

LIC Vector Kits

High efficiency directional cloning into expression vectors

Ligation-independent cloning (LIC) was developed for directional cloning of PCR products without restriction enzyme digestion or ligation reactions (1, 2). LIC vectors are created by treating a linearized backbone with T4 DNA polymerase in the presence of only one dNTP. The 3' → 5' exonuclease activity of T4 DNA polymerase removes nucleotides until it encounters a residue corresponding to the single dNTP present in the reaction mix. At this point, the 5' → 3' polymerase activity of the enzyme counteracts the exonuclease activity to effectively prevent further excision. Plasmid sequences adjacent to the site of linearization are typically designed to produce specific non-complementary 12–15 base single stranded overhangs in the LIC vector. PCR products with complementary overhangs are created by building appropriate 5' extensions into the primers. The PCR product is purified to remove dNTPs (and original plasmid if it was used as template) and is then treated with T4 DNA polymerase in the presence of the appropriate dNTP to generate the specific vector-compatible overhangs. The proper conversion of the insert ends for ligation-independent cloning requires a specific balance between the exonuclease and polymerase activities of T4 DNA polymerase. Novagen's T4 DNA polymerase has been qualified for ligation-independent cloning using a unique assay to verify its performance.

Cloning is very efficient because only the desired product is formed by annealing. The annealed LIC vector and insert are transformed into competent *E. coli* cells. Covalent bond formation at the vector-insert junctions occurs within the cell to yield circular plasmid.

Two Different Protease Cleavage Sites

Two families of multipurpose ligation-independent cloning vectors are available: enterokinase (Ek/LIC) and Factor Xa (Xa/LIC). The Ek/LIC vectors are for directional cloning of PCR products into pET, pBAC™, or pT7Blue-2 vectors. Xa/LIC vectors are for directional cloning of PCR products into pET or pBAC vectors. With both types of vector, the LIC site is designed to enable removal of all vector-encoded sequences from the target protein by digestion with either Recombinant Enterokinase or Factor Xa.

The Ek/LIC and Xa/LIC vector kits provide the necessary reagents for creating single stranded overhangs, annealing with the vector, and transforming competent *E. coli* cells. Each kit provides enough reagents for 20 annealings and transformations. A control insert is included to verify performance.

- Aslanidis, C. and de Jong, P.J. (1990) *Nucleic Acids Res.* **18**, 6069–6074.
- Haun, R.S., Servanti, I.M., and Moss, J. (1992) *BioTechniques* **13**, 515–518.

Primer design for expression of inserts in Ek/LIC and Xa/LIC Vectors

Ek/LIC for enterokinase cleavage

The Ek/LIC site in Ek/LIC vectors has a 13-base single stranded overhang on the left side and a 14-base single stranded overhang on the right side. The left side of the Ek/LIC site is designed to encode the recognition site for enterokinase (DDDDK↓). This feature enables removal of all of the vector encoded fusion sequences from expressed proteins by cleavage with enterokinase. The following sequences must be added to the 5' end of the target gene PCR primers to generate vector-compatible overhangs:

Sense primer: 5'-GAC GAC GAC AAG ATX*
Antisense primer: 5'-GAG GAG AAG CCC GGT

The antisense primer may encode a stop codon or allow read-through to the vector-encoded stop codon present after the C-terminal His•Tag® sequence.

* The first nucleotide of the insert-specific sequence must complete the codon ATX to give Ile (X = A, C or T) or Met (X = G).

Xa/LIC for Factor Xa cleavage

The Xa/LIC site in Xa/LIC vectors has a 12-base single stranded overhang on the left side and a 15-base single stranded overhang on the right side. The left side of the Xa/LIC site is designed to encode the recognition site for Factor Xa (IEGR↓). Like enterokinase, Factor Xa cleaves on the C-terminal side of its recognition site, thus a protein possessing any desired amino-terminus (except proline) can be generated by Factor Xa cleavage using these vectors. The following sequences must be added to the 5' end of the target gene PCR primers to generate vector-compatible overhangs:

Sense primer: 5'-GGT ATT GAG GGT CGC
Antisense primer: 5'-AGA GGA GAG TTA GAG CC

The sense primer encodes the Factor Xa recognition site and is in-frame with the open reading frame (ORF) defined by the vector. Note that the first three nucleotides of the insert-specific sequence must maintain this ORF and will encode the N-terminal amino acid of the protein following cleavage with Factor Xa. The antisense primer may encode a stop codon or allow read-through to the vector-encoded stop codon present after the C-terminal His•Tag sequence.

LIC Vectors for many needs

E. coli Expression

Ek/LIC vectors

- pET-30
- pET-32
- pET-34
- pET-36
- all 4 pET Ek/LIC vectors are included in the pET Ek/LIC Combo Kit

Xa/LIC vectors

- pET-30
- pET-32
- pET-35
- pET-37

Baculovirus Expression

Ek/LIC vectors

- pBAC-2cp
- pBACgus-2cp
- pBAC-7

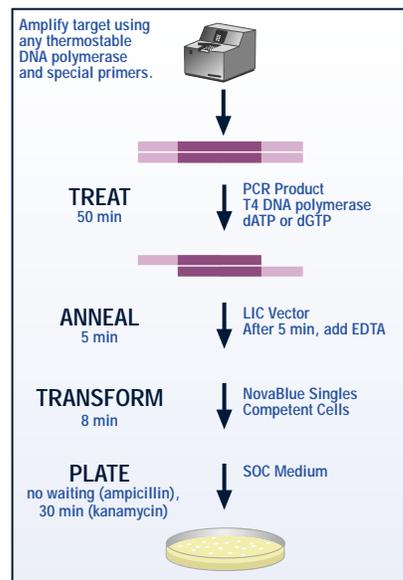
Xa/LIC vector

- pBAC-8

General Cloning, Optimized In Vitro Transcription/Translation

Ek/LIC vector

- pT7Blue-2



Ek/LIC and Xa/LIC Vector Kit Configurations

Kit Component	Ek/LIC & Xa/LIC Kits	pET Ek/LIC Combo Kit	Ek/LIC & Xa/LIC Vectors
	20 rxn	40 rxn	20 rxn
Ek/LIC or Xa/LIC Vector	1 µg	4 × 0.5 µg ¹	1 µg
Ek/LIC or Xa/LIC Control Insert	10 µl	10 µl	10 µl
T4 DNA Polymerase, LIC-qualified	25 U	2 × 25 U	
10X T4 DNA Polymerase Buffer	50 µl	2 × 50 µl	
100 mM DTT	100 µl	100 µl	
25 mM EDTA	50 µl	50 µl	
25 mM dATP or dGTP	40 µl	2 × 40 µl	
Nuclease-free Water	1.5 ml	1.5 ml	
NovaBlue Singles™ Competent Cells	22 × 50 µl	44 × 50 µl	
BL21(DE3) Competent Cells*	0.2 ml	2 × 0.2 ml	
BL21(DE3)pLysS Competent Cells*	0.2 ml	2 × 0.2 ml	
SOC Medium	5 × 2 ml	8 × 2 ml	
Test Plasmid	10 µl	10 µl	

¹ The pET Ek/LIC Combo Kit includes 0.5 µg each of pET-30, pET-32, pET-34 and pET-36 Ek/LIC vectors (10 reactions each)

* BL21(DE3) and BL21(DE3)pLysS Competent Cells are not included in pT7Blue-2 or pBAC LIC Vector Kits

Powerful Tools for Functional Genomics

Novagen's LIC Directional Cloning Systems represent an important addition to the functional genomics toolbox. With this strategy, one preparation of a PCR-generated ORF can be rapidly cloned into several bacterial, baculovirus, and *in vitro* expression vectors. The vectors encode a variety of sequences and peptide tags that enhance expression and facilitate purification and detection of fusion proteins. The system also allows the recovery of native ORFs by removing all vector-encoded sequences with protease treatment. The LIC system thus represents the fastest and most convenient method to optimize the expression of a given ORF.

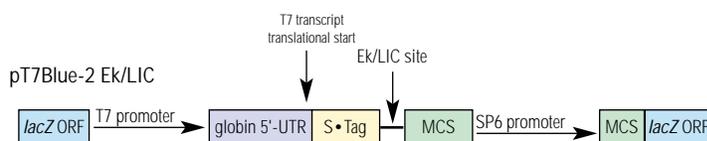
pT7Blue-2 LIC Vector Kit

Directional cloning for optimal *in vitro* expression

The pT7Blue-2 Ek/LIC Vector is a general cloning vector also designed for optimal expression of cloned inserts by *in vitro* transcription and translation. Inserts can also be expressed in *E. coli* from the *lac* promoter. The vector also has many features that make it an excellent choice for many other cloning applications.

- High-copy origin of replication for high plasmid yields and phage f1 origin for single stranded DNA production
- T7 promoter for analysis of Ek/LIC inserts by *in vitro* transcription and translation with the Single Tube Protein™ System 3
- *Xenopus* β-globin 5' UTR translation enhancer (1, 2) and optimal translation initiation site
- N-terminal 15 aa S•Tag™ fusion for quantitative detection and purification
- Extensive multiple cloning region
- Enterokinase cleavage site enables removal of all vector encoded fusion sequences

1. Krieg, P.A. and Melton, D.A. (1984) *Nucleic Acids Res.* **12**, 7057–7070.
2. Falcone, D. and Andrews, D.W. (1991) *Mol. Cell. Biol.* **11**, 2656–2664.



Product	Size	Cat. No.
pT7Blue-2 Ek/LIC Vector Kit	20 rxn	69085-3
Available separately:		
Product	Size	Cat. No.
pT7Blue-2 Ek/LIC Vector (linearized vector)	1 µg	69084-3
T4 DNA Polymerase, LIC-qualified	250 U	70099-3
NovaBlue Singles™ Competent Cells	11 rxn / 22 rxn	70181-3 / 70181-4

Related Products	Page
pT7Blue-2 Perfectly Blunt™ Kit	20
Antibiotics	38
Single Tube Protein™ System 3	91
S•Tag™ Rapid Assay Kit	100
FRETWorks™ S•Tag Assay Kit	101
S•Tag Western Blot Kits	102
S•Tag Purification Kits	136
Recombinant Enterokinase	142

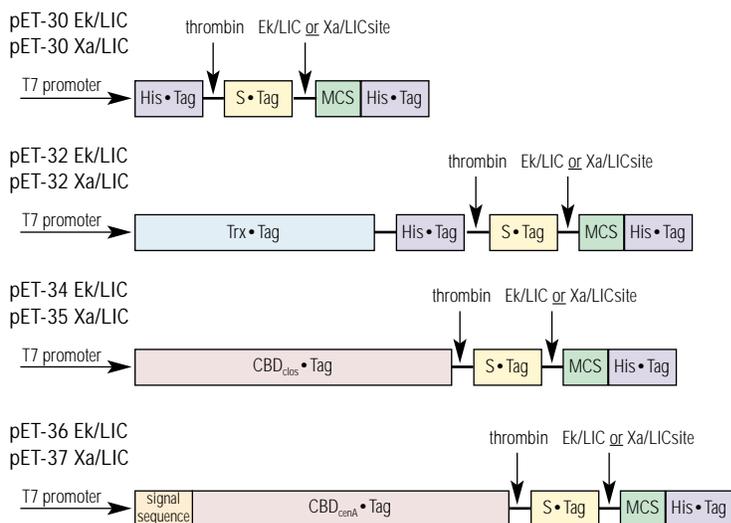
Additional Information Available	
Ek/LIC Vector Kit Protocol	TB163
pT7Blue-2 map	TB135
pT7Blue-2 cloning region	Appendix p. 177
<i>inNovations</i>	No. 5
Vector map	available on the web: www.novagen.com

pET LIC Vector Kits

Directional PCR cloning into the most powerful *E. coli* expression vectors

The various pET Ek/LIC and Xa/LIC vector configurations are shown below. Note that all of the pET LIC vectors carry the kanamycin resistance gene, except for pET-32 Ek/LIC and Xa/LIC vectors, which carry ampicillin resistance.

Please refer to page 22 for additional information about the LIC method and primer design, and to page 52 for a complete description of the pET expression system.



Ek/LIC cloning efficiency with two different inserts

Ek/LIC Vector	Ek/LIC Insert	Vector Plated (ng)	Colonies	Vector Efficiency*	% Recombinants
pT7Blue-2 Ek/LIC	β-gal	1.25 ng	606	4.8×10^5	ND
pT7Blue-2 Ek/LIC	gus	1.25 ng	531	4.2×10^5	100%
pT7Blue-2 Ek/LIC	none	1.25 ng	11	background	NA
pET-32 Ek/LIC	β-gal	1.25 ng	414	3.3×10^5	100%
pET-32 Ek/LIC	gus	1.25 ng	339	2.7×10^5	ND
pET-32 Ek/LIC	none	1.25 ng	8	background	NA

* Expressed as colony forming units (cfu)/μg vector. The transformation efficiency of the competent cells was $> 2 \times 10^8$ cfu/μg. ND = not determined; NA = not applicable. β-gal is 3.1 kbp and gus is 1.86 kbp in size.

Product	Size	Cat. No.
pET-30 Ek/LIC Vector Kit	20 rxn	69077-3
pET-30 Xa/LIC Vector Kit	20 rxn	70073-3
pET-32 Ek/LIC Vector Kit	20 rxn	69076-3
pET-32 Xa/LIC Vector Kit	20 rxn	70072-3
pET-34 Ek/LIC Vector Kit	20 rxn	70114-3
pET-36 Ek/LIC Vector Kit	20 rxn	70145-3
pET-35 Xa/LIC Vector Kit	20 rxn	70115-3
pET-37 Xa/LIC Vector Kit	20 rxn	70153-3
pET Ek/LIC Combo Kit	40 rxn	70255-3

Please see the table on page 23 for kit components and configurations.

Available separately:

Product	Size	Cat. No.
pET-30 Ek/LIC Vector (linearized vector)	1 μg	69024-3
pET-30 Xa/LIC Vector (linearized vector)	1 μg	70022-3
pET-32 Ek/LIC Vector (linearized vector)	1 μg	69023-3
pET-32 Xa/LIC Vector (linearized vector)	1 μg	70023-3
pET-34 Ek/LIC Vector (linearized vector)	1 μg	70100-3
pET-36 Ek/LIC Vector (linearized vector)	1 μg	70134-3
pET-35 Xa/LIC Vector (linearized vector)	1 μg	70101-3
pET-37 Xa/LIC Vector (linearized vector)	1 μg	70136-3
T4 DNA Polymerase, LIC-qualified	250 U	70099-3
NovaBlue Singles™ Competent Cells	11 rxn 22 rxn	70181-3 70181-4

Related Products	Page
Recombinant Enterokinase & Factor Xa	142, 143
S•Tag™ System	100–102, 136
His•Bind® Purification Kits	132
CBIND™ Purification Kits	137

Additional Information Available

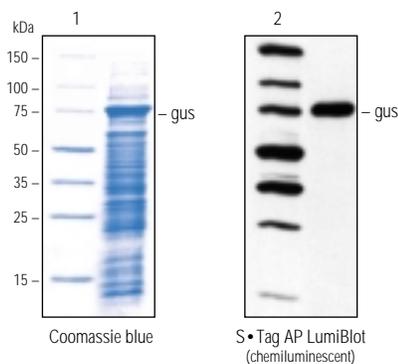
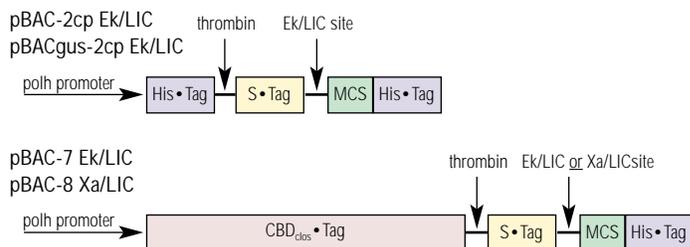
Ek/LIC Vector Kit Protocol	TB163
Xa/LIC Vector Kit Protocol	TB184
Vector Cloning Region Sequences	Appendix
<i>inNovations</i>	No. 5
Vector maps	available on the web: www.novagen.com

pBAC™ LIC Vector Kits

Directional PCR cloning into advanced baculovirus transfer plasmids

The various pBAC™ Ek/LIC and Xa/LIC vector configurations are shown below. Note that all of the pBAC LIC vectors carry the ampicillin resistance gene.

Please refer to page 22 for additional information about the LIC method and primer design, and to page 72 for a complete description of the BacVector™ Expression System.



1 Perfect Protein™ Markers
2 Perfect Protein Western Markers

Baculovirus expression of β -glucuronidase (gus)

β -glucuronidase expressed from a pBAC-2cp Ek/LIC recombinant was detected in total cell extracts by SDS-PAGE and staining with Coomassie blue (left panel) and by Western blotting using Novagen's S•Tag AP LumiBlot™ Kit (Cat. No. 69099-3)

Product	Size	Cat. No.
pBAC™-2cp <i>E. coli</i> Ek/LIC Vector Kit	20 rxn	70021-3
pBACgus-2cp <i>E. coli</i> Ek/LIC Vector Kit	20 rxn	70051-3
pBAC-7 <i>E. coli</i> Ek/LIC Vector Kit	20 rxn	70116-3
pBAC-8 <i>E. coli</i> Xa/LIC Vector Kit	20 rxn	70117-3

Please see the table on page 23 for kit components and configurations.

Available separately:

Product	Size	Cat. No.
pBAC-2cp Ek/LIC Plasmid (linearized vector)	1 μ g	70020-3
pBACgus-2cp Ek/LIC Plasmid (linearized vector)	1 μ g	70050-3
pBAC-7 Ek/LIC Plasmid (linearized vector)	1 μ g	70108-3
pBAC-8 Xa/LIC Plasmid (linearized vector)	1 μ g	70109-3
T4 DNA Polymerase, LIC-qualified	250 U	70099-3
NovaBlue Singles™ Competent Cells	11 rxn 22 rxn	70181-3 70181-4

Related Products	Page
Antibiotics	38
BacVector™ Transfection Kits	75
S•Tag™ System	100–102, 136
S•Tag AP LumiBlot™ Kit	102
His•Bind® Purification Kits	132
CBinD™ Purification Kits	137
Recombinant Enterokinase	142
Factor Xa	143

Additional Information Available

Ek/LIC Vector Kit Protocol	TB163
Xa/LIC Vector Kit Protocol	TB184
<i>inNovations</i>	No. 4a, 7
Vector Cloning Region Sequences	Appendix
Vector maps	available on the web: www.novagen.com