

PRG2010 Research Study

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.



Research • Technology
Communication • Education

PRG2010

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

- * **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

- 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

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

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

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

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

- **PRG2009:** A targeted relative protein quantification study relevant for a biomarker validation project.
- **PRG2008:** Identify differences between two samples that have different lengths of the same protein sequence
- **PRG2007:** Relative abundance of 12 proteins spiked into an *E. coli* lysate between 3 different samples
- **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

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

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

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

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

- **PRG2010:** Tackling unforeseen problems in otherwise straight-forward proteomics analyses.
- **PRG2009:** A targeted relative protein quantification study relevant for a biomarker validation project.
- **PRG2008:** Identify differences between two samples that have different lengths of the same protein sequence
- **PRG2007:** Relative abundance of 12 proteins spiked into an *E. coli* lysate between 3 different samples

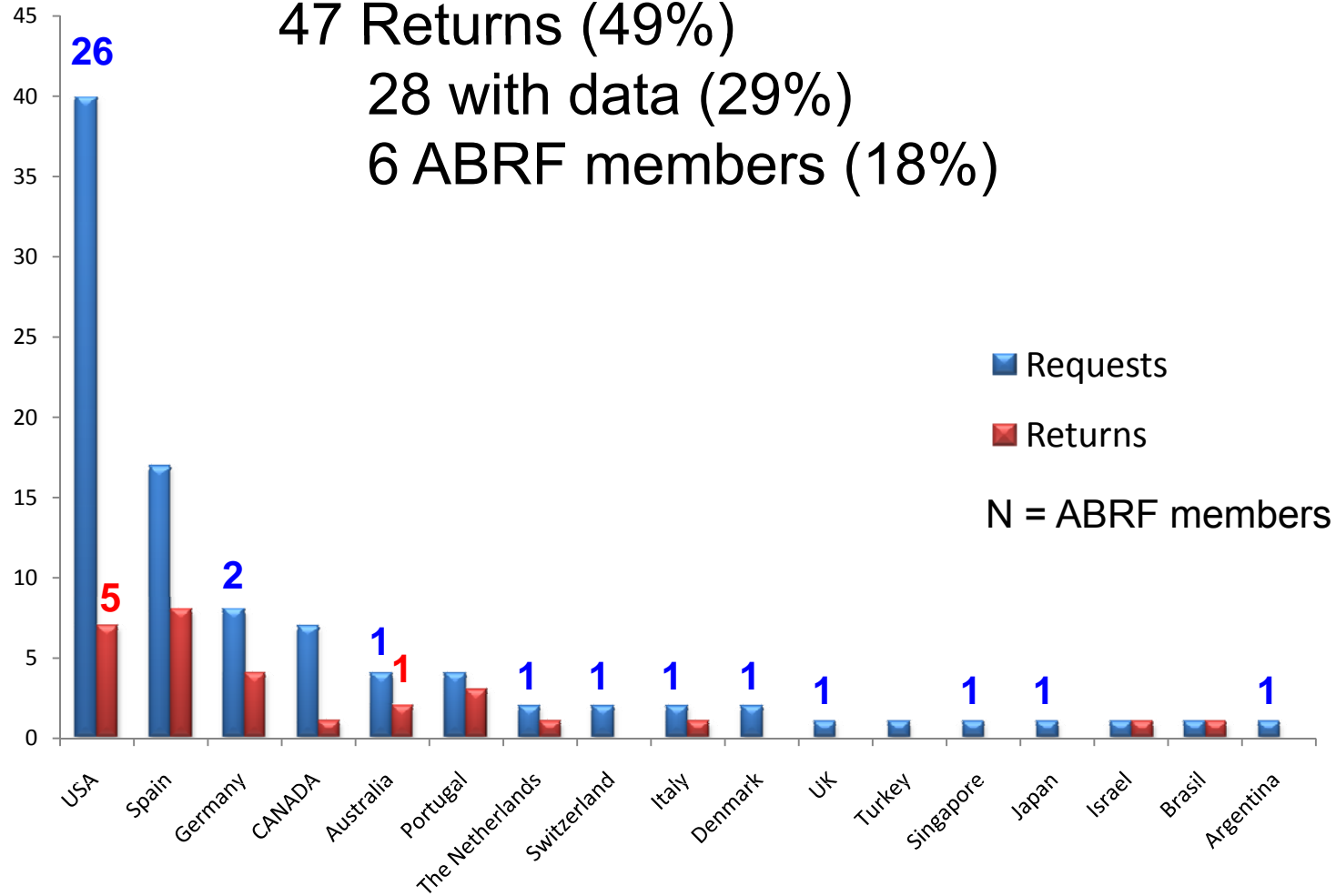
Requests & Returns

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

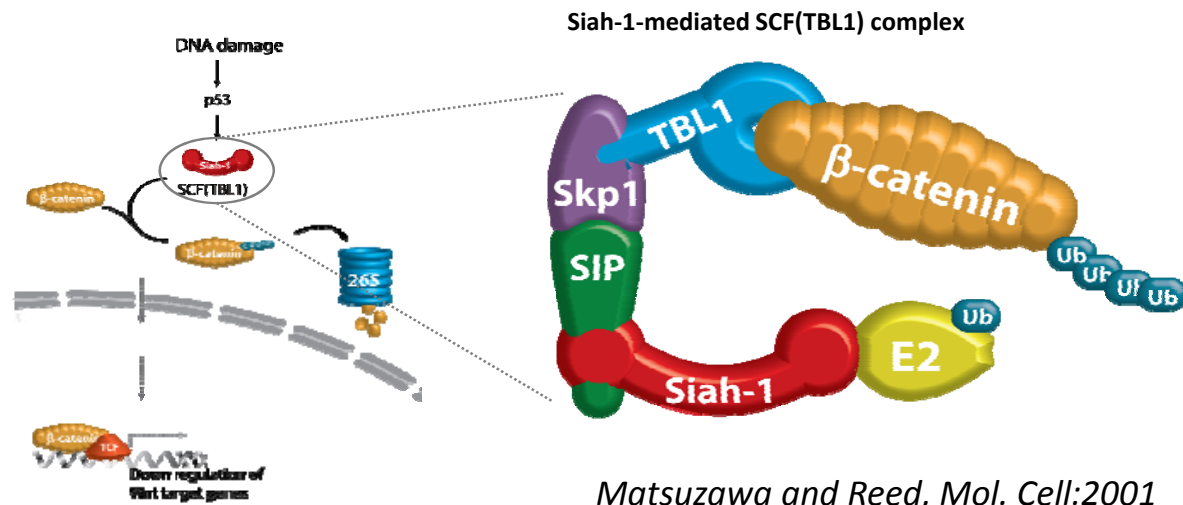
Tbl1 (Transducin β -like 1)

Skp1

SIP (Siah1-interacting protein, CYBP)

S100-A6

ubiquitin



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).



Research • Technology
Communication • Education

PRG2010

Extensive Sample Testing

CTNB1_HUMAN (134-781, upper)

```

1 MATQADLMEL DMAMEPDRKA AVSHWQQQSY LDSGIHSGAT TTAPSLSGKG
51 NPEEEDVDTS QVLYEWEQGF SQSFTQEQVA DIDGQYAMTR AQRVRAAMFP
101 ETLDEGMQIP STQFDAAHPT NVQRLAEP SQ MLKHAVVNLI NYQDDAELAT
151 RAIPELTKLL NDEDQVWNK AAVMVHQLSK KEASRHAIMR SPQMVAIVR
201 TMQNTNDVET ARCTAGTLHN LSHHREGLLA IFKSGGIPAL VKMLGSPVDS
251 VLFYAITTLH NLLLHQEGAK MAVRLAGGLQ KMWALLNKTN VKFLAITTDC
301 LQILAYGNQE SKLILILASGG PQALVNMIRT YTYEKLLWTT SRVLKVLVSC
351 SSNKPAIVEA GGMQALGLHL TDPQRSQVQN CLWTLRNLSD AATKQEGMEG
401 LLGTLVQLLG SDDINVTCA AGILSNLTCN NYKNRMVQC VGGIEALVRT
451 VLRAGDREDI TEPAICALRH LISRHOEAE AQNAVRLHYG LPVVVKLLHP
501 PSHWPLIKAT VGLIRNLALC PANHAPLREQ GAIPRLVQLL VRAHQDTQRR
551 TSMGGTQQQF VEGVRMEEIV EGCTGALHIL ARDVHNRIVI RGLNTIPLFV
601 QLLYSPIENI QRVAAGVLC E LAQDKEAEEA IEAEGATAPI TELLSRNEG
651 VATYAAAVLF RMSDFKPDY KRRLSVELTS SLFRTEPMAW NETADLGLDI
701 GAQGEPLGYR QDDPSYRSFH SGGYQDALG MDPMEHEMG GHHPGADYFV
751 DGLPDLGHAQ DLM DGLPDPG SNQLAWFDTD L

```

CTNB1_HUMAN (134-781, lower)

```

1 MATQADLMEL DMAMEPDRKA AVSHWQQQSY LDSGIHSGAT TTAPSLSGKG
51 NPEEEDVDTS QVLYEWEQGF SQSFTQEQVA DIDGQYAMTR AQRVRAAMFP
101 ETLDEGMQIP STQFDAAHPT NVQRLAEP SQ MLKHAVVNLI NYQDDAELAT
151 RAIPELTKLL NDEDQVWNK AAVMVHQLSK KEASRHAIMR SPQMVAIVR
201 TMQNTNDVET ARCTAGTLHN LSHHREGLLA IFKSGGIPAL VKMLGSPVDS
251 VLFYAITTLH NLLLHQEGAK MAVRLAGGLQ KMWALLNKTN VKFLAITTDC
301 LQILAYGNQE SKLILILASGG PQALVNMIRT YTYEKLLWTT SRVLKVLVSC
351 SSNKPAIVEA GGMQALGLHL TDPQRSQVQN CLWTLRNLSD AATKQEGMEG
401 LLGTLVQLLG SDDINVTCA AGILSNLTCN NYKNRMVQC VGGIEALVRT
451 VLRAGDREDI TEPAICALRH LISRHOEAE AQNAVRLHYG LPVVVKLLHP
501 PSHWPLIKAT VGLIRNLALC PANHAPLREQ GAIPRLVQLL VRAHQDTQRR
551 TSMGGTQQQF VEGVRMEEIV EGCTGALHIL ARDVHNRIVI RGLNTIPLFV
601 QLLYSPIENI QRVAAGVLC E LAQDKEAEEA IEAEGATAPI TELLSRNEG
651 VATYAAAVLF RMSDFKPDY KRRLSVELTS SLFRTEPMAW NETADLGLDI
701 GAQGEPLGYR QDDPSYRSFH SGGYQDALG MDPMEHEMG GHHPGADYFV
751 DGLPDLGHAQ DLM DGLPDPG SNQLAWFDTD L

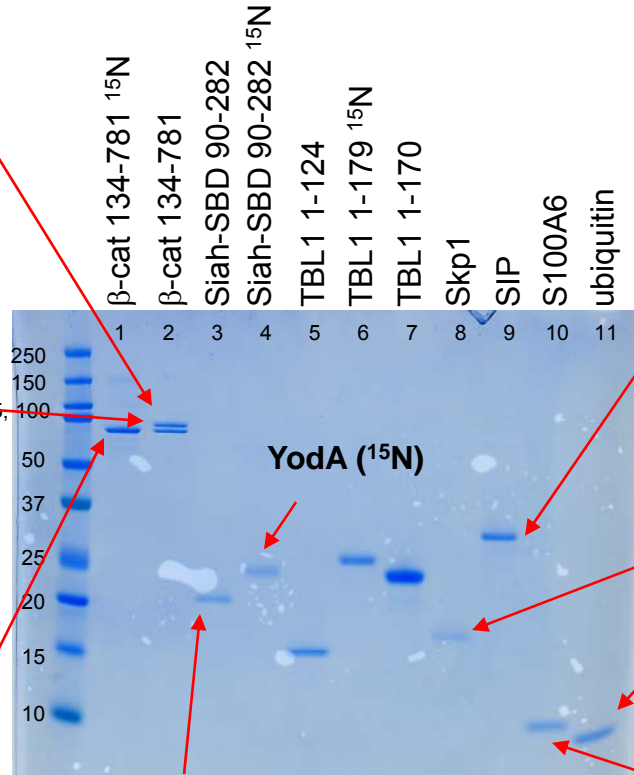
```

CTNB1_HUMAN (¹⁵N, 134-781)

```

1 MATQADLMEL DMAMEPDRKA AVSHWQQQSY LDSGIHSGAT TTAPSLSGKG
51 NPEEEDVDTS QVLYEWEQGF SQSFTQEQVA DIDGQYAMTR AQRVRAAMFP
101 ETLDEGMQIP STQFDAAHPT NVQRLAEP SQ MLKHAVVNLI NYQDDAELAT
151 RAIPELTKLL NDEDQVWNK AAVMVHQLSK KEASRHAIMR SPQMVAIVR
201 TMQNTNDVET ARCTAGTLHN LSHHREGLLA IFKSGGIPAL VKMLGSPVDS
251 VLFYAITTLH NLLLHQEGAK MAVRLAGGLQ KMWALLNKTN VKFLAITTDC
301 LQILAYGNQE SKLILILASGG PQALVNMIRT YTYEKLLWTT SRVLKVLVSC
351 SSNKPAIVEA GGMQALGLHL TDPQRSQVQN CLWTLRNLSD AATKQEGMEG
401 LLGTLVQLLG SDDINVTCA AGILSNLTCN NYKNRMVQC VGGIEALVRT
451 VLRAGDREDI TEPAICALRH LISRHOEAE AQNAVRLHYG LPVVVKLLHP
501 PSHWPLIKAT VGLIRNLALC PANHAPLREQ GAIPRLVQLL VRAHQDTQRR
551 TSMGGTQQQF VEGVRMEEIV EGCTGALHIL ARDVHNRIVI RGLNTIPLFV
601 QLLYSPIENI QRVAAGVLC E LAQDKEAEEA IEAEGATAPI TELLSRNEG
651 VATYAAAVLF RMSDFKPDY KRRLSVELTS SLFRTEPMAW NETADLGLDI
701 GAQGEPLGYR QDDPSYRSFH SGGYQDALG MDPMEHEMG GHHPGADYFV
751 DGLPDLGHAQ DLM DGLPDPG SNQLAWFDTD L

```



SiahH1_HUMAN 90-282 (¹⁴N) E3 Ub-ligase

```

1 MSRQTATALP TGISKCPSPQ RVPALIGITA SNNDLASLEE CPVCFDYLVP
51 PILQQCSGHL VCSNCRPKLT CCPTCRGPLG SIRNLAMEKV ANSVLFPCY
101 ASSGCEITLP HTEKADHEEL CEFPRYSCPC PGASCKWQGS LDVMPHLMH
151 OHKSITTLQ EDIVFLATDI NLPGAVDVMW MQSCFGFHEM LVLEKQEKYD
201 GHQOFFAIVQ LIGTRKQAE N FAYRLELNHG RRRLTWEATP RSIHEGIATA
251 IMNSDCIVFD TSIQLFREN GNLGINVIIS MC

```

CYBP_MOUSE (SIP)

```

1 MASVLEELQK DLEEVKVLLE KSTRKRLRDT LTSEKSKIEI ELKNKMQQKS
51 QKPELDNEK PAAVVAPLTT GYTVKISNYG WQSDKPKVI YITLTGVHCV
101 PTENVQVHFT ERSFDLLVKN LNKGNYSMIV NLLKLPISVE SSSKPKVTDI
151 VILLCRKKAE NTRWDYLTQV EKECKEKEKP SYDTEADPSE GLMNVLKIY
201 EDGDDDMKRT INKAWVESRE KQAREDETF

```

Skp1_HUMAN

```

1 MPSIKLQSSD GEIFEVDVEI AKQSVIITKM LEDLGMDDG DDDPVPLPNV
51 NAAILKKVIQ WCTHHKDDPF PPEDDENKEK RTDDIPVWDQ EFLKVDQGITL
101 FELILAAANYL DIKGLLDVTC KTVANMIKCK TPEEIRKTFN IKNDFTEEE
151 AQVRKENQWC EEK

```

ubiquitin

```

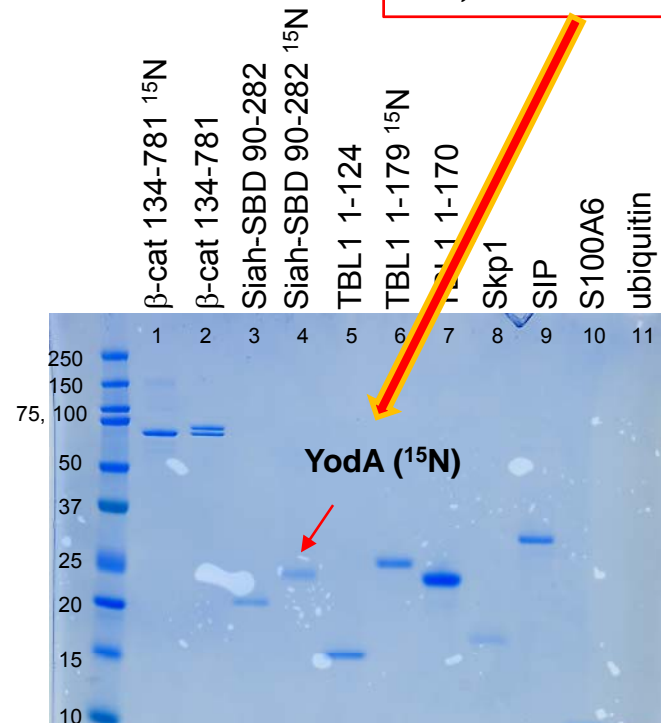
1 MQIFVKLTIG KTIITLEVPS DTIENVKAKI QDKEGIPDPQ QRLIPAGKQL
51 EDGRITLSDYN IQKESTLHLV LRLRGG

```

S100-A6

Extensive Sample Testing

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

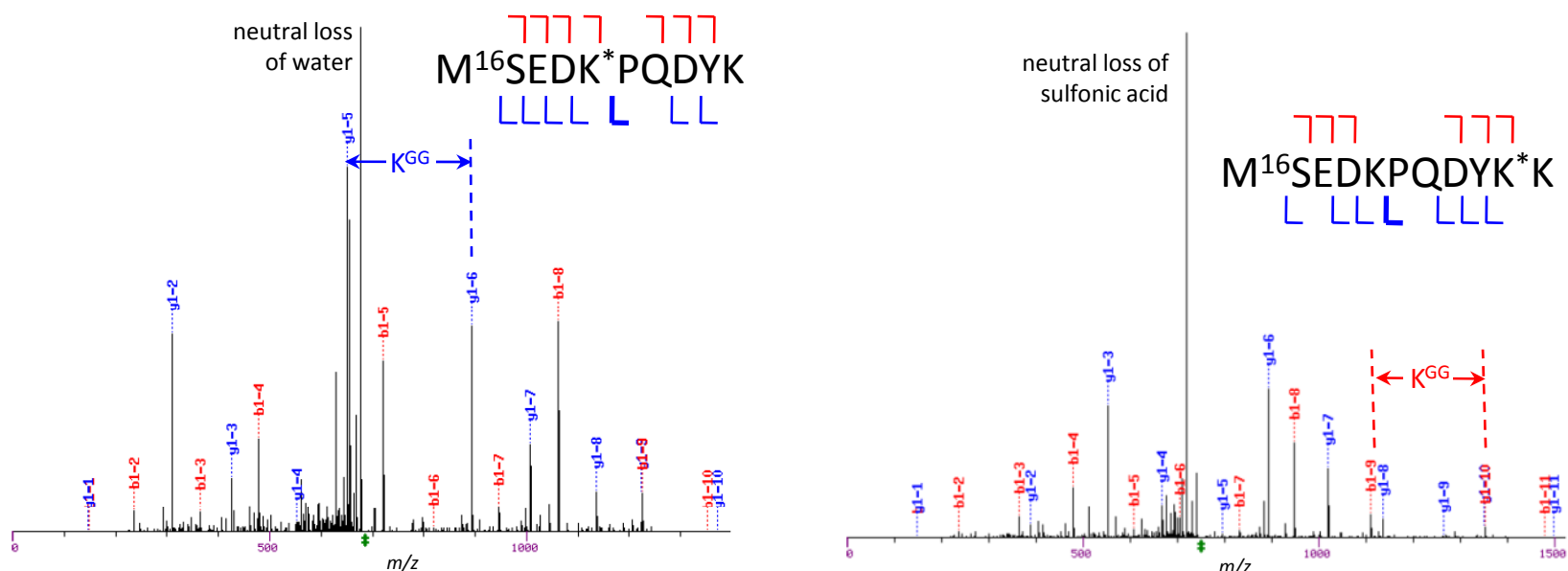


Biochimica et Biophysica Acta 1760 (2006) 1304 – 1313

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

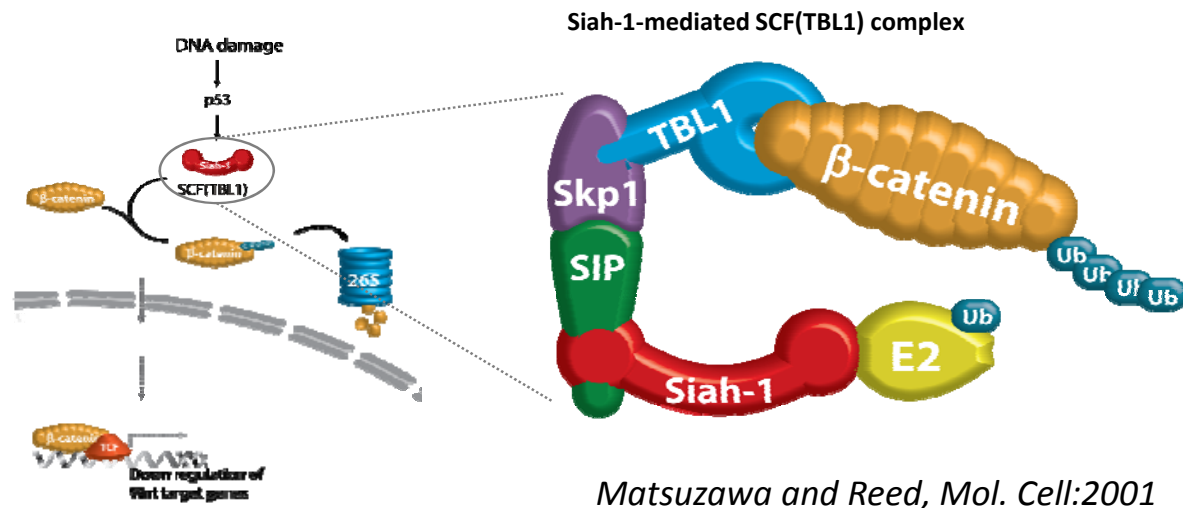


Note from presentation: This slide demonstrates that the proteins actually DO form an active complex, but due to the scarcity of this sample, we could not include this aspect into the study. The samples sent out either “could or could not produce an active complex”, but none of the samples contained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

The Saga of Dr. Quickhands

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.



The Saga of Dr. Quickhands

“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

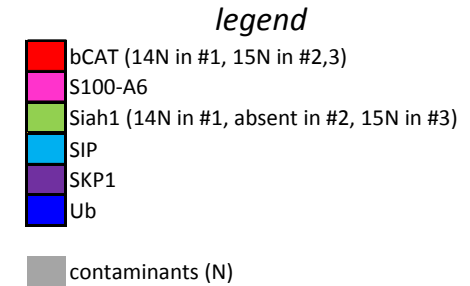
Protein	Coverage	Peptides	Spectral Counts
CTNB1_HUMAN	52	33	44
Cntm_P35527 K1C9_HUMAN	25	10	10
UBIQ_HUMAN	78	9	13
Cntm_P04264 K2C1_HUMAN	13	8	8
CYBP_HUMAN	27	10	14
Cntm_P13645 K1C10_HUMAN	12	6	6
SKP1_HUMAN	35	7	15
SIAH1_HUMAN	19	7	10
Cntm_P13647 K2C5_HUMAN	3	3	3
S10A6_HUMAN*	31	3	4

**more challenging to detect!*

Results: Tube 1 – simple ID

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

participant	Sample 1							# IDs Tube 1
	bCAT	S100-A6	Siah1	SIP	SKP1	Ub	contaminants (N)	
20091	1	1	1	1	1	1	2	6
12358	2	1	1	1	1	1		6
71415	1	1	1	1	1	1		6
73648	1	1	1	1	1	1	1	6
34621	1	1	1	1	1	1		6
72327	1	1	1	1	1	1		6
18018	2		1	1	1	1	1	5
97319	2		2	2	2	2		5
26402			1	1	1	1		4
14125	1		1	1	1	1	1	5
v11297	1		1	1	1	1		5
20032	1		1	1	1	1	5	5
71613	1		1	1	1	1		5
36918	1		1	1	1	1	5	5
27479	2		1	1	1	1		3
20139	1	2	1	1	1	1	2	6
30603	1	1	1		1	1	4	5
28475	1		1	1	1	1	2	5
12727	1		1	1	1	1		5
29850	1		1	1	1	1		5
29754	2	1		2	1	1		4
15973	1		1	1	1	1		4
40385	1		1			1		3
27774							10	0
46012								



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

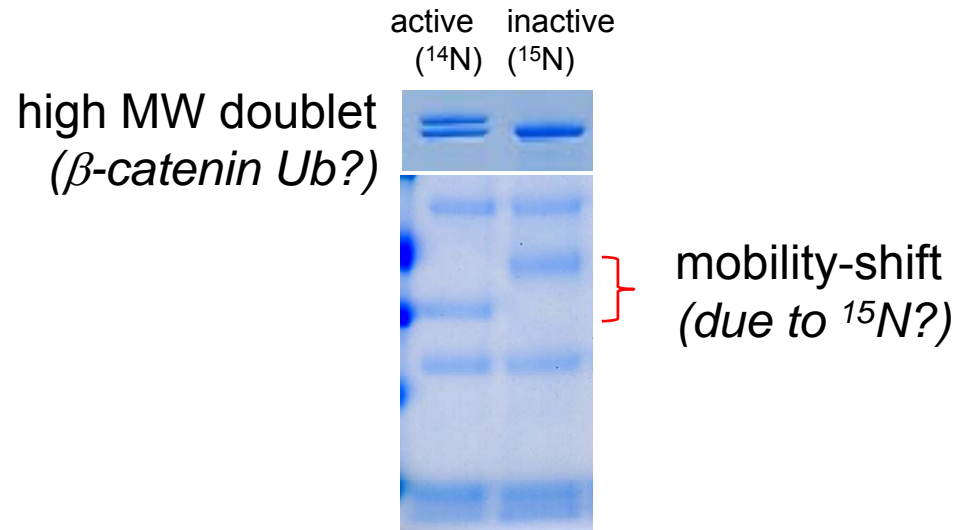
The Saga of Dr. Quickhands

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 2-¹⁵N and a contaminant

PRG2010

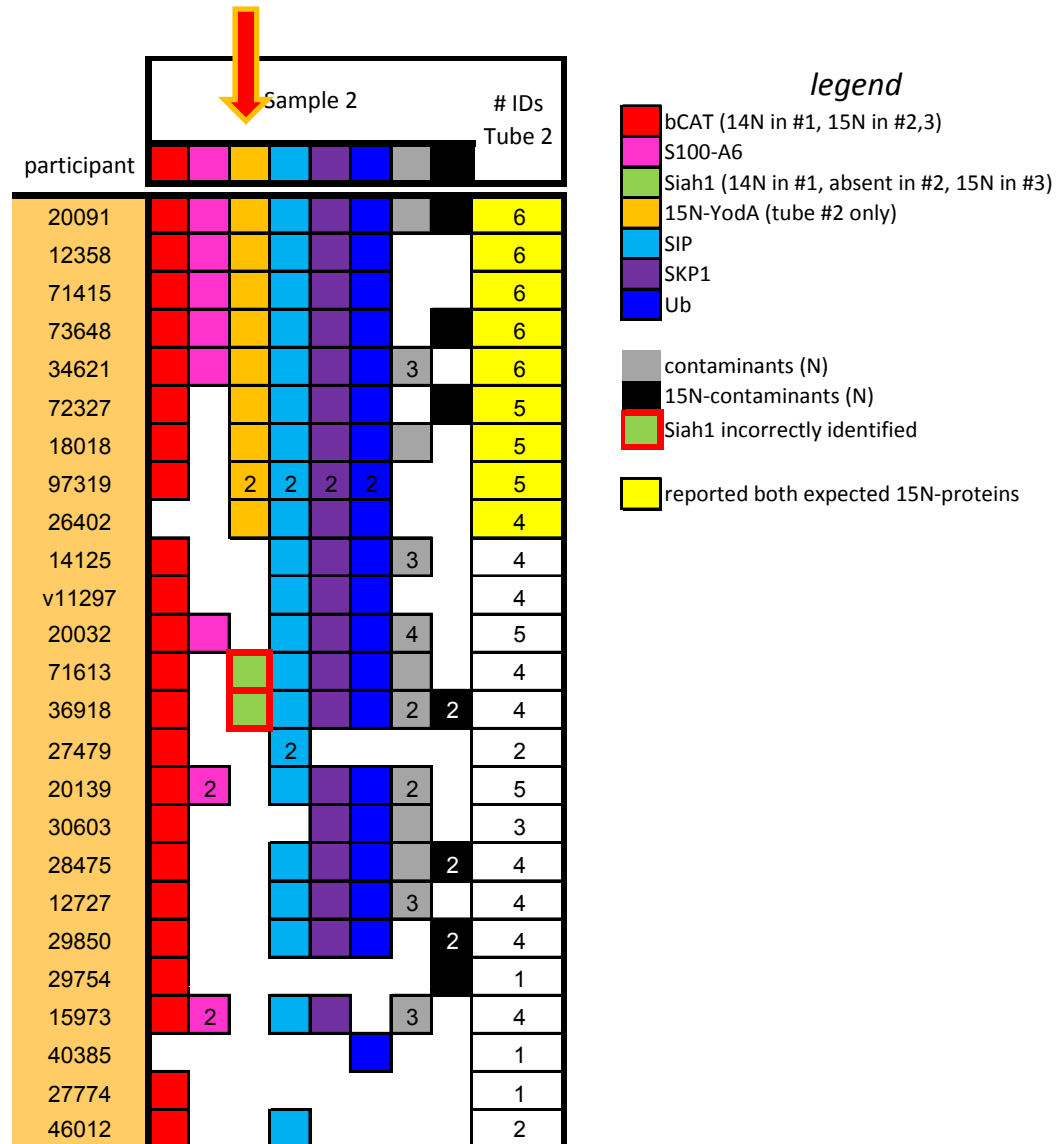
“Dr. Quickhands now makes ¹⁵N β-catenin and Siah1 for NMR and repeats assay, but now the isolated complex is **INACTIVE** for the *in vitro* ubiquitination assay”



¹⁵N β-catenin



But ¹⁵N Siah1 is really ¹⁵N *E. coli* YodA



Notes From Presentation:

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

Results: Tube 1 – the Doublet

PRG2010

CTNB1_HUMAN

```

1 MATQADLMEL DMAMEPDRKA AVSHWQQSY LDSGIHSGAT TTAPSLSGKG
51 NPEEEDVDTS QVLYEWEQGF SQSFTQEQVA DIDGOYAMTR AQRVRAAMFP
101 ETLDEGMQIP STQFDAAHPT NVQRLAEPQ MLKHAVVNLI NYQDDAELAT
151 RAIPELTKLL NDEDQVVVVK AAVMVHQLSK KEASKRRAIK SPQMVSAIVR
201 TMQNTNDVET ARCTAGTLHN LSHHREGLLA IFKSGGIPAL VKMLGSPVDS
251 VLFYAITTLH NLLHQEGAK MAVRLAGGLQ KMWALLNKTN VKFLAITTDC
301 LQILAYGNQE SKLIILASGG PQALVNIMRT YTYEKLWTT SRVLKVLVSV
351 SSNKPAIVEA GGMQALGLHL TDPSQRLVQN CLWTLRNLSD AATKQEGMEG
401 LLGTLVQLLG SDDINVVTC AAGILSNLTCN NYKNKMMVQC VGGIEALVRT
451 VLRAGDREDI TEPICALRH LISRHQEAE AQNAVRLHYG LPVVVKLLHP
501 PSHWPLIKAT VGLIRNLALC PANHAPLREQ GAIPRLVQLL VRAHQDTQRR
551 TSMGGTQQQF VEGVRMEEIV EGCTGALHIL ARDVHNRIVI RGLNTIPLFV
601 QLLYSPINI QRVAAGVLC LAQDKAAEA IEAEGATPL TELLSRNEG
651 VATYAAAVLF RMSEDKPDY KKRLSVELTS SLFRTEPMAW NETADLGLDI
701 GAQGEPLGYR QDDPSYRSFH SGGYGQDALG MDPMMEHEMG GHHPGADYVP
751 DGLPDLGHAQ DLMDGLPPGD SNQLAWFDTD L
  
```

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	✗

HAVVNLIYWDDELATR

full-length

active (14N) inactive (15N)

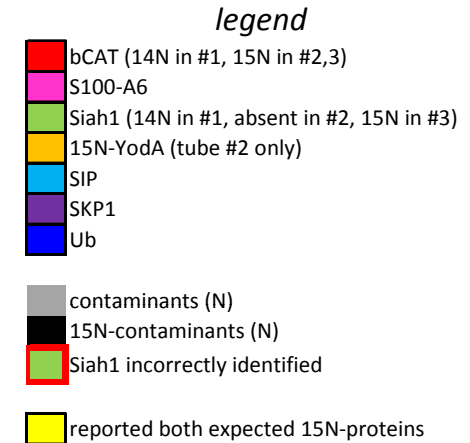
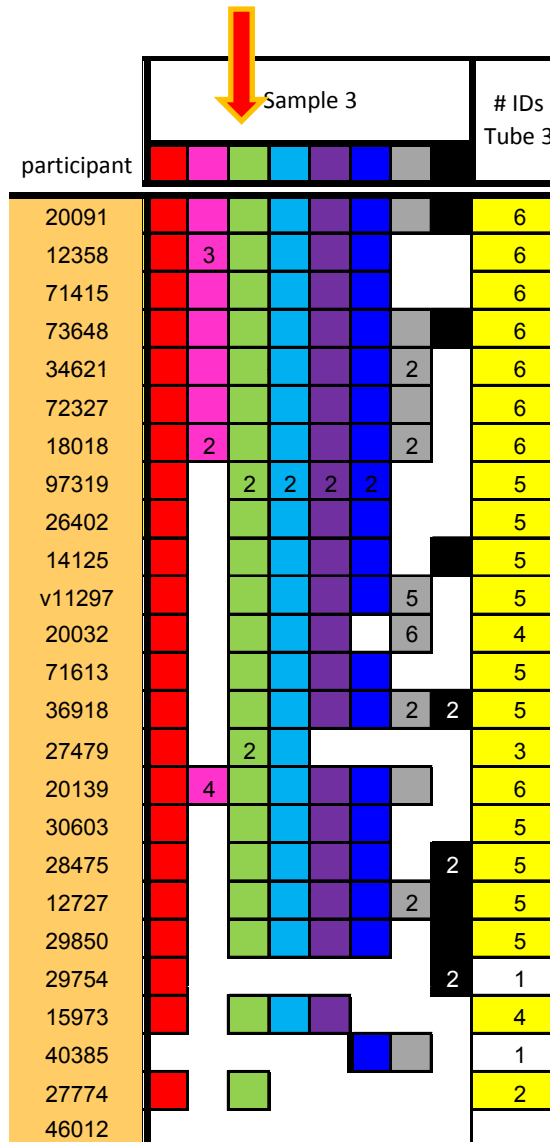


β-cat 134-781 fusion

GGILHAVVNLIYWDDELATR

Results: Tube 3 – All Restored

*“Third time is the charm!
Dr. Quickhand’s new ¹⁵N-labeled β -catenin, Siah1 complex is now ACTIVE!*



Results: Main Challenges

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 happened in Tube 2 that is now restored in Tube 3? in each tube (with the ¹⁵N contaminant twist)?

Properly Identify S100-A6?

Results: Self-Evaluation

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 happened in Tube 2 that is now restored in Tube 3? in each tube (with the ¹⁵N contaminant twist)?

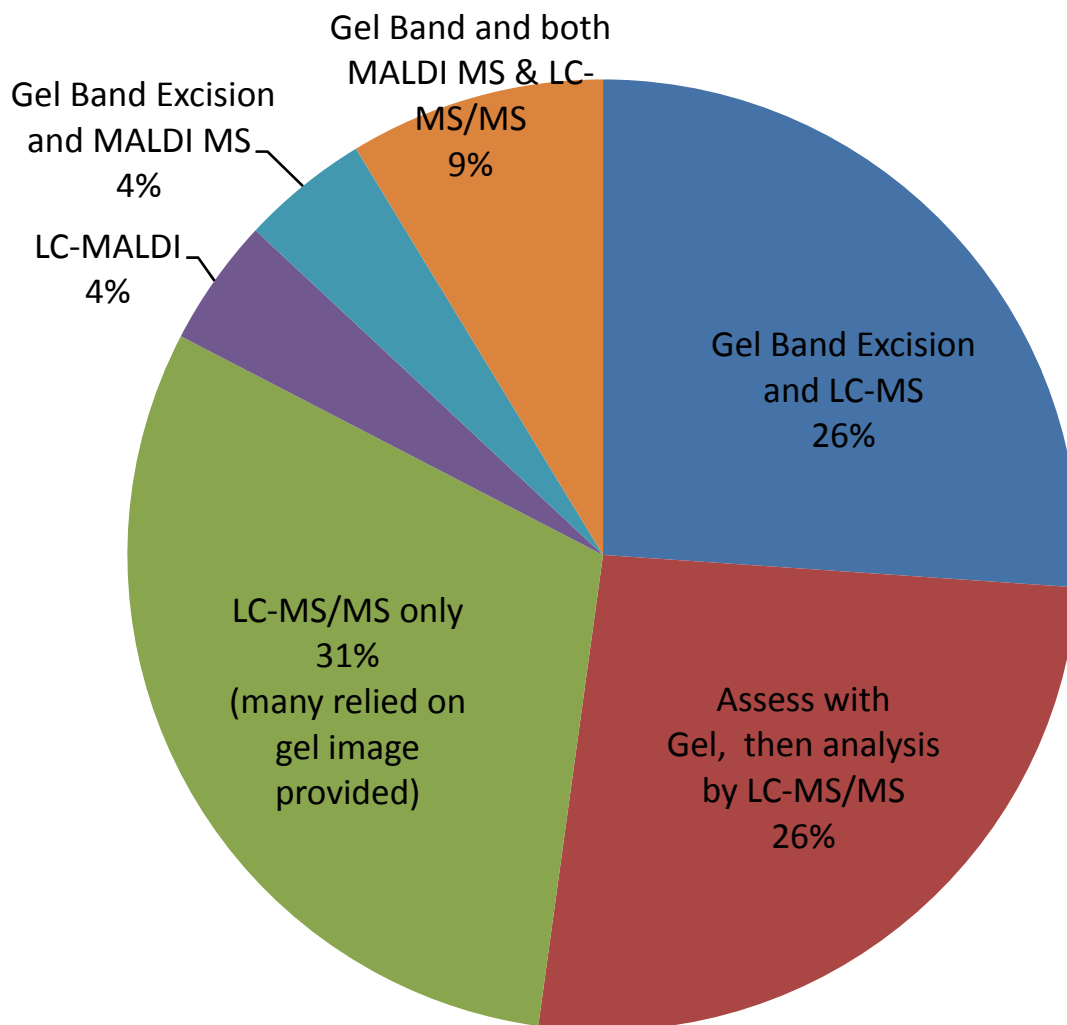
Properly Identify S100-A6?

Results: Self-Evaluation

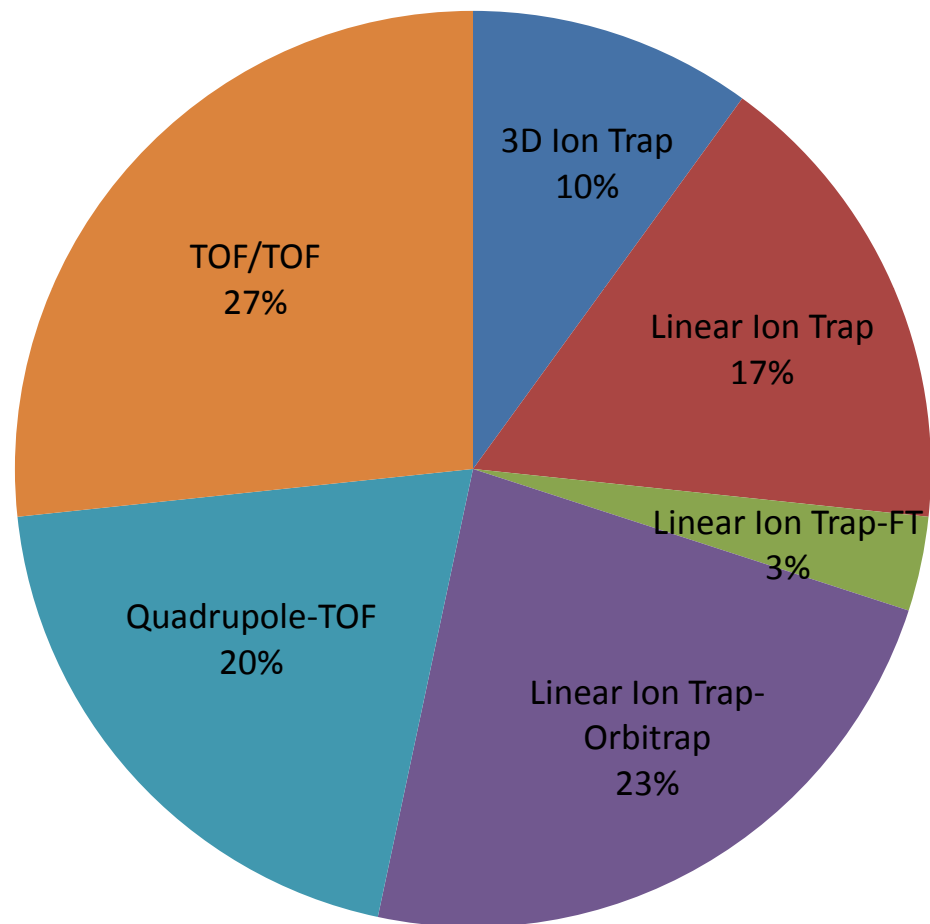
participant	DOUBLET	Yoda	S100A6	Difficulty	Experience	Time to complete study	General approach	Mass spectrometer used
20091	✓	✓	✓	Moderate	<6 months	2-4 days	Gel + LC-MS	Quadrupole-TOF
12358	✓	✓	!	Moderate	3-5 years	5-7 days	Gel + ESI-MS	3D Ion Trap
71415	✗	✓	✓	Moderate	3-5 years	2-4 days	Gel + LC-MS	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		✗	✗	Hard	5-10 years	2-4 days	Gel + MALDI MS	TOF/TOF

General Experimental Approach

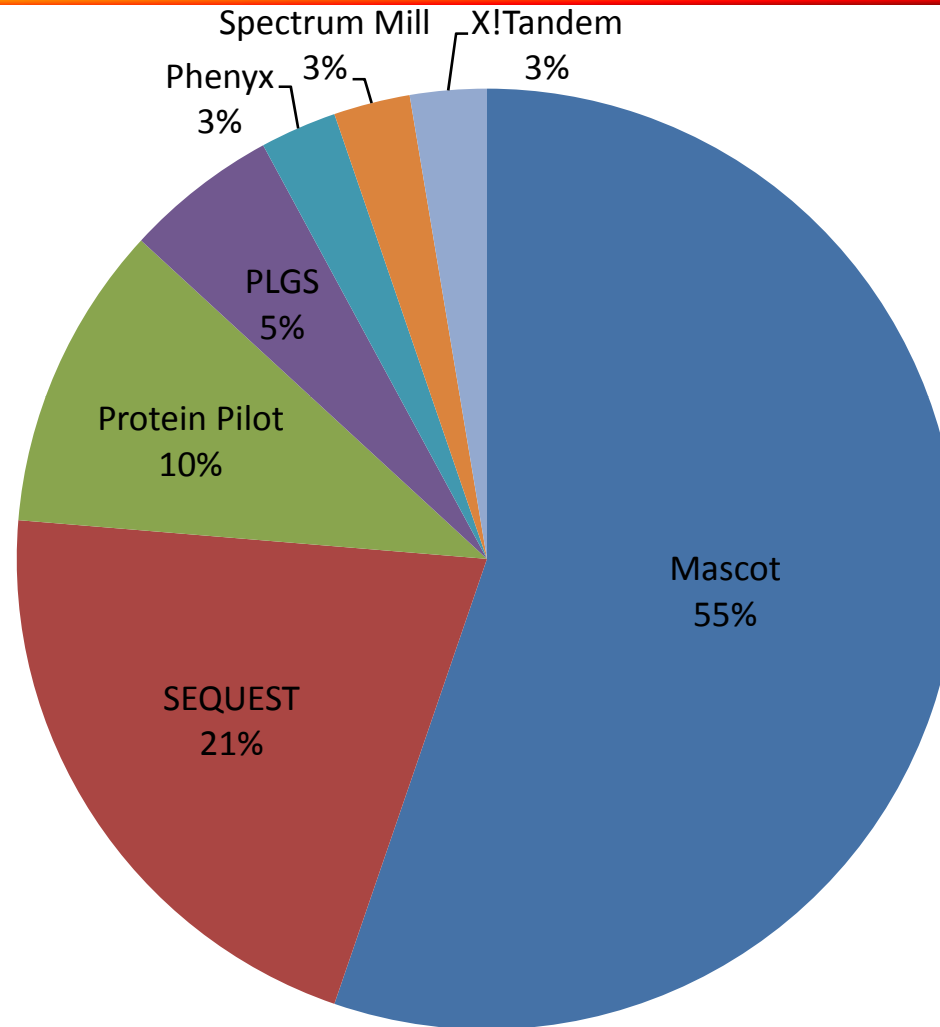
PRG2010



Use of Mass Spectrometry

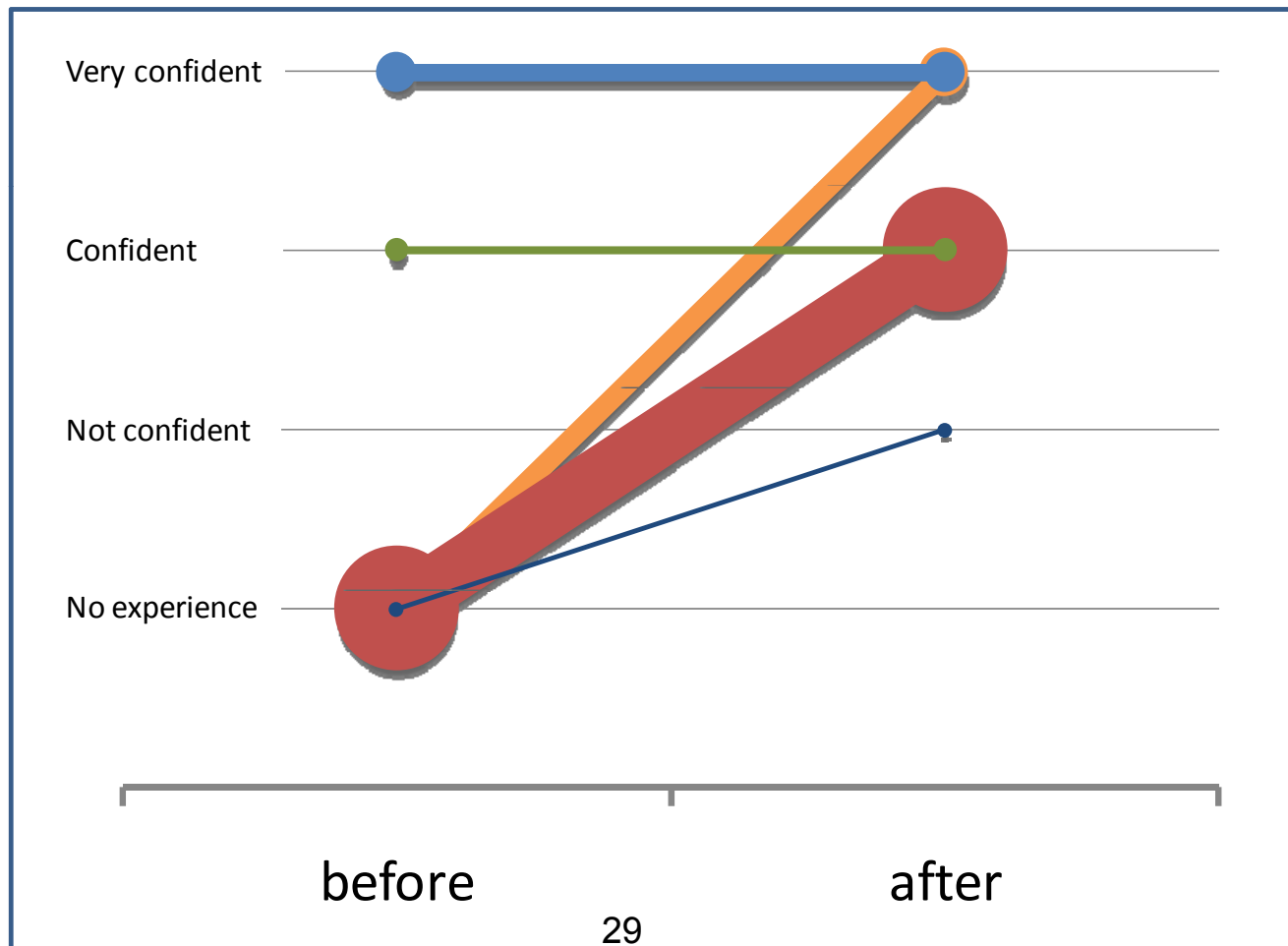


Results: Software Used

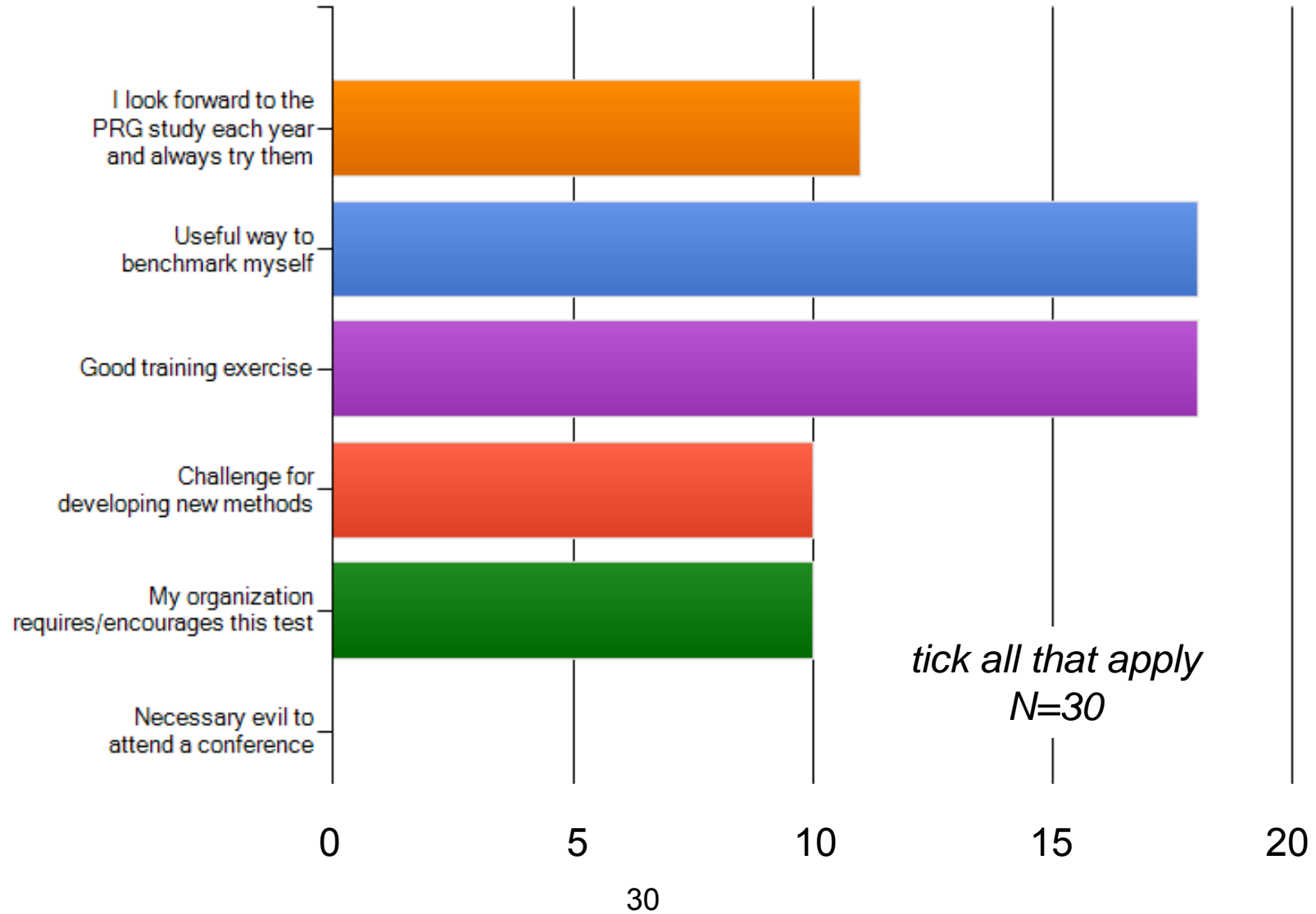


Results: Confidence for ¹⁵N

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

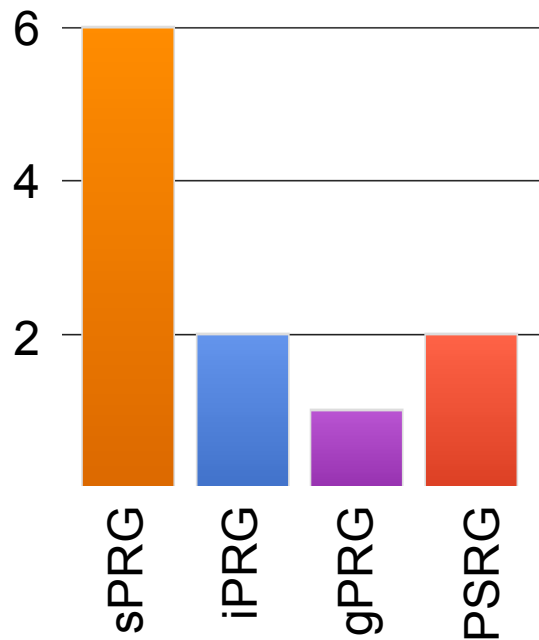


Motivation

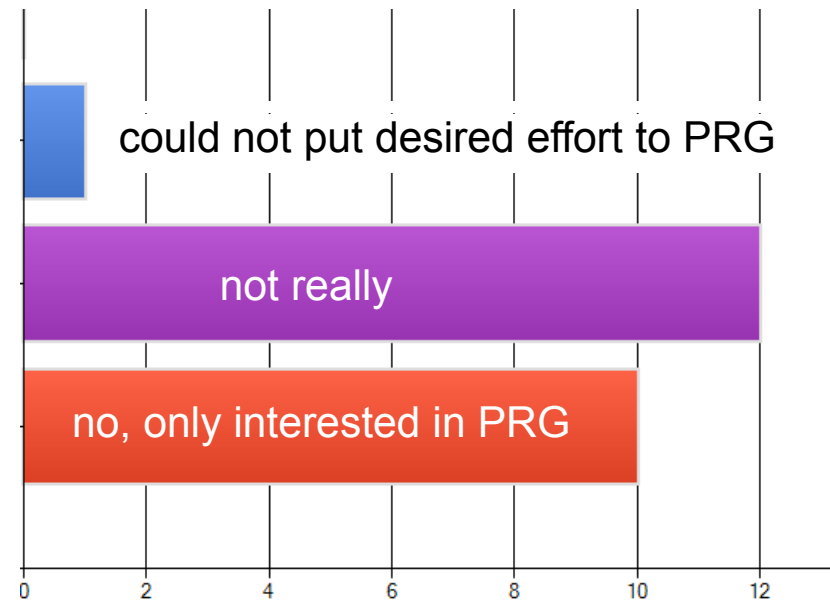


Other RG Studies

other RG participation
N=10

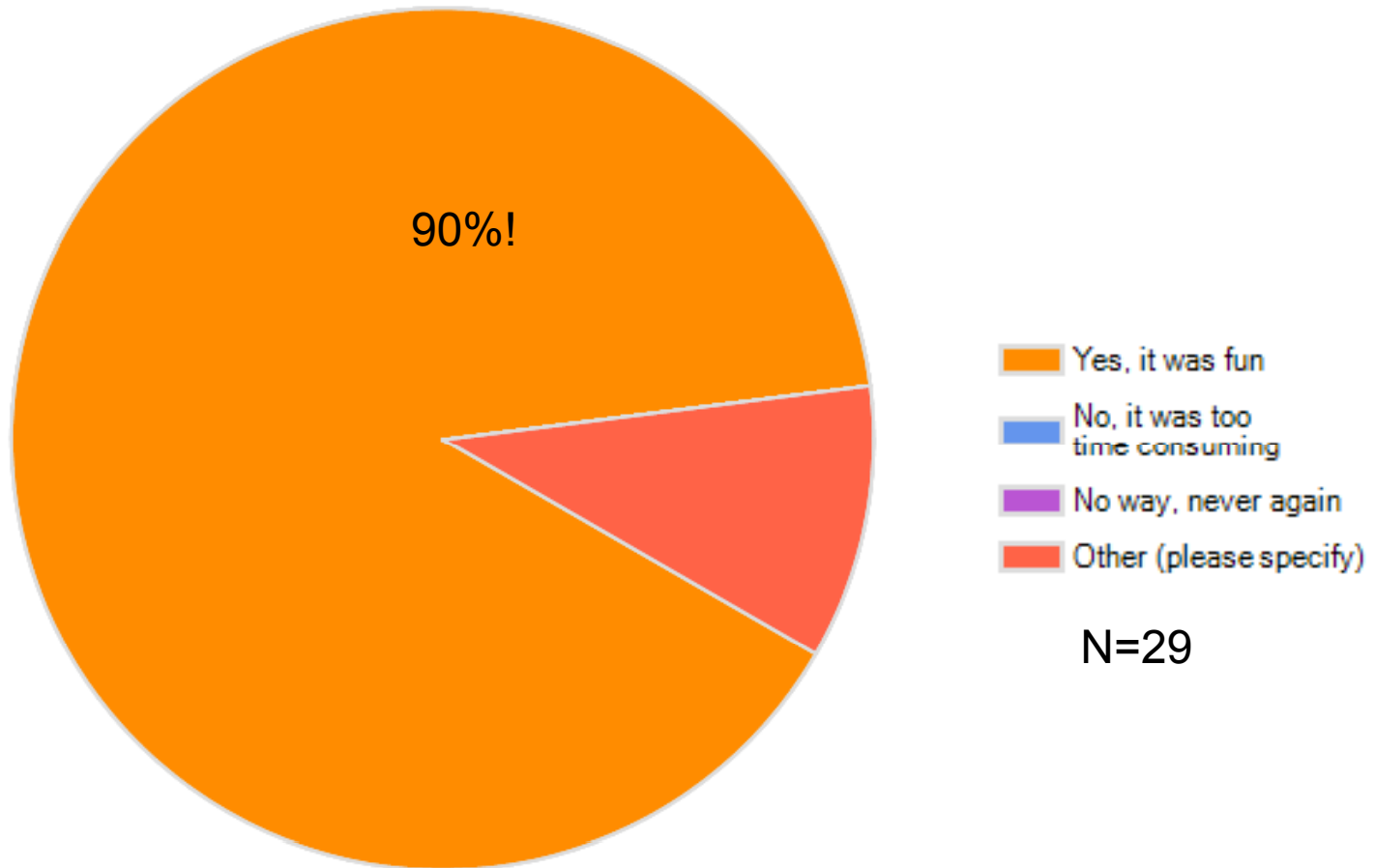


impact on PRG effort?
N=23



Do It Again?

Would you try and do this type of experiment again?



Participants Comments

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?

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DOH! I totally forgot!! (0)

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

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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

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Members of the ABRF MIRG

Thank You!