Prokaryotic Expression Overview

Protein Expression in Bacteria

For many researchers around the world, Novagen's pET System has become the overwhelming choice for protein expression in *E. coli*. A primary reason for the success of this system is that target genes are cloned under the control of the T7 promoter, which is not recognized by *E. coli* RNA polymerase, and therefore virtually no expression occurs until a source of T7 RNA polymerase is provided. Genes cloned in pET vectors 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. Alternatively, T7 RNA polymerase can be provided by infection of the original cloning host with λCE6. Many genes that have been difficult to establish in *E. coli* promoter-based systems (e.g., *tac*, *lac*, *trc*, p_L) have been stably cloned and expressed in the pET System. 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 a few hours after induction. Many other advantages of Novagen's pET System are discussed in the following sections.

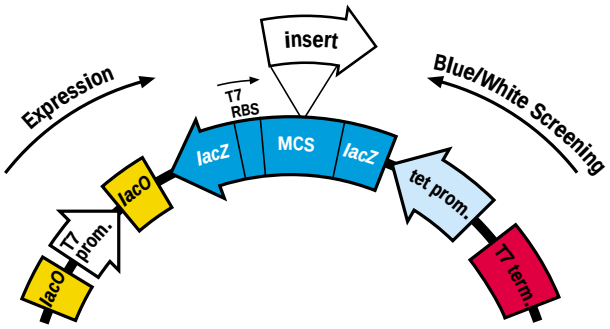
The newest development in T7-driven expression technology is represented by the pETBlue™ System. The pETBlue vectors incorporate all of the advantages of pET vectors for expression, while also increasing ease-of-use for target gene cloning and manipulation of plasmid DNA.

pETBlue™ System: The New Generation of T7 Expression Vectors

The pETBlue vectors represent a fundamentally new type of expression vector, combining the most desirable features of popular cloning vectors with the full power of T7-driven protein expression. Blue/white visual screening of recombinants is enabled by insertion of target genes into the *lacZ* α-peptide coding region in an antisense orientation relative to a modified *E. coli tet* promoter. Expression is made possible by T7 transcription and translation signals correctly positioned upstream from the sense orientation of the target gene. As with standard pET vectors, target proteins are produced by transfer into a λDE3 lysogen followed by IPTG induction or by infection of the original host with λCE6.

Advantages

- Blue/white screening for easy cloning
- High copy number for high plasmid DNA yields
- Available as **A**ccep**T**or™ Vectors or Perfectly Blunt® Vectors for rapid PCR cloning
- No basal expression of target genes; eliminates plasmid instability associated with toxic gene products
- Same expression levels as classic pET vectors
- True "rheostat" control of expression levels with Tuner™(DE3)pLacI host strain

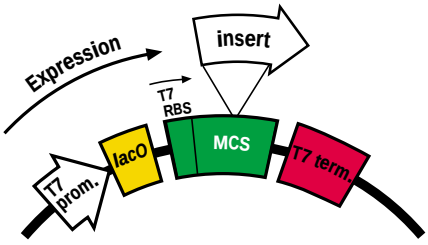


pET System: The Gold Standard for Protein Expression in *E. coli*

In pET vectors, target genes are cloned under control of strong bacteriophage T7 transcription and translation signals, and expression is induced by providing a source of T7 RNA polymerase in the host cell. Novagen's pET System has continuously expanded to offer new technologies and options for expression, and includes over 36 pET vector types, 15 different host strains and many other companion products designed for efficient detection and purification of target proteins.

Advantages

- #1 cited system for prokaryotic protein expression
- Lowest basal expression levels of any *E. coli* expression system
- True "rheostat" control for modulating expression levels if desired
- Widest variety of fusion tags and configurations of any expression system
- Specialized vectors and hosts for production of soluble proteins, disulfide bond formation, protein export, peptide production, etc.
- Many vectors available as LIC Vector Kits for rapid, directional cloning of PCR products
- Most host strains available as competent cells, ready for transformation



pETBlue Vectors

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Vector	Fusion Tags N-terminal	Fusion Tags C-terminal	Protease Cleavage Sites	Special Features/Applications
pETBlue-1	none	none	none	Cloning/expression of target genes having their own ATG start codons +/- fusion tags
pETBlue-2	none	HSV•Tag® His•Tag®	none	Cloning/expression from vector-encoded ATG (like most pET vectors); optional C-terminal tags

Common features:

Tightly controlled, T7 dual *lacO* promoter

Ampicillin resistance marker

High copy number plasmid origin of replication

f1 origin of replication

Blue/white screening

Available as **A**ccep**T**or™ Vectors (pETBlue-1) or Perfectly Blunt® Vectors for cloning PCR products

pET Vectors

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Vector	Promoter	Selection	Fusion Tags N-terminal	Fusion Tags C-terminal	Protease Cleavage Sites	Special Features/Applications
pET-3a-d	T7	Ap	T7•Tag	none	none	Basic pET vectors, offer single *Bam*H I cloning site in 3 frames, except for pET-5 series, which adds *Eco*R I, and pET-17b and pET-17xb, which have multiple cloning sites in one frame.
pET-5a-c	T7	Ap	T7•Tag	none	none	
pET-9a-d	T7	Kan	T7•Tag	none	none	
pET-11a-d	T7*lac*	Ap	T7•Tag	none	none	
pET-17b	T7	Ap	T7•Tag	none	none	
pET-17xb	T7	Ap	T7•Tag (260aa)	none	none	
pET-12a-c	T7	Ap	*ompT*	none	SP	Produce signal sequence fusions to facilitate export of target proteins to the periplasm. Signal sequence cleaved by signal peptidase (SP) concomitant with export.
pET-20b(+)	T7	Ap	*pelB*	His•Tag	SP	
pET-22b(+)	T7*lac*	Ap	*pelB*	His•Tag	SP	
pET-25b(+)	T7*lac*	Ap	*pelB*	HSV•Tag/His•Tag	SP	
pET-26b(+)	T7*lac*	Kan	*pelB*	His•Tag	SP	
pET-27b(+)	T7*lac*	Kan	*pelB*	HSV•Tag/His•Tag	SP	
pET-14b	T7	Ap	His•Tag	none	Tb	Basic cleavable N-terminal His•Tag® fusion vectors, single frame with 3 cloning sites.
pET-15b	T7*lac*	Ap	His•Tag	none	Tb	
pET-16b	T7*lac*	Ap	His•Tag	none	Xa	
pET-19b	T7*lac*	Ap	His•Tag	none	Ek	
pET-21a-d(+)	T7*lac*	Ap	T7•Tag	His•Tag	none	Combination of N-terminal T7•Tag® epitope and N- or C-terminal His•Tag sequence. Multiple cloning sites in 3 frames.
pET-23a-d(+)	T7	Ap	T7•Tag	His•Tag	none	
pET-24a-d(+)	T7*lac*	Kan	T7•Tag	His•Tag	none	
pET-28a-c(+)	T7*lac*	Kan	His•Tag/T7•Tag	His•Tag	Tb	
pET-29a-c(+)	T7*lac*	Kan	S•Tag	His•Tag	Tb	Cleavable N-terminal S•Tag™/His•Tag, and C-terminal His•Tag. Multiple cloning sites in 3 frames. Ek/LIC and Xa/LIC versions for PCR cloning (as LIC kits) and removal of all N-terminal tag aa.
pET-30a-c(+)	T7*lac*	Kan	His•Tag/S•Tag	His•Tag	Tb, Ek	
pET-30 Ek/LIC	T7*lac*	Kan	His•Tag/S•Tag	His•Tag	Tb, Ek	
pET-30 Xa/LIC	T7*lac*	Kan	His•Tag/S•Tag	His•Tag	Tb, Xa	
pET-31b(+)	T7*lac*	Ap	KSI	His•Tag	none	KSI fusions for high expression levels in inclusion bodies. Ideal for peptide production. *Alw*N I cut vector available.
pET-32a-c(+)	T7*lac*	Ap	Trx•Tag/His•Tag/ S•Tag	His•Tag	Tb, Ek	Cleavable Trx•Tag™ increases solubility of target proteins. Multiple cloning sites in 3 frames. Ek/LIC and Xa/LIC versions for PCR cloning (as LIC kits) and removal of all N-terminal tag aa.
pET-32 Ek/LIC	T7*lac*	Ap	Trx•Tag/His•Tag/ S•Tag	His•Tag	Tb, Ek	
pET-32 Xa/LIC	T7*lac*	Ap	Trx•Tag/His•Tag/ S•Tag	His•Tag	Tb, Xa	
pET-33b(+)	T7*lac*	Kan	His•Tag/PKA/ T7•Tag	His•Tag	Tb	PKA site for *in vitro* ^{32}P labeling of target proteins with PKAce™ Kit
pET-34b(+)	T7*lac*	Kan	CBD_{clos}•Tag/S•Tag	His•Tag	Tb, Ek	Cleavable CBD fusion sequences. CBD_{cenA} and CBD_{cex} constructs provide export signals. Ek/LIC and Xa/LIC versions for PCR cloning (as LIC kits) and removal of all N-terminal tag aa.
pET-35b(+)	T7*lac*	Kan	CBD_{clos}•Tag/S•Tag	His•Tag	Tb, Xa	
pET-36b(+)	T7*lac*	Kan	CBD_{cenA}•Tag/S•Tag	His•Tag	SP, Tb, Ek	
pET-37b(+)	T7*lac*	Kan	CBD_{cenA}•Tag/S•Tag	His•Tag	SP, Tb, Xa	
pET-38b(+)	T7*lac*	Kan	CBD_{cex} signal	CBD_{cex}•Tag/His•Tag	SP, Tb	
pET-39b(+)	T7*lac*	Kan	DsbA•Tag/His•Tag/ S•Tag	His•Tag	SP, Tb, Ek	Cleavable Dsb sequences for export and proper folding in periplasm.
pET-40b(+)	T7*lac*	Kan	DsbC•Tag/His•Tag/ S•Tag	His•Tag	SP. Tb, Ek	
pET-41b(+)	T7*lac*	Kan	GST•Tag/His•Tag/ S•Tag	His•Tag	Tb, Ek	Cleavable N-terminal GST•Tag™/His•Tag/ S•Tag, and C-terminal His•Tag.
pET-42b(+)	T7*lac*	Kan	GST•Tag/His•Tag/ S•Tag	His•Tag	Tb, Xa	
pET-43.1a-c(+)	T7*lac*	Ap	Nus•Tag/His•Tag/ S•Tag	HSV•Tag/His•Tag	Tb, Ek	Cleavable Nus•Tag™ sequence increases solubility of target proteins. Multiple cloning sites in 3 frames

Common features:

pBR322 plasmid origin of replication

f1 origin of replication [in (+) vectors]

All pET vectors listed here provide ATG start codons and ATG cloning sites (*Nde* I or *Nco* I). pET-21(+), pET-23(+) and pET-24(+) do not provide RBS or ATG start codons.