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FEATURE ARTICLE

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The pET System: Your Choice for Expression

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Over the past few years, Novagen's pET System has become recognized as one of the most powerful approaches available for producing recombinant proteins. Based on the T7 promoter-driven system originally developed by Studier and colleagues (1–3), the pET System has been greatly expanded and now includes over 23 vector types, 11 different *E. coli* host strains and many other companion products designed for efficient detection and purification of target proteins.

The various vectors and hosts provide important options for maximizing the yield of different types of proteins. In addition, several vector-encoded affinity tags, including 11aa and 260aa T7•Tag™, N-terminal and C-terminal His•Tag®, 15aa S•Tag™, and 11aa HSV•Tag™ sequences are available to assay expression levels and purify proteins by several independent strategies (see Table 1). Whereas systems available from other sources tend to offer few options for cloning and expression, Novagen has made the commitment to offer a wide selection of strategies for taking advantage of the power of the T7 promoter. These options are necessary since no one strategy or condition is suitable for every target protein. This article highlights some of the unique advantages of the pET System and the reasons for "tuning" the vector/host combination to optimize the expression of specific target proteins.

Control Over Basal Expression Levels

A surprising percentage of cloned sequences slow the growth of *E. coli* when expressed as protein. Genes whose products severely affect the host cell's growth rate at low concentrations are considered to be toxic and can be difficult to maintain as stable plasmids if even only weakly transcribed by the host. In these cases, cells carrying non-expressing mutants often are the only clones recovered from transformations. Therefore,

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Table 1. Fusion Tags Available for pET Constructs

Tag	N/C Terminal	Size (aa)	Basis for Detection and/or Purification	Applications	pET Vector Series
T7•Tag™	N	11 or 260	monoclonal antibody	western blot immunoprecipitation immunofluorescence	3, 3x, 5, 9, 11, 17, 17x, 21, 23, 24, 28, pTOPE™, λEX*lox*®
S•Tag™	N	15	S•protein (103aa)	western blot quantitative assay purification	29, 30
His•Tag®	N or C	6 or 10	metal chelation chromatography	His•Bind® resin purification (native or denaturing)	14, 15, 16, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30
HSV•Tag™	C	11	monoclonal antibody	western blot immunoprecipitation immunofluorescence	25, 27
pelB/ompT	N	20/22	potential periplasmic localization	protein export/folding	12, 20, 22, 25, 26, 27

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when choosing an expression system it is important to consider the level of basal transcription that occurs in the absence of induction ("leakiness"). The amount of basal transcription varies with different *E. coli* promoters, but it can be significant enough even with the most tightly controlled *E. coli* promoter systems to prevent the stable cloning of toxic genes.

Unlike systems based on *E. coli* promoters (*e.g.*, *lac*, *tac*, p_L), the pET System uses the bacteriophage T7 promoter to direct the expression of target genes. Since *E. coli* RNA polymerase does not recognize the T7 promoter, there is virtually no transcription of the target gene in the absence of a source of T7 RNA polymerase and the cloning step is thus effectively uncoupled from the expression step. Many genes that have been difficult to establish in *E. coli* promoter-based systems have been stably cloned and expressed in the pET System.

Choice of Host Strains

After plasmids are established in a non-expression host, they are most often transformed into a host bearing the T7 RNA polymerase gene (λDE3 lysogen) for expression of target proteins. The pET System uniquely provides a choice of three types of expression hosts that suppress basal transcription to varying degrees. Figure 1 illustrates in schematic form the host and vector elements available for control of T7 RNA polymerase levels and the subsequent transcription of a target gene in a pET vector. In λDE3 lysogens the T7 RNA polymerase gene is under the control of the *lacUV5* promoter, which allows some degree of transcription in the uninduced state and in the absence of further controls is suitable for expression of many genes whose products have innocuous effects on host cell growth. For more stringent control, hosts carrying either pLysS or pLysE are available. The pLys plasmids encode T7 lysozyme, which is a natural inhibitor of T7 RNA polymerase, and thus reduces its ability to transcribe target genes in uninduced cells. pLysS hosts produce low amounts of T7 lysozyme, while pLysE hosts produce much more enzyme and, therefore, represent the most stringent control available in λDE3 lysogens (4).

The pET System also provides a choice of expression host backgrounds, all available as λDE3 lysogens. The most widely used host is BL21, which has the advantage of being deficient in both *lon* and *ompT* proteases. Novagen has recently introduced two derivatives of BL21 designed for special purposes. The B834 series is methionine deficient and, therefore, allows high specific activity labeling of target proteins with ^{35}S-methionine. The BLR strain is a *recA* derivative that improves plasmid monomer yields and may help stabilize target plasmids containing repetitive sequences. Other available strain backgrounds include the K-12 strains HMS174 and NovaBlue. NovaBlue is potentially useful as a stringent host due to the presence of the high affinity *lacI*^{q} repressor encoded by the F'. In addition, Novagen offers the λDE3 Lysogenization Kit for making new expression hosts with other genetic backgrounds.

An alternative for expressing extremely toxic genes or preparing a new λDE3 lysogen is to provide T7 RNA polymerase by

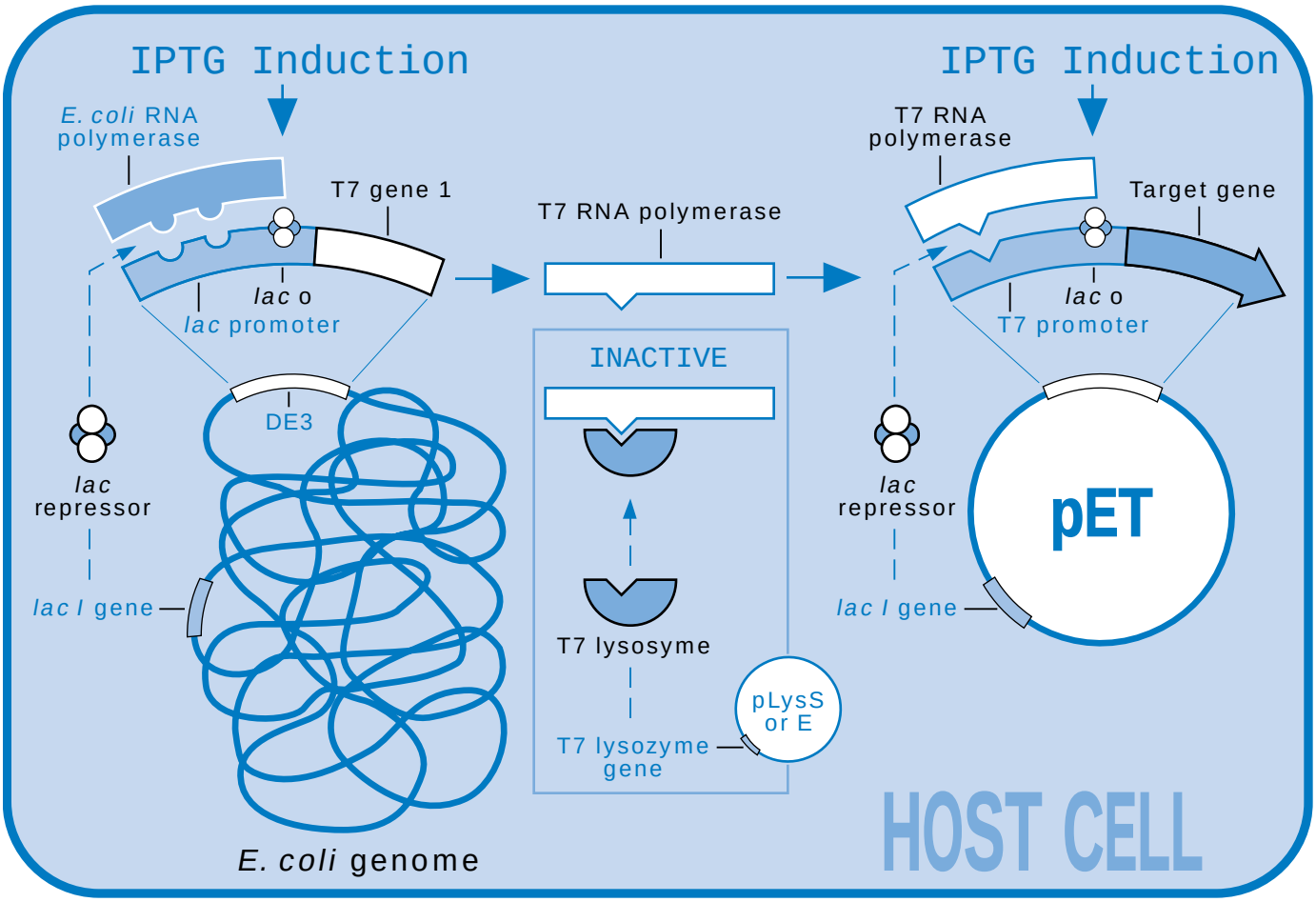

Figure 1. Control elements of the pET System.

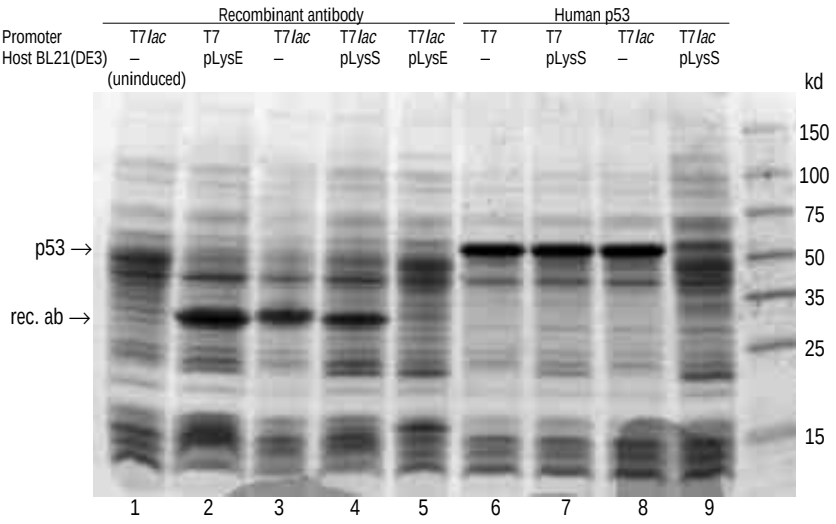

Figure 2. Effect of vector/host combination on expression levels of two proteins.

The indicated cell cultures were grown at 37°C to OD_{600} of approximately 0.8 and expression induced with 1mM IPTG for 2.5hr. Total cell protein samples were run along with Novagen's Perfect Protein™ Markers on a 4–20% SDS polyacrylamide gradient gel followed by staining with Coomassie blue. Vectors were pET-20b(+) and pET-22b(+) for the recombinant antibody and pET-23b(+) and pET-21b(+) for p53.

infection with λCE6. Although not as convenient as inducing a λDE3 lysogen with IPTG, this strategy may be preferred for certain applications.

High Stringency T7*lac* Promoter

In addition to affording a choice of three basic expression stringencies at the host level, the pET system provides two different stringency options at the level of the T7 promoter itself: the "plain" T7 promoter and the more recently developed T7*lac* promoter (5; also shown in Fig. 1). The T7*lac* promoter contains a 25bp *lac* operator sequence immediately downstream from the 17bp promoter region. Binding of the *lac* repressor at this site effectively reduces transcription by T7 RNA polymerase, thus providing a second *lacI*-based mechanism (besides the repression at *lacUV5*) to suppress basal expression in λDE3 lysogens. 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.

"Tunable" Expression

In-house experiments have compared the basal expression levels of *E. coli* β-galactosidase cloned behind the plain T7 promoter, the T7*lac* promoter, and in various host backgrounds. Since β-galactosidase is expressed almost exclusively as soluble active protein, its expression level can easily be measured by assaying activity in the crude soluble protein fraction. Table 2 shows that the relative basal expression of β-gal is suppressed 100-fold from the least stringent condition (plain T7 promoter in BL26(DE3)) to the most stringent condition (T7*lac* promoter in BL26(DE3)pLysE), with intermediate levels in other vector/host combinations. (BL26 is a *lacZ*-deleted derivative of BL21 and was used for this experiment because it has no endogenous β-gal activity.)

Table 2. Relative Basal (Uninduced) Expression Levels of Cloned β-Galactosidase with Various Vector/Host Combinations

Promoter	**T7**	**T7**	**T7**	**T7*lac***	**T7*lac***	**T7*lac***
Host*	(DE3)	(DE3) pLysS	(DE3) pLysE	(DE3)	(DE3) pLysS	(DE3) pLysE
Activity	100	30	10	10	3	1

* BL26, *lacZ*-deleted derivative of BL21

Table 2 demonstrates a general trend toward stringency that in principle can be applied to any protein expressed in the pET System. However, it is extremely important to note that the induced expression level and thus the yield of a given protein can decrease if basal expression is suppressed below that which is necessary for plasmid stability. In practice, it appears that most toxic proteins are expressed well with the T7*lac* promoter in BL21(DE3) and rarely require the use of pLysS or pLysE hosts. Less toxic proteins may be induced to higher levels under less stringent conditions.

Figure 2 illustrates these points by comparing the induced expression levels of two different proteins in several vector/host combinations. The relatively innocuous protein p53 was highly expressed under non-stringent conditions (plain T7 promoter, BL21(DE3), lane 6) or moderately stringent conditions (plain promoter + pLysS or T7*lac* promoter in BL21(DE3), lanes 7 and 8). However, expression was barely detectable from the T7*lac* promoter in the presence of pLysS (lane 9).

In contrast, the pattern of expression was very different for a toxic protein (recombinant antibody, Fig. 2). In this case, constructs using the plain T7 promoter were unstable in BL21(DE3) or BL21(DE3) pLysS (*i.e.*, no recombinants were obtained); expression from the plain T7 promoter occurred only in the presence of pLysE (lane 2). Expression was also obtained from the T7*lac* promoter, without or with pLysS (lanes 3 and 4), but not with the most stringent T7*lac*/pLysE combination (lane 5).

In addition to modulation of basal expression levels, other factors, such as the solubility and localization of the target protein and the growth characteristics of the cells, may be overriding considerations in optimizing the vector/host combination for a given target protein. Therefore, to obtain the best possible yields of a protein in its desired form, it is usually worthwhile to test several different vector/host combinations. The ability to modulate basal expression levels, host cell backgrounds, and vector-encoded fusion sequences is unique to the pET System, and can make a dramatic difference in the success of an expression project. By using the system ourselves, receiving constant feedback from customers and watching for new applications and technologies, we will continue to incorporate advanced features to further increase its usefulness.

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