



NEW YORK CONSORTIUM ON MEMBRANE PROTEIN STRUCTURE

## **Midi-scale Protein Expression Using the GNFermenter January 2010**

### **Summary:**

While we are able to obtain enough purified polyhistidine-tagged membrane protein from 0.5ml of culture to identify targets that express, little or no protein remains for further analysis. Methods for expressing enough protein for detergent stability analysis, for example, seemed cumbersome and time-consuming. One could imagine simultaneously growing, inducing and harvesting up to 96 individual samples in 25ml media in 100ml shake flasks, but there would still be no guarantee of obtaining enough purified protein for any biochemical characterization. Similarly, one could grow 0.5L of culture, in 2L culture flasks, of each of the targets. This almost certainly would provide enough material for analysis, but even with the capacity to grow 36 such flasks at the same time, it would take several days to grow such a large number of targets. We have found the solution to this problem to be the use of the Novartis Genomics Foundation GNFermenter. The GNFermenter allows the simultaneous expression of 96 individual samples, each in a volume of ~65ml. The amount of material obtained is easily enough to confirm scaled-up expression by SDS-PAGE and staining with Coomassie Blue and to perform detergent-stability assays with four or more different detergents.

### **Overview:**

Prepare overnight cultures and grow to saturation  
Bring cultures to volume by the addition of fresh rich media  
Grow cells and induce protein expression by the addition of IPTG  
Harvest

### **Materials:**

2xTY media (EMD Chemicals catalog number 71755)  
Terrific Broth (EMD Chemicals catalog number 71754)  
Antifoam 204 (Sigma catalog number A6426)

IPTG (GoldBio catalog number 12481C)  
MOPS  
glycerol

**Equipment:**

96-pronged inoculating tool (EnzyScreen)  
Hot plate capable to reaching 300°C  
Corning Costar 96 well, 2ml assay blocks (Costar number 3960)  
GNFermenter  
50ml screw-cap centrifuge tubes (VWR catalog number 21008-242)  
Refrigerated centrifuge with swinging bucket rotor

**Protocol:**

Heat hot plate to 300°C.

Fill a deep well block with 1ml 2xTY + Kanamycin and Chloramphenicol.

Scrub inoculating tool with brush under distilled water.

While still wet, place inoculating tool on to hot plate. Leave for five minutes. (Longer times may damage inoculating tool!)

Remove tool from plate and allow to cool on its side for 12 minutes.

Remove glycerol stock from -80°C freezer. Place on dry ice, or work quickly next to freezer to prevent the block from thawing.

Place inoculating tool into glycerol stock and leave for five seconds.

Transfer inoculating tool to deep well block containing media. Allow to stand for up to a few minutes.

Remove inoculating tool from media. Cover deep well block with a porous seal and place in 37°C shaker for ~one hour to allow cells to recover and begin growth.

Fill each tube of the fermenter with 8-10ml TB + 2x Kanamycin and Chloramphenicol. Place in 37°C shaker to pre-warm.

Transfer 1ml of media from deep well block to appropriate fermenter tube in rack.

Load entire rack into 37°C shaker and incubate overnight at 225 RPM.

-----

Pre-warm base media for expression to 37°C. Prepare complete media just before inoculation.

Expression media: Terrific Broth (+ Antifoam!)  
100mM MOPS, pH 7.6  
2mM MgSO<sub>4</sub>  
2x Kanamycin  
Chloramphenicol

Remove fermenter rack from shaking incubator and add ~55ml complete expression media to each tube using a peristaltic pump.

Load rack into circulating water bath of the GNFermenter. Insert manifold and connect gas lines.

Begin expression protocol:

60 minutes with air

90 minutes linear gradient from 0 to 100% O<sub>2</sub>

Induce protein expression with the addition of 4ml 8mM IPTG in 5% glycerol (0.5mM IPTG final)

180 minutes with 100% O<sub>2</sub>

Feed cultures with 4ml 5% glycerol/tube at 90 and 270 minutes.

Transfer cultures to top of 50ml screw-top centrifuge tube and pellet cells by centrifugation at 6000xg for 10 minutes.

Pour off supernatant and store cell pellets at -80°C.