

Quiz on protein expression (Chiu lecture 3) Aug 26, 2008

Name:

1) In the bacterial cell line BL21(DE3):

a) What is the purpose of the lac Repressor?

The lac repressor binds to the lac O operator of the lac promoter, thereby preventing E. coli RNA polymerase from binding to the lac promoter that is in front of the T7 polymerase gene. It also binds to the lac O operator that is part of the T7 promoter in front of the gene in pET vectors, which prevents T7 RNA polymerase from binding to the T7 promoter.

b) Is the gene for T7 Polymerase encoded on the host or vector DNA? host

c) What is the purpose of IPTG?

IPTG competes with the lac repressor for binding to the lac O operator. Addition of IPTG then knocks the lac repressor off both the E. coli lac and T7 promoters, allowing their respective polymerases to bind, thereby allowing their genes to be transcribed, resulting in expression of the protein of interest.

2) What is the purpose of the pRARE2 plasmid that is encoded in Rosetta2 cells ?

The pRARE2 plasmid encodes rare tRNA's to compensate for codon usage differences among bacterial and non-bacterial cells, allowing proteins of non-bacterial origins to be expressed in bacterial cells.

3) How do you control leaky expression in T7-promoter based bacterial cells?

Co-transform in a pLysS vector which encodes for T7 lysozyme, a natural inhibitor of T7 RNA polymerase.

4) What is the purpose of having an antibiotic resistance gene on an expression plasmid?

To survive in growth media containing antibiotic, the bacterial cells need to retain the plasmid which confers antibiotic resistance.

5) What is the general advantage of the Gateway cloning system from Invitrogen?

You generate one ENTRY clone, from which you can recombine with many different DESTINATION vectors designed for specific purposes (expression in different organisms, as fusion proteins, etc.)

6) In a PCR reaction, how many copies of the gene are generated after 4 cycles?

$$2^4 - 1 = 15$$

7) What is the general advantage of expressing a protein as a fusion protein?

The fusion partner is usually well behaved and expressed at high levels by itself. Fusing a protein of interest to it generally allow the protein of interest to be expressed at higher levels and more in the soluble fraction than if the protein of interest were expressed by itself.

8) In the protein fusion system MBP_protease-site1_His-tag_protease-site2_protein-of-interest, what is the reason you would want to delay the expression of protease1 relative to induction of the fusion protein?

Delayed expression of protease1 allows the solubilizing/chaperoning effect of MBP to help the protein-of-interest to fold properly, leading to more of the protein of interest in the soluble fraction.