Sunday, January 23, 2005

Q. What's the difference betweem DH5α and Rosetta competent cells?

A. Both are *E. coli* strains but one is only a cloning host cell (DH5α) while the second is both cloning/expression host cell (i.e. Rosetta bears T7 RNA polymerase gene (*λDE3* lysogen) for expression of target proteins.

Remember that adjacent to the gene, the regions of promoter and operator do the following:

Promoter: a region before the gene where RNA polymerase can bind to start the transcription.

Operator: a region comes after the promoter and before the gene where a repressor or inducer bind to and this affect the gene regulation.

Unlike systems that use *E. coli* promoters (lac, tac, …), the pET system uses **bacteriophage T7 promoter** to direct the expression of target proteins. Because the endogenous *E. coli* RNA polymerase does not recognize T7 promoter, hence, no virtual transcription of the target gene should occur in the absence of T7 RNA polymerase (i.e. without induction).

The gene responsible for bacteriophage T7 RNA polymerase is *λDE3* lysogen. *λDE3* lysogen has lac promoter and lac operator adjacent to it and thus, it is under *lacI* (lac repressor) control. When *lacI* is expressed, its product, the lac-repressor will bind in the lac operator and avoid T7 gene of DE3 to be expressed (i.e. no synthesis of T7 RNA polymerase and thus the target gene in pET vector can not be expressed.

IPTG acts as an inducer which binds to lac operator (instead of lac repressor) and allows RNA polymerase to bind and transcription to occur. Thus, once IPTG is added, DE3 gene becomes active (since lac repressor is kicked out), and is transcribed and bacteriophage T7 RNA polymerase is formed. This T7 polymerase can bind the T7 promoter in the pET vector and directs the expression of the target gene.

Q. How to control transcription leakiness in vector/host cells?

There are three ways:

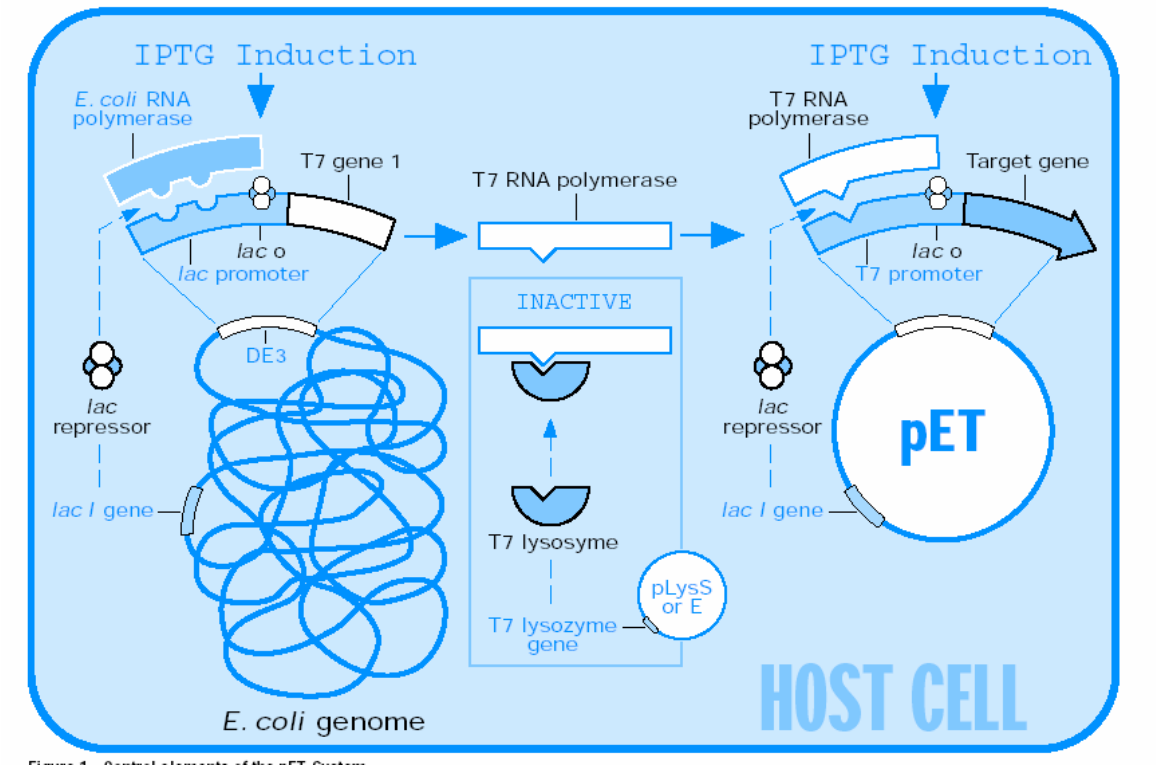
(1) The presence of lac operator in both DE3 gene of the host bacterial cell (e.g. Rosetta) and the after T7 promoter in the pET vector (see plain T7 vs T7*lac* pET vectors in the next page). Thus, lac repressor is always occupying the operator unless IPTG is added.

(2) pET vectors use bacteriophage T7 RNA polymerase promoter, which is unrecognized by *E. coli* RNA polymerase. Thus, a transcription of target gene happens only when T7 polymerase is synthesized. The latter is made when its gene is expressed and only when IPTG is present.

(3) Some host cells carry pLys gene (either **pLysS** or **pLysE**). This gene encodes T7 lysozyme, a natural inhibitor of T7 RNA polymerase. pLysS produces low while pLysE produces high amount of lysozyme to control T7 polymerase in uninduced cells.

Plain T7 promoter vs T7*lac* promoter:

Some pET vectors contain only the 17bp T7 RNA polymerase sequence and called plain T7 vectors. In contrast, other pET vectors have 25bp lac operator downstream from the 17bp promoter and called T7*lac* promoter. the latter provides an additional control of target gene expression in uninduced cells. Plasmids with the T7*lac* promoter also carry their own copy of *lacI* to ensure that enough repressor is made to titrate all available operator sites.



http://www.emdbiosciences.com/html/NVG/pETTable.html