ABRF 2009:
Optimization and Application of Existing and Emerging Biotechnologies
February 7-10, 2009 – Memphis, Tennessee

SATELLITE EDUCATIONAL WORKSHOP PROGRAM
(sw3) Recombinant Protein Laboratory
(current as of 12/1/08)

February 6-7, 2009
8:00 am – 4:00 pm
St Jude Children's Research Hospital.

Practical Aspects of Recombinant Protein Expression and Purification
John Hawes, Miami University (organizer),
Richard Heath, St. Jude Children's Research Hospital (co-organizer),
James Bryson, Bristol-Myers Squibb, Preston Hensley, Pfizer,
Cynthia Kinsland, Cornell University, and Francis Rajamohan, Pfizer

FRIDAY, FEBRUARY 6, 2009 (DAY 1)

7:00 am - 12:00 pm **REGISTRATION OPEN**

7:00 - 8:00 am **CONTINENTAL BREAKFAST** – *St Jude, DTRC Room T4*

8:00 - 9:00 am **INTRODUCTION: Basics of Protein Expression –** *St. Jude, E1004*

A. Choice of a Host System
1. Bacteria, yeast, insect and mammalian culture
2. Equipment needed for core lab

B. Biology of Heterologous Protein Overexpression in Bacteria
1. Overview of the pET system
2. The biggest failure in bacteria: insoluble protein
3. Tips for shifting the balance to soluble expression

C. Details of Lab Work
1. Growth of *E. coli* in shake flasks
2. Introduction to 10 L fermentors

9:15 am - 12:00 pm **LAB WORK: Grow and Induce Recombinant Cultures –** *St. Jude, Lab E8066*
1. Hands on lab work in groups of 3
2. Demonstration of 10 L bench top fermentor

12:00 - 1:00 pm **LUNCH** – *St. Jude, E1004*

1:00 – 1:30 pm **SOLUBILITY: Screening For Suitable Buffers –** *St. Jude, E1004*

A. Use of Prefilled 96 Well Trays to Select Optimum Buffers for Purified Proteins
B. Details of Lab Work

1:30 – 4:00 pm **LAB WORK –** *St. Jude, Lab E8066*

A. Solubility Screening
1. Set up solubility screens using supplied purified protein

B. Growth and Harvest of Cultures
1. Continuation of morning growths
2. Harvest by centrifugation

SATURDAY, FEBRUARY 7, 2009 (DAY 2)

7:00 am - 12:00 pm **REGISTRATION OPEN**

7:00 - 8:00 am **CONTINENTAL BREAKFAST** – *St. Jude, E1004*

8:00 - 9:00 am **BASICS AND TIPS OF AFFINITY PURIFICATION**

1. Introduction to metal chelation affinity chromatography
2. Other chromatography methods
3. Benchtop versus AKTA

9:15 am - 12:00 pm **LAB WORK –** *St. Jude, Lab E8066*

A. Cell Lysis

1. Mechanical (demonstration)
2. Detergent-based

B. Protein Purification

1. Running a HisTrap column on an AKTA (demonstration)
2. Bench-top purification using MCAC resins

12:00 - 1:00 pm **LUNCH** – *St. Jude, E1004*

1:00 - 1:30 pm **ASK THE EXPERTS SESSION –** *St. Jude, E1004*

Round-table discussion on protein expression/purification as a shared resource

1:30 - 4:00 pm **LAB WORK –** *St. Jude, Lab E8066*

A. SDS-PAGE Analysis of Purified Protein

B. Biochemical Assay of Activity