April 2006 . vol. 1

NEB expressions

a scientific update from New England Biolabs

It is with great pleasure that we introduce NEB Expressions, a scientific update from New England Biolabs, Inc. As we celebrate our 30th year of providing tools that have helped shape today's biotechnology industry, we maintain our dedication to the highest standards of quality and value. As always, we invite your feedback on our products, services and corporate philosophy.

inside:

NEW ENGLAND BioLabs® Inc.
the leader in enzyme technology

Competent Cells

superior competent E. coli strains for cloning and protein expression

New England Biolabs is pleased to introduce our own line of chemically competent *E. coli* cells for cloning and protein expression. These strains have been optimized and used by our staff scientists for many years. They are all backed by the high quality you have come to expect from NEB. Now when you are looking for a versatile cloning strain, rapid colony growth, or tight control of expression, you can benefit from the superior performance of these strains.

Advantages

- Ready to transform - packaged in single-use transformation tubes (20 x 0.05 ml)
- Free of animal products
- 5 minute transformation protocols
- Supplied with outgrowth media and control DNA

	NEB Turbo	NEB 5-alpha	T7 Express	T7 Express I^q	dam⁻/ dcm⁻
Transformation Efficiency (cfu/µg)	$>10^9$	$1\text{-}3 \times 10^9$	$2\text{-}6 \times 10^8$	$2\text{-}6 \times 10^8$	$>2 \times 10^6$
Strain	K12	K12	B	B	K12
T1 Phage Resistant	✓	✓	✓	✓	✓
Blue/White Screening	✓	✓	–	–	–
lac I^q	✓	✓	–	✓	–
Colonies Visible after 8 hours	✓	–	–	–	–
Endonuclease I Deficient	✓	✓	✓	✓	✓
Protease Deficient	–	–	✓	✓	–
Restriction Deficient	✓	✓	✓	✓	✓
M13 Phage Capable (F')	✓	✓	–	✓	–
RecA Deficient	–	✓	–	–	–

NEB Turbo Competent *E. coli*
Ligate, transform, plate and pick colonies in one day
#C2984H 20 transformation rxns

NEB 5-alpha Competent *E. coli*
Versatile cloning strain
#C2991H 20 transformation rxns

T7 Express Competent *E. coli*†
High efficiency transformation and protein expression
#C2566H 20 transformation rxns

T7 Express I^q Competent *E. coli*†
Tight control of protein expression
#C2833H 20 transformation rxns

dam⁻/dcm⁻ Competent *E. coli*
Grow plasmids free of dam and dcm methylation
#C2925H 20 transformation rxns

† **Notice to Buyer/User:** The buyer and user have a non exclusive license to use this system or any component thereof for **RESEARCH PURPOSES ONLY.** See Assurance Letter and Statement on www.neb.com for details on terms of the license granted hereunder.

Taq DNA Polymerase – Convenient Formats

robust and reliable reactions

For high yield PCR reactions, we invite you to try recombinant *Taq* DNA Polymerase from NEB. Known for robust and reliable reactions, this enzyme is the industry standard for routine PCR. NEB provides high quality, recombinant *Taq* at exceptional value in terms of cost per unit. For your convenience, this versatile enzyme is now available in a variety of formats.

Advantages

- Value – industry standard for PCR at low price
- Versatility – tolerates a wide range of templates
- Flexibility – incorporates dUTP, dITP
- Choice – three easy-to-use formats available

Taq with Standard or ThermoPol Reaction Buffer

Use in your existing PCR Protocols with buffers to match your performance needs

Taq DNA Polymerase with ThermoPol Buffer* RR
#M0267S 400 units
#M0267L 2,000 units
*uniquely formulated to promote high product yields

Taq DNA Polymerase with Standard Taq Buffer* RR
#M0273S 400 units
#M0273L 2,000 units
*detergent free for high throughput applications

Taq 2X Master Mix

Just add template and primers

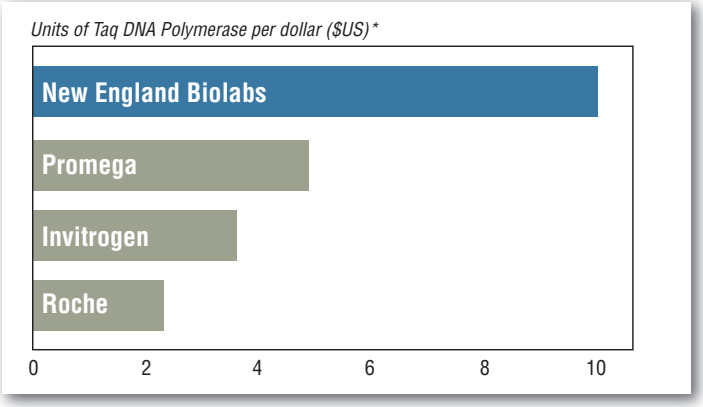
Taq 2X Master Mix RR
#M0270S 100 reactions
#M0270L 500 reactions

Taq PCR Kits

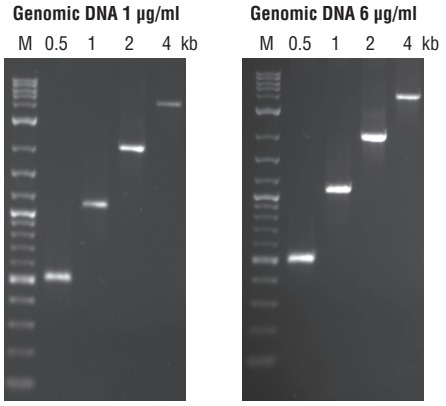
All you need to perform 200 PCR reactions

Taq PCR Kit RR
#E5000S

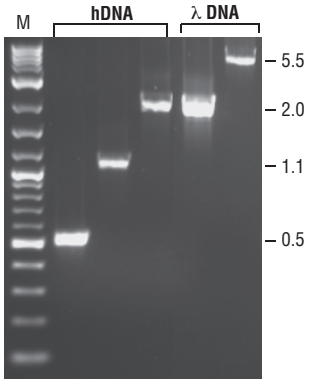
Taq PCR Kit with Controls RR
#E5100S



NEB provides more high quality Taq per dollar than the other leading suppliers.
** Units/$ data was calculated on 1/12/06 from published web site prices for each supplier's largest available package size. International customers, please check with your local NEB subsidiary or distributor for pricing and availability.*



Choose Taq DNA Polymerase for robustness and reliability: *Specific amplicons of 0.5, 1, 2, and 4 kb from human genomic DNA were amplified by Taq DNA Polymerase with Standard Buffer for 30 cycles. Marker (M) shown is 2-Log DNA Ladder (NEB #N3200).*



Versatility of the Taq 2X Master Mix. *30 ng human genomic DNA (hDNA) or 0.1 ng lambda DNA (λ DNA) was amplified in the presence of 200 nM primers in a 25 µl volume. Marker (M) shown is 2-Log DNA Ladder (NEB #N3200).*

ProtoScript® II RT-PCR Kit

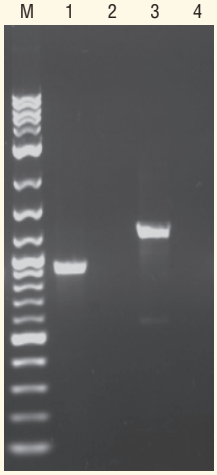
optimized for enhanced sensitivity and higher yield

The ProtoScript® II RT-PCR Kit is designed for the sensitive detection of mRNAs in a two-step process. In the first step, M-MuLV Reverse Transcriptase (RT) is used to extend a random nonamer primer, anchored oligo-dT primer or gene-specific primer annealed to an RNA sample. In the second step, PCR amplification is performed in a separate tube in the presence of gene-specific primers using the robust *Taq* 2X Master Mix (NEB #M0270).

ProtoScript® II RT-PCR Kit
#E6400S 30 reactions

Advantages

- Sensitivity – detect transcript as low as 20 pg total RNA
- Flexibility – transcription of long cDNA products (up to 15 kb)
- Choice – separate RT step maximizes primer choice
- Robust Reactions – *Taq* 2X Master Mix simplifies amplification process

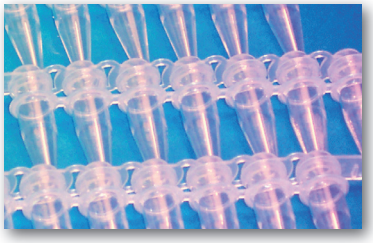


Amplification of different regions of human guanine nucleotide exchange factor p532 (15,164 bp, GenBank accession number U50078). *Approximately 200 ng of human spleen total RNA was reverse transcribed using $dT_{23}VN$. Reactions were setup with and without M-MuLV Reverse Transcriptase (control without RT). After 30 cycles of amplification using 1/20th of the cDNA product, 5 µl was analyzed on a 1% agarose gel. Lane M: 2-Log DNA Ladder. Lane 1: 0.9 kb fragment approx. 1.1 kb from 3´ end. Lane 2: control without RT of 0.9 kb fragment. Lane 3: 1.2 kb fragment approx. 15 kb from 3´ end. Lane 4: control without RT of 1.2 kb fragment.*

Properties of PCR Polymerases from NEB

For over 30 years, NEB has been a world leader in the discovery and production of reagents for the life science industry. NEB offers a wide range of DNA polymerases, several of which can be used for PCR. Through our commitment to research, we ensure the development of innovative and high quality tools for PCR and related applications.

Several factors influence the choice of an optimal enzyme for a specific PCR application. The following chart highlights the most significant properties to be considered when choosing a DNA polymerase from NEB for PCR.



	Fidelity vs. *Taq*	Optimal Extension Range	Difficult Templates	Resulting DNA ends	3´→5´ exo	PCR Kit/ Master Mix Available	Units/50 µl Reaction	Advantages
Deep Vent DNA Polymerase	5X	≤ 6 kb	★★	Blunt	Yes	No/No	0.2–1.0	High-fidelity (5X greater than *Taq*) Extremely high thermostability (half-life of 23 hr at 95°C) Ideal for GC-rich or looped sequences
Vent DNA Polymerase	5X	≤ 6 kb	★	Blunt	Yes	No/No	0.2–1.0	High-fidelity (5X greater than *Taq*) High thermostability (half-life of 6.7 hr at 95°C)
***Taq* DNA Polymerase**	1X	≤ 3 kb	–	3´ A	No	Yes/Yes	0.5–2.0	Industry standard for routine PCR Choice of formats and reaction buffers to accomodate a variety of PCR applications Tolerates a wide range of templates Incorporates dUTP, dITP and flourescently-labeled nucleotides

Taq DNA Polymerase: Some applications in which these products can be used may be covered by patents issued and applicable in the United States and certain other countries. Because purchase of these products does not include a license to perform any patented application, users of these products may be required to obtain a patent license depending upon the particular application in which the product is used. The PCR process is the subject of European Patent Nos. 201,184 and 200,262 owned by Hoffman-LaRoche, which expired on March 28, 2006. The corresponding PCR process patents in the United States expired on March 29, 2005.

Deep Vent® DNA Polymerase

Deep Vent® DNA Polymerase is a recombinant, high-fidelity DNA polymerase with unsurpassed thermostability. This feature makes Deep Vent an ideal choice for PCR amplification of DNA targets with a high degree of secondary structure, even in the absence of additives. It has an error rate 5-fold lower than *Taq* DNA Polymerase, a characteristic derived in part from an integral 3´→5´ proofreading exonuclease. Deep Vent's combination of extreme thermostability and high-fidelity make it an excellent choice for accurate PCR amplification of GC-rich sequences or templates with secondary structures.

Deep Vent® DNA Polymerase R
#M0258S 200 units
#M0258L 1,000 units

Deep Vent® (exo⁻) DNA Polymerase R
#M0259S 200 units
#M0259L 1,000 units

Vent® DNA Polymerase

Vent® DNA Polymerase is a recombinant, high-fidelity thermophilic DNA polymerase with the lowest cost per reaction of any high-fidelity PCR polymerase. It has an error rate 5-fold lower than *Taq* DNA Polymerase, a characteristic derived in part from an integral 3´→5´ proofreading exonuclease. In addition, greater than 90% of the polymerase activity remains following a 1 hour incubation at 95°C, ensuring maximal activity over the course of the PCR reaction. For high-fidelity amplification of routine targets, Vent DNA Polymerase is an exceptional value.

Vent® DNA Polymerase R
#M0254S 200 units
#M0254L 1,000 units

Vent® (exo⁻) DNA Polymerase R
#M0257S 200 units
#M0257L 1,000 units

R = Recombinant

K. lactis Protein Expression Kit

complete system for high yield protein expression in yeast with proven advantages over Pichia pastoris

The *K. lactis* Protein Expression Kit provides an easy method for expressing a gene of interest in the yeast *Kluyveromyces lactis* (Figure 1). Proteins may be produced intracellularly or secreted using the supplied integrative expression vector pKLAC1 (Figure 2). To achieve protein secretion, a gene of interest is cloned downstream of the *K. lactis* α-mating factor secretion domain (α-MF; Figure 2B) which is eventually processed in the Golgi resulting in secreted protein (Figure 1).

The *K. lactis* system offers several advantages over other yeast and bacterial expression systems. Abundant overexpression of protein is achieved through high culture densities as well as the ability to integrate multiple copies of the vector (Figure 3). pKLAC1 uses a strong *LAC4* promoter, which has been modified to lack expression in *E. coli*, making this system useful for expressing toxic genes. The high transformation efficiency of the supplied *K. lactis* cells makes the system suitable for methods that require large numbers of transformants, such as expression cloning using cDNA libraries. Selection of yeast transformants uses a unique antibiotic-free method in which acetamide acts as the sole nitrogen source. In addition, no methanol is required in growth media. Finally, the *K. lactis* system can express post-translationally modified proteins, making it a useful alternative to other expression systems.

Advantages

- High yield protein expression
- Clone and express genes toxic to *E. coli*
- No expensive antibiotics or highly flammable methanol required
- Easy-to-use protocols for those inexperienced with yeast systems
- Attractive commercial sublicensing

Kit includes:

pKLAC1 Vector and pKLAC1-*malE* Control Plasmid (20 µg)

Sac II (2,000 units)

Integration Primer Set

K. lactis GG799 Competent Cells

Transformation Reagent

Selective Yeast Media

K. lactis Protein Expression Kit
#E1000S

Reagents Sold Separately:

pKLAC1 Vector
#N3740S 20 µg

K. lactis GG799 Competent Cells
#C1001S 5 rxns

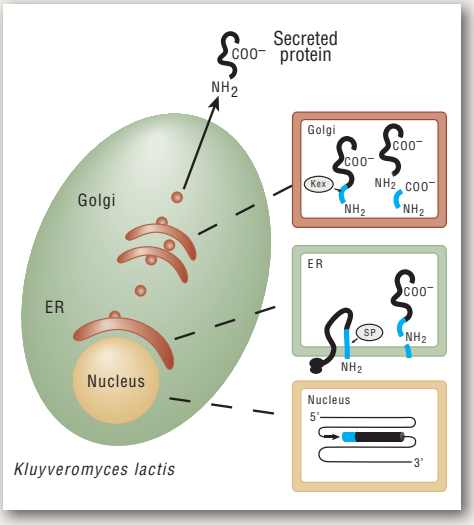


Figure 1: Secreted protein processing. *In the nucleus, an integrated expression vector encoding a fusion between the α-MF domain (blue) and a desired protein (black) is expressed. A signal peptide in the α-MF domain directs entry of the fusion protein into the endoplasmic reticulum (ER) and is removed by signal peptidase (SP). The fusion protein is transported to the Golgi where the Kex protease removes the α-MF domain. The protein of interest is then secreted from the cell.*

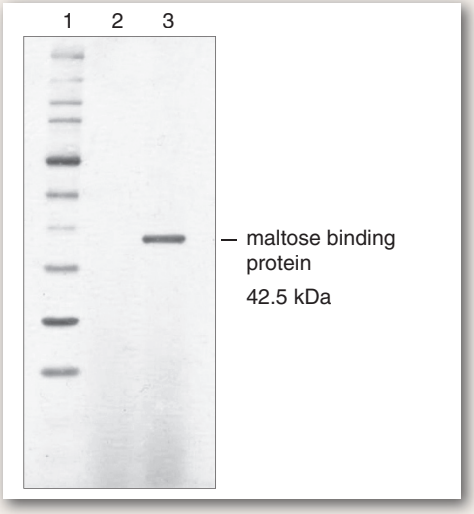


Figure 3: High Yield Protein Expression in K. lactis. *SDS-polyacrylamide gel electrophoresis separation of secreted recombinant maltose binding protein (MBP) detected directly in peptone rich growth medium by Coomassie staining. Lane 1: Protein Marker, Broad Range (NEB #P7702). Lane 2: spent culture medium (15 µl) from wild-type K. lactis cells. Lane 3: spent culture medium (15 µl) from K. lactis cells harboring an integrated expression cassette containing the E. coli malE gene.*

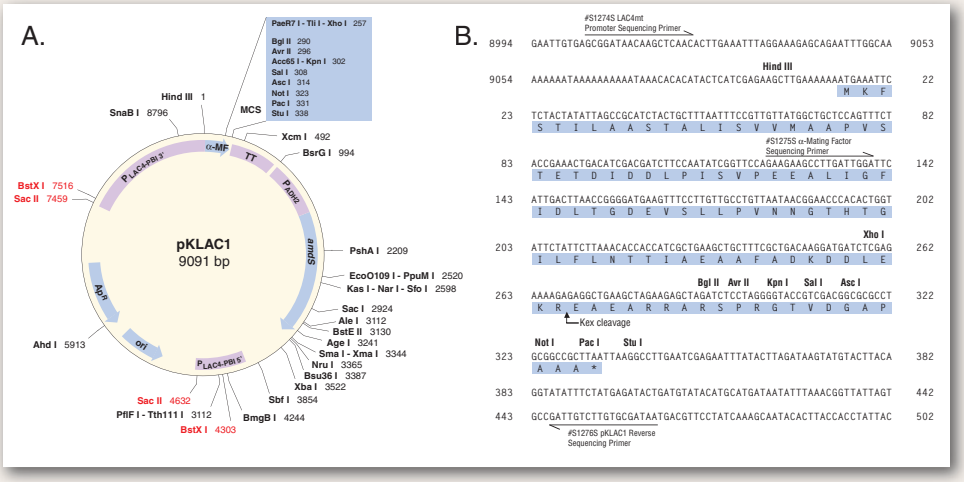


Figure 2: The pKLAC1 expression vector. *(A) pKLAC1 (9091 bp) contains the 5´ and 3´ ends of the LAC4 promoter ($P_{LAC4\text{-}PBI}$) separated by DNA encoding β-lactamase (Ap^R) and the pMB1 origin (ori) to allow for its propagation in E. coli. The K. lactis α-mating factor secretion leader sequence (α-MF), multiple cloning site (MCS), and the LAC4 transcription terminator (TT) lie immediately downstream of 3´ $P_{LAC4\text{-}PBI'}$. The yeast ADH2 promoter (P_{ADH2}) drives expression of an acetamidase selectable marker gene (amdS). The vector can be linearized by digestion with Sac II or BstX I for integration into the native LAC4 promoter region of the K. lactis genome. (B) The pKLAC1 multiple cloning site, including the K. lactis α-mating factor secretion leader sequence (blue background) and a polylinker immediately downstream of the $P_{LAC4\text{-}PBI}$ promoter. Unique polylinker restriction sites are indicated. Half-arrows show the positions of pKLAC1-specific sequencing primers available from New England Biolabs.*

Yeast Expression: Why Choose the *K. lactis* Protein Expression Kit?

Many types of protein can be secreted from yeast cells. As a general rule, those that tend to secrete best are proteins that are also secreted by their native host (e.g. glycosidases, serum albumins, cytokines, etc). However, there are numerous examples in the literature of normally non-secreted proteins that have been successfully secreted from various yeasts. When in doubt, it is always best to try secreted expression. Intracellular protein expression in yeast is also possible for a wide range of proteins and is a great alternative to bacterial protein expression.

K. lactis strain GG799 (as supplied in the NEB Kit) is a haploid (α) wild-type industrial isolate that has no genetic markers. It was originally chosen as a host strain in the food industry because of its ability to grow to very high cell density and to efficiently secrete heterologous proteins.

Avoid toxicity problems in *E. coli*

The *K. lactis* Protein Expression Kit vector (pKLAC1) contains a variant of the strong *K. lactis LAC4* promoter (PLAC4-PBI) for expression of a desired gene in *K. lactis*. The major advantage of the PLAC4-PBI promoter is that it is transcriptionally silent while in *E. coli*. In contrast, the wild-type PLAC4 promoter shows background transcriptional activity in *E. coli* which can be detrimental to the process of assembling or amplifying expression constructs in *E. coli* prior to their introduction into yeast cells. This is especially problematic if the cloned gene of interest encodes a translated product that is toxic to *E. coli* cells. Therefore, pKLAC1 is well-suited for the cloning and yeast expression of genes encoding proteins that are toxic or otherwise detrimental to bacteria.

High yield, lower cost protein expression

Integrative expression with no auxotrophic markers: pKLAC1 is an integrative expression vector that inserts into the promoter region of the *LAC4* locus of the *K. lactis* genome upon its introduction into *K. lactis* cells. While *K. lactis* episomal plasmids do exist, they can present some problems for large-scale protein production. For example, plasmids are easily lost by cells in the absence of a selection. For large-scale fermentation, plasmid selection using antibiotics can be too costly, and selection using an auxotrophic marker can reduce yields. While auxotrophic markers have historically been used for genetic manipulation of yeasts, they are not always desirable to achieve maximum protein expression. In some cases, an auxotrophy (e.g. uracil) can cause a significant reduction in the strain's ability to produce a heterologous protein even if exogenous uracil or uridine is provided in the growth medium. Integrative expression vectors are attractive because they insert into the genome, thus becoming part of the host chromosome, and are therefore quite stable even in the absence of selection.

Acetamide selection: There are two main advantages to acetamide selection: cost and selection of multiple integrants. Acetamide is significantly less expensive than antibiotics. Additionally, acetamide selection enriches transformant populations for cells that have integrated multiple tandem copies of the pKLAC1 expression vector. Multi-copy integrants are desirable because they often produce more recombinant protein than single integrants. Acetamide acts as a nitrogen source. A cell transformation mixture (containing a population of cells that are either transformed or untransformed by vector pKLAC1) is spread onto yeast carbon base (YCB) medium agar containing 5 mM acetamide. YCB medium contains all of the nutrients and carbon source required for *K. lactis* cells to grow, but lacks a nitrogen source. The acetamide provided in the medium can be utilized as a source of nitrogen only if it is broken down to ammonia by the enzyme acetamidase (expressed from the amdS gene present on pKLAC1). Therefore, only transformed cells are able to grow into colonies.

Flexible cloning strategies

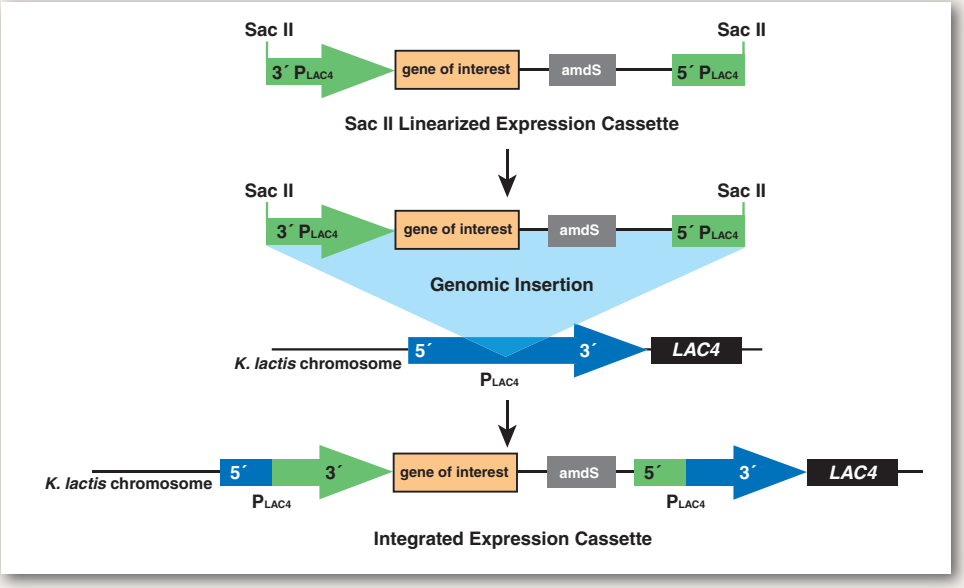
For cytosolic protein expression: The gene of interest can be cloned into pKLAC1 in a manner that places it downstream of PLAC4-PBI without the α–mating factor secretion domain being present. Genes expressed in this manner must include a methionine as their first codon, to initiate translation.

For tagged proteins: A PCR method for the addition of a carboxy-terminal HA tag to a secreted protein is described in the kit manual. This example can be adapted for the addition of other antibody epitope tags e.g. FLAG, c-myc or His tag. Additionally, a chitin-binding domain has been used as a tag for capture of secreted proteins onto inexpensive chitin beads directly in spent medium (1).

For an amino-terminal antibody epitope tag: A new forward PCR primer is used that contains an Xho I restriction site, the Kex protease cleavage site, the desired tag's sequence and DNA homologous to the 5´ end of the desired gene or cDNA. After Kex protease processing of the expressed protein in the Golgi, a protein bearing the desired tag at its amino-terminus is produced and secreted.

Reference

1. Colussi, P.A., Specht, C.A. and Taron, C.H. (2005). Characterization of a nucleus-encoded chitinase from the yeast *Kluyveromyces lactis*. *Appl Environ. Microbiol.* 71:2862–2869.



Genomic integration of a linear expression cassette. *Vector pKLAC1 containing the gene of interest is digested with either Sac II or BstX I (Sac II shown) and introduced into K. lactis cells. The 5´ P_{LAC4} and 3´ P_{LAC4} sequences direct insertion of the cassette into the promoter region of the LAC4 locus in the K. lactis genome.*

NEB Essentials™

a selection of our most popular reagents at an amazing price

Over the years, NEB has received repeated requests for certain items that bring the benefits of convenience, value and function to our customers. These items include a cluster of the most commonly used reagents for cloning and other basic techniques, a convenient triple pack of the popular NEB floaties and a custom designed storage rack that allows scientists to keep their reagent vials cold at the bench. We are proud to launch these items as part or our current efforts to celebrate the 30th anniversary of the founding of NEB.

With NEB Essentials, amplify your gene of interest using *Taq* DNA Polymerase and the Deoxynucleotide Solution Mix. Digest your PCR product and vector of choice with BamH I, EcoR I or Hind III. Modify your DNA with DNA Polymerase I (Klenow) or Antarctic Phosphatase. DNA Polymerase I (Klenow) removes 3´ overhangs or fills-in 5´overhangs to form blunt ends. Antarctic Phosphatase is a dephosphorylase that is heat inactivated in just 5 minutes and outperforms other alkaline phosphatases under proper reaction conditions.

Ligate with T4 DNA Ligase at room temperature or 16°C. The NEB Cool storage rack may be stored in the freezer at –20°C and will retain low temperatures for short periods of time on the bench. This cluster of popular products is available at a reduced rate when compared to the cost of purchasing each item separately. For more information on the products offered in NEB Essentials, see www.neb.com.

NEB Essentials contains:

Product		Size
BamH I	RI	10,000 units
EcoR I	RI	10,000 units
Hind III	RI	10,000 units
T4 DNA Ligase	RI	20,000 units
DNA Polymerase I, (Klenow)	RI	200 units
Taq DNA Polymerase	RI	400 units
Antarctic Phosphatase	RI	1,000 units
Prestained Protein Marker*		8 lanes
1 kb DNA Ladder		100 µg
100 bp DNA Ladder		50 µg
Deoxynucleotide Solution Mix		8 µmol of each
NEB Cool Storage Rack		

RI = Recombinant
* Special size-only available in NEB Essentials.

NEB Essentials™
#T0500S

NEB Cool™
#T0400S 20 tube capacity

NEB Floatie™ Pack
#T0300S 3 per pack

SPECIAL INTRODUCTORY OFFER
For a limited time, order NEB Essentials and you will receive a free additional NEB Cool storage rack as well as an NEB Floatie Pack.

Magnetic Matrices

selective purification of proteins and nucleic acids

Magnetic matrices are a convenient and efficient tool for the purification of proteins and nucleic acids. NEB offers a variety of magnetic beads for immunomagnetic isolations, protein purification, and biotin-labeled isolation. To accommodate different sample sizes, we offer magnetic racks that can be used with 1.5 ml or 50 ml tubes.

All of our magnetic separation racks pull the magnetic beads to the side of the tube rather than the bottom reducing sample loss. (the 50 ml Magnetic Separation Rack is shown)

Magnetic Racks Available from NEB. *From left to right: 12-Tube Magnetic Separation Rack, 6-Tube Magnetic Separation Rack, and 50 ml Magnetic Separation Rack*

Advantages

- No centrifugation required
- Matrix can be regenerated without loss of binding capacity
- Minimal sample loss during pipetting because magnetic beads concentrate at the side of the tube instead of the bottom

Biotin-labeled Isolation

Streptavidin Magnetic Beads
#S1420S 5 ml

Immunoglobulin purification

Protein A Magnetic Beads
#S1425S 1 ml

Protein G Magnetic Beads
#S1430S 1 ml

Protein Purification

Chitin Magnetic Beads
#E8036S 20 ml
#E8036L 100 ml

Amylose Magnetic Beads
#E8035S 25 mg

Anti-MBP Magnetic Beads
#E8037S 10 mg

Immobilized Secondary Antibodies

Goat Anti-Rabbit IgG Magnetic Beads
#S1432S 20 mg

Goat Anti-Mouse IgG Magnetic Beads
#S1431S 20 mg

Separation Racks

6-Tube Magnetic Separation Rack
#S1506S holds 6 tubes

12-Tube Magnetic Separation Rack
#S1509S holds 12 tubes

50 ml Magnetic Separation Rack
#S1507S holds 4 tubes

Antarctic Phosphatase

100% heat inactivated in 5 minutes at 65°C

Antarctic Phosphatase catalyzes the removal of 5´phosphate groups from DNA and RNA. Since phosphatase-treated fragments lack the 5´phosphoryl termini required by ligases, they cannot self ligate (1). This can be beneficial to cloning strategies because it decreases vector background. Antarctic phosphatase works best under mildly acidic conditions and when used under the proper reaction conditions will outperform other alkaline phosphatases. In addition, this superior reagent is 100% heat labile in just 5 minutes, eliminating the need to purify vector DNA prior to ligation.

Advantages

- 100% heat inactivated in 5 minutes
- Ligate without purifying vector DNA
- Recombinant enzyme for unsurpassed purity and consistency; no nuclease contamination
- Active on DNA, RNA, protein, dNTPs and pyrophosphate

Vector Dephosphorylation Protocol:

1. Add 1/10 volume of 10X Antarctic Phosphatase Reaction Buffer to 1 µg of linearized vector DNA
2. Add 1 µl Antarctic Phosphatase and mix
3. Incubate 15 minutes at 37°C for 5´ extensions or blunt-ends, 60 minutes for 3´ extensions
4. Heat inactivate for 5 minutes at 65°C (or as required to inactivate the restriction enzyme)
5. Proceed with ligation

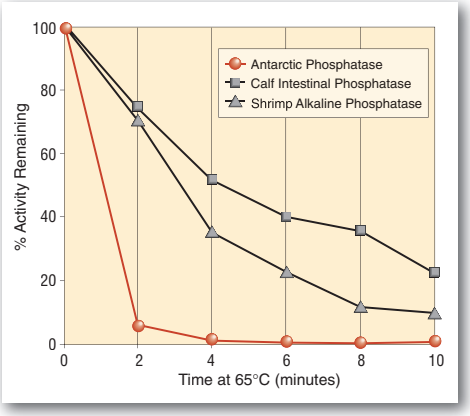
Applications

- Removing 5´ phosphate from nucleic acids
- Preparing templates for 5´end labeling
- Preventing self ligation of fragments
- Dephosphorylation of proteins
- Removal of dNTPs and pyrophosphate from PCR reactions

Antarctic Phosphatase	RR
#M0289S	1,000 units
#M0289L	5,000 units

Reference

1. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, (2nd ed.), (p. 5.72). Cold Spring Harbor Laboratory Press.

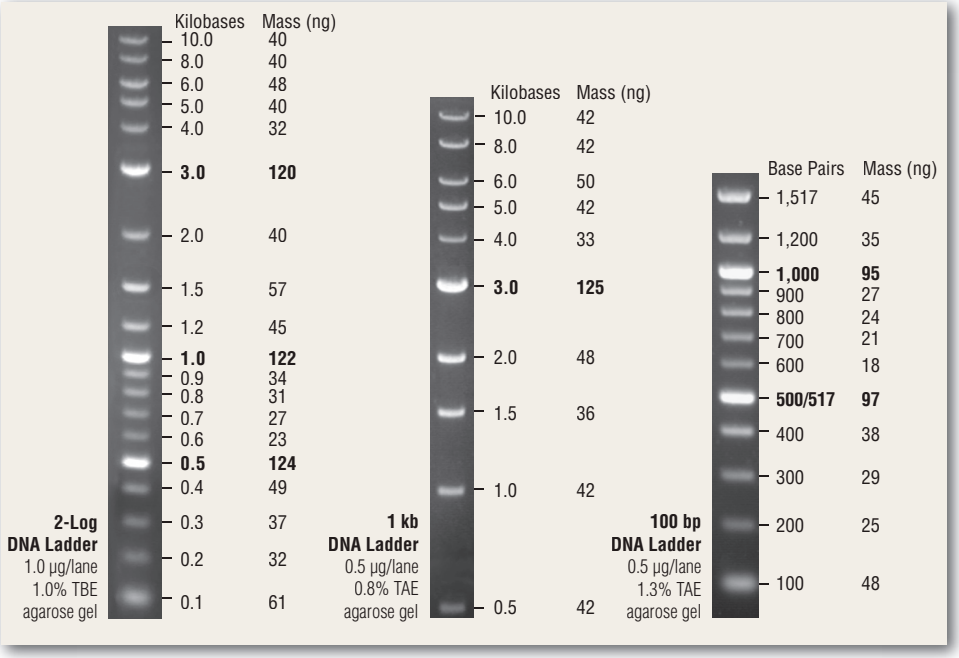


Antarctic Phosphatase – 100% heat inactivation in 5 minutes: *10 units of each phosphatase were incubated under recommended reaction conditions (including DNA) for 30 minutes and then heated at 65°C. Remaining phosphatase activity was measured by p-nitrophenylphosphate (pNPP) assay.*

DNA Ladders from NEB

now available in a variety of convenient formats

Our 2-Log, 1 kb and 100 bp DNA Ladders are now offered in three formats. Choose from the conventional ladder, the Quick-Load version using bromophenol blue as a tracking dye, or TriDye containing three dyes to facilitate monitoring of gel migration. Our ladders include only DNA fragments that are part of the ladder with no "extra" backbone DNA – you pay for the ladder and nothing else.



2-Log DNA Ladder Formats

2-Log DNA Ladder (0.1–10.0 kb)
#N3200S 100 µg
#N3200L 500 µg

Biotinylated 2-Log DNA Ladder
#N7554S 25 µg

Quick-Load™ 2-Log DNA Ladder
#N0469S 125 gel lanes

TriDye™ 2-Log DNA Ladder
#N3270S 125 gel lanes

1 kb DNA Ladder Formats

1 kb DNA Ladder
#N3232S 100 µg
#N3232L 500 µg

Quick-Load™ 1 kb DNA Ladder
#N0468S 125 gel lanes

TriDye™ 1 kb DNA Ladder
#N3272S 125 gel lanes

100 bp DNA Ladder Formats

100 bp DNA Ladder
#N3231S 50 µg
#N3231L 250 µg

Quick-Load™ 100 bp DNA Ladder
#N0467S 125 gel lanes

TriDye™ 100 bp DNA Ladder
#N3271S 125 gel lanes

NEW ENGLAND BioLabs® Inc.
the leader in enzyme technology

New England Biolabs, Inc.
240 County Road
Ipswich, MA 01938-2723
1-800-NEB-LABS
Tel. (978) 927-5054
www.neb.com