Use of *E. coli* HB101 (+pGlo) and Spectravis plus to study induction of the arbinose operon

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Introduction

These experiments were designed to determine the applicability of using the SpectraVis plus (Vernier) to measure induction of the arabinose operon in whole cells of HB101 pGlo+ strain of *Escherichia coli* (BioRad). We measured the presence of GFP using the fluorescence mode of the SpectraVis plus.

Things we did:
We noticed some background fluorescence with LB and Nutrient broth so we used M9, a mineral media, supplemented with ampicillin and arabinose as required in the induction experiments. I suspect any mineral media would work.

Materials and Methods

Organism. The *E. coli* HB101 pGlo+ strain was a gift from Biorad.

Media preparation. Media stocks used for Induction Experiments:

M9 Minimal Media 100 ml
1.128 g M9 Minimal Salts 5X (Sigma) per 100 ml Nanopure water (NPW) (1X solution)
Autoclave, cool and add 1ml $MgSO_4$ stock and 1ml $CaCl_2$ stock.
Add Amp and carbon source as needed

$MgSO_4$ stock – 100X
493mg $MgSO_4$, to 10ml NPW. Filter sterilize.
Add 1ml stock per 100ml media

$CaCl_2$ stock – 100X
14.7mg CaCl2, to 10ml NPW. Filter sterilize.
Add 1ml stock per 100ml media

LB Media
1.55g Difco Luria Broth Base – Miller per 100ml NPW. Filter sterilize.
Add Amp and carbon source as needed.

Amp Stock – 100X

100mg Ampicillin Sodium Salt (Sigma) in 10ml NPW. Filter sterilize.
Add 1ml per 100ml media

Arabinose Stock – 50X
3g L-arabinose in 10ml NPW. Filter sterilize.
Add 2ml per 100ml media

We used LB broth and agar to culture pGlo E. coli in the lab (Sigma). This was supplemented with ampicillin and arabinose as required at the concentrations suggested by BioRad.

Experiments.

A. Initial Induction Experiment:

Liquid cultures of *E. coli* HB101 pGlo+ strain were made by picking a colony from a culture dish (LB agar with ampicillin and arabinose) placing the colony in 5mL of LB broth with ampicillin and arabinose (LB/AMP/Arab) and incubating them at 37°C for 1-3 days. Liquid culture was made by adding 0.5mL of the incubated 5mL culture to 100 ml liquid LB/AMP media and incubating at 37°C and 150 rpm, overnight on a gyrotary shaker.

The culture was separated into two 50mL Centrifuge tubes and centrifuged for 15 minutes at 9,000g to obtain a pellet of cells in each tube. One pellet was re-suspended in 50mL of M9 solution with $MgSO_4$, $CaCl_2$, and Ampicillin to create an un-induced culture. The second pellet was re-suspended in 50mL of M9 solution with $MgSO_4$, $CaCl_2$, Ampicillin, and Arabinose to create an induced culture. These cultures were then incubated at 37 C and 1 ml removed to a cuvette every hour. Induced and un-induced cultures were excited at 405nm and fluorescence was read 509nm. Each culture was read at sensitivities of 100ms, 250ms, and 500ms for fluorescence. The optical densities of both cultures were read at 600nm at a sensitivity of 100ms for cell biomass.

Induced liquid culture was grown at 37°C and 150 rpm on. 0.5mL of cells were taken from a 5mL liquid culture prepared on July 6, 2012 and placed in 100mL of LB/AMP/Arab media to make the culture. This culture was used on July 10, 2012 to make dilutions. The culture was diluted at a 1:2 ratio from 1:2 to 1:1024 in 4mL of 0.9%NaCl solution. Dilutions were read for optical density and fluorescence in the same manner as before. 0.5mL of the 1:32 dilution was taken and placed in 4.5mL of 0.9% NaCl. This solution was diluted from 10^{-1} to 10^{-10} by pipetting 0.5mL of solution from the previous tube into the next. After all the dilutions were made, 100µL were taken from each tube and spread on an LB/AMP agar plate in order to do cell counts. These plates were incubated at 37°C overnight. Cells counts were done on July 11, 2012.