Center for Eukaryotic Structural Genomics

Technology Dissemination Report

CESG Tech Report No.	006
Title	Factorial Evolved Auto-Induction Medium
Research Unit	Small-Scale Expression Testing and Large-Scale Protein Production
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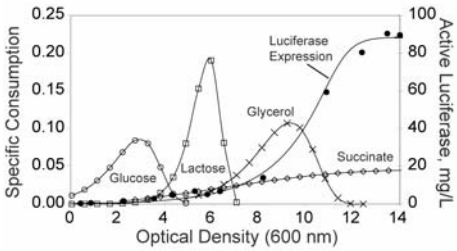
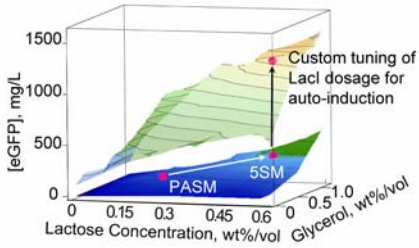
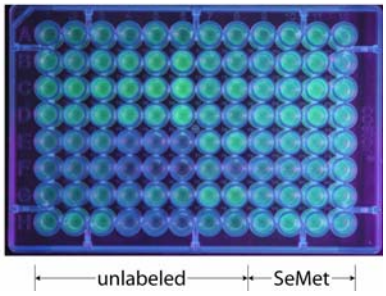
A. Diauxy during auto-induction



B. Medium and vector effects



C. Medium composition array



Summary

The auto-induction method of protein expression in *E. coli* is based on diauxic growth resulting from dynamic function of *lac* operon regulatory elements (*lacO* and LacI) in mixtures of glucose, glycerol, and lactose. Our results show that successful execution of auto-induction is strongly dependent on the plasmid promoter and repressor construction, on the oxygenation state of the culture, and on the composition of the auto-induction medium. Thus expression hosts expressing high levels of LacI during aerobic growth exhibit reduced ability to effectively complete the auto-induction process. Manipulation of the promoter to decrease the expression of LacI altered the preference for lactose consumption in a manner that led to increased protein expression and partially relieved the sensitivity of the auto-induction process to the oxygenation state of the culture. Factorial design methods were used to optimize the chemically defined growth medium used for expression of two model proteins, *Photinus* luciferase and enhanced green fluorescent protein, including variations for production of both unlabeled and selenomethionine-labeled samples [1]. The optimization included studies of the expression from T7 and T7-*lacI* promoter plasmids and from T5 phage promoter plasmids expressing two levels of LacI. Upon the basis of the analysis of over 500 independent expression results, combinations of optimized expression media and expression plasmids that gave protein yields of greater than 1000 µg/mL of expression culture were identified.

These approaches are incorporated into CESG Technology Dissemination Reports 010, 020, 021, and 022.

Publication:

[1] Blommel, P.G., Becker, K.J., Duvnjak, P. and Fox, B.G. (2007) Enhanced bacterial protein expression during auto-induction obtained by alteration of lac repressor dosage and medium composition. *Biotechnol Prog* 23(3):585-98.

Acquiring the Technology	See publication.
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