GTL P.I. Workshop, Washington, D.C.
Breakout Group: Protein Production Facility
March 1, 2004

Breakout Leaders: Michelle Buchanan (Oak Ridge National Laboratory) and Andrew Bradbury (Los Alamos National Laboratory)

Discussion Topics

Attendees expressed high interest in a wide array of gene and protein synthesis capabilities, especially the availability of high-quality proteins and antibodies (about 50:50) that would be produced by this facility. Other requested reagents included labeled proteins and cofactors (isotope, fluorescent), clones, and affinity-tagged proteins. Specific comments from the breakout session are listed below.

Andrew Bradbury (LANL)

- Not sufficient to produce proteins only. Genomics may move away from the storage of specific clones, so a gene-synthesis capability is essential.
- Sequencing capacity at the facility also will be needed.
- Different users will have different requirements. The Joint Genome Institute is a good model; requests validated by SAB would ensure the facility's value to users and also ensure that the facility can learn from users.
- In vitro translation should be explored, but it has not yet fulfilled its promise.

Steve Kennel (ORNL)

- Antibody-like reagents for IP, complex pulldowns, high affinity, and easy elution (e.g., soft tag) would avoid problems that can occur with overexpression of tagged endogenous proteins and the interference of function tags.
- The facility is likely to be most appropriate for creating sets of bacteria in which each gene is deleted (null mutations).
- Allowing users to come and express proteins or select antibodies will provide two-way information flow, and the facility also will learn from visitors.

Bill Studier (BNL)

- Antibodies also are useful for chromatin precipitation.
- Coexpression of protein pairs should be part of facility; use bioinformatics to identify potential partners. Look at protein interaction networks and coexpress new combinations. Coexpressing all possible pairs would not be necessary but only those identified as potentially interacting on the basis of previous work.
- Protocols that provide information on purification also should deal with protein study conditions.

Rob Siegel (PNNL)

- Can proteins be used in place of antibodies for pulldowns? Deriving antibodies is expensive; Santa Cruz antibodies are cheap because they provide little additional

information with their reagents. Complete libraries of antibodies would be very valuable for pulldowns of protein complexes.

- Selectivity with proteins is not sufficient for highly confident protein complex pulldowns.

Greg Hurst (ORNL)

Alternative labeled protein forms required:

- Isotopically labeled versions of proteins for quantitative MS measurements, SANS, NMR, and neutron studies.
- Fluorescently labeled tags for imaging and other detection schemes.
- H/D labeled for protein mobility.
- Seleno methionine labeled for structure studies.

Oznat Herzberg (University of Maryland)

- More than 5 mg pure protein required for structural studies. Facility should provide proteins on demand; P.I. would send clone and protocol, and facility would make it.

Additional Unattributed Comments

Facility should provide the following:

- Individual clones with specific protocols about expression (e.g., “kits”) to the end user.
- Clone sets.
- Complete suites of proteins, such as all bacteriorhodsins (e.g., from Sargasso Sea).
- Posttranslationally modified (PTM)–specific antibodies.
- PTM–modified standards.
- Panels of bar-coded knockouts for individual specific organisms.
- Evolved proteins that do not need partners.
- Protein and antibody arrays.

Characterization of proteins produced easily could overcome facility capacity. In the early stages, attendees suggested, many different tests should be carried out on proteins produced. Examples include:

- Mass spectrometry: Molecular weight
- Aggregation state: Small-angle X-ray scattering, light scattering, SANS
- Circular dichroism
- FTIR
- Conditions: pH, buffers
- Specific activity
- How isolated
- DS page gels

Storage condition
Native gels
Gel chromatography

After experience with such tests in high throughput, the facility should settle on a few tests that produce key maximum data for minimum effort. Information provided with commercial restriction enzymes could serve as a guide to the minimum reagent-specific information required. In the longer term, some proteins such as membranes will be more difficult to characterize.

Additional Comments

- Facility should work with proteins from organisms grown under native conditions (e.g., transcription factors).

- In vitro translation can be used to label proteins with unnatural amino acids (e.g., fluorescent).

Michelle Buchanan closed by noting that the facility typically would not provide "one-off" production of proteins or reagents. Rather, it would focus on high-throughput production of proteins and reagents for the other GTL facilities (especially the Facility for the Characterization and Imaging of Molecular Machines) and on comprehensive sets of reagents for the biological community that would be unavailable from commercial vendors.