

The pET System

macplasmid # 448

Our lab has the following pET vectors:

- 448 pET11a - has no His tag
- 766 pET15a - has His tag

Target genes are cloned in pET plasmids under control of strong bacteriophage T7 transcription and translation signals; expression is induced by providing a source of T7 RNA polymerase in the host cell. T7 RNA polymerase is so selective and active that almost all of the cell's resources are converted to target gene expression; the desired product can comprise more than 50% of the total cell protein after a few hours of induction. Perhaps just as important as the strength of the T7 promoter is the ability of the system to maintain target genes transcriptionally silent in the uninduced state. Target genes are initially cloned using hosts that do not contain the T7 RNA polymerase gene, so they are virtually "off" and cannot cause plasmid instability due to the production of proteins potentially toxic to the host cell. Once established, plasmids are transferred into expression hosts containing a chromosomal copy of the t7 RNA polymerase gene under lacUV5 control, and expression is induced by the addition of IPTG. Several hosts that differ in their stringency of suppressing basal expression levels are available. These controls provide the greatest amount of flexibility to optimize the expression of a wide variety of target genes.