laboratories doing mass spectrometry based protein analysis at your institution where you or users of your facility can have samples analyzed?			
		Response Percent	Response Total
Yes		62.2%	28
No		37.8%	17
Total Respondents			45
(skipped this question)			6

31. Which, if any, of the following mass spectrometry based protein analysis techniques does your lab perform; check all that apply			
		Response Percent	Response Total
Do not perform mass spectrometry protein analysis		18.6%	8
MALDI-TOF peptide mass fingerprinting protein id		55.8%	24
MALDI-TOF protein/peptide mass analysis		72.1%	31
ESI-MS mass analysis		65.1%	28
ESI-tandem mass spectrometry protein identification		51.2%	22
MALDI-tandem mass spectrometry protein id		25.6%	11
FT-ICR accurate mass determination		4.7%	2
Other (please specify)		4.7%	2
Total Respondents			43
(skipped this question)			8

32. Are you or anyone in your laboratory members of the Association of Biomolecular Resource Facilities (ABRF)?			
		Response Percent	Response Total
Yes		80%	36
No		20%	9
Total Respondents			45
(skipped this question)			6

33. Has your laboratory participated in past ABRF Edman Sequencing Research Group or Sequencing Research Group studies			
		Response Percent	Response Total
Yes		86.7%	39
No		13.3%	6
Not aware of Edman Research Group studies		0%	0
Total Respondents			45
(skipped this question)			6

34. Are you or any members of your lab aware of the information available about Edman sequencing in the Research Group section of the ABRF website (www.ABRF.org)?			
		Response Percent	Response Total
Yes		95.6%	43
No		4.4%	2
Total Respondents			45
(skipped this question)			6

35. What kinds of ESGR study samples would you be interested in running and what information regarding Edman sequencing would you find useful to have posted on the ESGR website.	
Total Respondents	15
(skipped this question)	36

Survey Comments:

2. What best describes your laboratory setting?

- a) For profit / Chemical Company

3. What best describes your laboratory function?

- a) Both facility and research lab
- b) Project development
- c) Analytical Development

5. What other services are offered or techniques performed in your laboratory? Check all that apply

- a) Secondary structure analysis by circular dichroism measurements, Fluorescence measurements
- b) Capillary electrophoresis, gc/ms
- c) CD
- d) DIGE and auto pickers, digestors and spotters are in the pipeline for early Jan 2005.
- e) SELDI
- f) Carbohydrate analysis Antibody production

9. If you have permanently shut down and no longer use any sequencers in your lab within the last 3 years, indicate the reasons below. Check all that apply.

- a) The non-functioning sequencer was donated to the lab at a time when an additional person was expected to be hired. That did not occur, and other demands left insufficient time to operate 2 sequencers.
- b) No longer supported by vendor
- c) Agilent has stopped supporting their protein sequencers.
- d) We were offered a trade-in discount

11. If you answered yes what is the most likely reason? Check all that apply

- a) Only if significantly improved
- b) HP G005 is obsolete with no support. Too unreliable to count on, yet steady if not increased sample demand.

12. Which describes your sequencer chemical and supply purchases. Select all that apply.

- a) Only PITC is purchased from a sequencer manufacturer.
- b) Purchase some chemicals from sequencer manufacturer and some from sources other than a sequencer manufacturer.
- c) Some homemade solutions and solvents.
- d) Purchase few chemicals from sequencer manufacturer and purchase and prepare all others from other sources.

13. If you purchase reagents from other sources indicate all reasons below:

- a) Adding additional chemical to improve the yield
- b) Make our own R2

14. Which describes your sequencer maintenance and repair habits? Select all that apply.

- a) I just dropped service coverage, too expensive!
- b) Make some repairs w/o company assistance
- c) Good thing considering obsolescence. Parts becoming short supply.
- d) Can make many repairs in-house - time permitting

31. Which, if any, of the following mass spectrometry based protein analysis techniques does your lab perform; check all that apply

- a) Nano-ESI de novo sequencing
- b) LC/M

35. What kinds of ESRG study samples would you be interested in running and what information regarding Edman sequencing would you find useful to have posted on the ESRG website.

- a) Obtaining sequence data from blocked proteins, identifying proteins when more than one sequence is present.
- b) What percentage of ABRF member need this study.
- c) Do sequence analysis of a mixture of 3 proteins that would need to be separated on a 1D gel, transfer, sequenced and identified.
- d) We would not be able to participate by running study samples -- sorry.
- e) Phosphorylated and glycosylated proteins/peptides
- f) In our lab Edman is most used to routinely check the N-term of transfections. If anything is odd about the data then Mass Spec is used to figure out what happened. It would be most useful to study simple competency of Edman Nts on regular proteins. A protein [with no tricks] that everyone should get like an antibody. How one would go about preparing the sample for sequencing to get the most info would be the most important aspect of the study. That is the real world of sequencing in our lab.
- g) (1) Low amount of protein sample (less than 2 pmol) (2) Entire protein with molecular Weight more than 50 KDa (3) Mixture of proteins. (4) very hydrophobic peptides. Troubleshooting are always useful.
- h) Anything realistic.
- i) How a Core Lab can avoid capping the Edman sequencer in future. Are there ways to keep the sequencers running, i.e. cost cutting or something like that.
- j) Sample with N-blocked group or posttranslational protein would be interested. currently, the experiences of sample deblocking treatment as well as the topic about alkylated cysteine's HPLC profile are extremely useful guide for me.
- k) It would be helpful to evaluate the yields of proteins applied to various brands of PVDF membranes, different stains, different post-transfer steps to determine the best transfer conditions.
- l) How about naturally occurring PTM's to add onto previous two year's studies. Quantitation of PTM. Example, naturally occurring histone with a free N-terminus.
- m) Recently there was a discussion on the ABRF list concerning severe Lag during Edman sequencing. It has been postulated that certain amino acids other than proline can be the cause. See the thread with the subject "Precise/Edman query" during November 2004. A synthetic peptide containing the appropriate amino acids or sequence motifs that are postulated to cause this lag might make for an interesting ESRG study.
- n) Low pmol samples
- o) Tips on unusual amino acid analysis.