PARAMETERS AFFECTING INDUCTION IN RICH AND DEFINED MEDIA

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Abstract

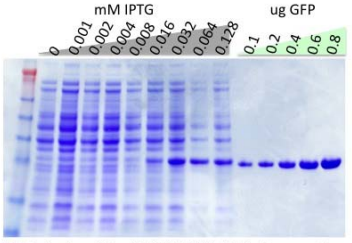
In this study we examine parameters affecting induction of various inducer systems in E.coli in both Rich and Defined growth media. It is found that in the T7 RNA polymerase/lac system expression is leaky, even in the absence of inducers except at high levels of glucose. On the other hand, both the Arabinose and Rhamnose systems are tightly regulated both by glucose and in the absence of inducers.

Background

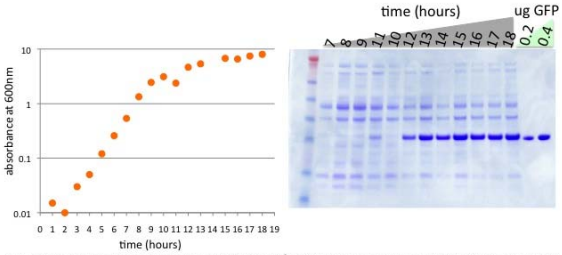
Induction is a convenient strategy for expressing cloned proteins in liquid media. It utilizes the catabolite repression effect of glucose on other sugar uptake systems to exclude inducers from entering the cells until the glucose in the media has been consumed and the cells have had sufficient time to grow to high concentrations. The strategy allows one to build cell mass before inducing genes that might be toxic or have a negative effect on the growth of the host cells. Use of the T7/lac promoter system for induction has been well described by Studier et al [1]. It has been found however that both yeast extract and soytone, common ingredients in Rich growth media, often cause induction in this system even in the presence of glucose except at relatively high levels. The Arabinose and Rhamnose systems appear to be unaffected by this phenomenon however.

Results

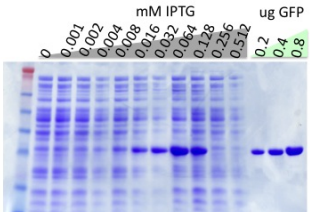
Two types of media were tested in this study, a highly rich media containing both yeast extract and soytone, herein referred to as Cinnabar Media, and a defined media based on an optimized version of the media originally published by Neihardt et al[2], herein referred to as Azure media (both media are available at Teknova, Inc.)

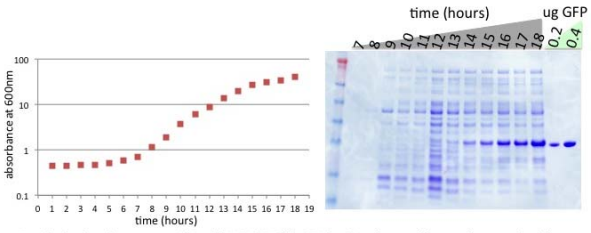


Induction of E. coli BL21 (DE3)/pGFP in Azure media supplemented with 0.1% glucose, 0.5% glycerol and varying amounts of IPTG. *Cells were grown at 30°C, 300rpm for 20 hours. Optimum induction occurred at 0.064 mM IPTG*

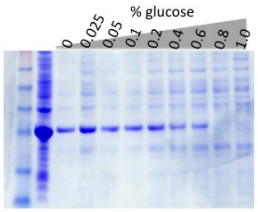


Induction timecourse of E. coli BL21 (DE3)/pGFP in Azure media supplemented with 0.8% glucose and 0.064mM IPTG. *Induction began at 11hrs (10 & 11 hr lanes were switched) and increased to optimal, steady levels from 13-18 hours.*

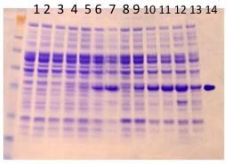




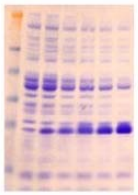
Induction timecourse of E. coli BL21 (DE3)/pGFP in Cinnabar media supplemented with 0.8% glucose and 0.064mM IPTG. *Induction began at 14hrs and increased up to 18 hours.*



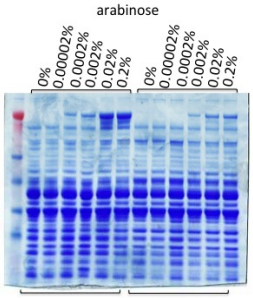
Glucose repression of E. coli BL21 (DE3)/pGFP in Cinnabar media supplemented with varying amounts of glucose. *Cells were grown at 37°C, 300rpm for 20 hours. Repression occurred at 0.8% glucose. Lane 2 is an un-normalized Magic Media GFP lysate.*



The effect of yeast extract on GFP Induction. Lanes 1-7 include yeast extract added to media at concentrations of 0.0, 0.2,0.4,0.8,1.6,3.2,6.4%. Lanes 8-12 are yeast extract from another manufacturer, similar range of concentrations. Lane 13 is a duplicate of lane 9 and lane 14 is purified GFP



Rhamnose induction
0.0 .02, .05, 0.1, 0.2, 0.5% rhamnose
No glucose is present. Some expression may be occuring in the absence of rhamnose



induction at 30°C for 20 hours
pBADhislacZ in Top10 cells

References

1 Protein production by induction in high-density shaking cultures
F. William Studier Protein Expression and Purification 41 (2005) 207–234

2 F. C. Neidhardt, P. L. Bloch, and D. F. Smith. 1974. Culture medium for enterobacteria. *J Bacteriol* 119(3): 736-747