

## Protein Expression Laboratory

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### Introduction

A new Protein Expression Laboratory (PEL) has been established at Michigan State University to manufacture proteins for structural biology studies. Proteins are being overexpressed by microbial hosts (e.g., *E. coli*) in computer-controlled bioreactors. Standard protocols are being developed to obtain high yields for a variety of proteins.

### Expression System

*E. coli*, which is commonly used to overexpress recombinant proteins, was selected as the standard expression host. Clients can provide genes coding for their proteins in a number of formats:

- Inserted into a plasmid of their choice
- Inserted into a plasmid provided by the PEL
- Already cloned into a microbial expression host

### Fermentations Conditions

Protein yield is strongly affected by the environmental conditions under which the cells are grown. The computer controlled bioreactors in the PEL enable key variables to be automatically controlled.

#### Variables Influencing Protein Yield

Media Composition	pH
Nutrient Feed Rate	Temperature
Inducer Concentration	Dissolved Oxygen
Induction Time	Harvest Time

The PEL is currently running two New Brunswick BioFlo 3000 bioreactors with BioCommand Plus supervisory software. 1L and 10L working volume vessels are currently available. Plans are underway to add a 100L working volume reactor.



### Bioreactor Control Strategies

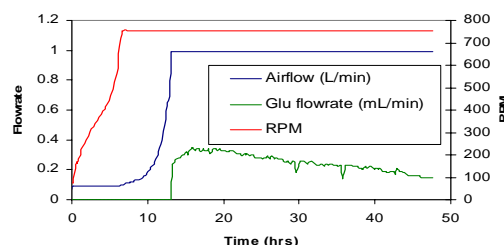
A control system has been developed to maintain physiological state variables (e.g., specific glucose uptake rate) on a desired trajectory throughout the fed-batch fermentation. The system uses:

- Glucose feed on demand
- Different control strategies during different growth phases
- Expert-system decision making
- Artificial neural networks to estimate product concentration

### Dissolved Oxygen (DO) Control

Tight control of DO is important in high-cell-density *E. coli* fermentations. The DO control system involves three stages:

- Stage 1: DO is controlled by adjusting impeller RPM
- Stage 2: DO is controlled by adjusting air flow rate
- Stage 3: DO is controlled by adjusting glucose feed rate

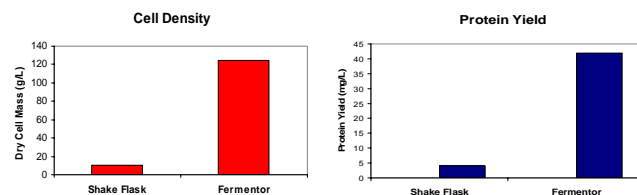


### GDP-mannose 4,6 dehydratase Expression

Expression system and optimized environmental conditions:

- Microbial Host: *E. coli*
- Expression vector: pQE30 (Qiagen Inc., Valencia, CA)
- Promoter: T5
- 6xHis tag for protein purification by affinity chromatography
- Induction: 0.5 mM IPTG added after 12 hours of growth
- Temperature: 37 C before induction; 25 C after induction
- Medium: M9 medium with glucose fed on demand.
- pH: 7.0

Cell density and protein yield 10 times those in shake flasks.



### Example Proteins Produced in the PEL

A standard set of conditions has been established for general protein production. Protein yield under these conditions is generally 10 times that achieved in shake flasks. Improvements are currently being researched using several green fluorescent protein producing constructs.

- Influenza Hemagglutinin HA2 domain - membrane fusion
- Human SNAP 50 and SNAP 190 – transcriptional regulation
- *T. ethanolicus* 2° Alcohol Dehydrogenase – thermophilic dehydrogenase
- Human phospholipid scramblase – phospholipid translocation
- *Arabidopsis thaliana* FtsH2 – chloroplast protease
- Tomato Allene Oxide Synthase – fatty acid metabolism