

NEB expressions

a scientific update from New England Biolabs

Welcome to the fall edition of *NEB Expressions*. In addition to new product announcements and technical tips, this issue highlights a range of products designed to accelerate your research. The feature article is an overview of several applications of NEB's unique phage display technology. The *New Student Starter Pack* is also featured, which is available to all new research students.

As always, we invite your feedback on our products, services and corporate philosophy.

inside:

Feature Article

- Recent applications of the Ph.D. Phage Display Peptide Libraries

New Products

- Ligases – 3 new ligases for DNA and RNA research

Technical Tips

- Maximizing Ligation Efficiencies
- Choosing a DNA or RNA Ligase for your application

Highlighted Products

- Products to accelerate your PCR reactions and cloning experiments
- Phage Display Products

Web Tool Focus

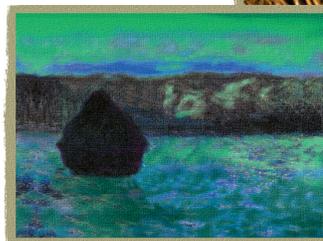
- Double Digest Finder

Of Needles and Haystacks

Recent Applications of the Ph.D. Phage Display Peptide Libraries

Beth M. Paschal and Christopher J. Noren,
New England Biolabs, Inc.

Phage display is a selection technique in which a library of peptide or protein variants is expressed as a genetic fusion to a bacteriophage coat protein. Each variant is displayed on the surface of a virion, while the DNA encoding the variant resides within the virion. This connection between genetic material and selectable function allows rapid identification of variants with desired properties by multiple rounds of affinity partitioning (panning) followed by phage amplification. Individual variants with the desired binding property can then be identified by simply sequencing the DNA contained within each selected phage. Combinatorial peptide libraries displayed on phage have allowed rapid screening of billions of different peptides against a wide variety of target molecules (reviewed in 1–3).



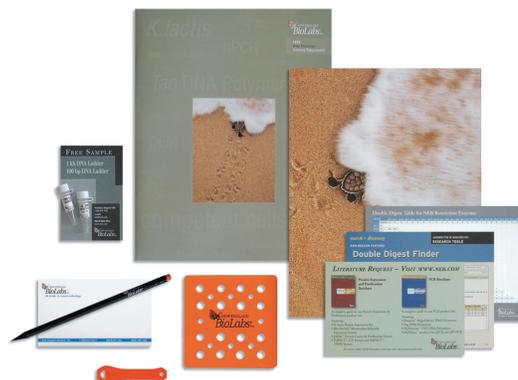
Over the last ten years, the Ph.D. libraries from New England Biolabs have become

the dominant tools in this field, with hundreds of publications describing applications including epitope mapping (4–10), mapping protein-protein contacts (11–16) and identification of peptide mimics of non-peptide ligands (17,18). Bioactive peptides, which can be used as cell-targeting or gene delivery agents, have been identified either by panning against purified receptors (19–24) or against intact cells or tissue samples, both *in vitro* and *in vivo* (25–33). It is apparent that applications of the Ph.D. kits have been limited only by the imagination of the scientific community.

(continued on page 2)

New Student Starter Pack

start your research off on the right path with a *FREE New Student Starter Pack*



Limited Time Offer

Available through
December 31, 2006

New Student Starter Pack Includes:

- NEB Catalog and Technical Reference
- Product Samples (DNA Ladders and *Taq* DNA Polymerase)
- Time-Saver™ Reference Sheet
- Technical Reference Cards
- Floatie and D-Cap It Tube Opener
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Post-it® is a registered trademark of 3M.

The NEB New Student Starter Pack is available to all new research students while supplies last. To register, please visit www.neb.com/starterpack. For additional information, please contact starterpack@neb.com.

Recent Applications of the Ph.D. Phage Display Peptide Libraries

(continued from page 1)

Cell-targeting peptides

Anti-angiogenic therapy is being investigated as a cancer treatment strategy. VEGF is an important angiogenic factor, regulated by kinase domain receptor KDR/Flk-1, which is involved in both physiological and pathological pathways. Hetian and co-workers (21) panned against recombinant KDR (sKDR) to screen for peptides that would bind to KDR and potentially block its interaction with VEGF. A selected peptide sequence was found to inhibit proliferation of primary human vein endothelial cells, to have antiangiogenic effects in chick embryos, and finally to reduce breast tumor growth and metastasis by 70% in mice. Blocking protein-protein interaction with peptides discovered by phage display will have broad clinical applications for treatment of metastasizing tumors.

In order to elucidate the physiological function of the cell surface glycosaminoglycan hyaluronan (HA), Mummert and co-workers (32) panned the Ph.D.-12 library against purified HA. The predominant selected peptide (Pep1) was determined to bind both free HA and HA expressed on cell surfaces, as well as to block HA binding to cell surfaces expressing the HA receptor, CD44. The activity of Pep1 facilitated studies asserting that HA is active in the trafficking of leukocytes at inflamed tissues. Highlighting the exceptional diversity of the Ph.D. libraries, neither alanine scanning nor scrambling of the Pep1 sequence resulted in clones with better activity than Pep1. Amazingly, when applied to skin, Pep1 prevented development of contact dermatitis upon exposure to a known allergen, underscoring connections between HA and immune responses to skin irritants, with broad clinical implications.

Genetic manipulation of dendritic cells (DC) could significantly improve our ability to control immune responses. Chamarthy and co-workers

(25) identified a peptide by whole-cell panning, which is able to recognize murine DCs and function as a gene delivery vehicle when conjugated to a short DNA-binding domain. Up to 25% of DCs could be transformed using this construct when appended to polymeric microspheres.

In vivo panning

By applying Ph.D.-C7C library phage to the skin of a live animal and harvesting self-transfecting phage from the circulating blood, Chen and co-workers (34) identified a peptide that mediates protein delivery through skin. The predominant phage from blood samples was used to design a cyclic synthetic peptide which delivered both insulin and human growth hormone across skin. Furthermore, assays with the full complement of alanine scanned derivatives of the peptide revealed that the originally selected sequence was the best performer, emphasizing the diversity of the library. This exciting work may lead to a new class of systemic drug delivery agents with broad application since there is no requirement for physical association of the peptide with the drug.

Using a combination of *in vitro* and *in vivo* panning, Böckmann and co-workers (35) identified a peptide that targets human thyroid cancer cells over lung cancer cells, human embryonic kidney cells and VH6 fibroblasts. The observed specific binding and internalization of the peptide are promising for adenoviral gene transfer into thyroid tumors. In related work using transgenic mice with thyroid cancer, a single phage clone injected into the animals showed a 2–4X higher titer from harvested tumor cells over other tissues like kidney, liver, lung and heart (36). One sequence appeared in both studies indicating that murine and human derived thyroid cancer tumors share characteristic surface markers.

Anti-viral/microbial peptides

In a search for molecules that will inhibit entry of HIV-1 into cells, Ferrer and Harrison (20) panned all three Ph.D. libraries against an HIV envelope glycoprotein, gp120. A peptide was identified which binds gp120 and inhibits binding to the host protein CD4. A synthetic peptide with this sequence was found to inhibit HIV infection *in vitro*, and represents a promising lead for further drug development (37).

A peptide discovered by Mourez and co-workers (38) provided the basis for a polyvalent inhibitor of anthrax toxin that was able to nearly eliminate toxicity of anthrax in rats. The Ph.D.-12 library was panned against immobilized heptameric PA domain of *B. anthracis* toxin, and peptides specific for inactive, unassembled PA were eliminated by washing with soluble monomeric

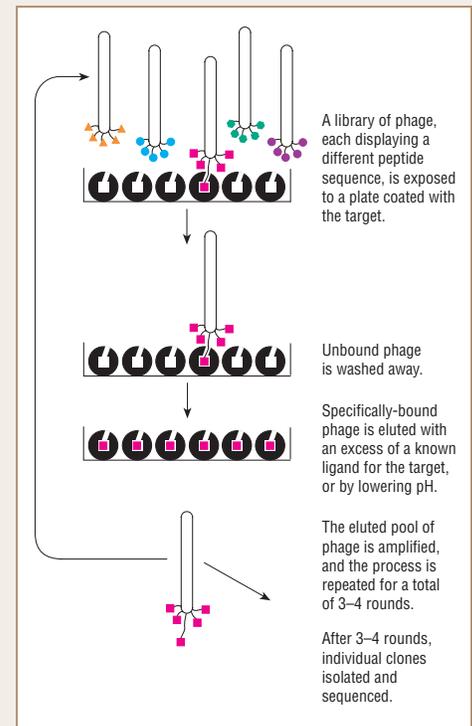


Figure 1: Panning with the Ph.D. peptide library.

PA. Peptides specific for the toxic, heptameric form of PA were eluted competitively with soluble PA heptamer. The resulting peptides were specific for the toxic heptameric form but bound weakly; affinity was improved by tetramerizing the peptides on a soluble scaffold.

Mapping protein-protein interactions

Phage display can be used as an alternative to conventional two-hybrid systems to study protein-protein interactions. Carter and co-workers (11) were able to map contacts between two proteins involved in iron transport in *E. coli*. Panning against one of the proteins, TonB, selected for a number of sequences that align with FhuA, an OM receptor known to bind TonB. By using both linear and constrained loop libraries, a number of binding and structural motifs were identified spanning two distinct regions of FhuA. Binding interactions were confirmed using peptide-MBP fusions generating using the pMal-pIII vector (NEB #N8101).

Mirror image phage display

Mirror image phage display is based on the idea that if phage-displayed peptides (natural L-symmetry) bind to a synthetic target with D-symmetry then it may be assumed the D-peptide analogs of the phage clones will bind to the natural target (L-symmetry). These D-peptides are more resistant to proteolysis in animal systems and thus would have improved bioavailability. Wiesehan and co-workers (39) used mirror image phage display to identify

Phage Display technology is being applied in a wide range of research areas, including:

- Cancer Treatment (21,46)
- Contact Dermatitis Prevention (32)
- Systemic Drug Delivery (34)
- HIV Antiviral Therapy (20,37)
- Anthrax Toxin Inhibition (38)
- Alzheimer's Disease Studies (39)
- Materials Science (40–45)
- Drug Target Discovery (46)

D-peptide ligands for the A β fibril plaques of Alzheimer's disease (AD). The target peptide for panning, A β (1–42), was synthesized entirely with D-amino acids. The predominant selected peptide was subsequently synthesized with D-amino acids and was shown to bind A β with micromolar affinity. Furthermore, in brain tissue sections from AD patients the D-peptide was able to stain plaques of natural A β fibrils much like Congo red dye, but unlike the dye did not stain plaques taken from five other non-AD samples. This work may lead to methods that monitor the disease progression *in vivo*, a crucial step in developing successful treatments.

Material-specific peptides

Angela Belcher has had a long-standing interest in harnessing the methods by which nature makes unique materials and surfaces for nanomaterials development. Her lab has carried out truly pioneering applications of the Ph.D. libraries for the synthesis and assembly of nanosized components into micrometer scale materials with unique electronic and/or optical properties. An initial publication described peptides discovered from panning against specific semiconductor surfaces which specifically bound to different crystalline compositions (e.g. GaAs(100) and Si) (40). In ensuing work, phage particles were engineered to express Ph.D.-selected CdS and ZnS binding peptides along their length via fusion to the pVIII major coat protein, resulting in spontaneous assembly of phage-templated nanowires (41). A viral film assembled in this way was stable at room temperature for seven months and phage were even infectious, demonstrating the reversibility of assembly (42,43). In yet another application, Sinesky and Belcher used the Ph.D. phage display to find peptides which detect surface defects in crystals (44). Surface defects are "inseparable/diffuse targets" but are essential knowledge for determining the quality

of a given material. Finally, in an astonishing application, gold-binding peptides selected from the Ph.D.-12 library were used as the basis for a lithium ion battery electrode consisting of phage-templated gold-cobalt nanowires (45).

Small molecule binding peptides

Paclitaxel (Taxol) is an anti-mitotic agent. To elucidate *in vivo* protein factors with which taxol may interact, biotinylated paclitaxel was screened against a Ph.D. library (46). Comparison of the selected sequences with protein databases pointed to a human anti-apoptotic protein, Bcl-2 as the natural binding partner of paclitaxel. ELISA and circular dichroism experiments confirmed that paclitaxel binds to purified Bcl-2, indicating that phage display peptide libraries can in fact be used to discover biologically relevant peptide ligands for small molecules.

Substrate phage

The Ph.D. libraries can also be used to easily and rapidly determine the substrate specificity of enzymes that modify proteins by panning against an affinity resin specific for the modification. Sugimura et al. (47) used this method to determine the substrate specificity of mammalian transglutaminases (TGase), which carry out cross-linking reactions between glutamine residues and primary amines. The library was incubated with enzyme and a substrate amine (which included a biotin moiety), followed by capture of modified phage on monomeric avidin. Biotin-elutable phage presumably displayed a substrate peptide sequence for the enzyme. Consensus substrate sequences could be rapidly identified for a number of different TGases using this method.

A growing list of literature references for applications of the Ph.D. libraries is available at <http://tinyurl.com/efc9l>. If you know of a reference that is not included on this list, please contact us at phd@neb.com.

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Phage Display Products Available from NEB

New England Biolabs offers three pre-made random peptide libraries as well as the cloning vector M13KE for construction of custom libraries.

The Ph.D. Kits Include:

- Sufficient phage display library for 10 separate panning experiments
- Two Sequencing Primers (100 pmol)
- Host *E. coli* strain ER2738
- Control Target (Streptavidin) and Elutant (Biotin)
- Detailed Protocols

Ph.D. Kits Available:

- Ph.D.-7™ Kit (linear heptapeptide)
#E8100S
- Ph.D.-12™ Kit (dodecapeptide)
#E8110S
- Ph.D.-C7C™ Kit
(disulfide-constrained heptapeptide)
#E8120S
- Components Sold Separately:
Ph.D.-7™ Library (50 experiments)
#E8102L

Ph.D.-12™ Library (50 experiments)
#E8111L

Ph.D.-C7C™ Library (50 experiments)
#E8121L

Companion Products:

Anti-M13 pIII Monoclonal Antibody
#E8033S

Ph.D. Peptide Display Cloning System
#E8101S



Accelerate your research without compromising quality

Choose from a range of products that can help you achieve more results from your valuable lab time.

For faster PCR reactions, choose the Quick-Load™ *Taq* 2X Master Mix. To speed up your cloning experiments, we offer Antarctic Phosphatase and the Quick Ligation™ Kit. For rapid colony growth, try NEB Turbo Competent Cells. Analyze your results more quickly using the Quick-Load™ DNA Ladders and our Time-Saver™ qualified enzymes. All of these products will allow you to achieve results faster, without compromising any quality of your reagents.

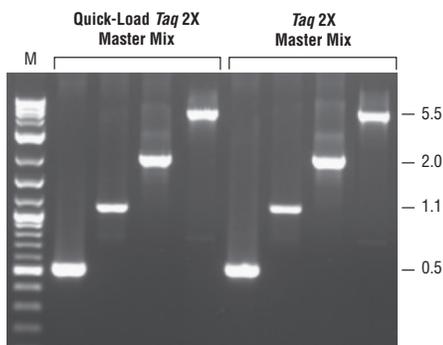
> Fast PCR

Quick-Load™ Taq 2X Master Mix

ready-to-use in your PCR reactions

Advantages

- Contains polymerase, dNTPs, MgCl₂, KCl, tracking dye and stabilizers
- Load PCR reactions directly onto agarose gels
- Contains dye for tracking
- High yield, robust and reliable reactions
- PCR from templates including pure DNA solutions, bacterial colonies and cDNA products



Versatility and yield with the Taq 2X Master Mixes. 40 ng human genomic DNA (hDNA) or 0.01 ng lambda DNA (λ DNA) was amplified in the presence of 200 nM primers in a 25 μ l volume. Marker (M) shown is 2-Log DNA Ladder (NEB #N3200). The 0.5, 1.1, and 2.0 kb fragments are amplified from hDNA, while the 5.5 kb fragment is from λ DNA.

Quick-Load Taq 2X Master Mix
#M0271S 100 reactions
#M0271L 500 reactions



RR = Recombinant

> Quick Cloning

Antarctic Phosphatase

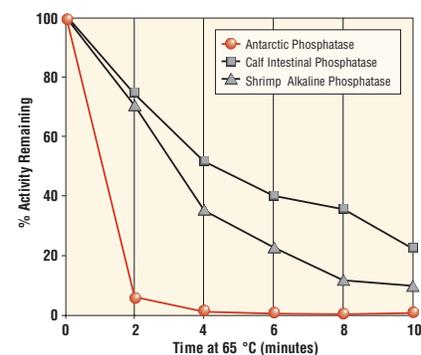
100% heat inactivated in 5 minutes

Advantages

- Ligate without purifying vector DNA
- Recombinant enzyme for unsurpassed purity and consistency
- Active on DNA, RNA, protein, dNTPs and pyrophosphate

Applications

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



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.

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



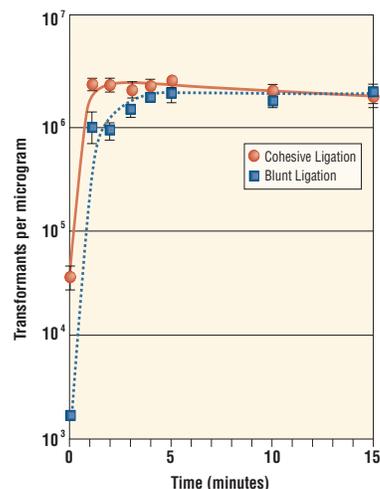
> Quick Cloning

Quick Ligation™ Kit

ligation in 5 minutes at room temperature

Advantages

- 5 minute reactions for cohesive or blunt ends
- Convenience of ligations performed at room temperature
- Suitable for all common ligation reactions



Complete ligation in 5 minutes with Quick Ligation Kit: Using the Quick Ligation Kit protocol, blunt and cohesive inserts were ligated into LITMUS 28 vector (NEB #N3628) cut with either *EcoRV* (blunt) or *HindIII* (cohesive), phosphatase treated and gel purified. Ligation products were transformed into chemically competent *E. coli* DH-5 α cells.

Quick Ligation Kit
#M2200S 30 reactions
#M2200L 150 reactions



> Faster Screening

Time-Saver™ Qualified Enzymes

digest your DNA in 5 minutes;
no special formulation or added expense

NEB restriction enzymes that are Time-Saver™ qualified will digest 1 µg of DNA in 5 minutes using 1 µl of enzyme under recommended reaction conditions. Unlike other suppliers, there is no special formulation, change in concentration or need to buy more expensive new lines of enzymes to achieve digestion in 5 minutes. In fact, 59% of our enzymes will digest 1 µg of DNA in 5 minutes, while 83% will fully digest in 15 minutes. That means >180 of our restriction enzymes have the power to get the job done fast.

Enzyme	Digests in 5 Minutes	Enzyme	Digests in 5 Minutes
BamHI	✓	SacI	✓
BglII	✓	SalI	✓
EcoRI	✓	SmaI	✓
HindIII	✓	SpeI	✓
NcoI	✓	SphI	✓
NotI	✓	XbaI	✓
PstI	✓	XhoI	✓

Time-Saver enzymes are determined based on their ability to digest 1 µg of the specified DNA in 5 minutes with 1 µl of enzyme under recommended reaction conditions. The DNA used for the reactions is also the unit assay substrate and is described in the current NEB catalog and on the web site.

> Rapid Transformation

NEB Turbo Competent *E. coli*

suitable for rapid colony growth

Advantages

- Highest growth rate on agar plates – visible colonies 8 hours after transformation
- Suitable for 5 minute transformation protocol with *Amp^r* plasmids
- Transformation efficiency >10⁹ cfu/µg
- Suitable for blue/white screening by α -complementation of the β -galactosidase gene
- Tight control of expression by *lacI^q* allows potentially toxic genes to be cloned
- Activity of nonspecific Endonuclease I (*endA*) eliminated for highest quality plasmid preparations
- Resistance to phage T1 (*fhuA2*)

NEB Turbo Competent *E. coli*
#C2984H 20 reactions

> Time-Saving Analysis

Quick-Load™ DNA Ladders

ready-to-load onto your agarose gels

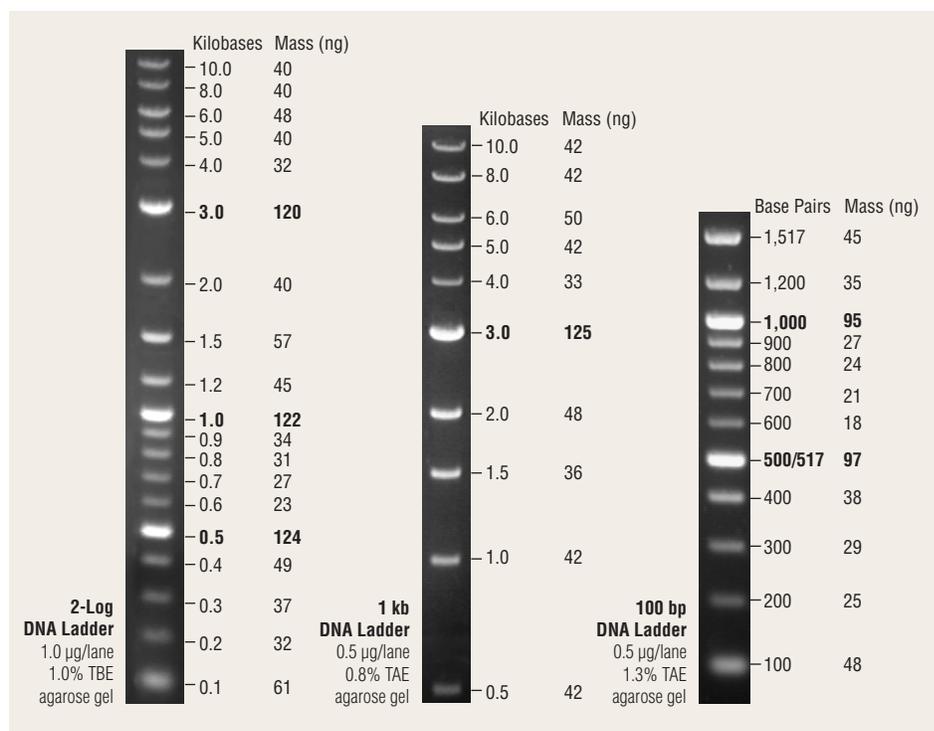
Advantages

- Contains Bromophenol Blue as tracking dye
- Store at 4°C – no need to thaw

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

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

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



New DNA and RNA Ligases

a growing selection of ligases to enhance your DNA and RNA research

NEB offers a variety of ligases for DNA and RNA research. Many of these enzymes are recombinant, and all offer the quality and value you have come to expect from all of our products. The following chart highlights the advantages for each of these ligases.

Ligase Selection Chart	T4 DNA Ligase	<i>E. coli</i> DNA Ligase	Taq DNA Ligase	New 9°N DNA Ligase	T4 RNA Ligase 1	New T4 RNA Ligase 2
Recombinant	✓		✓	✓	✓	✓
Thermostable			✓	✓		
Recommended for cloning	✓					
Ligation of cohesive DNA ends	✓	✓				
Ligation of blunt DNA ends	✓					
Nicks in dsDNA	✓	✓	✓	✓		
Nicks in dsRNA*						✓
Joining of RNA & DNA in a ds-structure*	✓				✓	✓
Labeling of 3' termini of RNA					✓	
Joining ssRNA and dsRNA oligos					✓	
Ligase Detection Reaction & Ligase Chain Reaction			✓	✓		

* For a detailed description of T4 RNA Ligase 1 and T4 RNA Ligase 2 capabilities, see chart to the right.

9°N™ DNA Ligase RR

9°N™ DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini of two adjacent oligonucleotides which are hybridized to a complementary target DNA. 9°N DNA Ligase is active at elevated temperatures (45–90°C).

Advantages

- Ligates nicks in DNA while incubating at high temperatures
- Extremely thermostable and can withstand PCR conditions
- Can be used for allele-specific gene detection using Ligase Detection Reaction and Ligase Chain Reaction
- Allows mutagenesis by incorporation of a phosphorylated oligonucleotide during PCR amplification

9°N DNA Ligase

#M0238S 2,500 units
#M0238L 12,500 units

T4 RNA Ligase 1 (ssRNA Ligase) RR

T4 RNA Ligase 1 catalyzes the ligation of a 5' phosphoryl-terminated nucleic acid donor to a 3' hydroxyl-terminated nucleic acid acceptor through the formation of a 3'–5' phosphodiester bond, with hydrolysis of ATP to AMP and PPi. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates.

Advantages

- Ligation of a ssRNA and DNA
- Labeling 3' ends of RNA with 5'-[³²P] pCp
- Synthesis of ss oligonucleotides
- Incorporates unnatural amino acids into proteins

T4 RNA Ligase 1

#M0204S 1,000 units
#M0204L 5,000 units

T4 RNA Ligase 2 (dsRNA Ligase) RR

T4 RNA Ligase 2 is much more active joining nicks on double stranded RNA than on joining the ends of single stranded RNA. The enzyme requires an adjacent 5' phosphate and 3' OH for ligation. The enzyme can also ligate the 3' OH of RNA to the 5' phosphate of DNA in a double stranded structure.

Advantages

- Both intermolecular and intramolecular RNA strand joining activity
- Ligate a nick in dsRNA

T4 RNA Ligase 2

#M0239S 150 units
#M0239L 750 units

Ligation of nicks in DNA and RNA – Which T4 ligase should you choose?

Courtesy of Dr. Richard Bowater, University of East Anglia, Norwich, UK. Original graph appears in Bullard D. and Bowater R. (2006) *Biochem J.* 398, 135–144.

Substrate Black = DNA Orange = RNA	Rate of Ligation at 37°C (mol nicks joined/mol protein/min)		
	T4 DNA Ligase	T4 RNA Ligase 1	T4 RNA Ligase 2
	–	+	+++
	–	–	+++
	–	–	+++
	+++	+	+++
	–	–	–
	–	–	–
	–	–	–
	+++	–	–

+++ rate is greater than 10
++ rate is 1–10
+ rate is .1–1
– rate is less than 1

Maximizing Ligation Efficiencies

Our T4 DNA Ligase is the most extensively used ligase for cloning-based experiments. Typically, a ligation reaction (blunt or cohesive ends) using traditional T4 DNA Ligase (NEB #M0202) involves incubation at 16°C using 0.1–1 μM DNA (5' termini) in 1X T4 DNA Ligase buffer. For your convenience, T4 DNA Ligase can also be used at room temperature, and is available in concentrated form (NEB #M0202T). Alternatively, NEB's Quick Ligation Kit (NEB #M2200) is uniquely formulated to ligate blunt or cohesive ends in 5 minutes at room temperature. The following tips will help to achieve maximum results from your ligation reactions.

Reaction Buffers

- T4 DNA Ligase Buffer should be thawed on the bench or in the palm of your hand, and not at 37°C (to prevent breakdown of ATP).
- Once thawed, T4 DNA Ligase Buffer should be placed on ice.
- BSA in the Ligase Buffer may precipitate when thawed. This will not affect ligation efficiency.
- Ligations can be performed in any of the four standard restriction endonuclease NEBuffers or in T4 Polynucleotide Kinase Buffer if they are supplemented with 1 mM ATP.
- When supplementing with ATP, use ribo ATP. Deoxyribo ATP will not work.
- Before ligation completely inactivate restriction enzyme by heat inactivation, spin column or Phenol/EtOH purification.

DNA

- Use purified DNA preparations without EDTA or high salt concentrations.
- Either heat inactivate (Antarctic Phosphatase) or remove phosphatase (CIP, BAP or SAP) before ligation.
- Keep total DNA concentration between 1–10 μg/ml.
- Insert:Vector molar ratios between 2:1 and 6:1 are optimal for single insertions.
- Insert:Vector molar ratio should be 6:1 to promote multiple inserts.
- If you are unsure of your DNA concentration, perform multiple ligations with varying ratios.

Ligase

- For most ligations (blunt or cohesive) traditional T4 DNA Ligase or the Quick Ligation Kit are recommended.

- For single base overhangs, it is recommended to use up to 5 μl concentrated ligase at 16°C overnight.
- For large inserts, reduce insert concentration and use concentrated ligase at 16°C overnight.
- T4 DNA Ligase can be heat inactivated at 65°C for 20 minutes.
- Do not heat inactivate if there is PEG in the reaction buffer because transformation will be inhibited. The Quick Ligation Kit contains PEG.

Transformation

- Add between 1–5 μl of ligation mixture to competent cells for transformation.
- Extended ligation with PEG causes a drop off in transformation efficiency (Quick Ligation Kit).
- Electroporation is recommended for large constructs (>10,000 bp). Dialyze sample or use a spin column to purify first.

30th Anniversary Contest (US offer only)

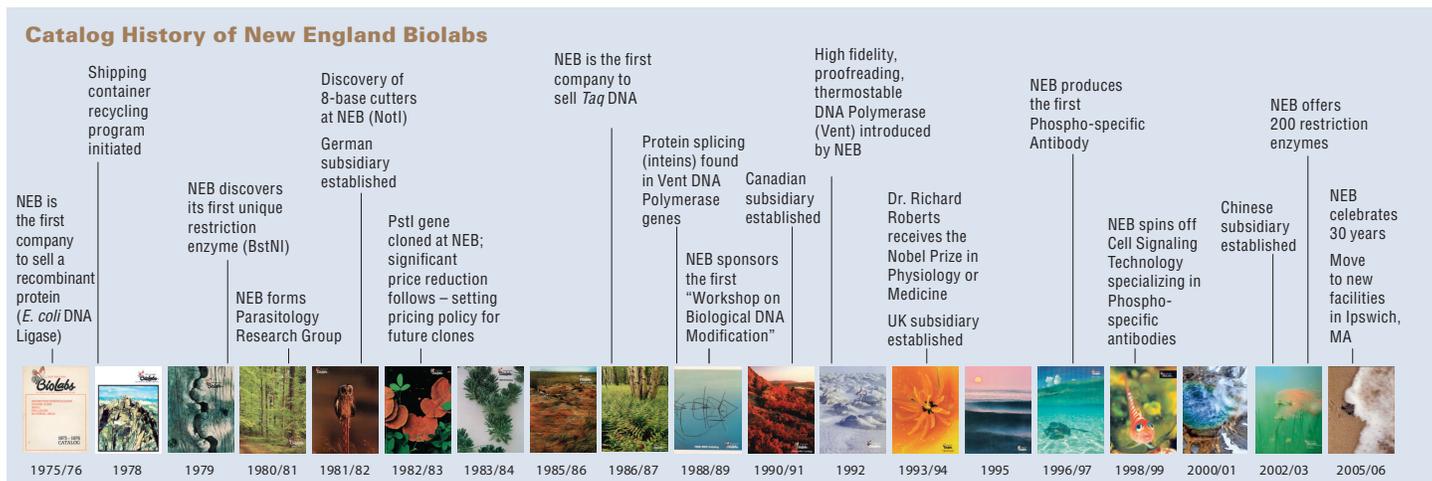
winners spend a day with NEB scientists at our new facility

This spring, NEB held a contest linking our catalog to the closing out of our 30th anniversary celebration. Customers were invited to submit a short statement sharing their thoughts about the NEB catalog. The contest winners ranged from post-docs to career scientists to high school educators and were flown to Boston to spend a summer day at the new NEB headquarters in Ipswich, MA. They were given a tour of the NEB campus, including our state-of-the-art research and production facility, ecologically-oriented wastewater treatment plant and the refurbished Victorian mansion that houses our administrative offices. They learned about the ongoing basic research projects at NEB from Dr. Richard J. Roberts, CSO, and participated in a roundtable discussion.

Thank you to everyone who submitted a contest entry.

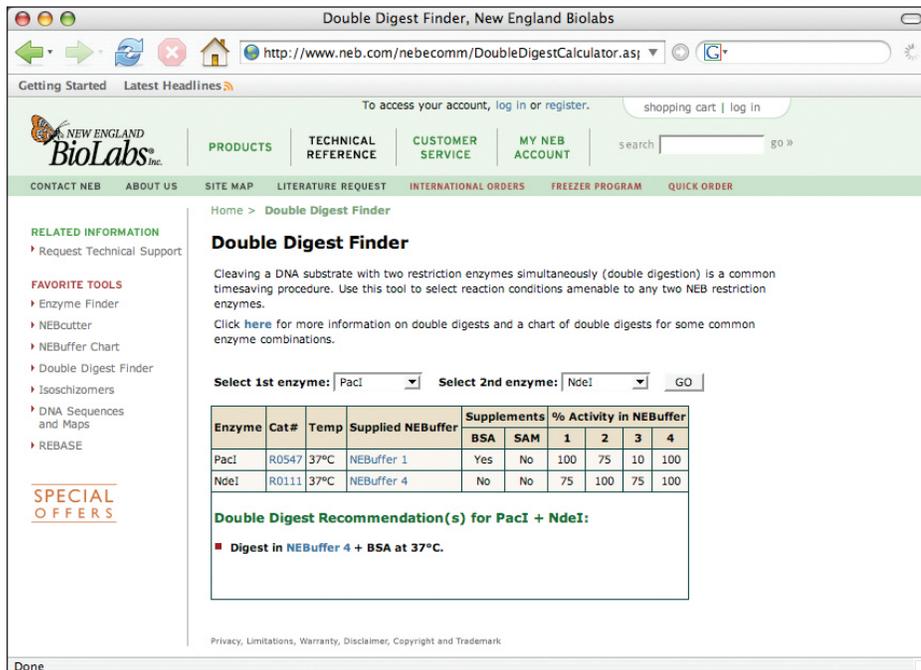


The 30th anniversary contest winners with Dr. Richard J. Roberts.



Double Digest Finder

The technical reference section of our website provides several web based programs to aid in experimental design. The Double Digest Finder allows you to select optimal conditions for a double digest, including buffer, incubation temperature, and supplement requirements.



- Gives recommended reaction conditions for any two NEB enzymes
- Displays recommendations for: appropriate NEBuffer, reaction temperature, BSA and/or SAM addition
- Alerts users if enzymes selected have overlapping recognition sequences

Double Digest Finder:
www.neb.com/nebcomm/doubledigestcalculator.asp

Recommended reaction conditions are displayed for the enzymes chosen.

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