



Association of Biomolecular Resource Facilities

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Dear PRG2010 Study Participant:

Thank you for agreeing to participate in the “PRG2010 Proteomics Study” conducted by the ABRF Proteomics Research Group (PRG). Accompanying this letter are three sample vials. If for some reason the samples did not arrive in good condition, please notify the PRG by sending an e-mail to **ABRF.PRG2010@gmail.com**. Results returned by January 8, 2010, will be included in the PRG presentation at the 2010 ABRF Meeting (March 20-23, 2010, Sacramento, CA) and will be posted electronically.

Please read the following scenario and related information carefully before planning your studies. Representative gel images and running conditions are available on the PRG study website, www.abrf.org/PRG. The following scenario was adapted from an actual study.

PRG2010 Study Scenario:

Dr. Quickhands has spent a great deal of time working out the purification of an active ubiquitination complex based on the Siah1 E3 ligase using beta-catenin as a substrate. Both Siah1 and beta-catenin are bacterially-expressed from fusion proteins (see below). Dr. Quickhands wants to determine the other components present in this active complex, as well as characterize the structure of the complex by NMR.

She first presents you with a preparation that yields high ubiquitination activity when used in her *in vitro* assay (Tube 1; lane 1 on gel images), and asks you to identify the other 3 to 4 proteins that she thinks are present in this active complex (“I sometimes see three or four extra bands on a gel, but they are weak”).

To prepare for the NMR structural studies, Dr. Quickhands next performs the same purification that led to the previous active complex in Tube 1, but this time uses ¹⁵N labeled Siah1 and ¹⁵N labeled beta-catenin. Alas, this time the complex (Tube 2; lane 2 on gel images) is not active! Dr. Quickhands runs a gel with the active and non-active complexes, and notices a slight band-shift for one protein (which she attributes to ¹⁵N labeling), and a high MW doublet that is present in Tube 1 but absent in Tube 2 (maybe responsible for activity?).

Dr. Quickhands then makes a third preparation (again with the ¹⁵N-labeled Siah1 and ¹⁵N-labeled beta-catenin) and now the complex is active again (Tube 3; lane 3 on gel images). She notices that the high MW SDS-PAGE doublet is still gone in the active Tube 3, so she assumes that it was not responsible for the activity in Tube 1. But she would still like to understand what this doublet may represent, as well as why the reaction in Tube 2 didn't work.

Perplexed (but happy that her assay is working again), Dr. Quickhands quickly runs down the hall and comes to you to help her figure out what is different between the three complexes.

Specific questions to focus on:

- (1) Identify the contents of Tube 1 (“I sometimes see three or four extra bands on a gel, but they are weak”).
- (2) Identify what is different in Tube 2 that might explain why the reaction failed.
- (3) Identify what is restored in Tube 3 that might explain why the reaction is functional again.
- (4) What is the nature of the unusual doublet that is present only in Tube 1, but does not seem to be related to function?

*** Note: these samples contain protein complexes that were either active or inactive when used in the *in vitro* ubiquitination assay, but beta-catenin should NOT be ubiquitinated in these samples.

*** Note: some samples contain proteins grown in ¹⁵N-medium. Please refer to the PRG study website, www.abrf.org/PRG, for information on database searching ¹⁵N-labeled proteins.

Additional Sample Information.

The study samples are supplied in three vials, labeled “Tube 1”, “Tube 2”, and “Tube 3”, and consist of separate preparations of related human proteins. Human Siah1 and beta-catenin were expressed in *Escherichia coli* (grown in either ¹⁴N- or ¹⁵N-medium); the expressed sequences are provided below (sequences in red were from the expression tags).

beta-catenin (CTNB1_HUMAN, P35222, sequences 134-781)

GGILHAVVNLINYQDDAELATRAIPELTKLLNDEDQVVVNKAAVMVHQLSKKEASRHAIMRSPQM
 VSAIVRTMQNTNDVETARCTAGTLHNLSSHREGLLAIFKSGGIPALVKMLGSPVDSVLFYAITTL
 HNLHLLHQEGAKMAVRLAGGLQKMVALLNKTNVKFLAITTDCQLILAYGNQESKLIILASGGPQAL
 VNIMRTYTYEKLLWTTSRVLKVLSSCNKPAIVEAGGMQALGLHLTDPSQRLVQNCWTLRNL
 DAATKQEGMEGLLGLTLVQLLGSDDINVVTCAAGILSNLTCNNYKNKMMVCQVGGIEALVRTVLRA
 GDREDITEPAICALRHLTSRHQEAEMAQNAVRLHYGLPVVVKLLHPPSHWPLIKATVGLIRNLAL
 CPANHAPLREQGAI PRLVQLLVRAHQDTQRRTSMGGTQQQFVEGVRMEEIVEGCTGALHILARDV
 HNRIVIRGLNTIPLFVQLLYSPIENIQRVAAGVLCELAQDKEAAEAIEAEGATAPL TELLHSRNE
 GVATYAAAVLFRMSSEDKPQDYKKRLSVELTSSLFRTPEMAWNETADLGLDIGAQGEPLGYRQDDP
 SYRSFHSGGYGQDALGMDPMEHEMGGHHPGADYPVDGLPDLGHAQDLMDGLPPGDSNQLAWFDT
 DL

Siah1 E3 ligase (Siah1_HUMAN, Q8IUQ4, sequences 90-282)

MGSSHHHHHHSSGLVPRGSHVANSVLFPCKYASSGCEITLPHTEKADHEELCEFRPYSCPCPGAS
 CKWQGS�DAVMPHLMHQHSITTLQGEDIVFLATDINLPGAVDWVMMQSCFGFHFMLVLEKQEKY
 DGHQQFFAIVQLIGTRKQAEINFAYRLELNHRRRLTWEATPRSIHEGIATAIMNSDCLVFDTSIA
 QLFAENGNLGINVTISMC

The samples we have provided were prepared from aqueous solutions that also contained small amounts of salts. To the best of our knowledge, there are no appreciable quantities of interfering

substances that contain primary amines and/or free thiols. Please consult the PRG website, www.abrf.org/PRG, for examples of sample reconstitution and representative 1-D SDS-PAGE gels.

Returning Results. Submission of results will be via an on-line survey, details for which will follow in a separate mailing in late-November. In addition, we are asking participants to voluntarily provide a short summary of the approach(es) used and the key results obtained, which will be anonymously available through our website after the study is completed. Based on these write-ups, exceptional strategies may be selected for inclusion in our presentation at the annual meeting in Sacramento in March, 2010.

PRG2010 Study Mission Statement. The main goals of the 2010 PRG study are for participants to assess their own capabilities relative to other labs performing similar tasks, as well as share best-practices and methods throughout the scientific community. The samples for this study attempt to represent a realistic experiment that might be submitted to a core facility or a collaborator, and should be quickly and successfully analyzed using a wide variety of proteomic methodologies performed by participants at a variety of skill levels. At the same time, we anticipate that the study will have some aspects that are challenging enough to expand the skills of the participants, regardless of expertise.

Any updates about the study will be posted on the ABRF PRG website: www.abrf.org/PRG. If you have any questions, please e-mail the PRG at ABRF.PR2010@gmail.com. If you wish to send an anonymous inquiry or comment, please email the PRG at ABRF.PR2010.anonymous@gmail.com. We thank you for your support of the ABRF and look forward to your participation in this study.

Please Consider Joining the ABRF! The ABRF is a voluntary international society that supports education through Research Group studies such as this one, as well as workshops, research, networking and scientific exchange with members and commercial vendors at the annual meeting and throughout the year. It relies on an active membership, which should include YOU! If you're not a member, please read all about membership [benefits](#) and other activities of the ABRF at www.abrf.org, and consider [joining today!](#)

Sincerely,

The ABRF Proteomics Research Group

David B. Friedman (Chair) - Vanderbilt University

Tracy M. Andacht – Centers for Disease Control and Prevention

Maureen K. Bungler – RTI International

Allis S. Chien - Stanford University

David Hawke – UT MD Anderson Cancer Center

Jeroen Krijgsveld - EMBL

Rob Moritz – Institute for Systems Biology

Bob Settlage – Virginia Bioinformatics Institute

Chris W. Turck (EB Liaison) - Max Planck Institute of Psychiatry

Vendors and Commercial Service Labs

PLEASE NOTE:

If you are a vendor or commercial service lab and wish to participate in this study, please read the following:

ABRF Research Group Studies are conducted for the benefit of our members and the field at large to help them evaluate their own technical level in comparison to their colleagues, to provide education in techniques and strategies to which they normally might not be exposed, and to give an overview of the current capabilities of the “average” lab in carrying out a challenging analysis.

The ABRF welcomes the participation of vendors and for-profit labs, provided that they abide by the ABRF guidelines for the use and distribution of data derived from these studies, as follows:

An ABRF Research Study is not a competition and under no circumstances should it be referred to as such. Words and phrasing that imply a competition – such as a ‘winner’, ‘best of’, etc., are strictly forbidden.

Representations and publications should not be deceptive and should fairly emphasize any differences between any data comparisons. For example, instrument reliability cannot be fairly concluded by comparing 5-year old instruments in the field used in the study with a vendor’s new instrument.

Any comparisons to or use of ABRF data should prominently indicate: the number of samples the vendor received, the number of runs performed by the vendor, and whether the actual characteristics of the sample were known by the vendor at the time the vendor’s analysis was performed.

Uses of or comparisons to ABRF data should specifically emphasize that many factors will affect analytical results and that the data obtained in the company’s R&D lab may exceed feasible expectations for an “average” resource or research facility under routine conditions.

Publications and presentations should contain a disclaimer stating that ABRF prepared and provided the sample to all members and vendors, but did not participate in the vendor’s study and does not endorse any specific manufacturer, instrument or strategy.

Vendors are strongly encouraged to distribute potential publications to the ABRF Executive Board and Research Group Chairperson for comments regarding compliance with these guidelines.

Recipient: We recommend that this document be distributed to the appropriate marketing and senior personnel in the company to ensure compliance. A copy of this document (Vendor ABRF Study Participation Guidelines.pdf) can be found at www.abrf.org under the Forms and Documents menu.