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SOP-109

B. subtilis Recombinant Protein Expression

Procedures for inducing target gene expression and recombinant protein production in *B. subtilis*.

Section 1

Materials and Reagents

37 C Incubators

Expression System

LB media

Antibiotics

- Ampicilian (100 mg / ml)
- Chloramphenical (20 mg / ml EtOH)
- Neomycin (50 mg / ml)

IPTG (1 M stock solution)

1 L Erlenmeyer flask

Sterile 10 ml culture tube

Section 2

Protocol

The pHT Vectors

All vectors use the strong promoter preceding the *groESL* operon of *Bacillus subtilis* fused to the *lac* operator allowing their induction by addition of ITPG. While the background level of expression of these expression cassettes is very low in the absence of the inducer, an induction factor of about 1,300 was measured using the *bga*B reporter gene (Phan et al., 2005). The amount of recombinant protein produced after addition of IPTG may represent 10 and 13%, respectively, of the total cellular protein (demonstrated when fusing the *htpG* and *pbpE* genes to the *groE* promoter; Phan et al., 2005). High level secretion of *amyQ* α-amylase and cellulase A and B of *Clostridium thermocellum* was demonstrated. An efficient Shine-Dalgarno (SD) sequence as well as a multiple cloning site (*Bam*H I, *Xba* I, *Aat* II, *Sma* I) were also inserted. To obtain secretion of recombinant proteins, the coding region for the signal peptide of the *amyQ* gene encoding an α- amylase was fused to the SD sequence of pHT01, thereby constructing pHT43.

The following protocol is based on using expression vector pHT43 which is designed to secrete target gene products into culture medium. Induction of target gene expression is performed by adding IPTG.

Suited for secretion vectors *B subtilis* strain used is:

WB800N: *nprE aprE epr bpr mpr* :: *ble nprB* :: *bsr .vpr wprA* :: *hyg cm* :: *neo*; NeoR (Please note that WB800N carries resistance to neomycin)

Specific expression system is *B. subtilis* WB800N(pHT43TVA-Mj) This system produces an amylase fusion enzyme.

General Recombinant Protein Expression Protocol

1. Inoculate 5 ml LB media supplemented with 50 ug / ml neomycin, 20 ug / ml chloramphenical and 100 ug / ml ampicilian with WB800N(pHT43TVA-Mj) and incubate with agitation at 37 C overnight.

2. Next day inoculate 500 ml LB media supplemented with 20 ug/ml chloramphenical with 5 ml of overnight seed culture.

3. Incubate culture at 37 C with agitation until an OD_{600} = 0.6 – 0.8.

4. Induce expression of amylase fusion enzyme by making culture 1 mM IPTG (500 ul of 1 M stock).

5. Allow culture to express protein for 2 h – 4 h.

6. Remove cells by centrifugation (10,000 x g 10 min.) and retain supernatant.

7. Store media at 4 C until ready for protein purification.

Section 3 References

Short Protocols in Molecular Biology. 4th Edition. Editors Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A Smith and Kevin Struhl. John Wiley & Sons, Inc. 1999

Nguyen, D.H., Nguyen, Q.A., Ferreira, R.C., Ferreira, L.C.S., Tran, L.T. and Schumann, W. (2005). Construction of plasmid-based expression vectors for *Bacillus subtilis*. Plasmid; in press.

Phan, T.T.P., Nguyen, H.D. and Schumann, W. (2005). Novel plasmid-based expression vectors for intra- and extracellular production of recombinant proteins in *Bacillus subtilis*. Protein Expr. Purif.; in press.