



*Proteomics Research
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PRG2006 Research Study

Relative Quantification



<http://www.abrf.org/prg>



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Members



Arnold M. Falick - *UC Berkeley HHMI*

Jeffrey A. Kowalak - *NIMH LNT*

William S. Lane (**EB Liaison**) - *Harvard University*

Kathryn S. Lilley (*ad hoc*) - *University of Cambridge*

Brett S. Phinney - *UC Davis*

Chris W. Turck (**Chair**) - *Max Planck Institute of Psychiatry*

Susan T. Weintraub - *UT Health Science Center at San Antonio*

Ewa Witkowska - *UC San Francisco*

Nathan Yates - *Merck Research Laboratories*



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Past Research Studies



- PRG2002: Identification of Proteins in a Simple Mixture
 - Task: Identify components of 5 protein mixture.
- PRG2003: Phosphorylation Site Determination
 - Task: Identify 2 phosphopeptides and sites of phosphorylation.
- PRG2004: Differentiation of Protein Isoforms
 - Task: Discrimination of 3 closely related proteins.
- PRG2005: Sequencing Unknown Peptides
 - Task: *De novo* sequence analysis of 5 peptide mixture.



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Mission

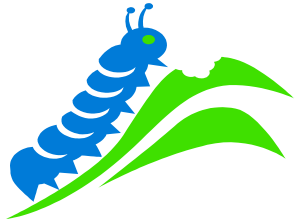


The mission of the Proteomics Research Group (PRG) is to assist ABRF members in evaluating their capabilities to identify "unknown" proteins in order to establish realistic expectations for this technology. New technologies related to identifying proteins or sites of modification will also be evaluated with the goal of both educating and introducing these technologies to the membership.



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Why a Quantitative Study?



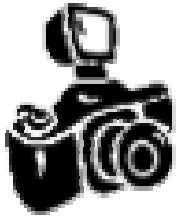
- Living systems are dynamic
- Importance of temporal change





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Why a Quantitative Study?



Systems Biology

- increasing requirement for quantitative data as an output for modeling
- ultimate goal: globally profile changes in protein abundances in biological systems



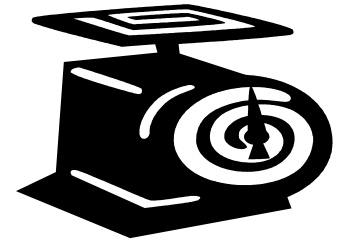


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Quantitative Proteomics

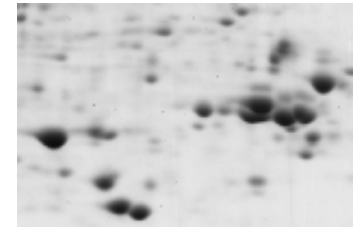
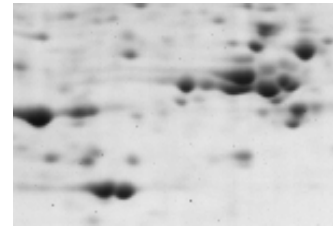


- Absolute amount of a protein in a sample
- Relative change in protein amount between two states



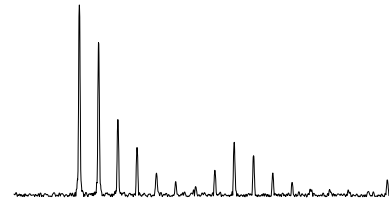


- 2D gel electrophoresis
 - staining intensity
 - fluorescence labeling



- Mass spectrometry

- extracted ion current



- stable isotope labeling (analog spiking, during enzyme digest, tag incorporation, metabolic labeling)



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PRG2006 Relative Quantification Study Objectives

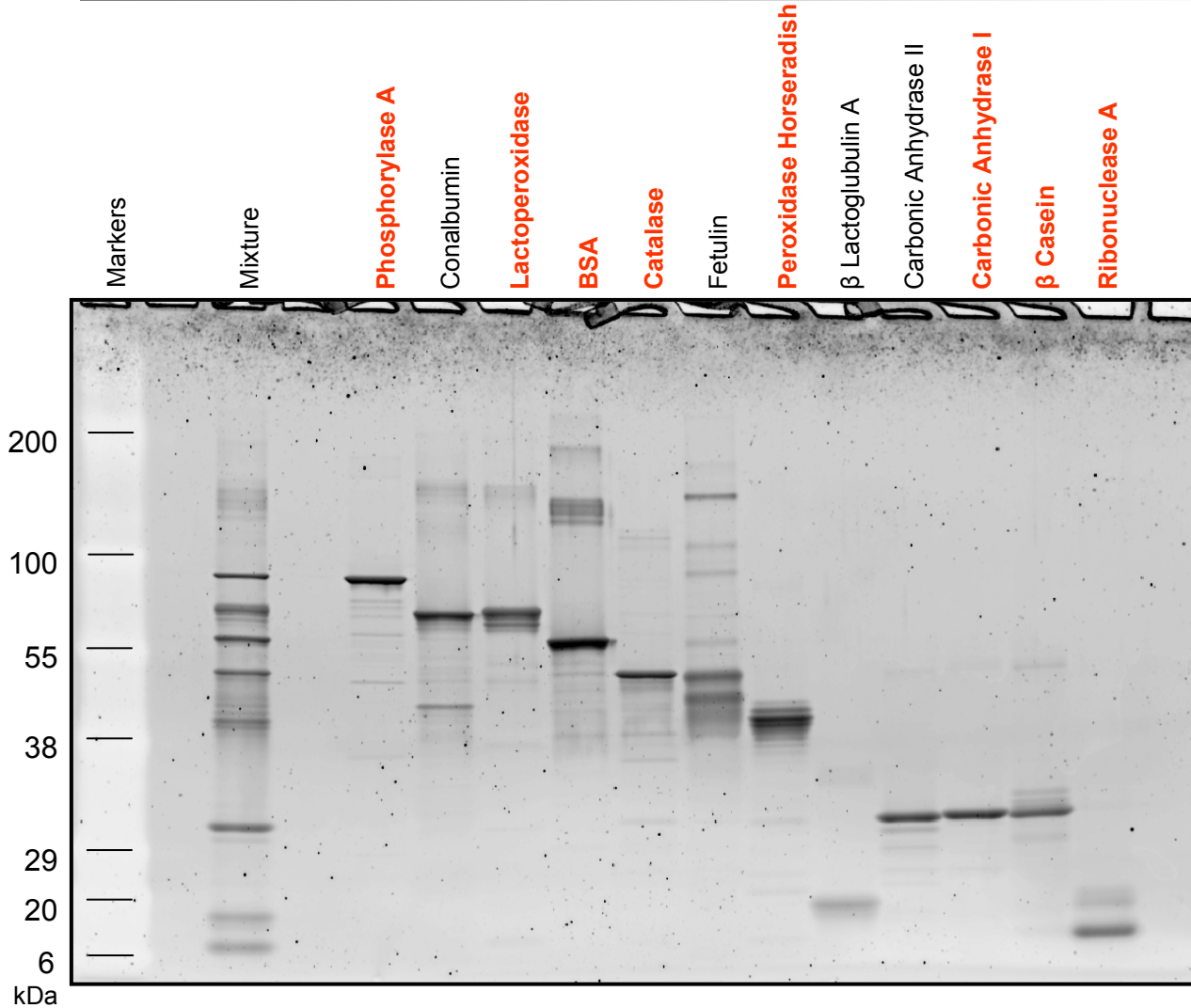


- What methods of relative quantification are used in the community?
- What is the success rate of the individual methods?
- How well established are quantitative methodologies in the community?
- What is the ability of software to determine quantitative differences across samples or help analyze data from quantitative experiments?
- What is the level of confidence and consistency in the quantification data achieved?
- What are the views of the participants in terms of the feasibility of relative protein quantification projects in core facilities?



PRG2006 Relative Quantification Study Sample

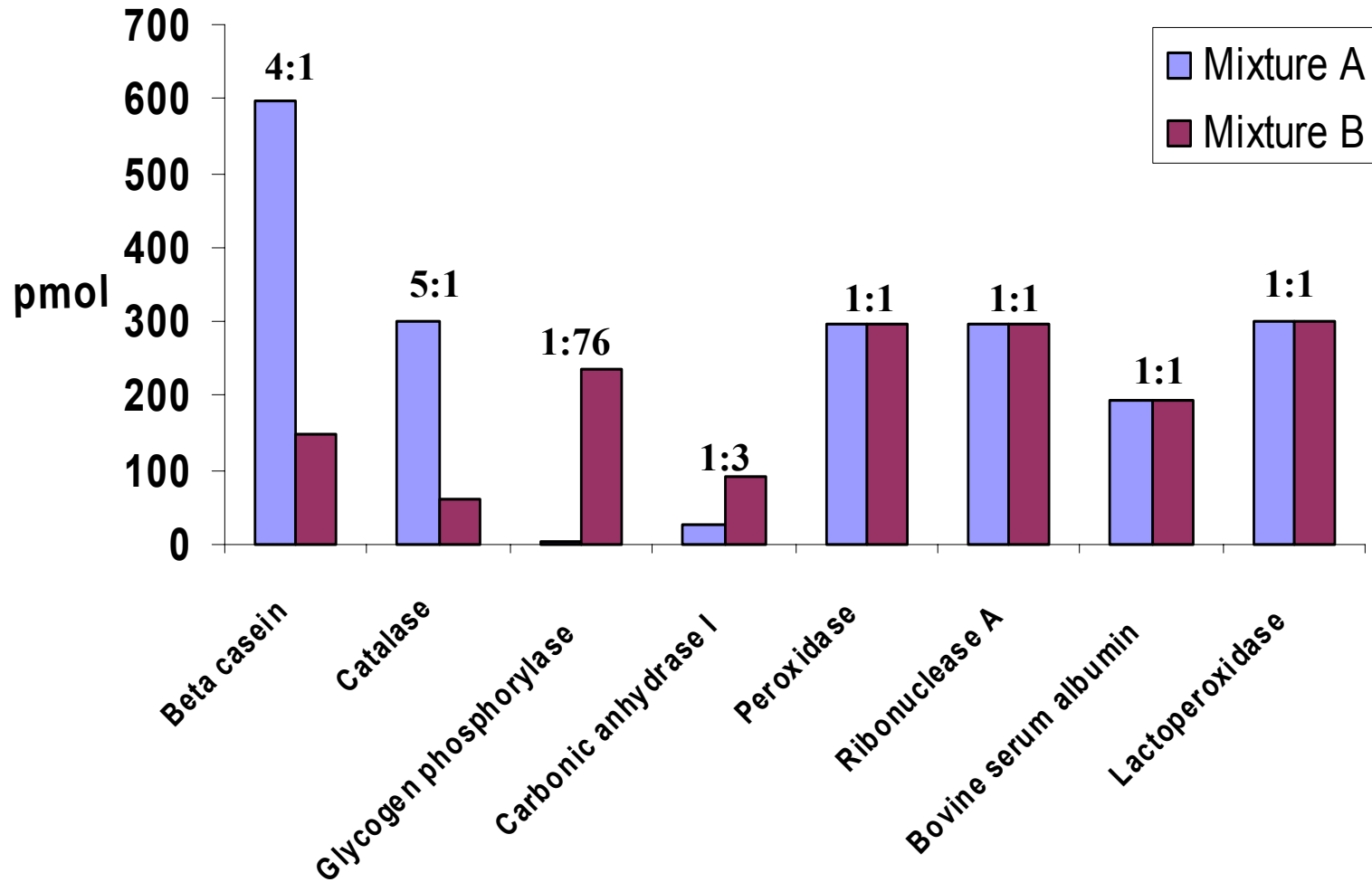
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PRG2006 Relative Quantification Study Sample

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Study Sample

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Protein	MW	pI	Mixture A	Mixture B	Mixture A	Mixture B	Ratio
			ug	ug	pmol	pmol	
Beta casein	23.4	5.0	14	3.5	598	150	4:1
Catalase bovine liver C 1345	57.5	6.4	17	3.4	299	59	5:1
Glycogen phosphorylase rabbit	97.1	6.8	0.3	22.8	3	228	1:76
Carbonic anhydrase I	28.9	6.6	0.8	2.4	28	84	1:3
Peroxidase horseradish P 6782	35.6	5.6	10.6	10.6	298	298	1:1
Ribonuclease A bovine R5500	13.5	5.8	4	4	296	296	1:1
Bovine serum albumin	66.3	5.6	12.9	12.9	195	195	1:1
Lactoperoxidase	80.4	8.4	24.1	24.1	300	300	1:1

TOTAL

83.7

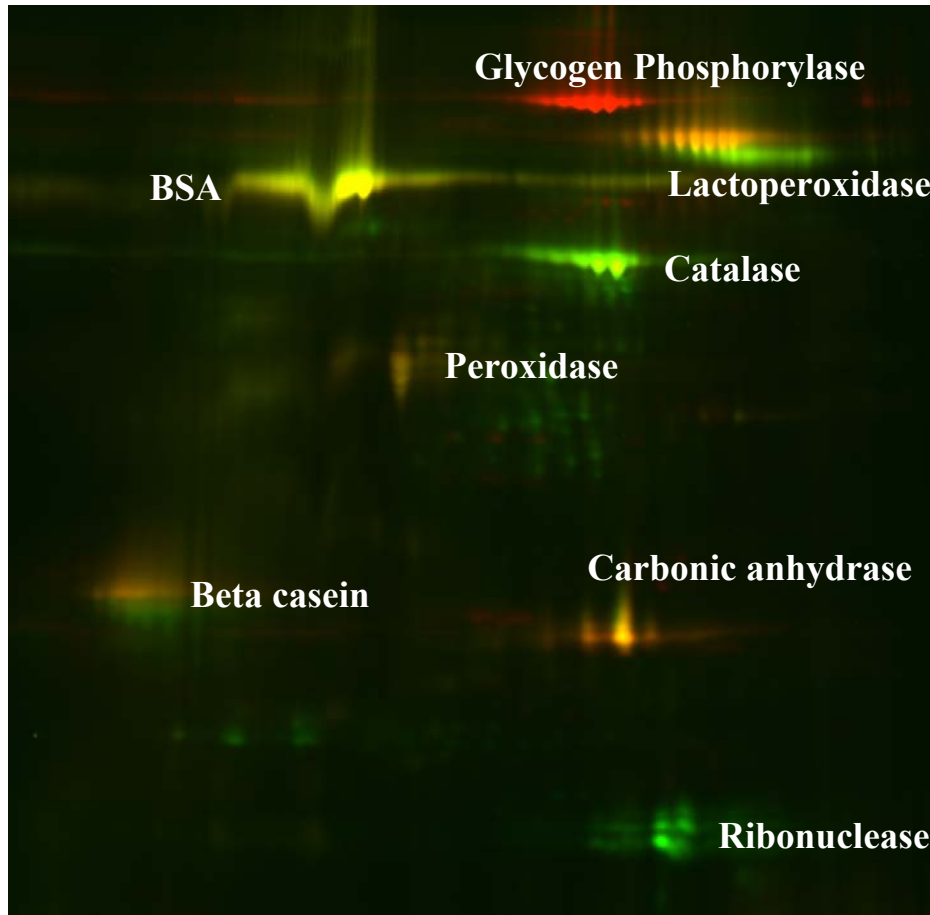
83.7



PRG2006 Relative Quantification

Study Sample

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Sample A = Cy3 (green)
Sample B = Cy5 (red)

3 → 10



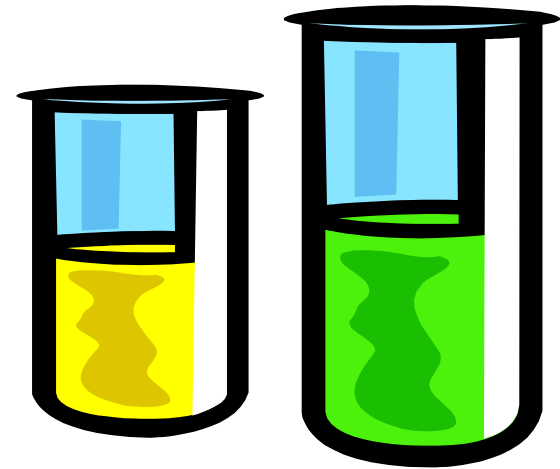
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Tasks



- Identify the components of the 8 protein mixture.
- Determine the relative amounts of the 8 proteins in two samples.





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Provided Sample Information



- Two mixtures, A and B.
- Both mixtures contain 8 major proteins each of various amounts.
- In some cases isoforms and/or contaminant(s) are present.
- The total amount of protein in each tube is approximately 80 μg .
- The individual proteins are present on average at 300 pmol, ranging from approximately 3 to 600 pmol.
- The ratios of proteins between mixtures A and B vary up to 1:100.
- Four of the eight proteins are present at a 1:1 ratio with bovine serum albumin being one of them.
- The proteins were mixed from aqueous solutions that also contained small amounts of salt and then dried.



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PRG2006 Relative Quantification Recommendations



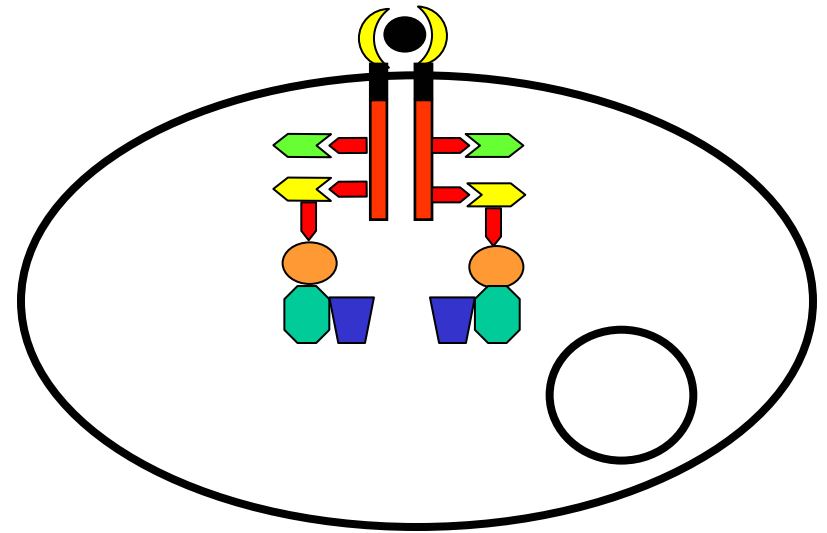
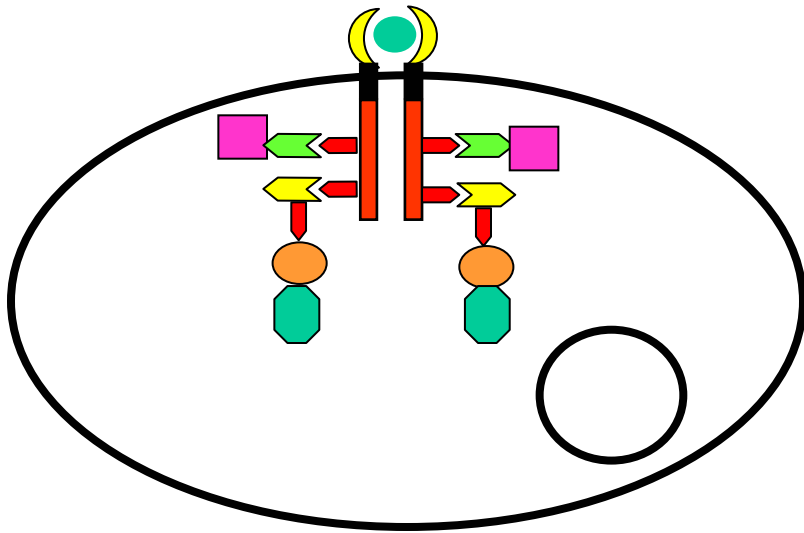
- Perform the analysis of the two mixtures more than once.
- Compare equal amounts of the same mixture (A or B).



PRG2006 Relative Quantification

Interaction Proteomics

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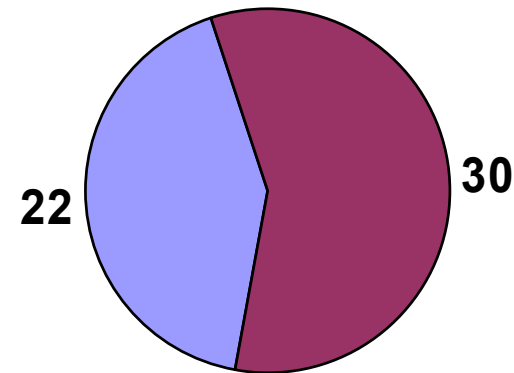
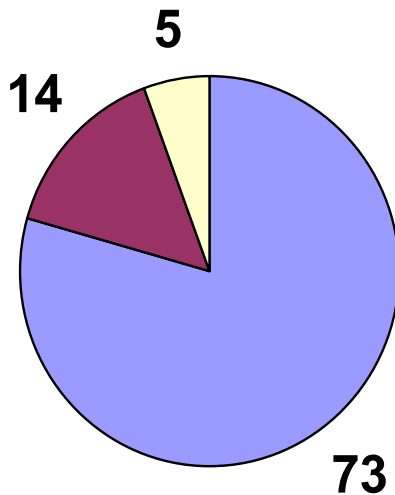




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Requesters/Participants

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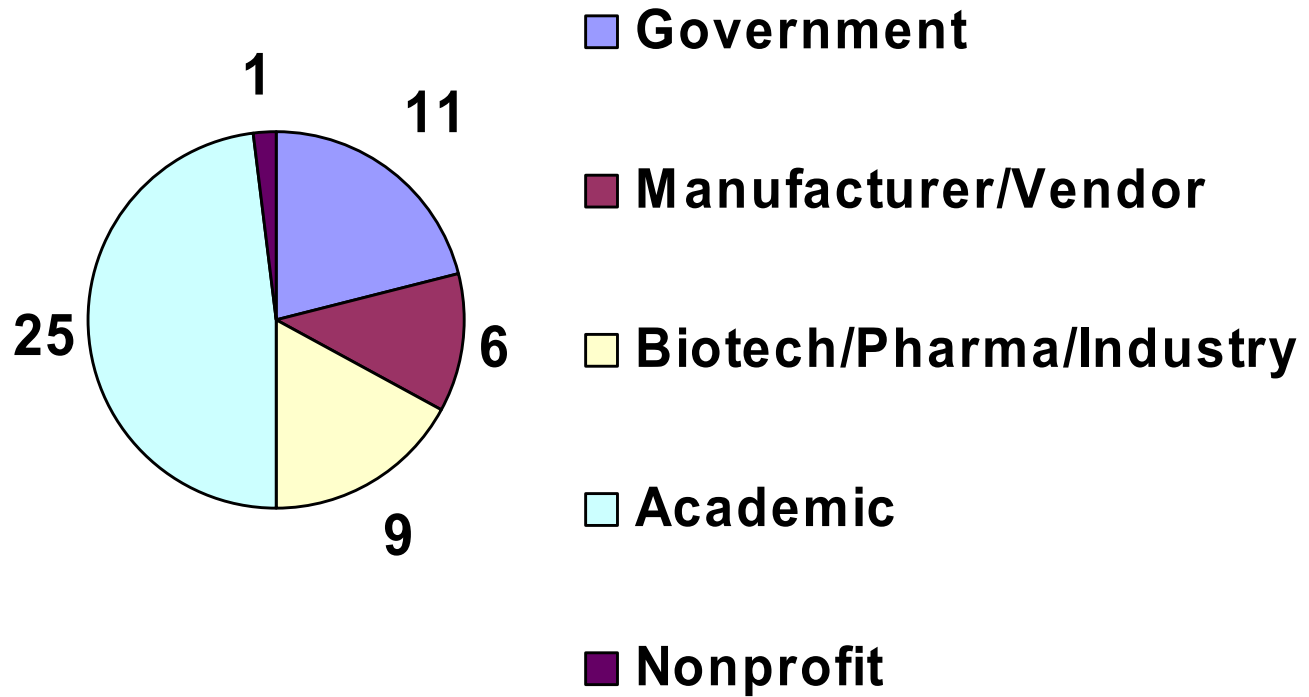
■ North America ■ Europe ■ Asia/Australia ■ Nonmember ■ ABRF Member

- 52 laboratories returned results (57% return rate !)



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PRG2006 Relative Quantification Participants





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Abbreviations

DIGE	Differential In-Gel Electrophoresis
ICPL	Isotope Coded Protein Label
iTRAQ	isobaric Tags for Relative and Absolute Quantitation
$^{16}\text{O}^{18}\text{O}$	Stable Oxygen Isotope Label



PRG2006 Relative Quantification

Methods Used

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Method of Quantitation	Number of Responses
------------------------	---------------------

1D Coomassie	4
2D Coomassie	4
2D silver	2
1D fluorescence	4
2D fluorescence	1
2D radioactivity	1
2D DIGE	3
Isotope ICPL	4
Isotope iTRAQ	12
Isotope O-methylisourea	1
Isotope $^{16}\text{O}^{18}\text{O}$	5
Spectral count	1
Ion current	10

36.5 %

63.5 %



PRG2006 Relative Quantification

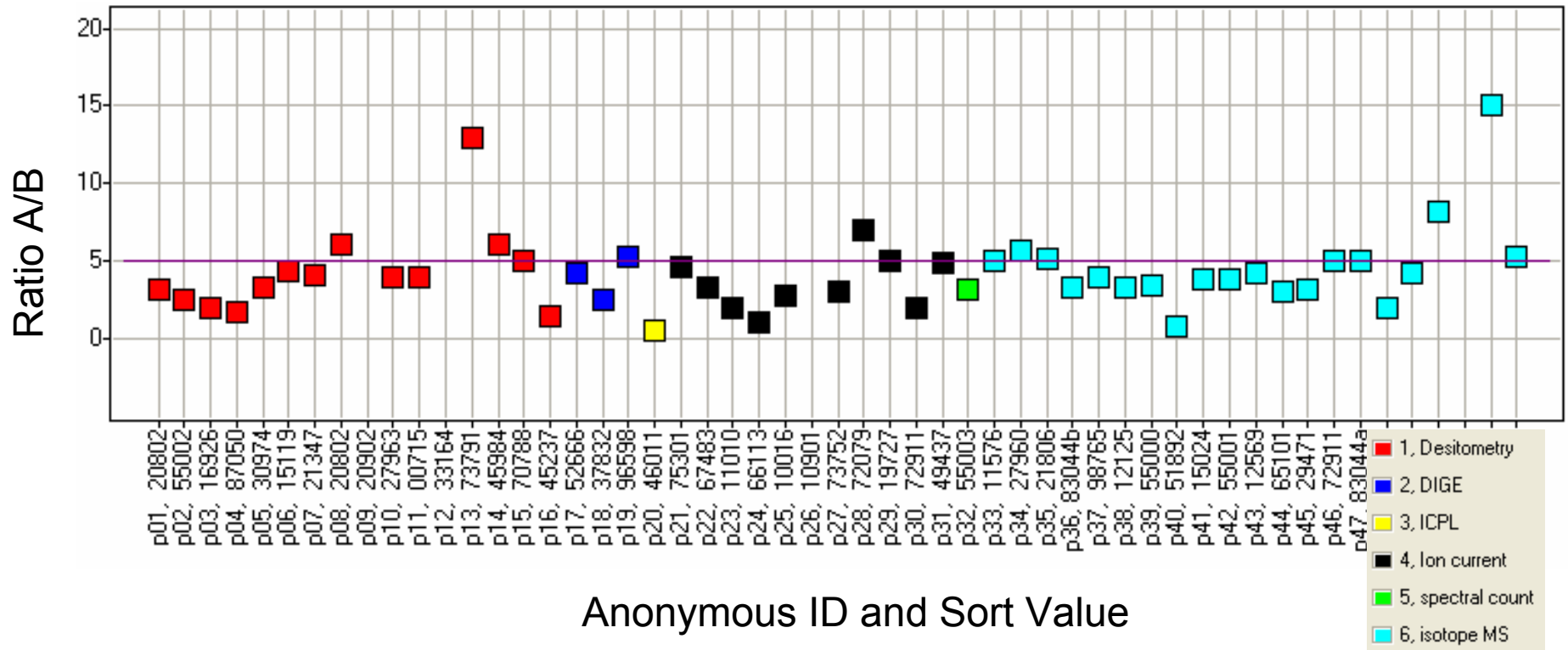
Reported Ratios – Catalase (5:1)

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Gel Methods

Gel Free Methods





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Data Evaluation

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$$[(\text{Expected Ratio} - \text{Observed Ratio}) / \text{Expected Ratio}] \times 100$$

=

“ % Error of Ratio ”

for example:

“Expected Ratio” = 5:1

“Observed Ratio” = 4:1 or 6:1

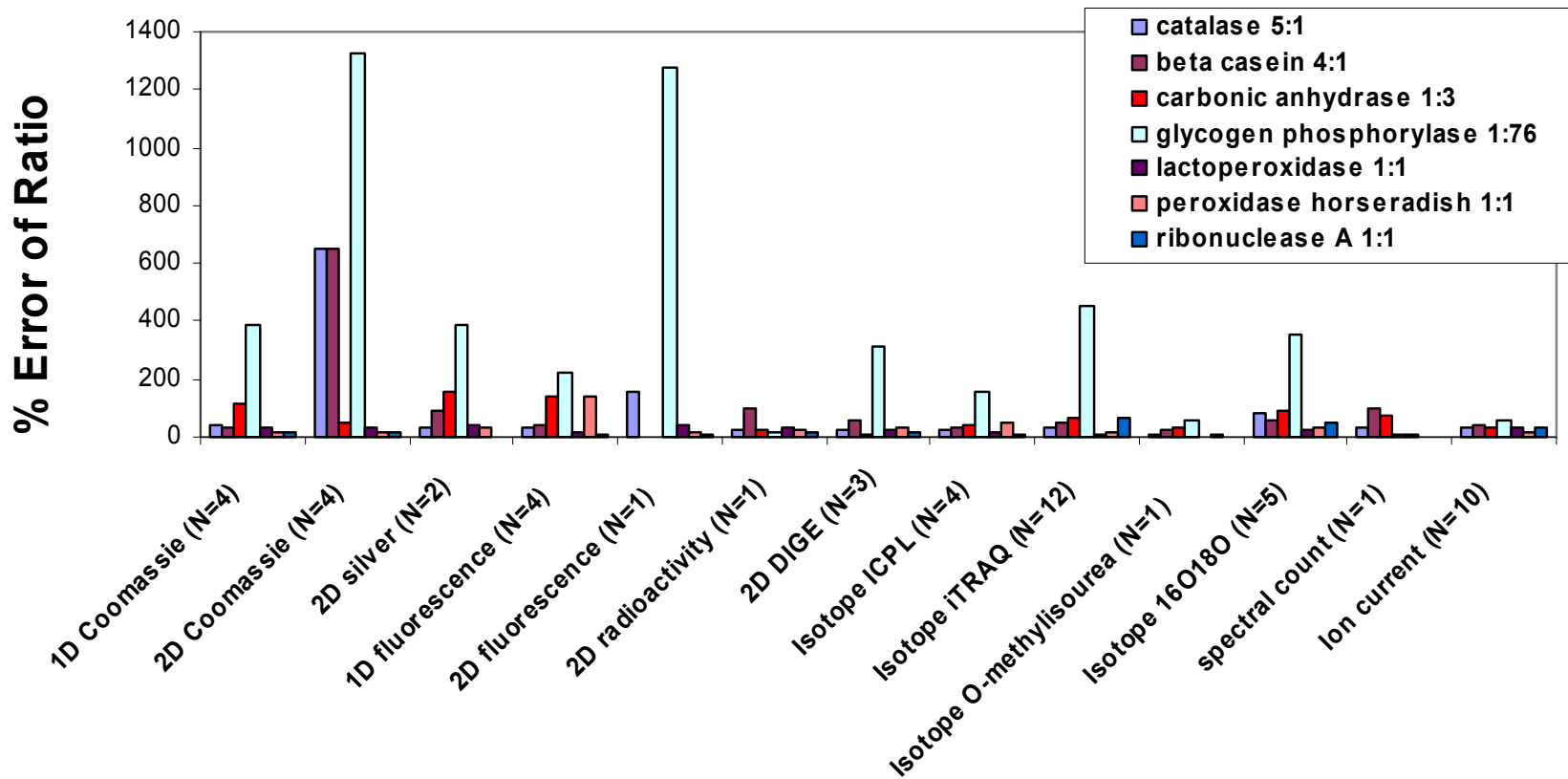
$$[(5 - 4) / 5] \text{ or } [(5 - 6) / 5] \times 100 = 20 \text{ “\% Error of Ratio”}$$



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Individual Methods - Individual Proteins (no BSA)

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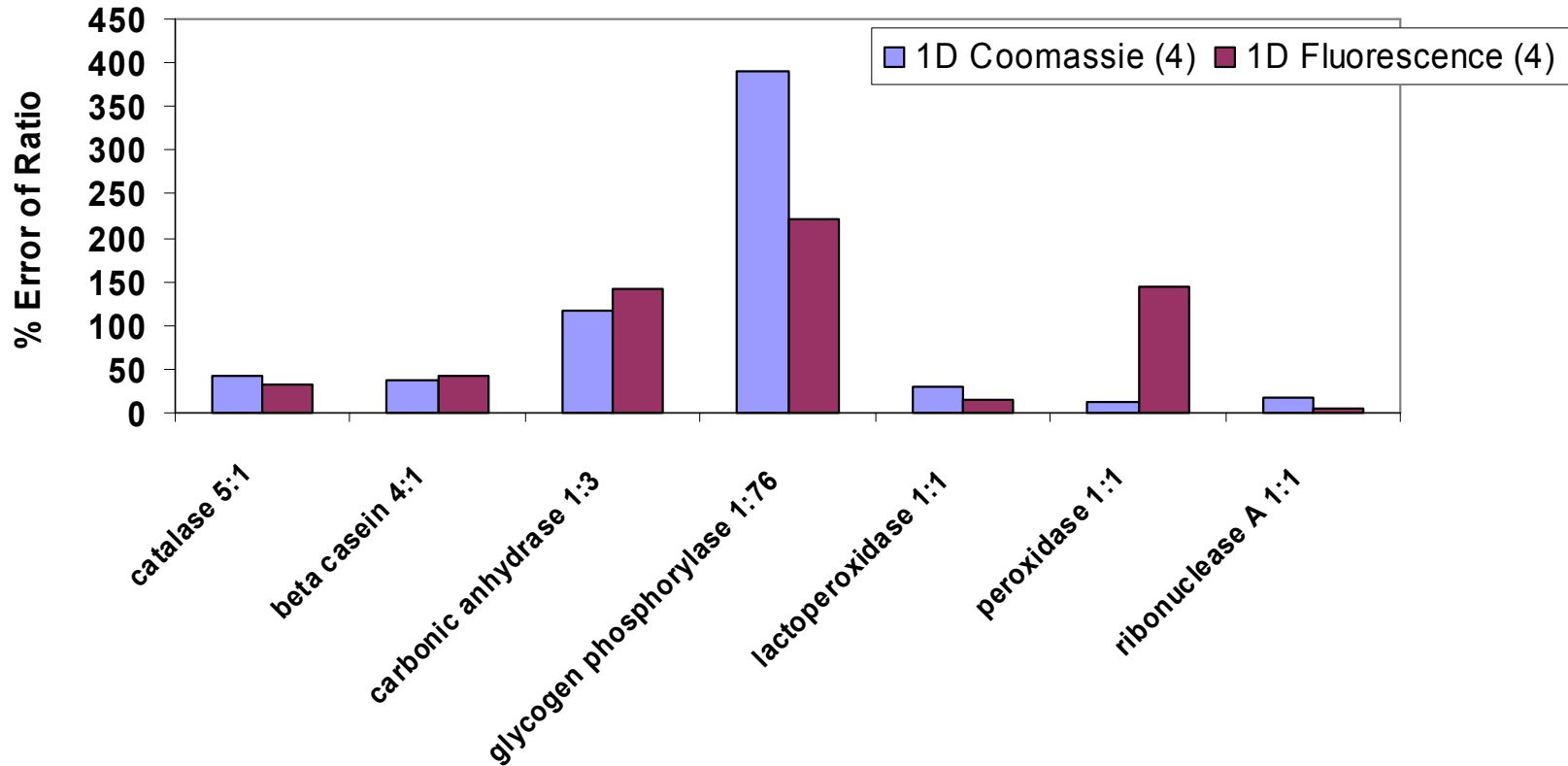




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Individual Proteins - 1 D Electrophoresis

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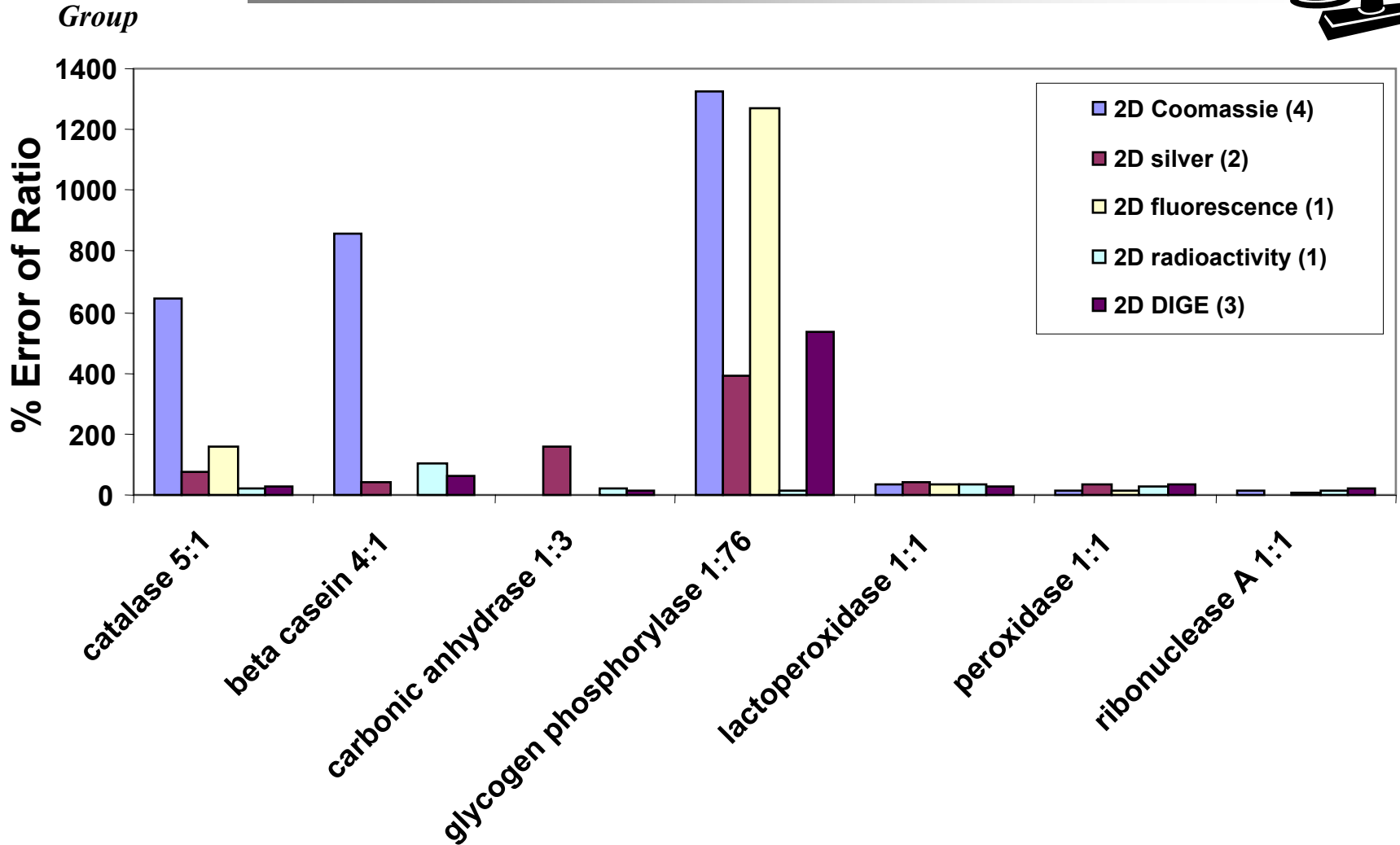




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Individual Proteins - 2 D Electrophoresis

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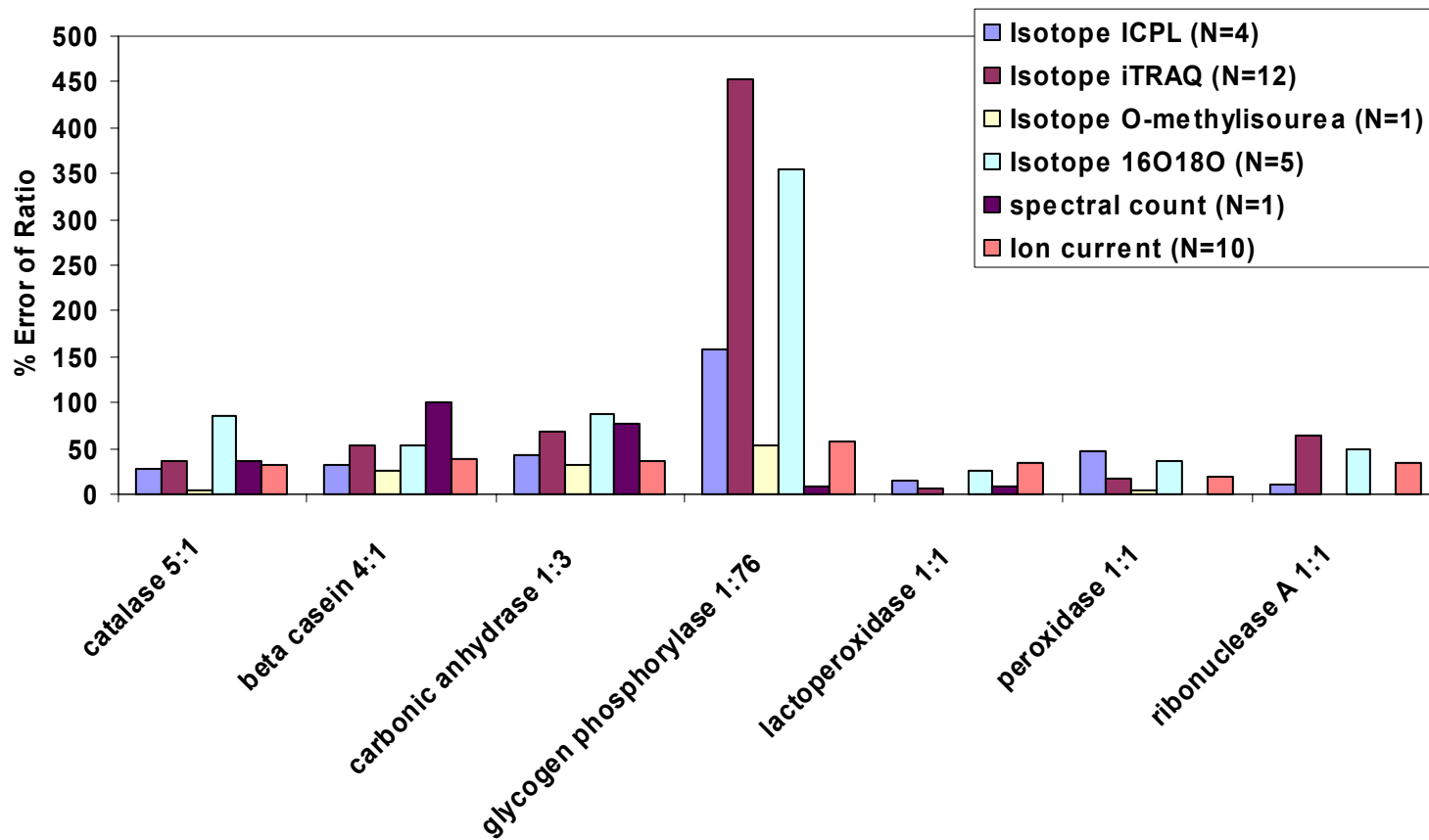




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Individual Proteins - Mass Spectrometry

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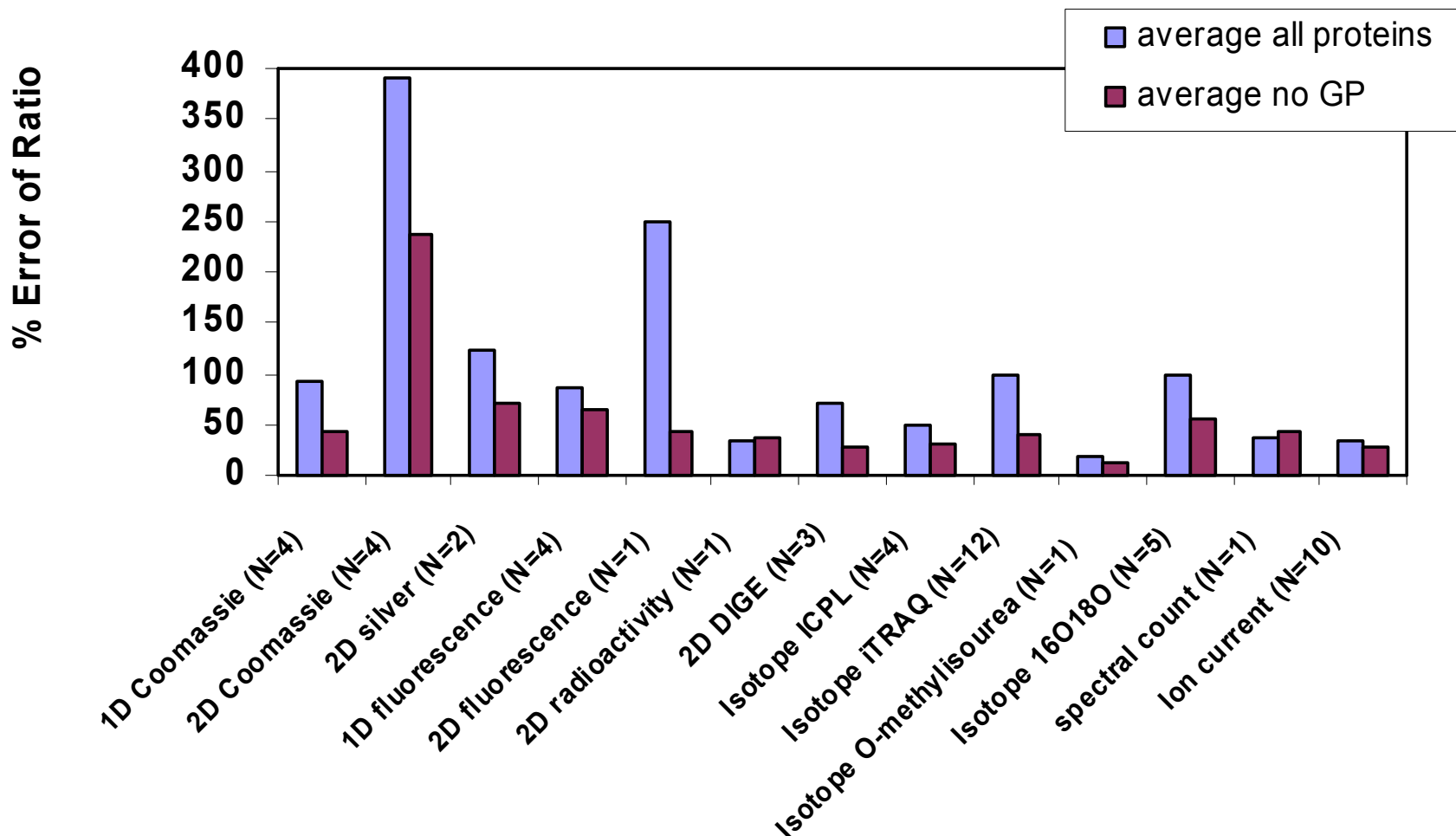




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Individual Methods - Protein Average

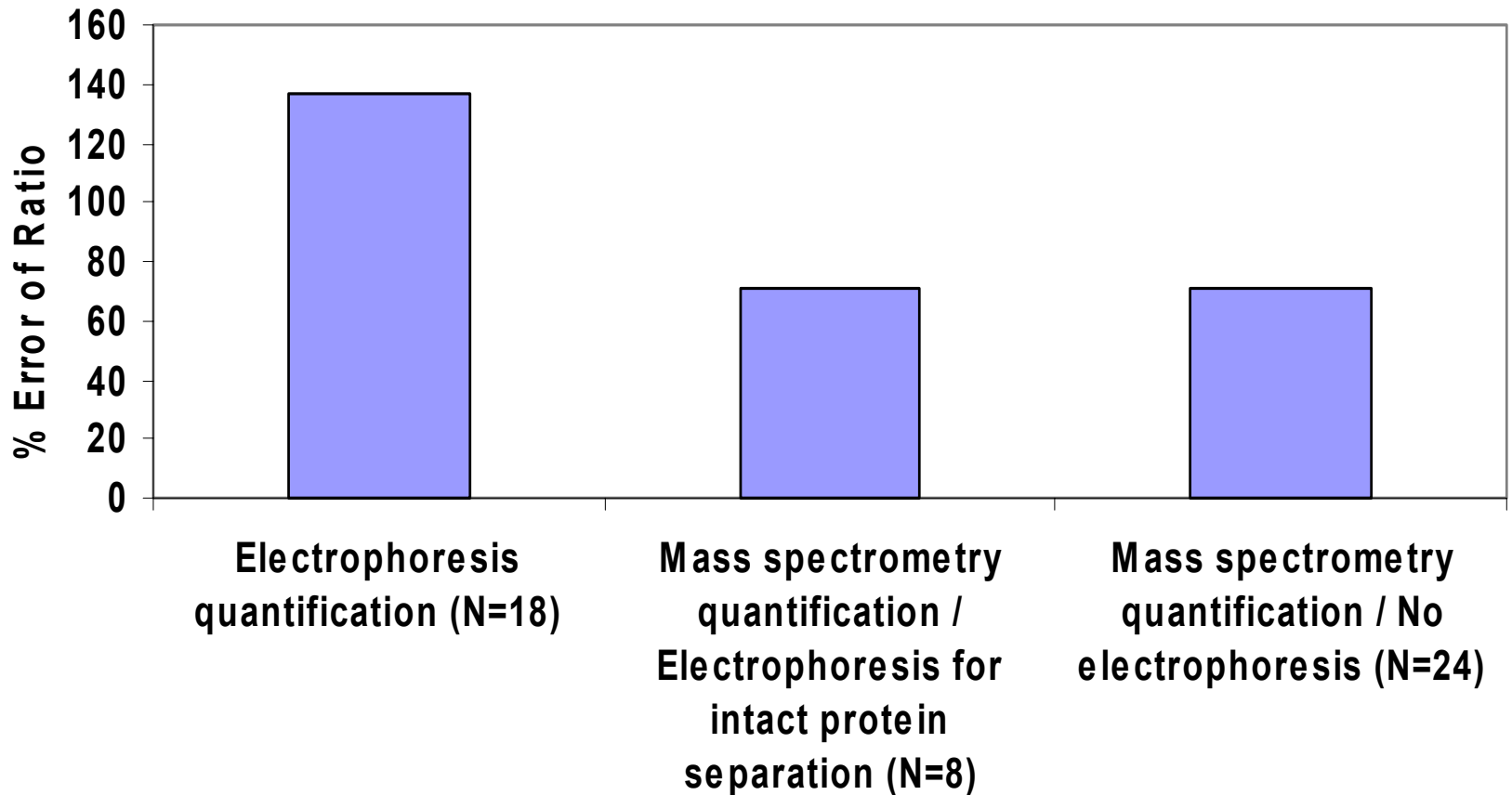
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PRG2006 Relative Quantification Use of Electrophoresis

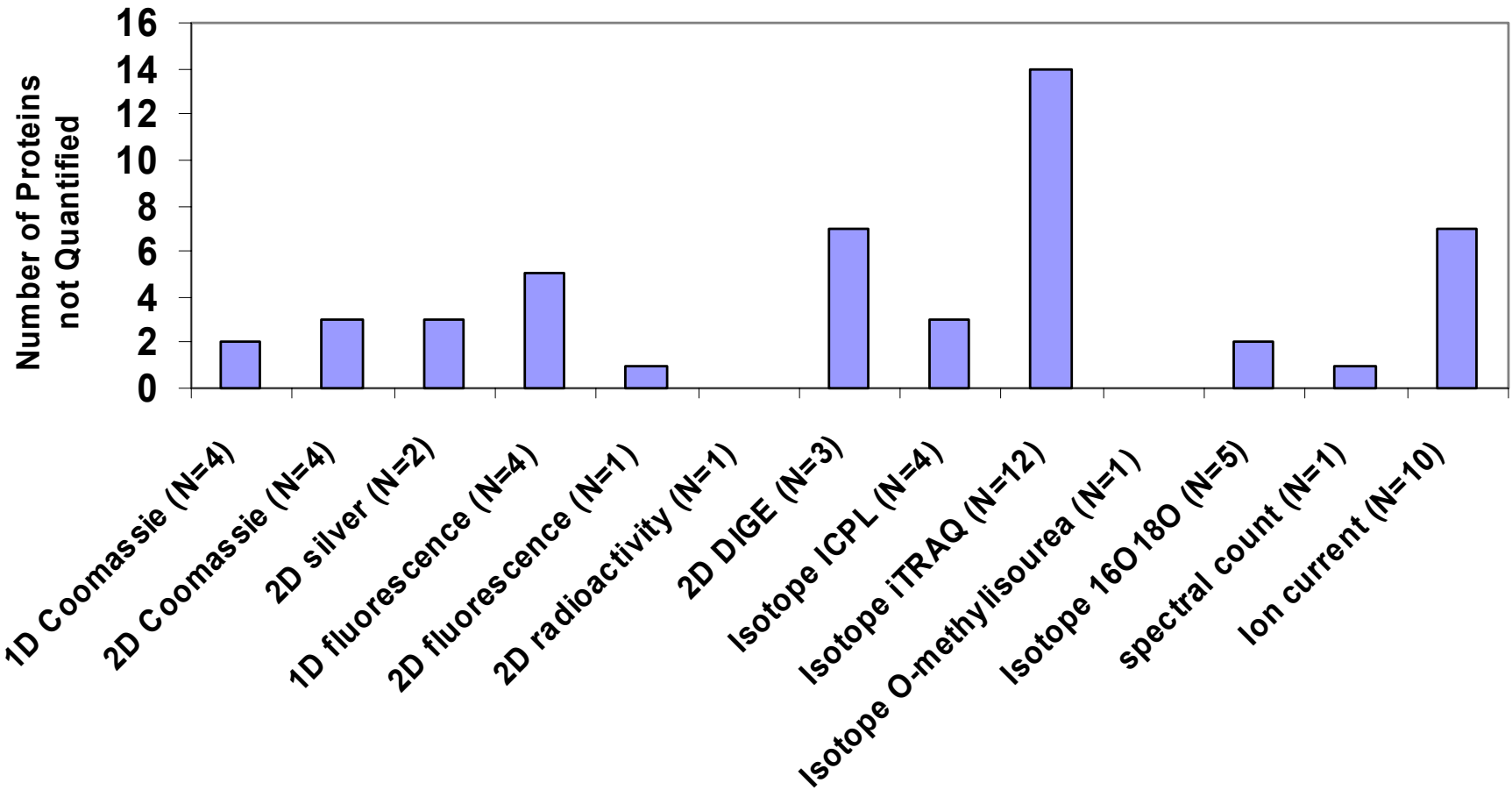




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Proteins not Quantified

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Experimental Schema

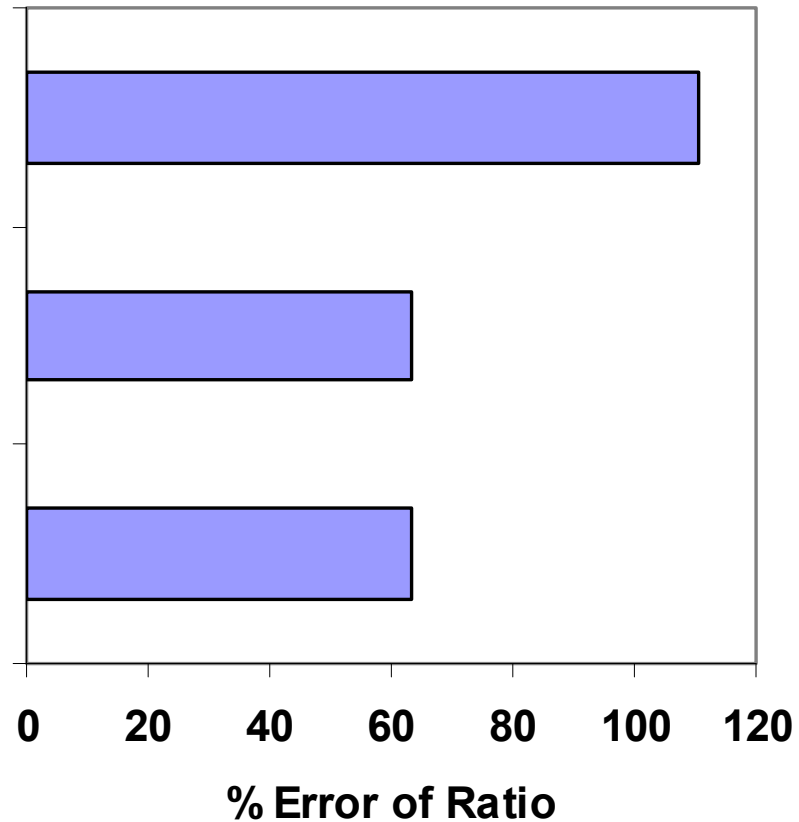
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**pairwise comparison of sample A
versus sample B (N=37)**

**multiplexed comparison of
replicates of samples A and B
(N=12)**

**other multiplexed comparisons
(N=3)**

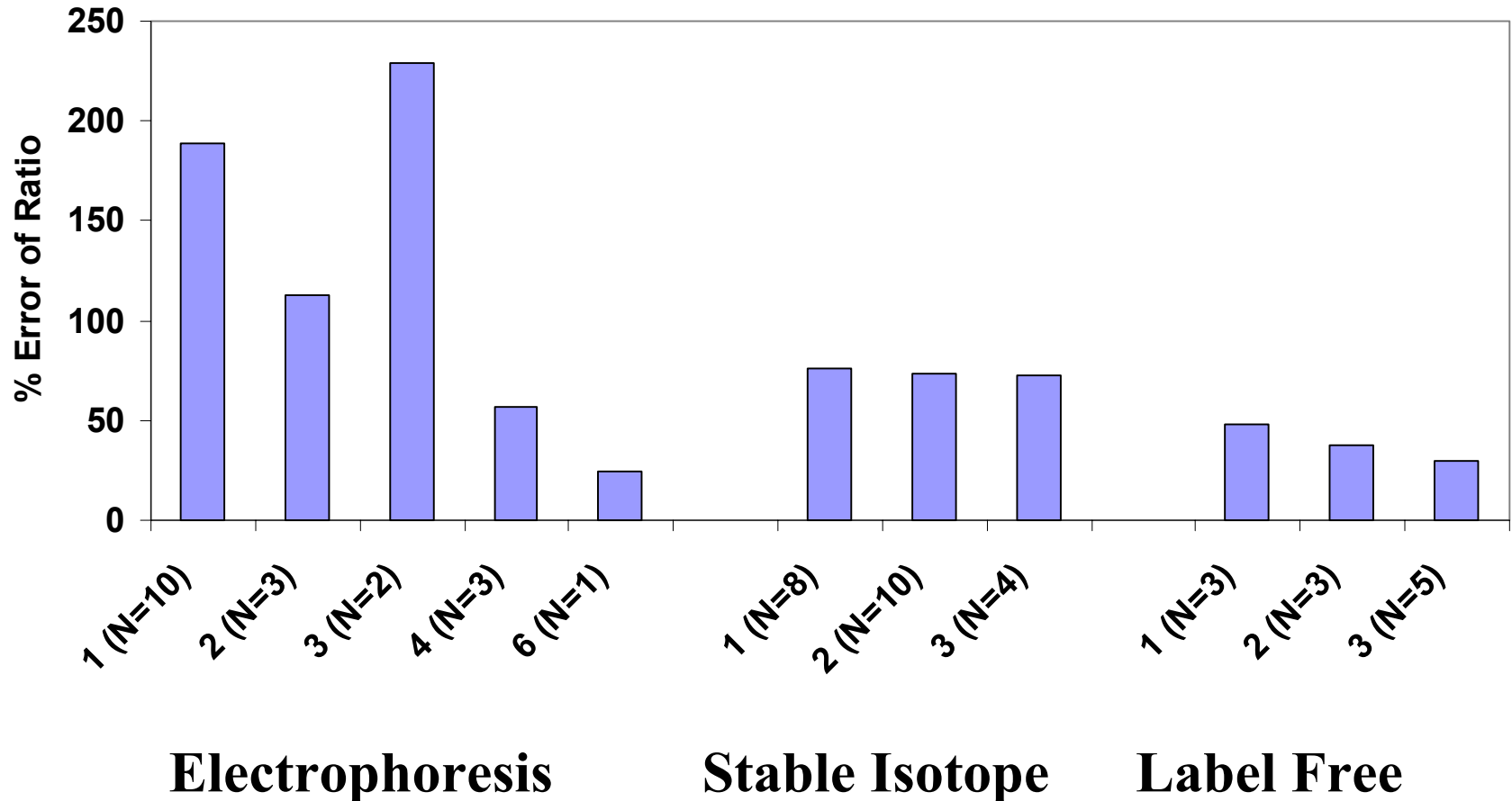




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Number of Analyses

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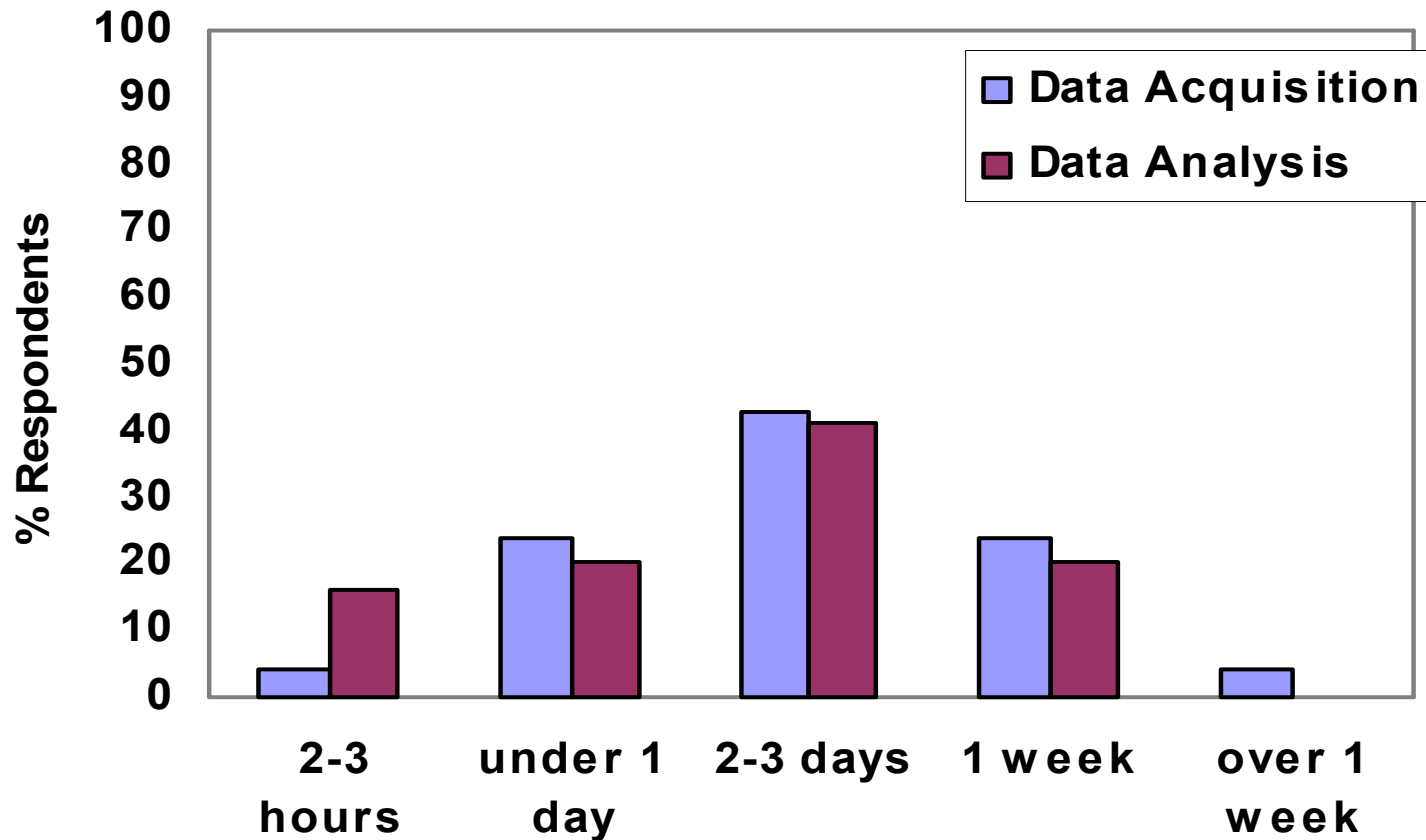




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Time for Data Acquisition and Analysis

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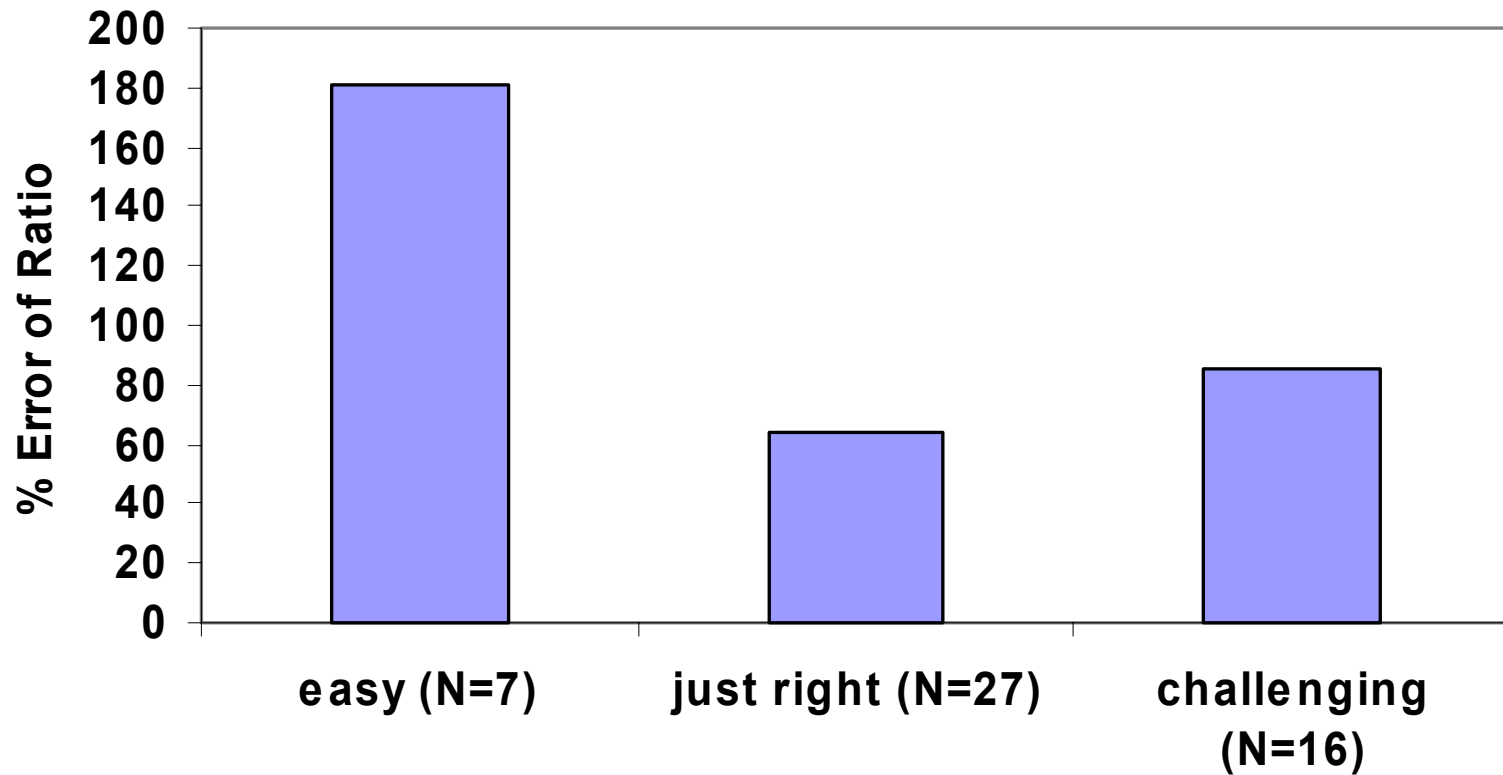




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Difficulty Rating

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Study Objectives

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- **What methods of relative quantification are used in the community?**
 - **1D/2D electrophoresis (N = 19)**
 - **Mass spectrometry, stable isotope labeling (N = 22)**
 - **Mass spectrometry, label free (N = 11)**



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Study Objectives



- **What is the success rate of the individual methods?**
 - **Most respondents were able to identify and report quantification data for all eight proteins.**
 - **For the majority of methods the greatest “% Error of ratio” was seen for glycogen phosphorylase (expected ratio of 1:76).**
 - **Mass spectrometry using ion current (N=10), spectral count (N=1) or isotope labeling with O-methylisourea (N=1) and 2D electrophoresis using radioactivity (N=1) yielded ratios closest to expected for glycogen phosphorylase.**



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Study Objectives



- **What is the success rate of the individual methods?**
 - **Overall, electrophoresis showed greater variability of “% Error of ratio” between proteins than mass spectrometry based methods.**
 - The results obtained for electrophoresis were closer to the expected values for proteins present at a 1:1 ratio than for proteins present at other ratios.
 - **For electrophoresis, the lowest “% Error of ratio” was seen for 2D DIGE (N=3) and 2D radioactivity (N=1) while 2D Coomassie (N=4) and 2D fluorescence (N=1) exhibited a relatively high “% Error of ratio”.**



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Study Objectives



- **What is the success rate of the individual methods?**
 - **Overall, mass spectrometry did better than electrophoresis.**
 - **Mass spectrometry using ion current (N=10) or spectral count (N=1) did as well or better than stable isotope labeling (N=22).**
 - **When mass spectrometry with stable isotope labeling was used, the “% Error of ratio” was evenly distributed for all proteins except glycogen phosphorylase.**
 - **When mass spectrometry with ion current or spectral count was used, the “% Error of ratio” was evenly distributed for all proteins.**



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Study Objectives



- **What is the success rate of the individual methods?**
 - **Quantification by mass spectrometry was not affected by prior separation of intact proteins by electrophoresis.**
 - **Repetition of an analysis yielded results that were closer to expected compared to analyses performed only once (particularly for electrophoresis).**



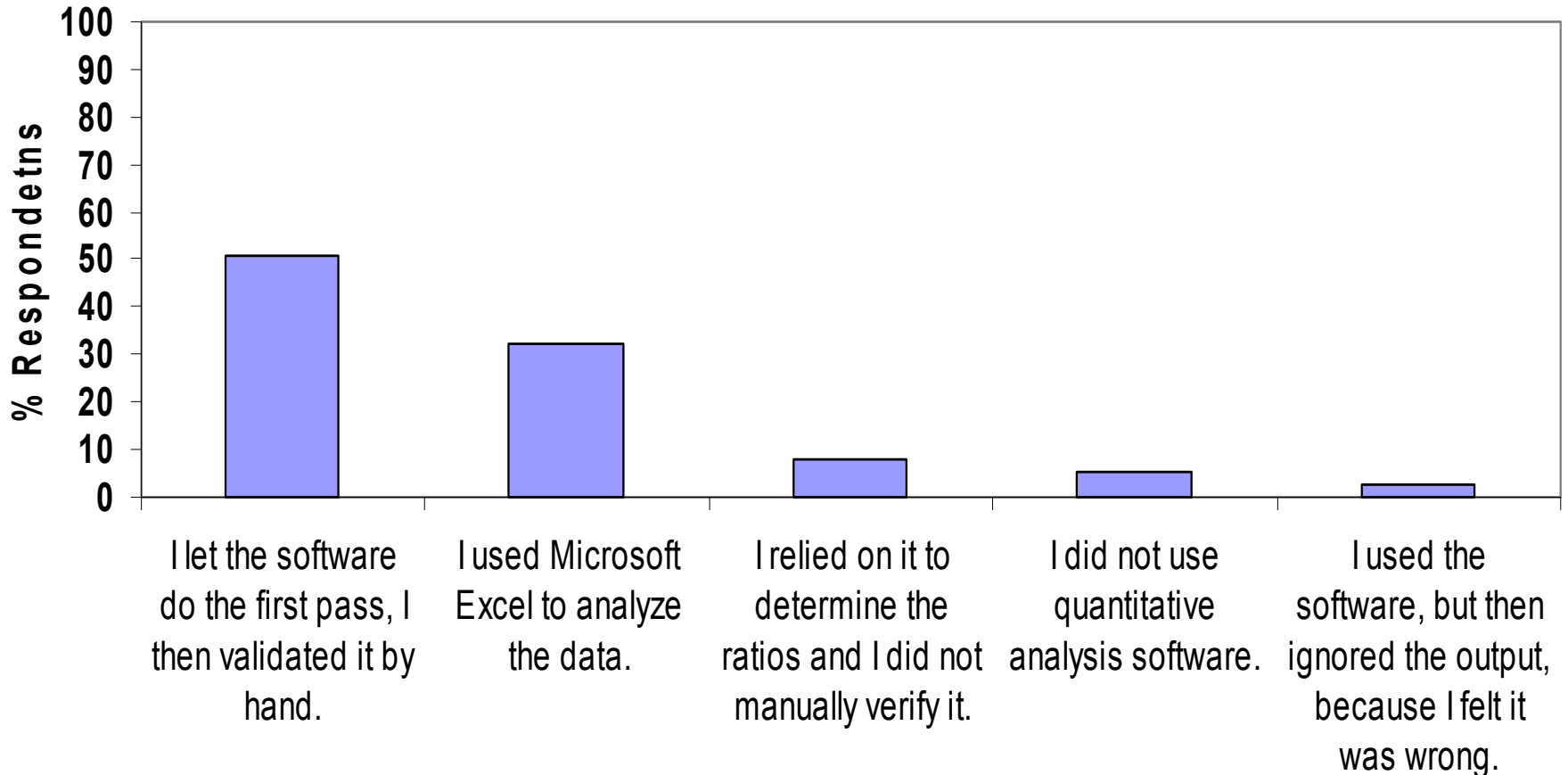
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Study Objectives

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- The ability of software to determine quantitative differences across samples or help analyze data from quantitative experiments.





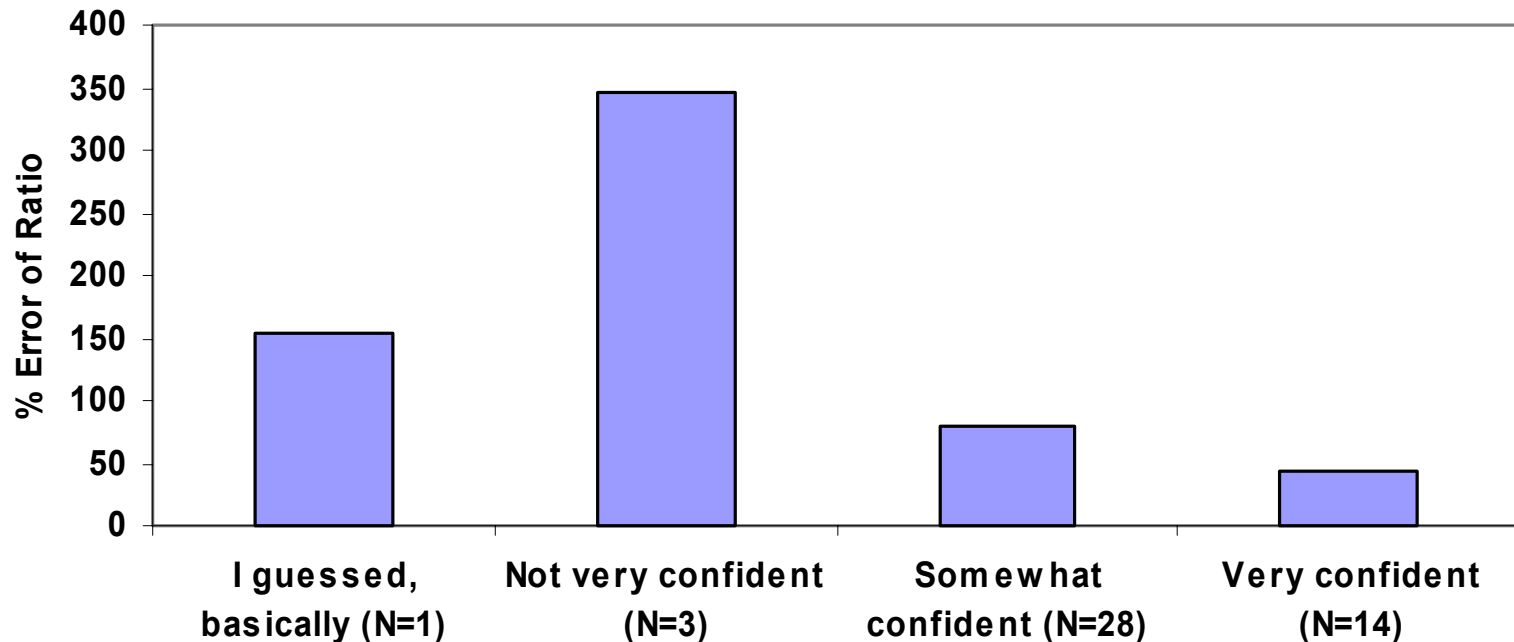
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Study Objectives

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- Assess the level of confidence and consistency in the quantification data achieved.





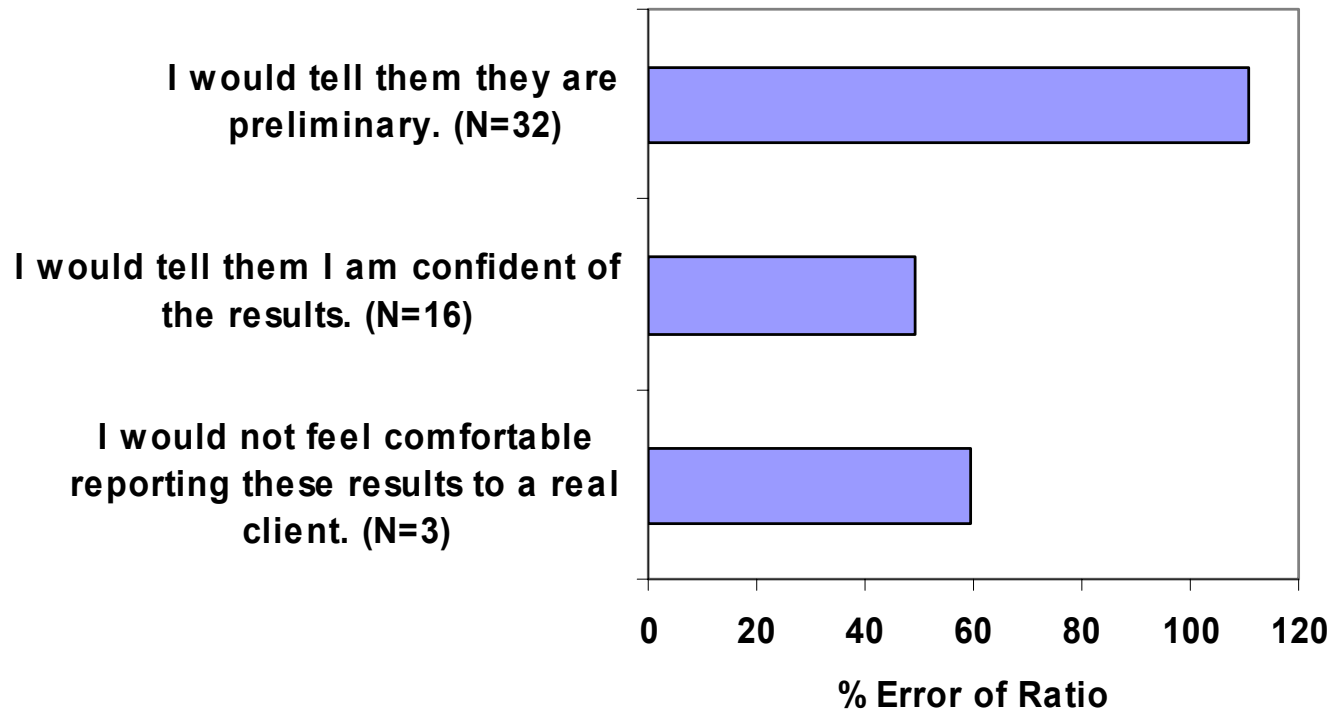
PRG2006 Relative Quantification

Study Objectives

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- **Assess the level of confidence and consistency in the quantification data achieved**
 - **Would you report the results you obtained to a real client?**





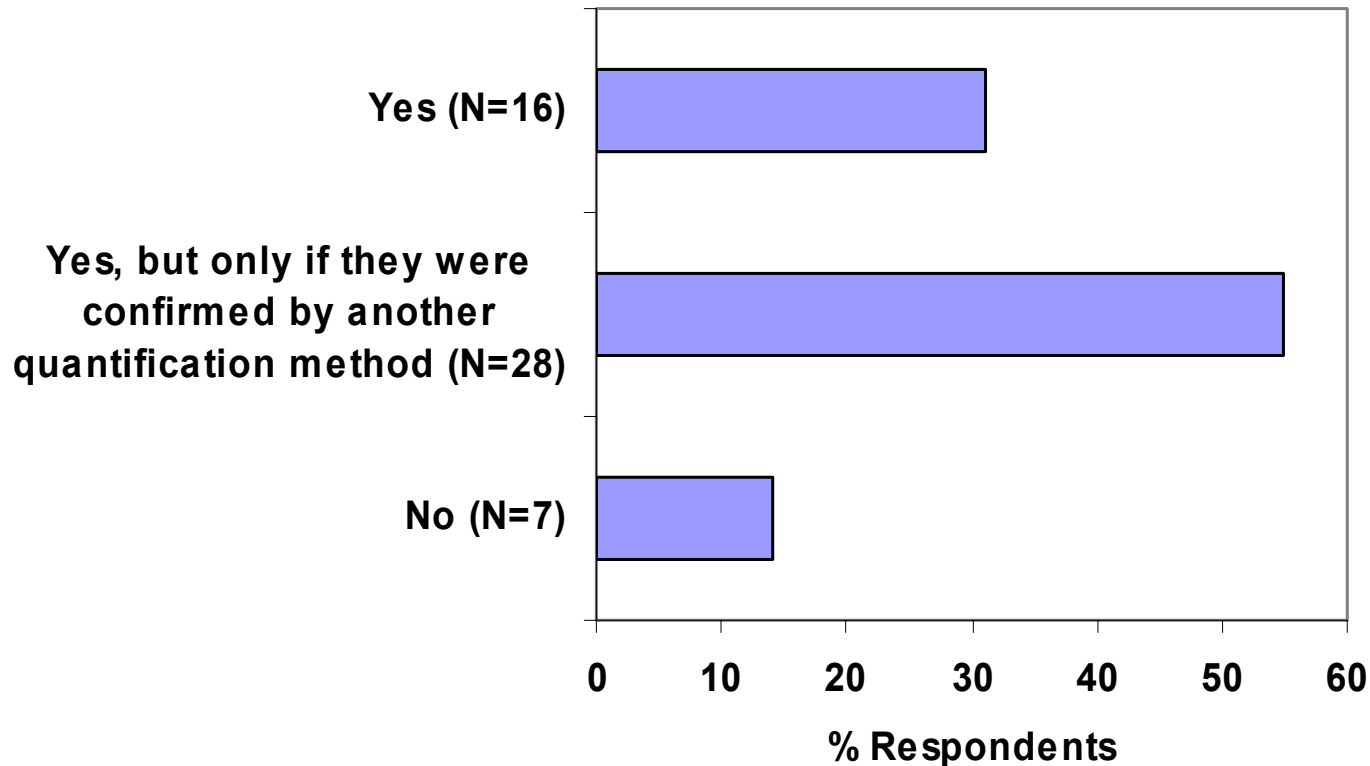
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Study Objectives

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- Assess the level of confidence and consistency in the quantification data achieved
 - Would you publish the results?





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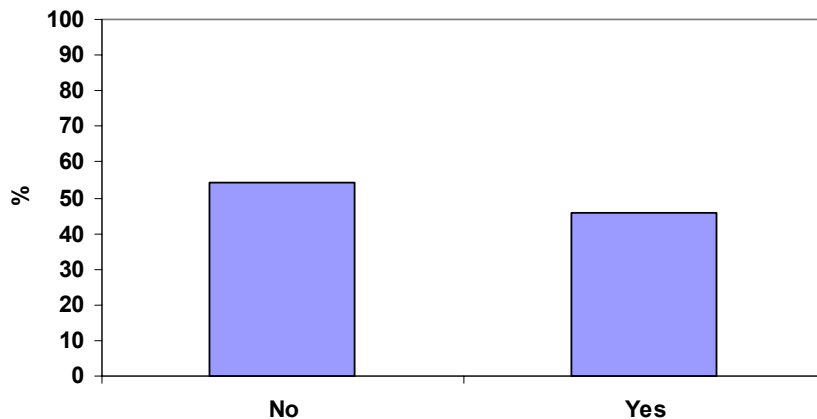
Study Objectives

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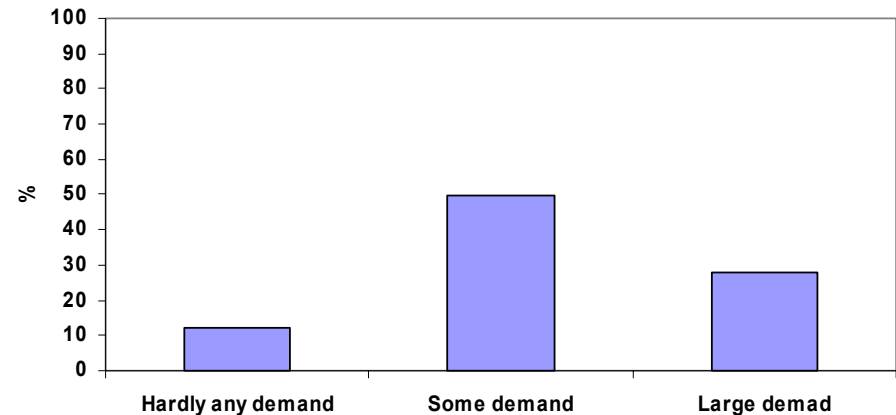


- What are the views of the participants in terms of the feasibility of relative protein quantification projects in core facilities?

Service Offered?



Demand in Core Facility?





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Quantitative Proteomics



- **Today there is no consensus on how to report statistical confidence.**
- **Acceptable limits of error depend on the analytes and design of the experiment, particularly the replicates.**



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Acknowledgements

Kevin Hakala - UTHSCSA

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Katrin Haegler - Max Planck Institute

Nikola Mueller - Max Planck Institute



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Thank You Study Participants !

49437	70788	10016	69318
27960	20802	14304	20802
98765	75301	55000	33164
11576	45984	46011	65101
65216	40508	715	45237
52666	66113	56285	29471
83044	10901	16926	73791
12125	72911	51892	19727
55003	73752	96598	11641
72079	20902	15024	
67483	55002	55001	
11010	27963	12569	
15119	21806	30974	
37832	21347	87050	



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Quantification or Quantitation?



- **'Quantification'** is the usual word. It was introduced by logicians in the mid 19th century, but from the late 19th century was adopted by mathematicians, scientists, and writers in a wide range of fields.
- **'Quantitation'** was introduced by statisticians in the late 19th century, on the grounds that it was slightly shorter, and avoided confusion with the concept of quantification in logic. However, it did not become so widely used, except in connection with certain kinds of scientific analysis, particularly in biochemistry and related fields, which is the context of all the examples cited in the complete Oxford English Dictionary. It is perhaps kept current in these fields because of its constant use alongside the related adjective 'quantitative'.
- The **actual meaning of the two words appears to be the same**, and some technical glossaries state explicitly that they are interchangeable.

Dr J. H. Marshall Associate Editor **Oxford English Dictionary**