

## Biotechnology Service Milestones

### **Molecular Biology Service**

#### **Generation of a recombinant expression construct via preexisting restriction sites**

- Insert fragment preparation and purification
- Vector fragment preparation and purification
- Ligation and transformation of bacteria
- Identification of insert-carrying clones
- Restriction mapping of insert-carrying clones
- Plasmid DNA preparation of selected product clone
- (Optional: sequence analysis of cloning junctions)

#### **Cloning of a PCR-amplified insert fragment**

- Design of PCR primer oligonucleotides
- PCR amplification of insert fragment from cloned template
- Insert fragment preparation and purification
- Vector fragment preparation and purification
- Ligation and transformation of bacteria
- Identification of insert-carrying clones
- Restriction mapping of insert-carrying clones
- Plasmid DNA preparation of selected product clone

Generally, sequence analysis of the product clone will be necessary to verify success of the PCR experiment and absence of PCR artifact mutations in the insert. This will be charged according to the required experimental effort.

## ***E. coli* Expression Service**

### **Expression analysis**

- Transfer of expression construct into *E. coli* expression strains
- Expression cultures at 100 ml scale
- Harvesting
- Fractionation of bacteria into soluble and insoluble/inclusion body components
- SDS -PAGE analysis of amount and localization of produced protein (combined with Western blot analysis if required, pending availability of antibody/antiserum)
- Enzyme activity assay (if applicable; charged extra, photometric assays only)
- Documentation

These services are charged according to the required amount of strain comparison and optimization work. The pricing is based on a setup fee for expression analysis, the number of different strains (possibly with different helper plasmid systems) and the number of different culture conditions tested.

### **Pilot expression for protein purification**

- Shaker-flask expression culture at 1-Liter scale
- Harvesting
- SDS-PAGE analysis of expression result
- Documentation

### **Inclusion Body preparation (insoluble proteins)**

- Lysis of bacteria from previous 1-Liter expression culture
- Determination of optimal wash and solubilization conditions
- Inclusion body isolation
- SDS-PAGE analysis of inclusion body purification and solubilization
- Documentation

### **Production Scale-Up**

Scale-up of *E. coli* expression cultures is possible with production in shaker-flask cultures (up to 14 liter culture volume per run) or alternatively by fermentation (through special arrangement), if larger product amounts are required. Quotations for these services are provided individually, based on detailed evaluation of project-specific requirements. Apart from the actual production cultures, scale-up projects can include flanking activities such as set-up of master and working stocks for seed culture generation or downstream processing steps (e.g. inclusion body preparation).

## **Baculovirus Expression Service**

### **Generation of recombinant Baculovirus**

- Lipofection
- Primary plaque assay
- Secondary plaque assay
- Amplification (1<sup>st</sup> round)
- Amplification (2<sup>nd</sup> round)
- Infection of approx.  $10^7$  Sf9 cells
- Determination of expression
- Choice of rec. Baculovirus

### **Generation of 0.5 Liter suspension culture**

- Virus amplification
- Generation of virus stock
- Infection of approx.  $6 \times 10^8$  Sf9 cells
- Harvesting (incl. PBS wash)
- Documentation

### **15-Liter Fermentation**

- Growth of Sf9 cells
- Generation of virus stock
- Titration of virus stock
- Infection of approx.  $2.4 \times 10^{10}$  Sf9 cells
- Harvesting (incl. PBS wash)
- Documentation (cells, virus and fermentation)

### **40-Liter Fermentation**

- Growth of Sf9 cells
- Generation of virus stock
- Titration of virus stock
- Infection of approx.  $6 \times 10^{10}$  Sf9 cells
- Harvesting (incl. PBS wash)
- Documentation (cells, virus and fermentation)

## Protein Purification Service (1 mg –1 g)

### Setup and preliminary tests

(considering important factors for determination of column material and size; e.g. expression rate, total amounts required, degree of purity, presence of affinity tag, cellular location)

Broken down according to the following:

### Preparation of cell extract

#### Buffer determination and preparation

- Buffering compound
- Stabilizing compounds
- Sulfhydryl reagents
- Protease inhibitors
- Analysis and documentation

#### Homogenization

- Dounce homogenizers
- Sonification
- Centrifugation
- Analysis and documentation

#### Solubilization (only membrane proteins)

- Determination of detergents
- Sonification
- Centrifugation
- Analysis and documentation

#### Preliminary Tests

- Test potential capture media
- Determine binding conditions
- Determine elution conditions
- Analysis and documentation

### Capture Step (IEX, GF, HIC, IMAC)

- Buffer preparation
- Column preparation
- Optimization
- Chromatography
- Analysis      SDS-PAGE, Western Blot (if antibodies are available)  
                  Enzyme activity (extra, photometric assays only)

## Intermediate Purification

- Buffer preparation
- Column preparation
- Optimization
- Chromatography
- Analysis      SDS-PAGE, Western Blot (if antibodies are available)  
                    Enzyme activity (extra, photometric assays only)

## Polishing Step

- Buffer preparation
- Column preparation
- Optimization
- Chromatography
- Analysis      SDS-PAGE, Western Blot (if antibodies are available)  
                    Enzyme activity (extra, photometric assays only)