

## The NIH Common Fund Protein Capture Reagents Consortium Meeting

December 15-16, 2011  
Hyatt Regency Bethesda, MD

### Executive Summary

#### Summary of Action Items:

- 1) Working groups: volunteer for chair, select members, decide on frequency of meetings for each of the following groups:
  - a. Target list WG – production centers
    - i. Develop a mechanism for soliciting community input into the priority list.
  - b. Target list WG – tech dev centers
  - c. Validation WG – all centers (maybe production and tech dev split at a later point)
  - d. Data WG
    - i. what data to collect and present for each reagent
    - ii. “user front end”/public portal
- 2) NIH personnel to explore options to close antigen center funding gap (NIH program staff)
- 3) Develop a branding/communication plan.
- 4) Develop an antibody production report format by February 15, 2012. (NIH/Production Centers)
- 5) Steering committee – bimonthly/quarterly calls? Fold in any WGs (Data?) into this call to minimize number of meetings/maximize efficiency? (Brian Kay)
- 6) Set NIH/ESP teleconference meeting in 6 months time. (Leslie)
- 7) Create logo (Tina)
- 8) Generate an organizational chart (Tina)
- 9) Develop 2 year milestones (production and tech dev centers separately)

#### Purpose and Meeting Goals

The initial NIH Common Fund Protein Capture Reagents Consortium Meeting, gathered the Principal Investigators (PIs) of the Protein Capture Reagents Program to present research efforts and plans, and to work with NIH program staff and members of the Protein Capture External Scientific Panel (ESP) to structure a successful long-term Protein Capture Reagents program.

Protein capture reagents have been widely used in basic research and clinical applications. The need for developing a large library of protein capture reagents and for improving the technology for generating these reagents has been expressed at large by the scientific community. While a number of approaches to generating protein affinity reagents exist; current limitations of quality, cost and

throughput represent significant roadblocks to the development of a comprehensive and broadly applicable resource of renewable affinity reagents to all human proteins.

The overall goal of the first phase of the Protein Capture Reagents program (<http://nihroadmap.nih.gov/proteincapture/>) is to pilot whether a community resource of low cost, high quality, renewable affinity reagents can be generated for all human proteins, and to develop technologies that will lead to next generation platforms for producing renewable protein capture reagents. This pilot effort includes the generation of affinity reagents against human transcription factors as a test case. Thus, this pilot on its own is intended to produce a resource of considerable utility to the community; as human TFs represent an important sub-proteome, with major efforts underway worldwide, particularly to understand transcriptional regulation of genes for fundamental biological and disease knowledge.

### Meeting Highlights

The Meeting agenda (see attached) organized the proceedings into four general categories: 1) program highlights from Center PIs, 2) invited speaker presentations, 3) general discussions for implementing the Protein Capture Reagents Research Network, and 4) comments from the ESP.

- 1) Center PIs: After an introduction to meeting goals by NIH staff, the PIs of the three U54 production centers and four UO1 technology development centers (<https://commonfund.nih.gov/proteincapture/fundedresearch.aspx>) gave presentations of their respective programs, introduced their teams and highlighted expected outcomes from their Center's work.
- 2) Invited speakers: A number of key individuals involved in related efforts to the Protein Capture Reagents Program were invited to speak and offer their perspectives. Individuals included:
  - a. **Mike Taussig**, coordinator for the EC Affinomics Project, provided an overview of this "collaborative large scale integrating project" which has an overarching goal to generate and validate protein binders for characterization of "all" human proteins. This includes development of novel application tools and creation of public database portals. The Project has 15 partners and is funded in this latest round for 5 years (start April 2010) for 11M€.
  - b. **Mike Snyder** discussed the challenges of validating reagents against human transcription factors from the perspective of the ENCODE program. A major component of ENCODE is to map the binding sites of all TFs using ChIP-Seq. ENCODE has rigorous antibody characterization requirements ([http://compbio.med.harvard.edu/antibodies/documents/30MAY2010\\_mod-ENCODE\\_TF\\_Chrom\\_Data\\_Standards.pdf](http://compbio.med.harvard.edu/antibodies/documents/30MAY2010_mod-ENCODE_TF_Chrom_Data_Standards.pdf)) which can be considered in developing characterization/validation procedures for the Protein Capture Reagents program.
  - c. **Tara Hiltke** gave an overview of NCI's Antibody Characterization Program and Antibody Portal. The portal includes characterization data and serves as a public resource of monoclonal antibodies made available by NCI to the scientific community.
  - d. **David Sol** is the director of the Developmental Studies Hybridoma Bank (DSHB). The mission of the DSHB is to bank, maintain and distribute hybridomas and the monoclonal antibodies

they produce to the general scientific community at cost. DSHB is non-profit and self-supported. DSHB claims no intellectual property rights. The contributor retains IP, and can sell/commercialize the hybridoma separately. DSHB retains lifetime distribution rights to basic scientists.

- e. **Jim Trimmer** is the director of the UC Davis/NIH NeuroMab Facility (neuromab.ucdavis.edu). The facility is funded through NINDS/NIMH and has generated a catalog of “neuro” mouse mAbs against ~300 targets with more in the pipeline. Some conclusions/opinions offered by Dr. Trimmer include:
- Screen directly for desired functionality, don’t infer from other assays.
  - Be responsible for quality control on final product to be distributed to end users as this is all that really matters to your overall impact (and reputation). End users are generally not interested in doing your QC for you.
  - Branding is related to quality of reagent.
  - Be careful in relying too much on results from outside labs, they may not be timely and/or reliable. You can control your own schedule and quality.
  - Don’t stop at “capture” as the only desirable outcome for the Common Fund reagents which represent a huge investment and potentially an invaluable resource to biologists and basic biomedical researchers for labeling targets in immunoblots, immunohistochemistry, and immunocytochemistry (e.g., transcription factors represent some of the best markers for cell lineages). At least archive all monospecific reagents for future validation.

### 3) Discussions on Implementing the Protein Capture Reagents Network:

#### *Sessions:*

##### *i. Structure of Collaborations*

##### *a. Between production centers*

Points of discussion (from agenda):

- *Consensus target list.* This issue was first addressed on a recent teleconference with the production centers and NIH staff. A decision was made to form a **working group to decide on a target list** – what TFs and what domains of TFs to include on the list.
- *Quantity vs. quality.* *Should the Ag/Ab Production Centers devote extra attention to a subset of "high impact" anti-TF affinity reagents?* The group consensus was to emphasize quality; do not sacrifice quality for quantity. There was extended discussion over the question of “how do the antibody groups know they are finished”? No closure was reached. Each center might need to have multiple endpoints (eg. ChIP-Seq; integrate with real groups doing ChIP-Seq?). Maybe two endpoint categories: one ChIP-Seq and one other?
- *Sample coordination: overlap?* The antigen group will provide complete set of antigens to both antibody groups with some exceptions.
- *Who will be responsible for the databasing and IT chores to keep track of the affinity capture reagents being generated (along with associated basic data and characterization info), and how will this be merged and/or coordinated between the centers? A data working group will be*

**formed to address databasing/IT.** Guy Montelione and Joel Bader volunteered to be on group. This group should contain not only IT savvy individuals by end-users as well. Tech dev groups should be a part of this IT working group.

- *Impact of antigen center ending before antibody centers?* Action item for NIH personnel to explore options to close antigen center funding gap.
- *Possibility of use of joint validation/characterization resources?* Consensus was that Jef Boeke's protein array would be a great joint validation resource.
- *Standard naming conventions to ease communication/"branding".* Work with technology development centers to carry out a similar brand.
- *Production metrics/goals – for NIH reporting.* Action item to **develop a production report format (for antibody centers)** by February 15, 2012.
- *Communication between centers: project website?* Not discussed.
- *Communication to community: project website? Reagent deposition.* **Action item: explore options for developing a user "front end".** Need to decide on what data should be provided along with the reagents.
- It was decided to **hold production conference calls once per month** – to include the production centers and NIH staff.

*b. Between technology development centers*

- *How to coordinate interaction amongst technology development centers?* **The tech dev centers with NIH staff will hold quarterly calls.**
- *Identifying a set of common antigens for all technology centers.* Consensus was to decide on 20 biotinylated proteins (10 "good"/10 "bad"). Biotin is the easiest to incorporate. List should include a few TFs. Send 1 mg to each group. Guy Montelione volunteered to do this. **A working group should be formed to develop this common antigen set.**
- *What can be common validation criteria?* A common detection mechanism is needed in order to compare different capture reagents.
- *Should an independent validation laboratory that can characterize the selected affinity reagents against the common targets be identified?* (Notes missing here – tg)
- *Milestones and goals.* **Develop two year milestones.**
- *Informatics needs.* It is not clear at the moment if a common LIMS is needed. The NESG LIMS is very portable. It is based on SESAME (<http://www.sesame.wisc.edu>) and is free, open-source. Tony Kossiakov and Josh LaBaer use this too. We should get a SESAME expert involved.

*ii. Further Network Structure and Working Groups*

- Interactions between production and technology development centers:
  - Monthly calls of production centers; quarterly calls of tech dev centers.

- Steering committee. Brian Kay is chair. Frequency of meetings?
- NIH/ESP meeting every 6 months.
- All hands meeting once a year
- Branding – “CFPC” proposed as common nomenclature
- Formation of working groups:
  - *For defining consensus target list, and antigen transfer.* Production and tech dev centers will form separate WGs.
  - *For validation.* Separate groups for production/tech dev centers.
  - *For data working group.* May be best for this WG to go across both arms – production and tech dev centers. Data WG to: decide on what data to collect, save and present; decide on annotation; include db developers and users. Possible paper – “Minimum Information About a Protein Affinity Reagent (MIAPAR)”?
  - *For outreach.* Not discussed.
  - Action item: nominate chairs of WGs and decide of frequency of meeting.

*iii. Dissemination and Outreach*

- a. *Branding* – suggested the use of prefix “CFPC” (Common Fund Protein Capture). Develop a branding/communication plan.
- b. *Must set bars for inclusion in collection.* For each different application, need clear criteria. What is NCI criteria? This could be good starting criteria for CFPC.
- c. *Public nomination of capture reagents?* One suggestion was the use of the NIH RFI mechanism to solicit community input. Maybe the nominators would have to offer to do validations?  
**Action item: Develop a mechanism for soliciting community input into the priority list.**
- d. *Common website* – where all CFPC reagents are posted
  - i. Ideally to have link out to individual centers.
  - ii. PSKB (Protein Structure Knowledgebase) and Antibodypedia – can only search one protein at a time. PSKB – can download entire database – will want this same capability for DFPC.
  - iii. Can have mirror site with AntibodyPedia.
  - iv. Consider Protein Data Bank ([www.pdb.org](http://www.pdb.org)) as model.
- e. *Informing community*
  - i. As above, common website with mirrors.
  - ii. Presence at meetings. Not just reagent meetings but cell biology, TF meetings too. There is an upcoming antibody meeting in China.
  - iii. Need to have logo.
- f. **Generate an organizational chart of research network.**

- g. Goal: get some useful reagents into hands of users early (but not exclusive).
- h. Timeline: ~16 months (~April 2012) to first assessment of entire program.

4) ESP comments (delivered by ESP chair Jim Trimmer):

- Tremendously exciting idea whose time has come, coupled with expert investigators leading cutting edge projects.
- Should work towards integrating two arms: production and tech development.
- Within tech development arm: working group should provide self-defined concrete criteria for success/completion, and cross-project validations.
- Within production arm: integrating two antibody groups: Requirement for fresh versus frozen protein - if former requires precise coordination between antigen production and reagent production group, this need to be determined ASAP whether approach being used by antigen production arm will yield high-quality reagents.
- “Binder” production: working group should provide self-defined concrete criteria for initial release of products, as well as for completion of project. Define best proxy assay? IP efficiency (IP versus depleted lysate), IB, shRNA knockdown, stress that all should be from endogenous proteins in native cells and not over-expressors.
- Huge impact potential; downside is potential for introduction of incorrect information into the field/literature.
- How to prioritize targets? Input from community? Biological impact vs. practicality? Existing/popular reagents vs. ones where reagents do not exist?
- How to make name for this initiative?
- Work to get community to buy in and use reagents to show that this is a good investment by NIH.

## AGENDA

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### Day 1: Thursday, December 15

8:00-8:30 a.m.           **Registration and Continental Breakfast**

8:30-8:50 a.m.           **SESSION 1**

**Welcome, Overview and Meeting Charge**

Adam Felsenfeld, NHGRI and Salvatore Sechi, NIDDK

8:50-3:20 p.m.           **SESSION 2**

**The Protein Capture Reagents Centers**

*Moderator: Tina Gatlin, NHGRI*

The PIs and their teams will highlight their projects, identify network strengths, weaknesses, expertise, and expected outcomes (*25 min presentation, 5 min discussion*).

*Production Centers*

8:50-9:20 a.m.           Stephen Anderson, Rutgers University  
"Human Transcription Factors Immunogens: Generation of a Complete Set"

9:20-9:50 a.m.           Jef Boeke, Johns Hopkins University  
"Monospecific Monoclonal Antibodies Against Human Transcription Factors"

9:50-10:20 a.m.         Tony Kossiakoff, University of Chicago  
"Recombinant Antibody Network"

10:20-10:30 a.m.        *Open discussion* with production centers PIs

10:30-10:45 a.m.        **BREAK**

*Technology Development Centers*

10:45-11:15 a.m.        Andrew Bradbury, Los Alamos National Laboratory  
"A High Throughput Pipeline to Select Renewable Recombinant Polyclonal Antibodies"

11:15-11:45 a.m.        John Chaput, Arizona State University  
"A Pipeline for Production of Bivalent Synthetic Antibodies to the Human Proteome"

11:45- 12:50 p.m.       **LUNCH (on your own)**

12:50-01:20 p.m. Brian Kay, University of Illinois-Chicago  
"Technology Development for Recombinant Affinity Reagents"

1:20-1:50 p.m. Tom Soh, UC Santa Barbara  
"QPASS: Quantitative Parallel Aptamer Selection System"

1:50-2:00 p.m. *Open discussion* with technology development centers Pls

2:00-2:10 p.m. **The NIH Common Fund**  
James Anderson, Director, DPCPSI

2:10-4:45 p.m. **SESSION 3**

**Implementing the Protein Capture Network –Structure of Collaborations**

2:10-2:40 p.m. **Keynotes:**  
Mike Taussig, Babraham Biosciences Technologies  
"Affinomics – an EC FP7 Collaborative Project"

2:40-3:10 p.m. Mike Snyder, Stanford University  
"Challenges in the Protein Capture Reagents Technology Development and in the Validation of Affinity Reagents Against Human Transcription Factors"

3:10-3:25 p.m. **BREAK/Consortia Photo**

3:25-4:45 p.m. **Breakouts**  
A. Collaboration between production centers  
*Moderators: Stephen Anderson and Nancy Freeman, NIDCD*

Points of Discussion:

- Consensus target list.
- Quantity vs. quality. Should the Ag/Ab Production Centers devote extra attention to a subset of "high impact" anti-TF affinity reagents?
- Sample coordination: overlap?
- Who will be responsible for the databasing and IT chores to keep track of the affinity capture reagents being generated (along with associated basic data and characterization info), and how will this be merged and/or coordinated between the two centers?
- Impact of antigen center ending before antibody centers?
- What data should be provided along with the reagents?
- Possibility of use of joint validation/characterization resources? How would this be handled?
- Standard naming conventions to ease communication/"branding".
- Production metrics (intermediate and final): where do they need to be different? Where common? How to compare mAbs to rAbs? What really needs to be reported?
- Production goals reporting: formats, frequency.
- Communication between centers: project website?



- Communication to community: project website? Reagent deposition.
- Working groups needed to deal with these issues on an ongoing basis?

B. Collaboration between technology development centers

*Moderators: Andrew Bradbury and Salvatore Sechi*

Points of Discussion:

- How to coordinate interaction amongst technology development centers?
- Development of common web site.
- Identifying a set of common antigens for all technology centers.
- Should an independent validation laboratory that can characterize the selected affinity reagents against the common targets be identified?
- What can be common validation criteria?
- Milestones and goals.
- What working groups are needed to deal with these issues on an ongoing basis?

4:45-5:45 p.m.

**SESSION 4**

**Implementing the Protein Capture Network – Further Network Structure and Working Groups**

*Moderators: Jef Boeke and Adam Felsenfeld*

Points of Discussion:

- Breakout reports from Session 3 moderators.
- Interactions between production and technology development centers:
  - Scheduling and prioritizing antigen production.
  - Identify personnel to be responsible for coordinating sample transfers at each Center.
  - Blanket MTA? Terms?
- Formation of working groups:
  - For defining consensus target list, and antigen transfer.
  - For website coordination.
  - For outreach.
  - For validation criteria.

5:45 p.m.

**General Session ADJOURNS**

5:45-6:45 p.m.

NIH – ESP executive session (closed session)

7:00 p.m.

**DINNER** (PI/ESP, post-doc dinners)

**Day 2: Friday, December 16**

7:30-8:30 a.m.

**Working Breakfast with Contact PIs and Key NIH Staff** (Contact PIs are PI presenters in Session 2; election of the Steering Committee Chair)

8:30-8:40 a.m. Renata Pasqualini, University of Texas MD Anderson Cancer Center  
*Comments from the ESP*

8:40-9:55 a.m. **SESSION 5**

**Other Affinity Reagent Programs**

*Moderator: Charles Edmonds, NIGMS*

8:40-8:55 a.m. Tara Hiltke, NCI  
"The NCI antibody resource"

8:55-9:10 a.m. David Soll, University of Iowa  
"The Hybridoma Bank at the University of Iowa"

9:10-9:25 a.m. Jim Trimmer, UC Davis  
"The UC Davis/NIH NeuroMab Facility"

9:25-9:40 a.m. *Open discussion with session speakers*

9:40-9:55 a.m. **BREAK**

9:55 – 11:05 a.m. **SESSION 6**

**Implementing the Protein Capture Network - Dissemination and Outreach**

*Moderators: Adam Felsenfeld; Joshua LaBaer, Arizona State University; Gaetano Montelione, Rutgers University*

9:55-10:55 a.m. Points of Discussion:

- Branding, deposition, dissemination.
- How to inform the community?
- How to initiate community feedback (e.g. reagent priority, quality)?
- New issues arising.
- Defining milestones.

10:55-11:00 a.m. **Remarks by the Protein Capture Reagents Steering Committee Chair**

11:00-11:05 a.m. **Closing remarks by Salvatore Sechi and Adam Felsenfeld**

11:05 a.m. **General Session ADJOURNS**

11:05-11:35 a.m. NIH – ESP executive session (closed)

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