Tackling Unforeseen Problems in Otherwise 'Straight-Forward' Proteomics Analyses.

- http://www.abrf.org/prg

These are the slides presented during the ABRF2010 Annual Meeting held in Sacramento, March 20-23. Notes from the presentation have been included here to convey ideas not apparent from the slides themselves. Please consult the study letters and support information for more details of the study.

Join the ABRF!

If you're consulting these slides because you're interested in these studies, but did not participate, then we strongly encourage you to give it a try next year.

Also, many participants are not ABRF members, but clearly value these studies every year. We encourage participating scientists to formally join the Association, as both stand to benefit!

PRG Members 2009-2010

PRG2010

* **David B. Friedman** (Chair) – *Vanderbilt University*
  Tracy M. Andacht – *Center for Disease Control and Prevention*
  Maureen K. Bunger – *Research Triangle Institute*
* **Allis S. Chien** - *Stanford University*
* **Jeroen Krijgsveld** – *EMBL Heidelberg*
  David Hawke (incoming Chair) – *MD Anderson Cancer Center*
  Robert E. Settlage – *VBI Virginia Tech*
  Robert L. Moritz – *Institute for Systems Biology*
  Chris Turck (EB Liaison) - *Max Planck Institute*

*outgoing members

Henrik Molina (incoming) – *CGR, Barcelona*
Larry Dangott (incoming) – *Texas A&M University*
Cory Bystrom (incoming) – *Quest Diagnostics*

Proteomics Research Group Mission

PRG2010

- The PRG is a volunteer scientific organization dedicated to sharing knowledge about the analysis of proteins.
- The PRG aims to assist protein scientists and resource facilities by sponsoring annual research studies that examine current techniques and capabilities.
- Through the promotion of broad participation and scientific excellence, the PRG aims to raise awareness, knowledge and education about modern methods of protein analysis.

Some Key Reminders

- These studies are not a competition
- These studies are meant to be challenging
- Studies are chosen to represent ‘real life’ examples of analyses that are requested of core facilities
- These studies are useful for laboratories to benchmark themselves against other groups.

Past Research Studies

PRG2010

49 requests
27 returns (55%)
30% members (8)

- **PRG2009:** A targeted relative protein quantification study relevant for a biomarker validation project.

114 requests
57 returns (50%)
42% members (24)

- **PRG2008:** Identify differences between two samples that have different lengths of the same protein sequence

87 requests
43 returns (49%)
51% members (22)

- **PRG2007:** Relative abundance of 12 proteins spiked into an *E. coli* lysate between 3 different samples

91 requests
52 returns (57%)
57% members (30)

- **PRG2006:** Relative abundance of 8 proteins between 2 different samples

PRG2010 Study Objectives

- Make study broader in scope to attract greater participation.
- Increase member participation.
- Design an experiment that was more realistic to a submission.
- Provide a thorough pre-evaluation of samples and sufficient information.
- Make survey easier by providing questions ahead of time.

Participation

PRG2010

96 requests (↑96%)
47 returns (49%)
28 with data (29%)
6 members (18%)

- **PRG2010**: Tackling unforeseen problems in otherwise straight-forward proteomics analyses.

49 requests
27 returns (55%)
8 members (30%)

- **PRG2009:** A targeted relative protein quantification study relevant for a biomarker validation project.

114 requests
57 returns (50%)
24 members (42%)

- **PRG2008:** Identify differences between two samples that have different lengths of the same protein sequence

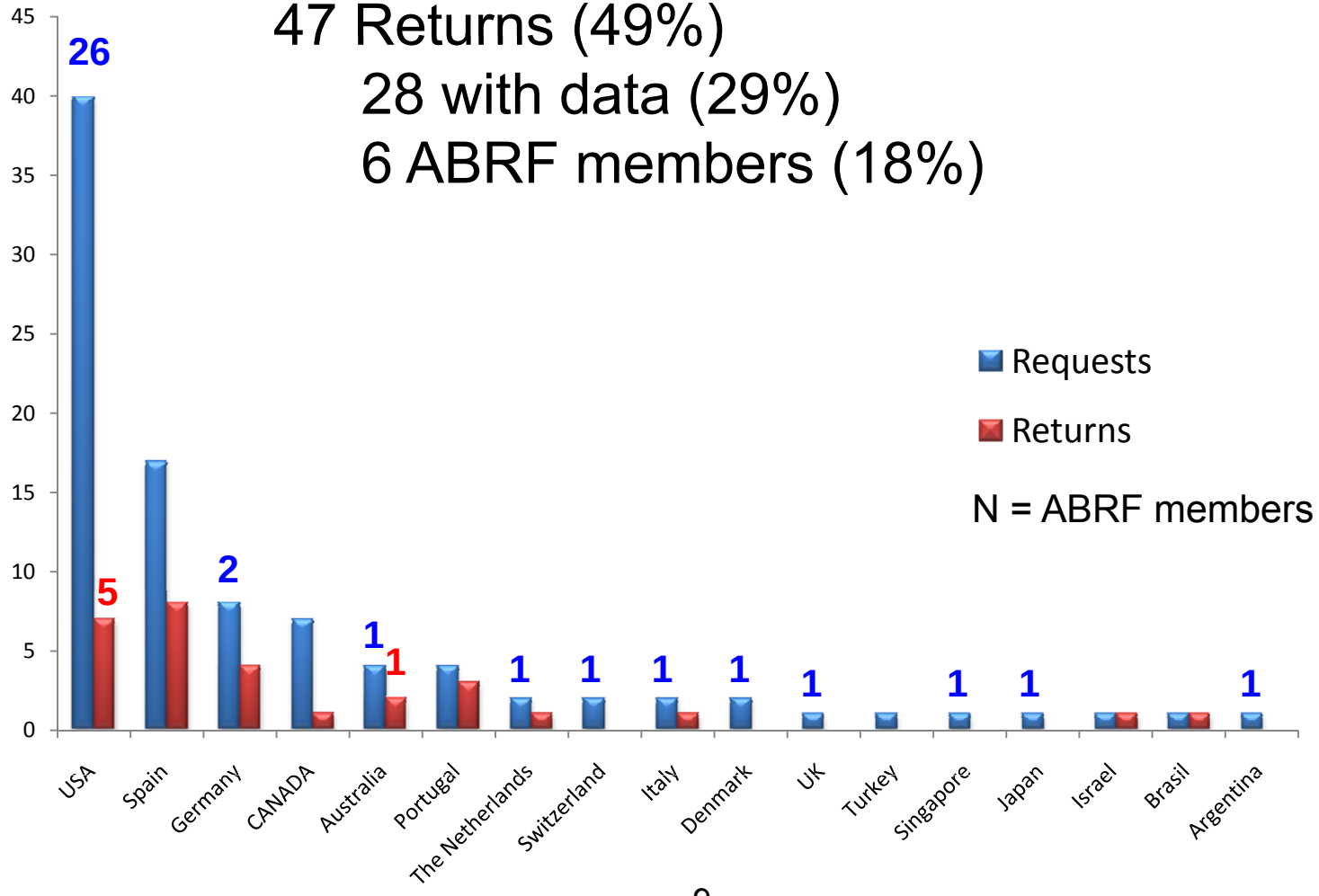
87 requests
43 returns (49%)
22 members (51%)

- **PRG2007:** Relative abundance of 12 proteins spiked into an *E. coli* lysate between 3 different samples

Requests & Returns

PRG2010

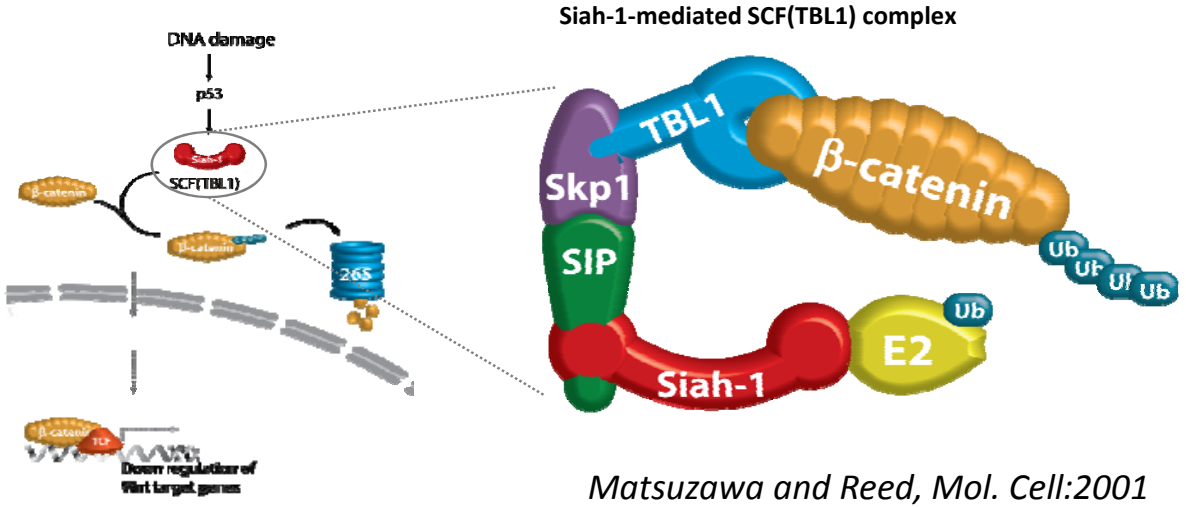
96 Requests (96% increase from 2009!)
47 Returns (49%)
28 with data (29%)
6 ABRF members (18%)



The Study Samples

Siah-1 mediated ubiquitination of β-catenin

β-catenin
Siah1 (Seven in absentia homolog 1) E3 ligase
Tbl1 (Transducin β-like 1)
Skp1
SIP (Siah1-interacting protein, CYBP)
S100-A6
ubiquitin



Matsuzawa and Reed, Mol. Cell:2001

Notes From Presentation:

All participants were given as much information as would be reasonably requested from any proteomics core facility. This included representative SDS-PAGE gel images, sequences of the recombinantly-expressed β-catenin and Siah1, as well as useful tips for searching ^{15}N-labeled MS data using the more commonly used search algorithms.

Our intention was to make this study as transparent as possible. This study was in fact mirrored after an actual study that came into one of our PRG labs in a very similar fashion with similar information. The study scenario was fabricated, but the building blocks were real and were used in a related study for ubiquitination of β-catenin which is now published (see acknowledgements).

Extensive Sample Testing

PRG2010

CTNB1_HUMAN (134-781, upper)

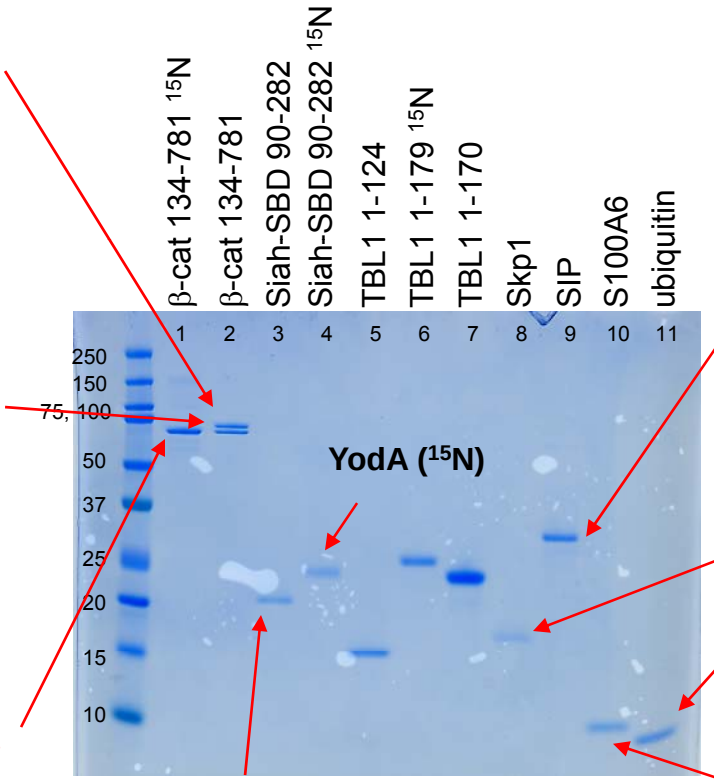
```
  1 MATQADLMEL DMAMEPDRKA AVSHWQQQSY LDSGIHSGAT TTAPSLSGKG
 51 NPEEEDVDTS QVLYEWEQGF SQSFTQEQVA DIDGQYAMTR AQRVRAAMFP
101 ETLDEGMQIP STQFDAAHPT NVQRLAEPSQ MLKHAVVNLI NYQDDAELAT
151 RAIPELTKLL NDEDQVVVNK AAVMVHQLSK KEASRHAIMR SPQMVSAIVR
201 TMQNTNDVET ARCTAGTLHN LSHHREGLLA IFKSGGIPAL VKMLGSPVDS
251 VLFYAITTLH NLLLHQEGAK MAVRLAGGLQ KMVALLNKTN VKFLAITTDC
301 LQILAYGNQE SKLIILASGG PQALVNIMRT YTYEKLLWTT SRVLKVLSVC
351 SSNKPAIVEA GGMQALGLHL TDPSQRLVQN CLWTLRNLSD AATKQEGMEG
401 LLGTLVQLLG SDDINVVTCA AGILSNLTCN NYKNKMMVCQ VGGIEALVRT
451 VLRAGDREDI TEPAICALRH LTSRHQEAEM AQNAVRLHYG LPVVVKLLHP
501 PSHWPLIKAT VGLIRNLALC PANHAPLREQ GAIPRLVQLL VRAHQDTQRR
551 TSMGGTQQQF VEGVRMEEIV EGCTGALHIL ARDVHNRIVI RGLNTIPLFV
601 QLLYSPIENI QRVAAGVLCE LAQDKEAAEA IEAEGATAPL TELLHSRNEG
651 VATYAAAVLF RMSEDKPQDY KKRLSVELTS SLFRTEPMAW NETADLGLDI
701 GAQGEPLGYR QDDPSYRSFH SGGYGQDALG MDPMMEHEMG GHHPGADYPV
751 DGLPDLGHAQ DLMDGLPPGD SNQLAWFDTD L
```

CTNB1_HUMAN (134-781, lower)

```
  1 MATQADLMEL DMAMEPDRKA AVSHWQQQSY LDSGIHSGAT TTAPSLSGKG
 51 NPEEEDVDTS QVLYEWEQGF SQSFTQEQVA DIDGQYAMTR AQRVRAAMFP
101 ETLDEGMQIP STQFDAAHPT NVQRLAEPSQ MLKHAVVNLI NYQDDAELAT
151 RAIPELTKLL NDEDQVVVNK AAVMVHQLSK KEASRHAIMR SPQMVSAIVR
201 TMQNTNDVET ARCTAGTLHN LSHHREGLLA IFKSGGIPAL VKMLGSPVDS
251 VLFYAITTLH NLLLHQEGAK MAVRLAGGLQ KMVALLNKTN VKFLAITTDC
301 LQILAYGNQE SKLIILASGG PQALVNIMRT YTYEKLLWTT SRVLKVLSVC
351 SSNKPAIVEA GGMQALGLHL TDPSQRLVQN CLWTLRNLSD AATKQEGMEG
401 LLGTLVQLLG SDDINVVTCA AGILSNLTCN NYKNKMMVCQ VGGIEALVRT
451 VLRAGDREDI TEPAICALRH LTSRHQEAEM AQNAVRLHYG LPVVVKLLHP
501 PSHWPLIKAT VGLIRNLALC PANHAPLREQ GAIPRLVQLL VRAHQDTQRR
551 TSMGGTQQQF VEGVRMEEIV EGCTGALHIL ARDVHNRIVI RGLNTIPLFV
601 QLLYSPIENI QRVAAGVLCE LAQDKEAAEA IEAEGATAPL TELLHSRNEG
651 VATYAAAVLF RMSEDKPQDY KKRLSVELTS SLFRTEPMAW NETADLGLDI
701 GAQGEPLGYR QDDPSYRSFH SGGYGQDALG MDPMMEHEMG GHHPGADYPV
751 DGLPDLGHAQ DLMDGLPPGD SNQLAWFDTD L
```

CTNB1_HUMAN (^{15}N, 134-781)

```
  1 MATQADLMEL DMAMEPDRKA AVSHWQQQSY LDSGIHSGAT TTAPSLSGKG
 51 NPEEEDVDTS QVLYEWEQGF SQSFTQEQVA DIDGQYAMTR AQRVRAAMFP
101 ETLDEGMQIP STQFDAAHPT NVQRLAEPSQ MLKHAVVNLI NYQDDAELAT
151 RAIPELTKLL NDEDQVVVNK AAVMVHQLSK KEASRHAIMR SPQMVSAIVR
201 TMQNTNDVET ARCTAGTLHN LSHHREGLLA IFKSGGIPAL VKMLGSPVDS
251 VLFYAITTLH NLLLHQEGAK MAVRLAGGLQ KMVALLNKTN VKFLAITTDC
301 LQILAYGNQE SKLIILASGG PQALVNIMRT YTYEKLLWTT SRVLKVLSVC
351 SSNKPAIVEA GGMQALGLHL TDPSQRLVQN CLWTLRNLSD AATKQEGMEG
401 LLGTLVQLLG SDDINVVTCA AGILSNLTCN NYKNKMMVCQ VGGIEALVRT
451 VLRAGDREDI TEPAICALRH LTSRHQEAEM AQNAVRLHYG LPVVVKLLHP
501 PSHWPLIKAT VGLIRNLALC PANHAPLREQ GAIPRLVQLL VRAHQDTQRR
551 TSMGGTQQQF VEGVRMEEIV EGCTGALHIL ARDVHNRIVI RGLNTIPLFV
601 QLLYSPIENI QRVAAGVLCE LAQDKEAAEA IEAEGATAPL TELLHSRNEG
651 VATYAAAVLF RMSEDKPQDY KKRLSVELTS SLFRTEPMAW NETADLGLDI
701 GAQGEPLGYR QDDPSYRSFH SGGYGQDALG MDPMMEHEMG GHHPGADYPV
751 DGLPDLGHAQ DLMDGLPPGD SNQLAWFDTD L
```



CYBP_MOUSE (SIP)

```
  1 MASVLEELQK DLEEVKVLLE KSTRKRLRDT LTSEKSKIET ELKNKMQQKS
 51 QKKPELDNEK PAAVVAPLTT GYTVKISNYG WDQSDKFVKI YITLTGVHQV
101 PTENVQVHFT ERSFDLLVKN LNGKNYSMIV NNLLKPISVE SSSKKVKTDT
151 VIILCRKKAE NTRWDYLTQV EKECKEKEKP SYDTEADPSE GLMNVLKKIY
201 EDGDDDMKRT INKAWVESRE KQAREDTEF
```

Skp1_HUMAN

```
  1 MPSIKLQSSD GEIFEVDVEI AKQSVTIKTM LEDLGMDDEG DDDPVPLPNV
 51 NAAILKKVIQ WCTHHKDDPP PPEDDENKEK RTDDIPVWDQ EFLKVDQGTL
101 FELILAANYL DIKGLLDVTC KTVANMIKGK TPEEIRKTFN IKNDFTEEEE
151 AQVRKENQWC EEK
```

ubiquitin

```
  1 MQIFVKTLTG KTITLEVEPS DTIENVKAKI QDKEGIPPDQ QRLIFAGKQL
 51 EDGRTLSDYN IQKESTLHLV LRLRGG
```

SiahH1_HUMAN 90-282 (^{14}N) E3 Ub-ligase

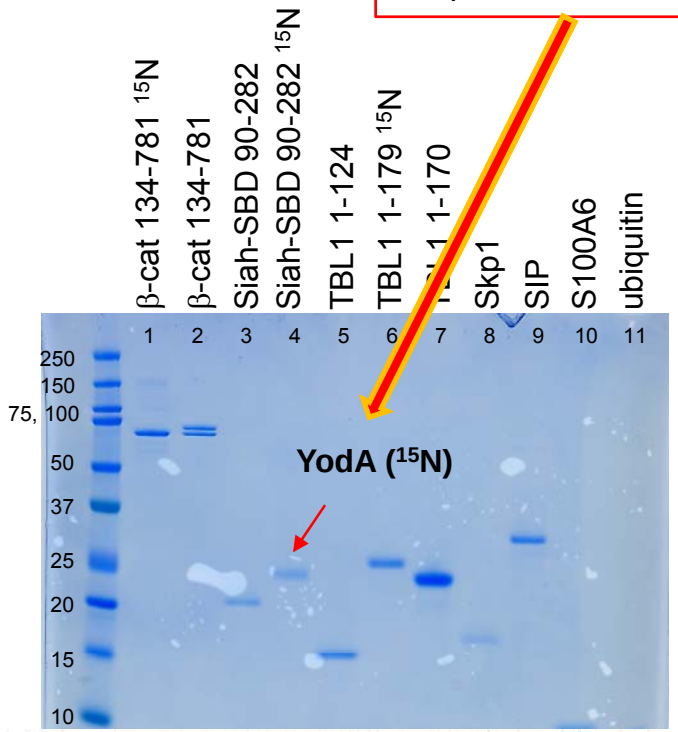
```
  1 MSRQTATALP TGTSKCPPSQ RVPALTGTTA SNNDLASLFE CPVCFDYVLP
 51 PILQCQSGHL VCSNCRPKLT CCPTCRGPLG SIRNLAMEKV ANSVLFPCKY
101 ASSGCEITLP HTEKADHEEL CEFRPYSCPC PGASCKWQGS LDAVMPHLMH
151 QHKSITTLQG EDIVFLATDI NLPGAVDWVM MQSCFGFHFM LVLEKQEKYD
201 GHQQFFAIVQ LIGTRKQAEN FAYRLELNGH RRRLTWEATP RSIHEGIATA
251 IMNSDCLVFD TSIAQLFAEN GNLGINVTIS MC
```

S100-A6

Extensive Sample Testing

PRG2010

YodA is expressed in response to low Zn, and Siah1 is a Zn-binding protein



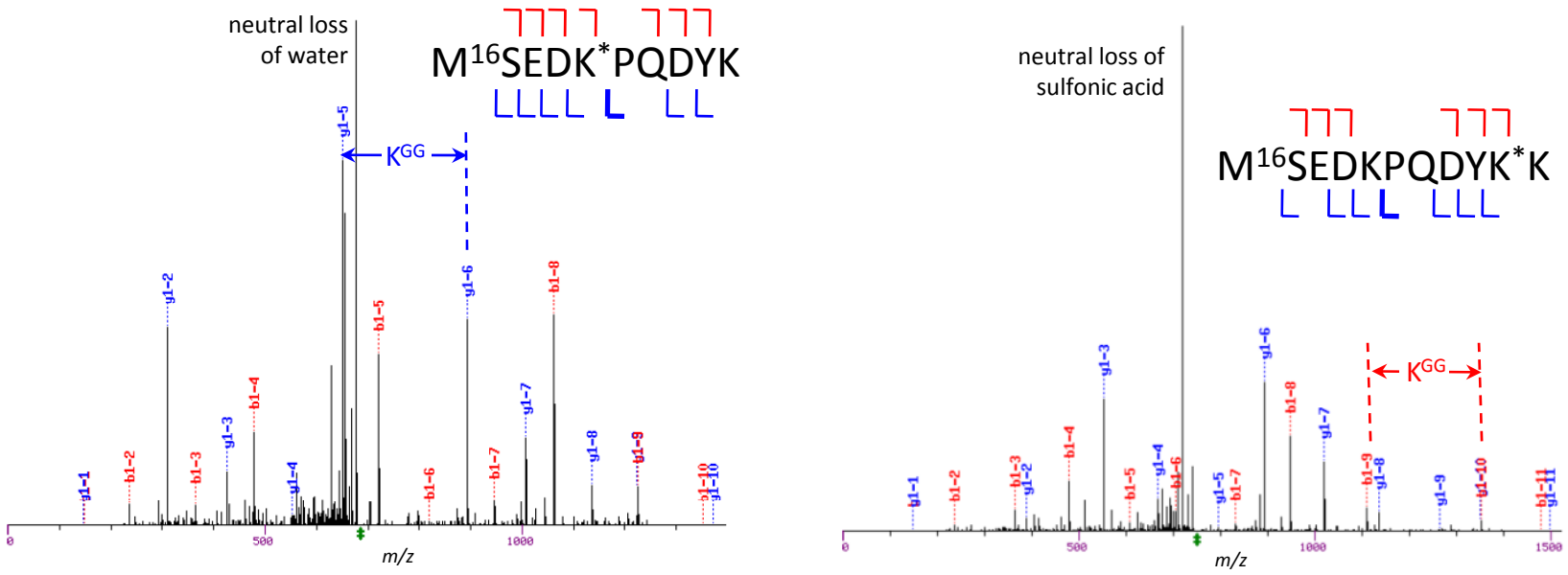
Biochimica et Biophysica Acta 1760 (2006) 1304 – 1313

Structural analysis and classification of native proteins from *E. coli* commonly co-purified by immobilised metal affinity chromatography

Victor Martin Bolanos-Garcia *, Owen Richard Davies

β-catenin is Ubiquitinated in Active Complex

PRG2010



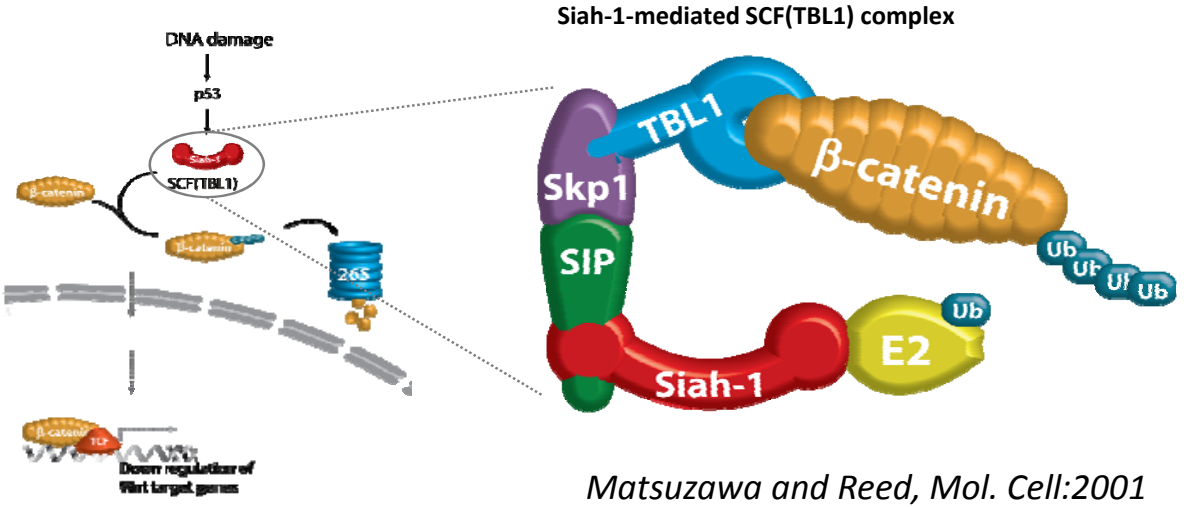
Note from presentation: This slide demonstrates that the proteins actually DO form an active complex, but due to the scarcity of this sample, we could not include this aspect into the study. The samples sent out either "could or could not produce an active complex", but none of the samples contained ubiquitinated products.

The Saga of Dr. Quickhands

PRG2010

Dr. Quickhands has worked out the purification of an active ubiquitination complex based on the Siah1 E3 ligase using β-catenin as a substrate.

- Siah1 and β-catenin are bacterially-expressed (fusion sequences provided).
- Dr. Quickhands wants to determine the other components present in this active complex.



Matsuzawa and Reed, Mol. Cell:2001

The Saga of Dr. Quickhands

PRG2010

"Dr. Quickhands has isolated a complex that is ACTIVE for the in vitro ubiquitination assay"

Tube 1: "Active" complex

challenges

Identify components (6)

confirmed by MALDI-TOF/TOF and ESI-LC/MS/MS

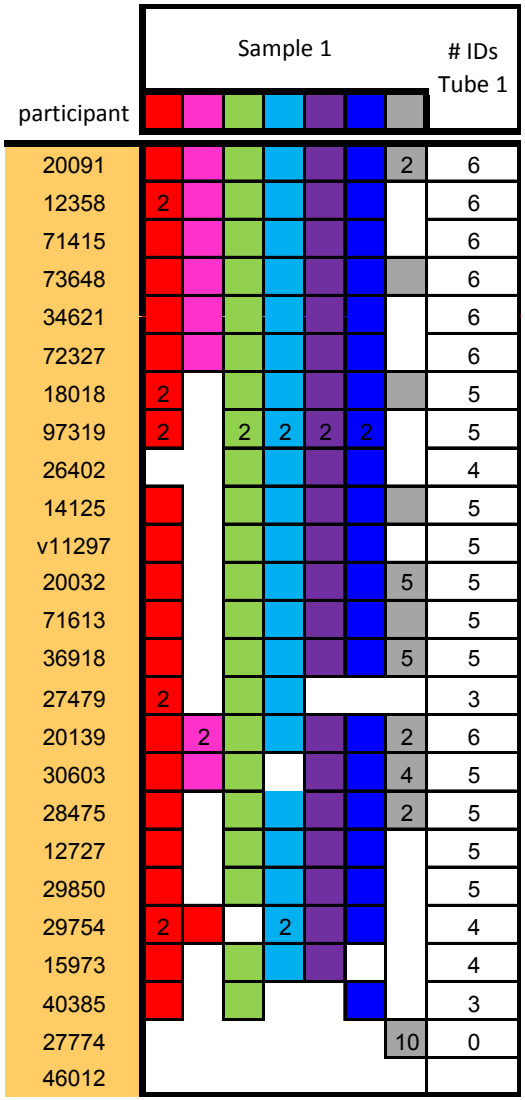
Protein	Coverage	Peptides	Spectral Counts
CTNB1_HUMAN	52	33	44
Cntm_P35527|K1C9_HUMAN	25	10	10
UBIQ_HUMAN	78	9	13
Cntm_P04264|K2C1_HUMAN	13	8	8
CYBP_HUMAN	27	10	14
Cntm_P13645|K1C10_HUMAN	12	6	6
SKP1_HUMAN	35	7	15
SIAH1_HUMAN	19	7	10
Cntm_P13647|K2C5_HUMAN	3	3	3
S10A6_HUMAN*	31	3	4

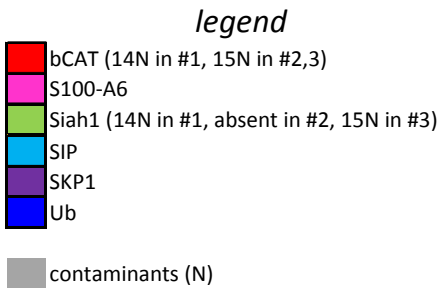
**more challenging to detect!*

Results: Tube 1 – simple ID

PRG2010

"Dr. Quickhands has isolated a complex that is ACTIVE for the in vitro ubiquitination assay"





Note from presentation: almost all respondants were able to identify the components of tube #1, but many struggled with identification of S100-A6 as expected.

The Saga of Dr. Quickhands

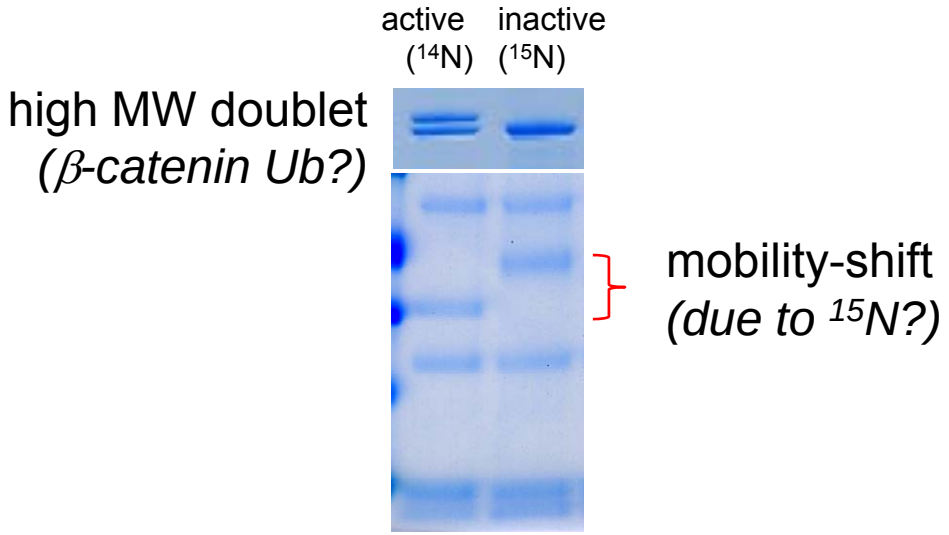
PRG2010

To prepare for the NMR structural studies, Dr. Quickhands repeats purification using ^{15}N labeled Siah1 and β-catenin:
*But the complex is now **INACTIVE**!*

Tube 2: "Inactive" complex

challenges:

- ^{15}N labeled proteins (sequences provided)
- ^{15}N Siah1 is actually an ^{15}N *E. coli* contaminant YodA (no sequence provided)
- Explain Doublet



participants were also given guidelines for performing searches with ^{15}N-masses.

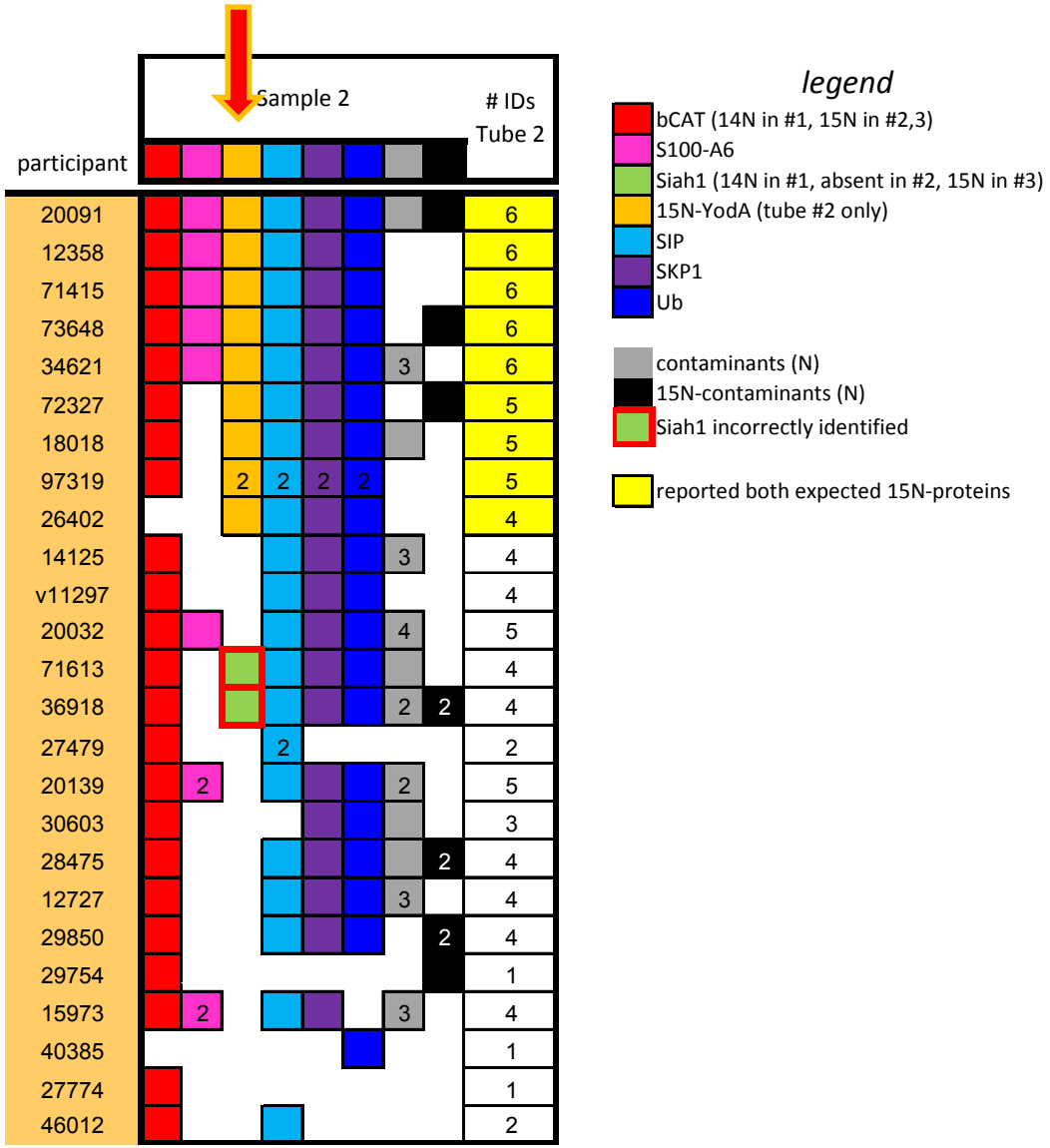
Results: Tube 2-^{15}N and a contaminant

PRG2010

"Dr. Quickhands now makes ^{15}N β-catenin and Siah1 for NMR and repeats assay, but now the isolated complex is INACTIVE for the in vitro ubiquitination assay"

^{15}N β-catenin

But ^{15}N Siah1 is really ^{15}N E. coli YodA



Notes From Presentation:

Although only some respondants correctly identified the ^{15}N-labeled *E. coli* contaminant YodA, most/all respondants did correctly tell us that ^{15}N Siah1 was missing from tube #2.

Results: Tube 1 – the Doublet

PRG2010

CTNB1_HUMAN

```
  1 MATQADLMEL DMAMEPDRKA AVSHWQQQSY LDSGIHSGAT TTAPSLSGKG
 51 NPEEEDVDTS QVLYEWEQGF SQSFTQEQVA DIDGQYAMTR AQRVRAAMFP
101 ETLDEGMQIP STQFDAAHPT NVQRLAEPSQ MLKHAVVNLI NYQDDAELAT
151 RAIPELTKLL NDEDQVVVNK AAVMVHQLSK KEASRHAIMR SPQMVSAIVR
201 TMQNTNDVET ARCTAGTLHN LSHHREGLLA IFKSGGIPAL VKMLGSPVDS
251 VLFYAITTLH NLLLHQEGAK MAVRLAGGLQ KMVALLNKTN VKFLAITTDC
301 LQILAYGNQE SKLIILASGG PQALVNIMRT YTYEKLLWTT SRVLKVLSVC
351 SSNKPAIVEA GGMQALGLHL TDPSQRLVQN CLWTLRNLSD AATKQEGMEG
401 LLGTLVQLLG SDDINVVTCA AGILSNLTCN NYKNKMMVCQ VGGIEALVRT
451 VLRAGDREDI TEPAICALRH LTSRHQEAEM AQNAVRLHYG LPVVVKLLHP
501 PSHWPLIKAT VGLIRNLALC PANHAPLREQ GAIPRLVQLL VRAHQDTQRR
551 TSMGGTQQQF VEGVRMEEIV EGCTGALHIL ARDVHNRIVI RGLNTIPLFV
601 QLLYSPIENI QRVAAGVLCE LAQDKEAAEA IEAEGATAPL TELLHSRNEG
651 VATYAAAVLF RMSEDKPQDY KKRLSVELTS SLFRTEPMAW NETADLGLDI
701 GAQGEPLGYR QDDPSYRSFH SGGYGQDALG MDPMMEHEMG GHHPGADYPV
751 DGLPDLGHAQ DLMDGLPPGD SNQLAWFDTD L
```

HAVVNLINYWDDELATR

full-length

β-cat 134-781 fusion

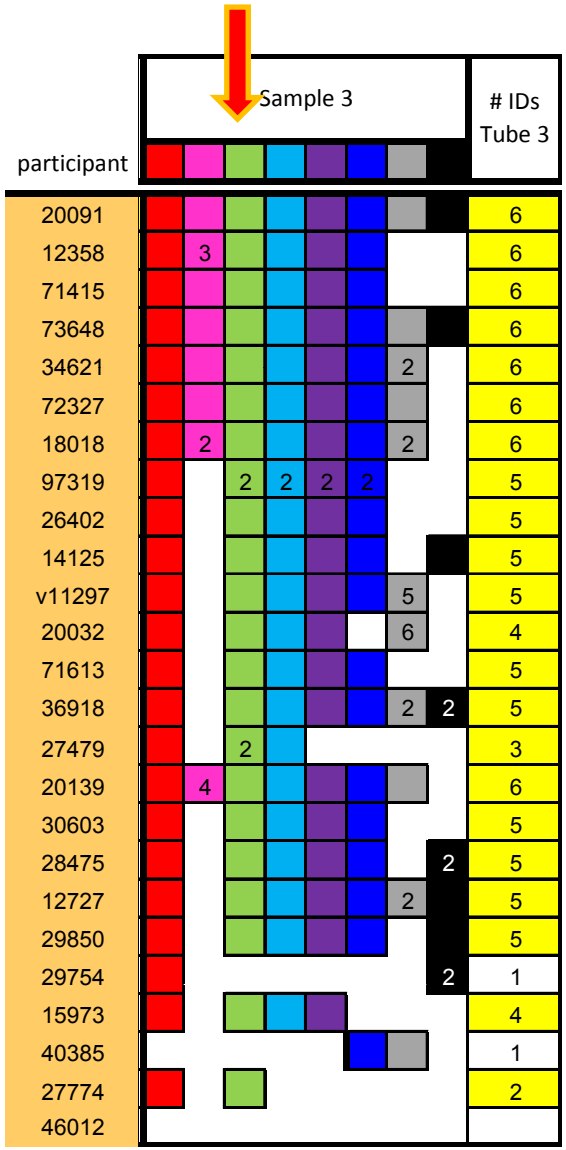
GGILHAVVNLINYWDDELATR

participant	DOUBLET
20091	✓
12358	✓
71415	✗
73648	
34621	
72327	
18018	✓
97319	✗
26402	✗
14125	✓
v11297	✓
20032	✓
71613	✓
36918	✓
27479	✓
20139	✗
30603	✗
28475	
12727	
29850	✗
29754	✗
15973	✗
40385	
27774	
46012	

Results: Tube 3 – All Restored

PRG2010

"Third time is the charm!
Dr. Quickhand's new ^{15}N-labeled β–catenin, Siah1 complex is now ACTIVE!



legend

- bCAT (14N in #1, 15N in #2,3)
- S100-A6
- Siah1 (14N in #1, absent in #2, 15N in #3)
- 15N-YodA (tube #2 only)
- SIP
- SKP1
- Ub
- contaminants (N)
- 15N-contaminants (N)
- Siah1 incorrectly identified
- reported both expected 15N-proteins

Results: Main Challenges

PRG2010

participant	DOUBLET	YodA	S100A6
20091	✓	✓	✓
12358	✓	✓	!
71415	✗	✓	✓
73648		✓	✓
34621		✓	✓
72327		✓	!
18018	✓	✓	!
97319	✗	✓	✗
26402	✗	✓	✗
14125	✓	✗	✗
v11297	✓	✗	✗
20032	✓	✗	!
71613	✓	✗	✗
36918	✓	✗	✗
27479	✓	✗	✗
20139	✗	✗	!
30603	✗	✗	!
28475		✗	✗
12727		✗	✗
29850	✗	✗	✗
29754	✗	✗	✗
15973	✗	✗	!
40385		✗	✗
27774		✗	✗
46012			

Simple protein identification.

What's the nature of the doublet in Tube 1?

What's happed in Tube 2 that is now restored in Tube 3? in each tube (with the ^{15}N contaminant twist)?

Properly Identify S100-A6?

Results: Self-Evaluation

PRG2010

participant	DOUBLET	YodA	S100A6	Difficulty
20091	✓	✓	✓	Moderate
12358	✓	✓	!	Moderate
71415	✗	✓	✓	Moderate
73648		✓	✓	Moderate
34621		✓	✓	Moderate
72327		✓	!	Moderate
18018	✓	✓	!	Moderate
97319	✗	✓	✗	Moderate
26402	✗	✓	✗	Moderate
14125	✓	✗	✗	Moderate
v11297	✓	✗	✗	Moderate
20032	✓	✗	!	Vy easy
71613	✓	✗	✗	Hard
36918	✓	✗	✗	Moderate
27479	✓	✗	✗	Moderate
20139	✗	✗	!	Moderate
30603	✗	✗	!	Hard
28475		✗	✗	
12727		✗	✗	Moderate
29850	✗	✗	✗	Easy
29754	✗	✗	✗	Moderate
15973	✗	✗	!	Easy
40385		✗	✗	Moderate
27774		✗	✗	Hard
46012				

Simple protein identification.

What's the nature of the doublet in Tube 1?

What's happed in Tube 2 that is now restored in Tube 3? in each tube (with the ^{15}N contaminant twist)?

Properly Identify S100-A6?

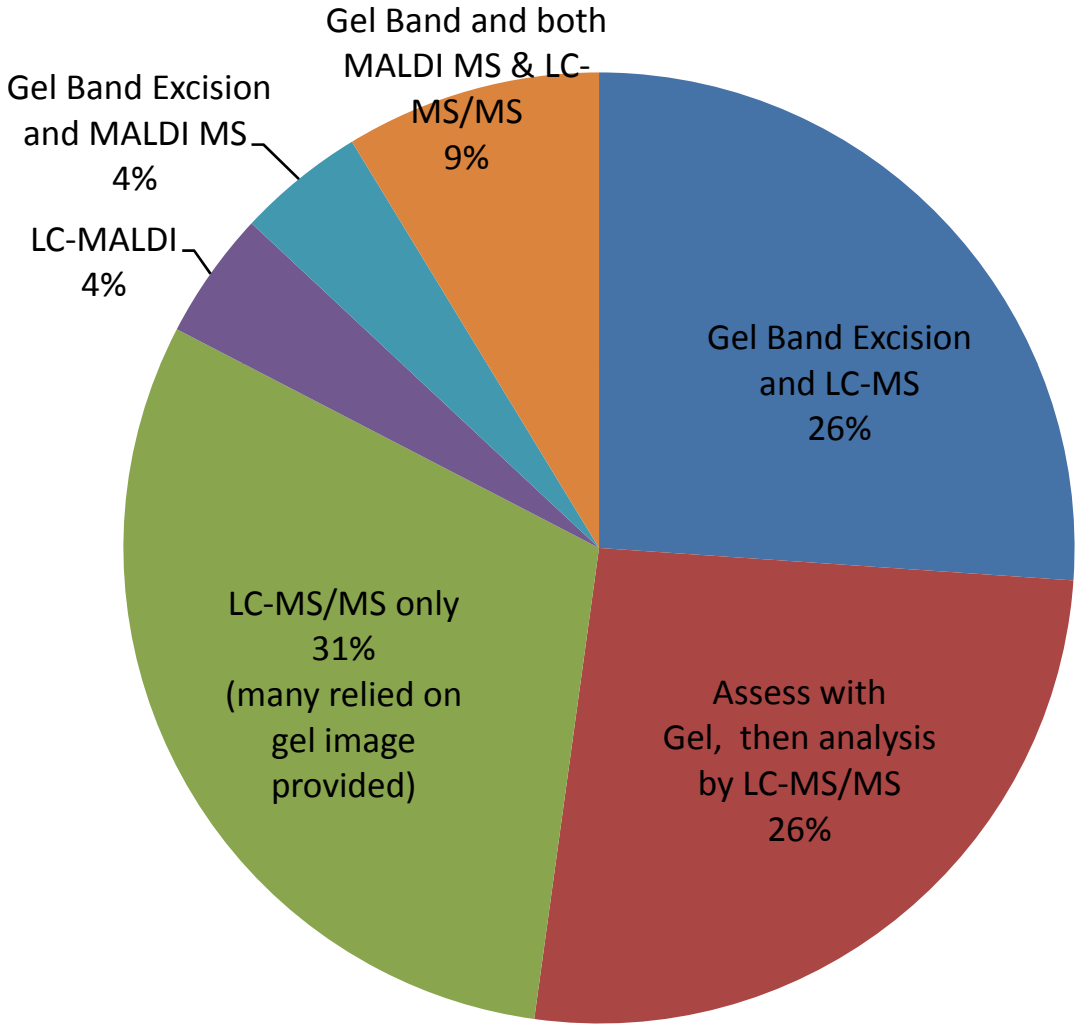
Results: Self-Evaluation

PRG2010

participant	DOUBLET	YodA	S100A6	Difficulty	Experience	Time to complete study	General approach	Mass spectrometer used
20091	✓	✓	✓	Moderate	<6 months	2-4 days	Gel + LC-MS	Quadrupole-TOF
12358	✓	✓	!	Moderate	3-5 years	5-7 days	Gel + ESI-MS	3D Ion Trap
71415	✗	✓	✓	Moderate	3-5 years	2-4 days	Gel + LC-MS	Quadrupole-TOF
73648		✓	✓	Moderate	5-10 years	2-4 days	LC-MS	Linear Ion Trap-Orbitrap
34621		✓	✓	Moderate	3-5 years	5-7 days	LC-MS	Quadrupole-TOF
72327		✓	!	Moderate	<6 months	2-4 days	LC-MS	Quadrupole-TOF
18018	✓	✓	!	Moderate	>10 years	5-7 days	Gel + LC-MS	Linear Ion Trap
97319	✗	✓	✗	Moderate	1-3 years	5-7 days	Gel + LC-MS	Linear Ion Trap
26402	✗	✓	✗	Moderate	5-10 years	2-4 days	Gel + MALDI MS + ESI-MS	3D Ion Trap + TOF/TOF
14125	✓	✗	✗	Moderate	5-10 years	2-4 days	Gel + ESI-MS	Linear Ion Trap-Orbitrap
v11297	✓	✗	✗	Moderate	5-10 years	2-4 days	LC-MS	Quadrupole-TOF
20032	✓	✗	!	Vy easy	>10 years	2-4 days	Gel + LC-MS	Linear Ion Trap-Orbitrap
71613	✓	✗	✗	Hard	5-10 years	8-10 days	Gel + LC-MALDI	TOF/TOF
36918	✓	✗	✗	Moderate	1-3 years	5-7 days	Gel + ESI-MS	Linear Ion Trap-Orbitrap
27479	✓	✗	✗	Moderate	3-5 years	8-10 days	Gel + MALDI MS + ESI-MS	TOF/TOF
20139	✗	✗	!	Moderate	3-5 years	2-4 days	Gel + ESI-MS	Linear Ion Trap-FT
30603	✗	✗	!	Hard	3-5 years	>2 weeks	Gel + LC-MALDI	TOF/TOF
28475		✗	✗		5-10 years	2-4 days	LC-MS	Linear Ion Trap-Orbitrap
12727		✗	✗	Moderate	5-10 years	2-4 days	LC-MALDI	TOF/TOF
29850	✗	✗	✗	Easy	5-10 years	2-4 days	LC-MS	Linear Ion Trap
29754	✗	✗	✗	Moderate	5-10 years	11-14 days	Gel + ESI-MS	3D Ion Trap
15973	✗	✗	!	Easy	3-5 years	1 day	LC-MS	Linear Ion Trap-Orbitrap
40385		✗	✗	Moderate	3-5 years	2-4 days	Gel + ESI-MS	Linear Ion Trap
27774		✗	✗	Hard	First time	5-7 days	Gel + LC-MS	Linear Ion Trap
46012					5-10 years	2-4 days	Gel + MALDI MS	TOF/TOF

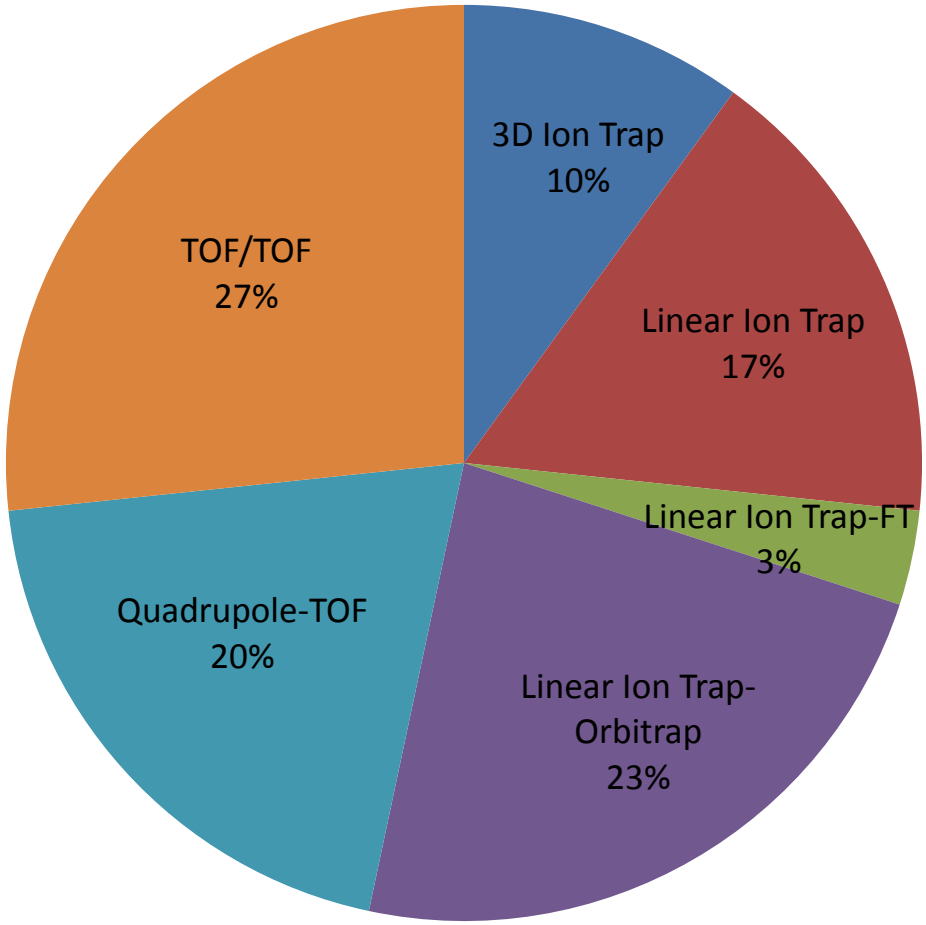
General Experimental Approach

PRG2010



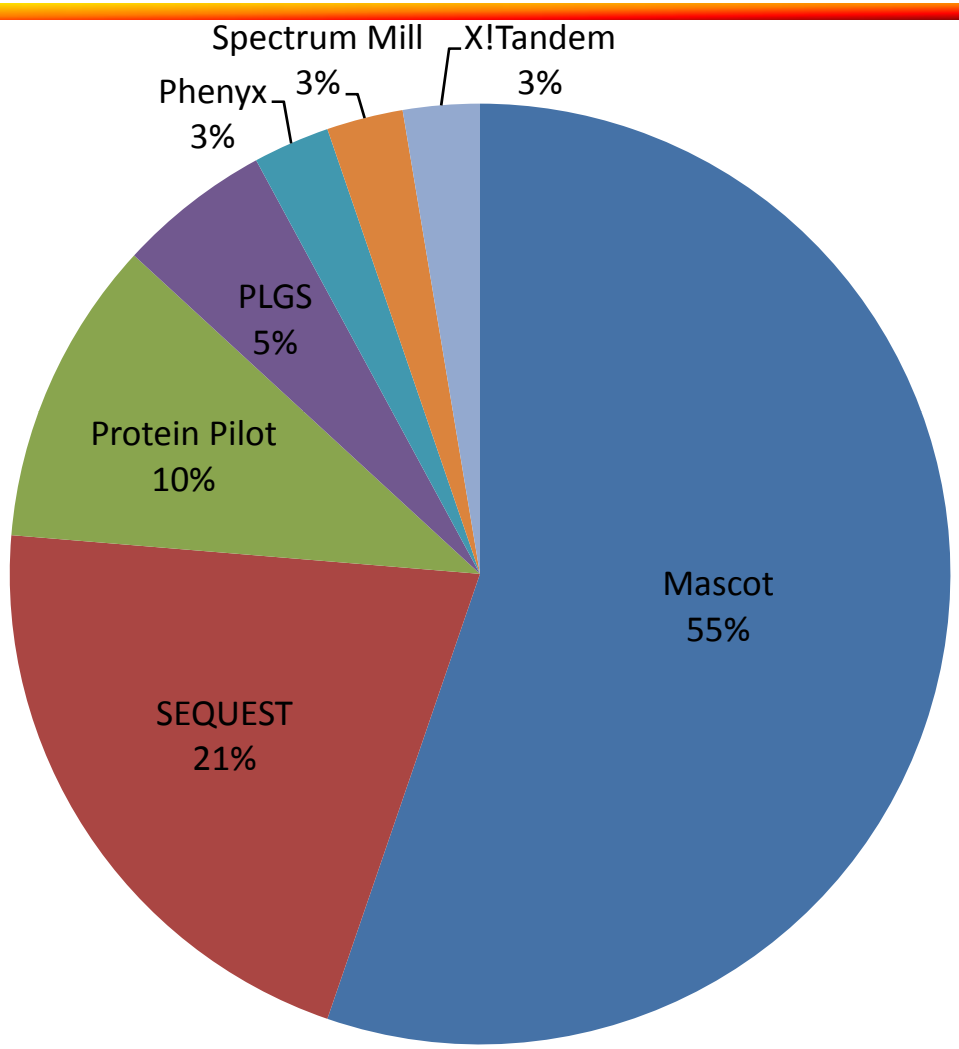
Use of Mass Spectrometry

PRG2010



Results: Software Used

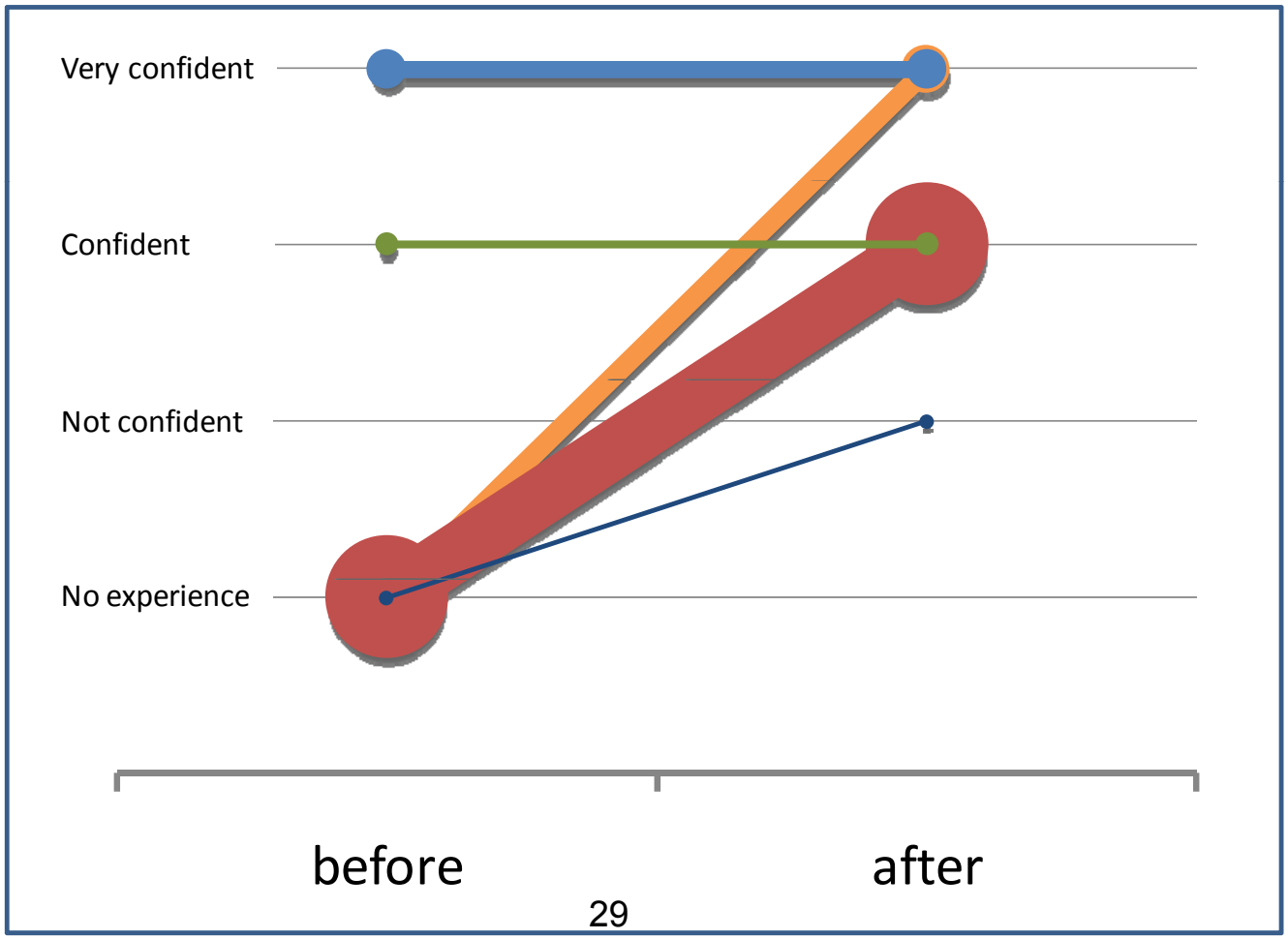
PRG2010



Results: Confidence for ^{15}N

PRG2010

Confidence in the identification of ^{15}N-labeled proteins before and after the study
- line thickness reflects participant response -



Motivation

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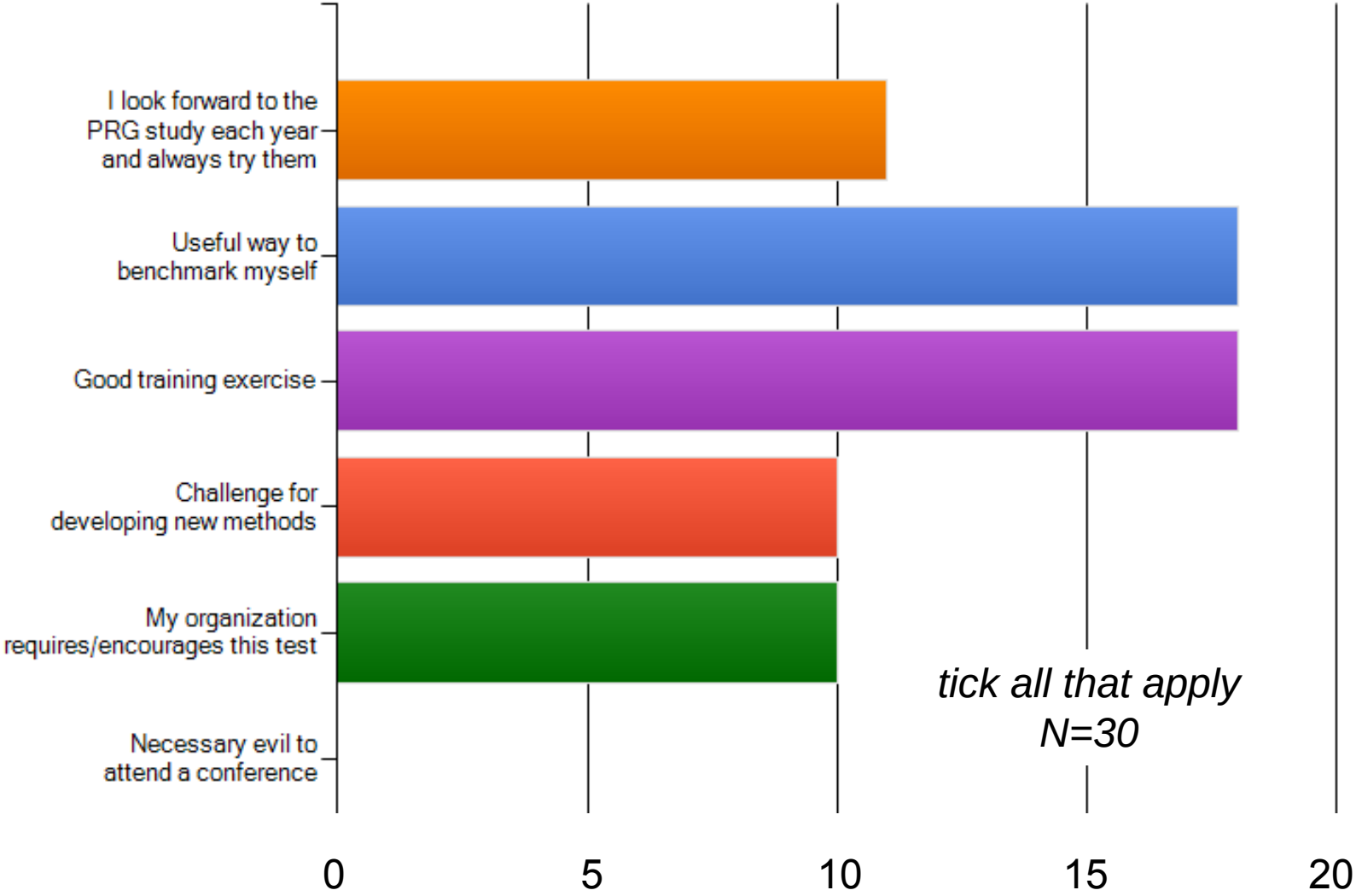
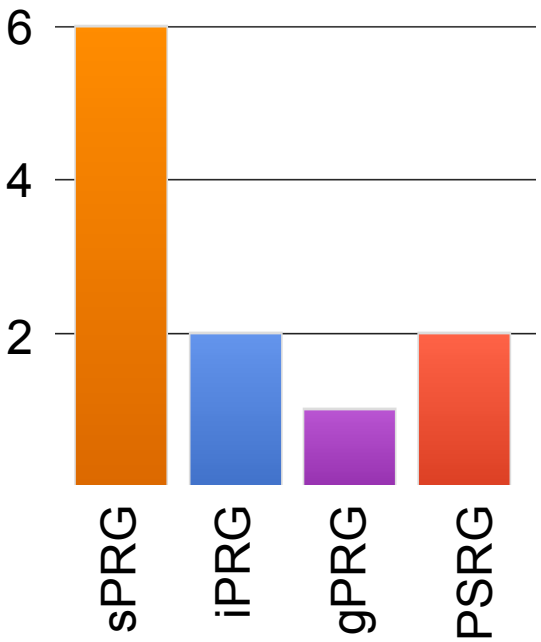


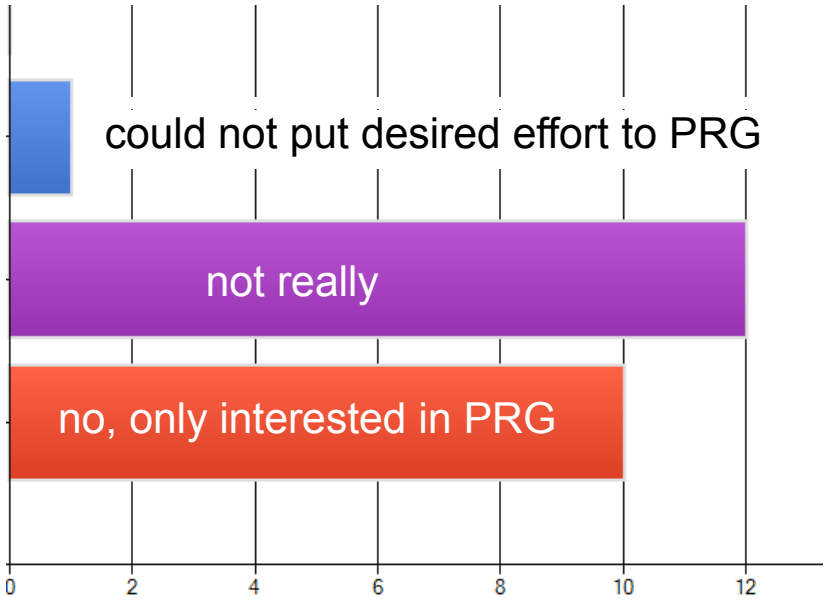
Other RG Studies

PRG2010

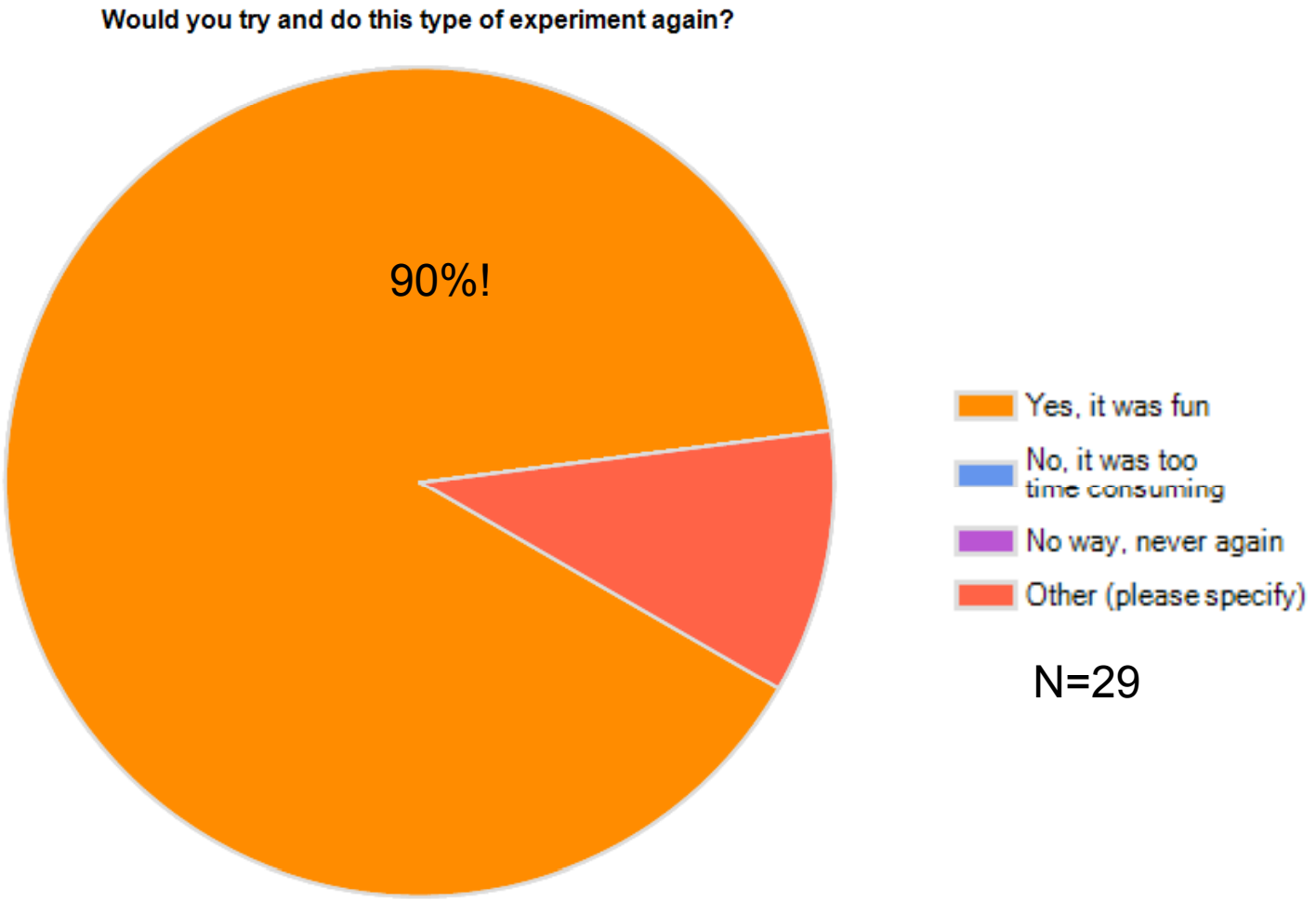
other RG participation
N=10



impact on PRG effort?
N=23



Do It Again?


Would you try and do this type of experiment again?
90%!
Yes, it was fun
No, it was too time consuming
No way, never again
Other (please specify)
N=29

Participants Comments

PRG2010

This year the organization was much better than other years. It was very helpful to have the survey in pdf. Congratulations!

Use a easier/more reliable survey software that saves entries and allows changes to be made.

In practice, I rarely accept a sample without consulting the client first.

Difficulty due to the low amount of proteins the protein bands were almost invisible even if the gels were stained with colloidal Coomassie for > 4 days.

If you did not complete or perform the survey, why not?

PRG2010

DOH! I totally forgot!! (0)

I really thought we were going to get a T-shirt or something ... (0)

Acknowledgements

PRG2010

Sample Supply
Yoana Dimitrova
Walter Chazin

JBC Papers in Press. Published on February 24, 2010 as Manuscript M109.049411
The latest version is at http://www.jbc.org/cgi/doi/10.1074/jbc.M109.049411

DIRECT UBIQUITINATION OF β-CATENIN BY SIAH-1 AND REGULATION BY THE EXCHANGE FACTOR TBL1

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Study Dedicate to Lidia Dimitrova

Vanderbilt
Sarah Stuart
Salisha Hill

Stanford
Maurizio Splendore

MD Anderson
Bih-Fang Pan

RTI
Xinxin Zhang

Members of the ABRF MIRG

Thank You!