



	RBS		6xHN tag
5317	AGA AGG AGA TAT ACC	ATG GGT CAT AAT CAT AAT CAT AAT CAT AAT CAT AAT	ATG GGT CAT AAT CAT AAT CAT AAT CAT AAT
		Met Gly His Asn His Asn His Asn His Asn His Asn	
		Enterokinase cleavage site	
	6xHN tag	PstI	StuI
5368	CAC AAC GCT GCA GGT GAT GAC GAT GAT AAG GCC TCT GTC GAC CAG ATC TCT		SalI
	His Asn Ala Ala Gly Asp Asp Asp Asp Lys Ala Ser Val Asp Gln Ile Ser		BglII
			PacI
	HindIII	EcoRI	NotI
5419	AAG CTT GCG AAT TCT GGC GGC CGC TTA ATT AAT TAA TCT AGA		XbaI
	Lys Leu Ala Asn Ser Gly Gly Arg Leu Ile Asn		*** Stop codon

pEcoli-Nterm 6xHN Vector Map and Multiple Cloning Site (MCS).

Description

pEcoli-Nterm 6xHN is a tightly regulated, yet highly inducible bacterial expression vector that allows you to express your protein of interest with an N-terminal his tag in *E. coli*. The resulting his-tagged fusion protein can be easily purified on his tag purification resins, such as TALON® Co or His60 Ni. The vector is based on the pET system vectors developed by William Studier and colleagues at Brookhaven National Laboratories (1–4). A derivative of pET11 (1), pEcoli-Nterm 6xHN contains a T7 *lac* hybrid promoter ($P_{T7 lac}$), which combines the strong T7 promoter with the *lac* operator. Basal expression of the protein of interest is repressed by the Lac repressor (*lacI*), which binds to the *lac* operator, preventing expression from the promoter in the absence of IPTG. High-level IPTG-inducible expression of the protein of interest is driven by the T7 promoter in the presence of T7 RNA polymerase.

pEcoli-Nterm 6xHN encodes a 6xHN tag composed of 6 repeating His-Asn subunits, (His-Asn)₆, and an enterokinase cleavage site, (Asp)₄-Lys, for subsequent his tag removal. The vector also contains an ampicillin resistance gene (Amp^r) and a pBR322 origin of replication, which maintains the vector at a low-copy-number to further reduce basal levels of the protein of interest.

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Use

pEcoli-Nterm 6xHN is included in Clontech's pEcoli Expression System (Cat. No. 631417). Subclone your gene of interest into the MCS. Depending on the restriction sites chosen, your gene of interest can be expressed with or without a 6xHN tag, and with or without an enterokinase cleavage site. Alternatively you may include any tag of your choice simply by incorporating the tag's sequence into your PCR primers. See the pEcoli Expression Systems User Manual (PT5018-1) for more information.

Notes:

- The MCS is designed with overlapping PacI sites at the 3' end. This ensures that all three reading frames contain a stop codon. If the PacI site is used for cloning, only one of the sites in the vector will be cut. Thus, be sure that your intended stop codon is found in the first PacI site. That way, the stop codon will be in frame regardless of which PacI site in the vector is digested.
- The XbaI site is downstream of the PacI sites, and is therefore not followed by stop codons. If you use the XbaI site for cloning, be sure that your insert contains its own stop codon.



The MCS in both pEcoli-Nterm 6xHN and pEcoli-Cterm 6xHN contains the restriction sites for StuI, SalI, BglII, HindIII, EcoRI and NotI in the same reading frame. If these sites are used for cloning, the same insert can be cloned into both vectors in parallel, which is convenient for comparing expression and purification of N-terminally-tagged versus C-terminally-tagged proteins. For convenient cloning using the SalI and HindIII sites, prelinearized versions of both pEcoli-Nterm 6xHN and pEcoli-Cterm 6xHN are included in our pEcoli Linear Expression System (Cat. No. 631418), which also includes our In-Fusion™ Dry-Down Mix.

His-tagged proteins can be readily detected with either our Universal His Western Blot Kit (Cat No. 635633) or our 6xHN Polyclonal Antibody (631207). Exceptionally pure his-tagged proteins can be obtained with our TALON Co resins (Cat. Nos. 635501–635507, 635509 & 635510) and columns (Cat. Nos. 635601–635603 and 635606). For routine use, we have a variety of high capacity His60 Ni resins available (Cat. Nos. 635659–635664). In addition, an assortment of starter kits containing your choice of TALON Co or His60 Ni resin, as well as pEcoli-Nterm/-Cterm 6xHN circular or linear vectors (Cat Nos. 631416, and 631419–631427) are also available.

Location of Features

- Amp^r (ampicillin resistance gene; β-lactamase): 210–1067
- pBR322 origin of replication: 1242–1856
- *lacI* (Lac repressor): 3779–4858 (complementary)
- $P_{T7\ lac}$ (T7 *lac* hybrid promoter):
 - T7 promoter: 5245–5261
 - lac* operator: 5264–5288 (complementary)
- RBS (ribosomal binding site): 5318–5324
- 6xHN tag ([His-Asn]₆): 5338–5373
- MCS (multiple cloning site): 5375–5460
- T7 terminator: 5499–5545
- Enterokinase cleavage site: 5383–5397
- T7 terminator: 5499–5545

Primer Locations

- T7-Up1 (forward) sequencing primer (5'-CGGCGTAGAGGATCGAG-3'): 5210–5226
- T7 terminator (reverse) sequencing primer (5'-CTAGTTATTGCTCAGCGG-3'): 5501–5484

Propagation in *E. coli*

- Suitable host strains for manipulation and propagation: DH5α and other general purpose strains
- Suitable host strains for protein expression: BL21 (DE3) and other DE3 lysogens.
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pBR322
- Copy number: low

References

1. Dubendorff, J. W. and Studier, F. W. (1991) Controlling basal expression in an inducible T7 expression system by blocking the target T7 promoter with *lac* repressor. *J. Mol. Biol.* **219**(1):45-59.
2. Moffatt, B. A. and Studier, F. W. (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189**(1):113-130.
3. Rosenberg, A. H. and Studier, F. W. (1987) T7 RNA polymerase can direct expression of influenza virus cap-binding protein (PB2) in *Escherichia coli*. *Gene* **59**(2-3):191-200.
4. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* **185**:60-89.

Note: The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

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