Spring 2007, vol. 2.1

NEB expressions

a scientific update from New England Biolabs

Welcome to the spring edition of NEB Expressions, in which we are pleased to introduce the 2007•08 NEB Catalog & Technical Reference. This issue highlights several important components of our award winning catalog, including new products, reference appendix updates and minireviews that focus on various environmental topics. The feature article overviews the impact of DNA damage, and we introduce the PreCR Repair Mix, a new product designed to repair damaged DNA prior to PCR.

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

inside:

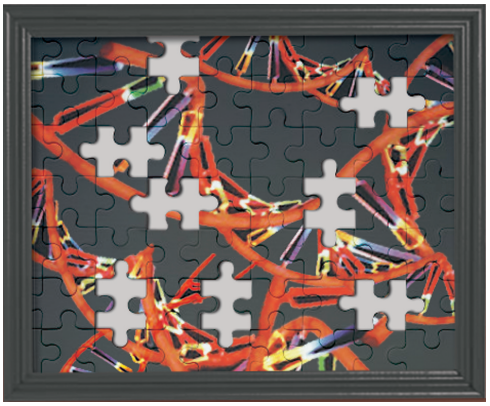
NEW ENGLAND BioLabs® Inc.
the leader in enzyme technology

DNA Damage

the major cause of missing pieces from the DNA puzzle

Thomas C. Evans, Jr.,
New England Biolabs, Inc.

DNA is nature's most widely used long-term information storage system. The elegant simplicity of the double helix belies the complex pathways that have evolved to copy, modify, and maintain the integrity of the genome. In the last 50 years insights into the structure of DNA and the enzymes that act upon it have led to the development of powerful tools for genetic analysis and engineering, including cloning and subcloning with restriction enzymes, DNA sequencing and amplification using PCR. As DNA methodologies have matured they have been applied to more diverse problems such as deriving genetic information from degraded samples, including blood and tissue samples. Gathering sequence information from these samples is critical in forensic identification (1), in the Consortium for the Barcode of Life initiative (2), in deducing the evolutionary relationships of living and extinct species (3), and in associative studies of tissue biopsy collections (4), to name a few. Such studies are experimentally limited by the quality and quantity of the DNA extracted from biological samples. In addition, compounds that inhibit the amplification of the small amounts of extracted



DNA often co-purify with the DNA sample, further complicating analysis. Damaged DNA has therefore become an important experimental issue in many areas of research.

There are many common types of DNA damage that impact accurate replication by DNA polymerases (5). Furthermore, the degree and spectrum of DNA damage depends on the sample source and the type of environment to which it was exposed. Some types of damage are ubiquitous and can potentially be present in all extracted DNA, while other types of damage are the result of exposure to a specific source (see Table 1). Under physiological conditions the most labile bond in DNA is the N-glycosyl bond that attaches the base to the deoxyribose backbone.

(continued on page 2)

PreCR™ Repair Mix

repair a broad range of DNA damage prior to PCR

Unlock genetic information that was previously inaccessible due to damaged DNA template. The PreCR Repair Mix is a blend of recombinant proteins designed to repair damaged DNA, and is ideal for:

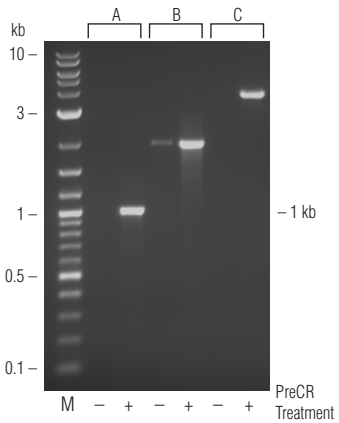
- Ancient DNA
- Environmental DNA
- Forensic samples
- Phenol/chloroform extracted DNA
- UV exposed DNA

The PreCR Repair Mix will not damage template DNA, and this simple 20 minute reaction may increase your chances for successful PCR amplification.

See page 3 for more information.

Limited Time Offer

Purchase the PreCR Repair Mix and receive a small pack of the Quick-Load™ 2-Log DNA Ladder (0.1 – 10 kb).



Repair of different types of DNA damage with the PreCR Repair Mix. *A: UV exposure (λDNA), B: heat treatment (λDNA), C: oxidation (plasmid).*

DNA Damage

(continued from page 1)

This is in contrast to RNA in which the phosphodiester bond in the backbone is the least stable under the same conditions. Hydrolysis of the N-glycosyl bond results in the loss of a base leaving an apurinic/apyrimidinic (AP) site that itself eventually decomposes into a nick. Because the reactive species is H_2O, AP sites are expected in all stored DNA samples. This includes lyophilized samples because it is very difficult to remove the final shell of H_2O molecules immediately adjacent to the DNA.

Under metabolically active conditions it is estimated that approximately 2,000-10,000 AP sites are formed in a single human cell genome each day (5). This rate will vary from sample to sample, especially in samples taken from a crime scene because the type of environmental exposure will vary.

The presence of AP sites in a DNA sample is problematic for two primary reasons. First, genetic information is lost because the AP site cannot form a base pair with an incoming nucleotide during DNA replication. Second, typical PCR polymerases stall at the AP site preventing further replication (6). If enough AP sites are present, amplification or sequencing reactions will simply fail. The breakdown of AP sites into nicks further compounds the problem as it eventually leads to the fragmentation of the DNA.

Another common type of DNA damage that occurs under physiological conditions is the hydrolytic deamination of cytosine to form uracil (5). Sequencing studies on DNA extracted from very old samples, termed ancient DNA, have determined that this is the major damage complicating data analysis (7,8). Cytosine deamination, like AP site formation, is caused by hydrolysis and is probably present in the DNA extracted from many sources. Interestingly, unlike depurination, the rate of cytosine deamination is slowed in double-stranded DNA as compared to single stranded DNA.

The effect of deaminated cytosine in the amplification or sequencing reaction is polymerase dependent. Some polymerases (e.g. *Taq* DNA Polymerase) are able to effectively extend past the deaminated cytosine (i.e. uracil), inserting an adenine residue opposite the uracil instead of a guanine. This generates a mutated daughter strand even though the polymerase was 100% accurate. Alternatively, common proof-reading polymerases, including archaeal polymerases (e.g. Vent, *Pfu*, 9°N DNA Polymerases), stall at deoxyuracil encountered in DNA templates (9). The active site of these polymerases contain a binding pocket that specifically recognizes deaminated cytosine (10). This prevents the damage from creating a permanent mutation in the daughter strand. Because deamination of cytosine may result in inhibition of PCR or mutagenic DNA products, this is a particularly important issue in methods where DNA sequence is crucial. In contrast, methods that rely on the amplicon length rather than the exact sequence (i.e. short tandem repeats used in human identification) are not impacted by the mutagenic effect of cytosine deamination.

A third and common type of DNA damage is oxidation. As in the case of hydrolytic damage, most DNA samples are susceptible to oxidation, as they are exposed to oxygen throughout storage. Many types of base modifications are created by oxidation, but the conversion of guanine to 8-oxo-guanine is one of the most common (5). 8-oxo-guanine can base pair with adenine and is therefore a mutagenic product. Such damage is prevalent in mitochondria and may be one of the factors in the aging process (11). In studies that quantify oxidative damage it has been shown that the DNA extraction process itself can introduce this modification and therefore must be carefully considered (12).

Other types of damage become prevalent only in certain circumstances. DNA-protein or DNA-DNA crosslinks are a specialized, but important, type of damage that blocks the genetic investigation of an enormous number of stored samples. In both the museum and medical research communities a large number of samples are either stored in formalin (formaldehyde) or exposed to formalin at some point. The formalin-induced cross-linking effectively preserves structural morphology, but it is extremely detrimental to subsequent DNA analysis because crosslinked bases stall polymerases and DNA-DNA crosslinks can inhibit denaturation. In addition, the pH of formalin solutions drop over time due to the formation of formic acid, increasing the rate of AP site formation and subsequent fragmentation (13).

Other well-studied lesions that occur only in certain instances are pyrimidine dimers (14). These form when DNA is exposed to UV light and are very effective at stalling DNA polymerases.

Source of DNA	Potential Damage	Comments	References
Ancient DNA	abasic sites, deaminated cytosine, oxidized bases, fragmentation, nicks	Cytosine deamination has been reported to be the most prevalent cause of sequencing artifacts in ancient DNA.	Gilbert, M.T. et al. (2007) *Nuc. Acid Res.,* 35, 1–10. Hofreiter, M. et al. (2001) *Nuc. Acid Res.,* 29, 4793.
Environmental DNA	fragmentation, nicks (plasmid or genomic)	Nicks and fragmentation can increase the formation of artifactual chimeric genes during amplification.	Qiu, X. et al. (2001) *Appl. Envir. Microbiol.,* 67, 880.
Source of Damage			
Exposure to Ionizing Radiation	abasic sites, oxidized bases, fragmentation, nicks	Ionizing radiation is used to sterilize samples.	Sutherland, B.M. et al. (2000) *Biochemistry,* 39, 8026.
Exposure to Heat	fragmentation, nicks, abasic sites, oxidized bases, deaminated cytosine, cyclopurine lesions	Heating DNA accelerates the hydrolytic and oxidative reactions in aqueous solutions.	Bruskov, V.I. (2002) *Nuc. Acids Res.,* 30, 1354.
Phenol/Chloroform Extraction	oxidized bases	Guanine is more sensitive to oxidation than the other bases and forms 8-oxo-G. 8-oxo-G can base pair with A making this damage potentially mutagenic.	Finnegan, M.T. (1995) *Biochem. Soc. Trans.,* 23, 403S.
Exposure to Light (UV)	thymine dimers, (cyclobutane pyrimidine dimers) pyrimidine (6–4) photo products	UV trans-illumination to visualize DNA causes thymine dimer formation.	Cadet, J. et al. (2005) *Mutat. Res.,* 571, 3–17. Pfeifer, G.P. et al. (2005) *Mutat. Res.,* 571, 19–31.
Mechanical Shearing	fragmentation, nicks	Normal DNA manipulations such as pipetting or mixing can shear or nick DNA.	
Dessication	fragmentation, nicks, oxidized bases		Mandrioli, M. et al. (2006) *Entomol. Exp. App.,* 120, 239.
Storage in Aqueous Solution	abasic sites, oxidized bases, deaminated cytosine, nicks, fragmentation	Long term storage in aqueous solution causes the accumulation of DNA damage.	Lindahl, T. et al. (1972) *Biochemistry,* 11, 3610 and 3618.
Exposure to Formalin	DNA-DNA crosslinks, DNA-protein crosslinks	Formaldehyde solution that has not been properly buffered becomes acidic, increasing abasic site formation.	Workshop on recovering DNA from formalin preserved biological samples. (2006) The National Academies Press.

Table 1: Types of DNA Damage
Note: The extent of damage caused by exposure to different reagents can vary, and its importance will depend on how the DNA is being used.

In conclusion, there is a wealth of DNA sequence information contained in degraded samples; however, extracting that information is sometimes difficult. Whether the major difficulty is the efficacy of DNA extraction, the presence of PCR inhibitors, or the extent of DNA damage has not been fully determined. It is most likely a case in which the most prevalent problem varies with the sample and techniques that address all three possibilities are needed. Studies determining what types of damage are present in degraded samples and new methodologies to overcome them will hopefully make previously hidden information accessible.

References:

1. Butler, J.M., (2006) *J. Forensic Sci.,* 51, 253–265.
2. Hajibabaei, M., et al. (2007) *Trends Genet.,* in press.
3. Noonan, J.P., et al. (2006) *Science,* 314, 1113–1118.
4. Thompson, E.R. et al. (2005) *Hum. Mutat.,* 26, 384–389.
5. Lindahl, T., (1993) *Nature,* 362, 709–715.
6. Sikorsky, J.A., et al. (2007) *Biochem. Biophys. Res. Comm.,* 355, 431–437.
7. Stiller, M., et al. (2006) *Proc. Natl. Acad. Sci. USA,* 103,13578–13584.
8. Gilbert, M.T., (2007) *Nucleic Acids Res.,* 35,1–10.
9. Lasken, R.S., et al. (1996) *J. Biol. Chem.,* 271,17692–17696.
10. Fogg, M.J., et al. (2002) *Nat. Struct. Biol.,* 9, 922–927.
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12. Hofer, T., et al. (2006) *Biol. Chem.,* 387, 103–111.
13. Kelly, K. (2006) *Path to Effective Recovering of DNA from Formalin-Fixed Samples in Natural History Collection*, (pp. 5–14). Washington, DC: The National Academies Press.
14. Sinha, R.P., et al. (2002) *Photochem. Photobiol. Sci.,* 1, 225–236.

Repair Enzymes

Detect types of damage in your DNA samples using a variety of techniques. NEB offers several DNA repair enzymes suitable for a variety of damaged DNA. Many of these recombinant enzymes can be produced on a large scale and are available for customized solutions.

- APE 1
- UDG
- *Afu* UDG
- Endo III
- Endo IV
- Endo V
- Endo VIII
- Fpg
- hOGG1
- T7 Endo I
- T4 PDG

PreCR™ Repair Mix

putting the pieces back in the puzzle

The PreCR™ Repair Mix is a cocktail of enzymes formulated to repair damaged DNA *in vitro* prior to PCR. The repair pre-treatment can be applied to techniques such as whole genome amplification, DNA sequencing and microarray analysis.

The PreCR Repair Mix uses enzymes in a coordinated process that emulates base excision repair. The heart of the mix is a combination of polymerase and ligase activities. The ligase chosen acts strongly on nicks but has very low activity on blunt ends to minimize the possibility of chimeric gene formation. Added to this core are enzymes that can recognize different DNA lesions.

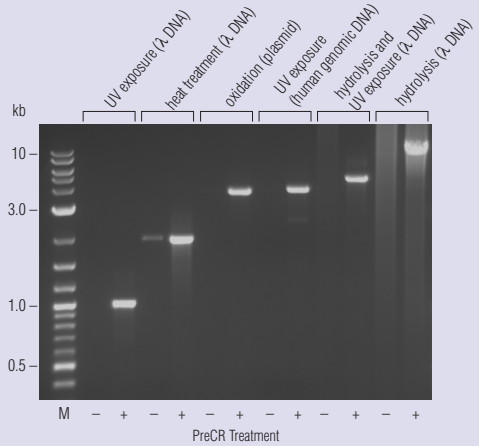


Figure 1: Repair of different types of DNA damage with the PreCR Repair Mix. *The gel shows trial amplifications from damaged DNA that was either not treated (–) or treated (+) with the PreCR Repair Mix. Type of DNA damage is shown. Note: heat treated DNA is incubated at 99°C for 3 minutes. Marker M is the 2-Log DNA Ladder (NEB #N3200).*

DNA Damage	Cause	Can it be repaired by PreCR Repair Mix?
abasic sites	hydrolysis	yes
nicks	hydrolysis nucleases shearing	yes
thymidine dimers	UV radiation	yes
blocked 3´-ends	multiple	yes
oxidized guanine	oxidation	yes
oxidized pyrimidines	oxidation	yes
deaminated cytosine	hydrolysis	yes
fragmentation	hydrolysis nucleases shearing	no
Protein-DNA crosslinks	formaldehyde	no

Table 2: Types of DNA Damage

Currently the PreCR Repair Mix can repair a wide range of damaged DNA, resulting from exposure to heat, low pH, oxygen, and/or UV light (Figure 1). The lesions repaired by the PreCR Repair Mix do not include all possible damages (Table 1). Currently, it cannot repair DNA crosslinks, such as those that occur during exposure to formalin, nor can the mix effectively repair highly fragmented DNA, a common, but difficult problem. If the DNA is highly damaged repair may not be possible.

The power of this repair mix is illustrated in Table 3, where sequence data from plasmid DNA that has been damaged and treated with the PreCR Repair Mix clearly shows that base pair changes incurred due to oxidative damage are corrected using PreCR.

Substitution	Number of Substitutions		
	Control DNA	Damaged DNA	PreCR Treated
A to G or T to C	0	0	0
A to T or T to A	0	0	0
A to C or T to G	0	0	0
C to T or G to A	1	3	0
C to G or G to C	0	0	0
C to A or G to T	0	40	0
error rate per 1,000 bp	0.03	1.4	0
total bp sequenced	30,128	30,128	15,064

Table 3: Analysis of base substitution errors in DNA that has been damaged and treated with the PreCR Repair Mix. *Plasmid DNA was treated with 25 µM Methylene Blue (induces oxidation) and then treated with the PreCR Repair Mix for 15 minutes at 37°C. A 3.7 kb fragment was amplified, cloned and sequenced. The results show that damage incurred upon oxidation was corrected by PreCR.*

PreCR Repair Mix
#M0309S 30 reactions
#M0309L 150 reactions

Introducing the 2007•08 NEB Catalog & Technical Reference

The NEB Catalog & Technical Reference contains many new reagents for PCR, cloning, expression and purification, as well as up-to-date technical charts and protocols to aid in your experimental design. Highlighted products include:

- 11 new restriction enzymes
- 17 restriction enzyme unit size increases
- A new Competent Cell chapter
- A new Epigenetics chapter
- A growing line of DNA Polymerases
- PreCR Repair Mix for damaged DNA template
- DNA Ladders in convenient formats
- A selection of expression systems (yeast, mammalian and *E. coli*)
- High efficiency transfection reagents
- Many new reagents for RNA research

Please make sure your mailing information is up-to-date by visiting www.neb.com.

Water Minireviews

Each NEB catalog contains several minireviews focusing on various environmental topics. This catalog features a collection of essays highlighting varied topics pertaining to water, our most abundant, important and precious natural resource.

Steve Nadis, a writer based in Cambridge, Massachusetts, wrote the water minireviews in the 2007•08 catalog. Steve has worked for the Union of Concerned Scientists, the World Resources Institute, the Woods Hole Oceanographic Institution, and WGBH/NOVA. He spent the 1997/98 academic year at MIT as a Knight Science Journalism Fellow. A Contributing Editor to Astronomy Magazine and a columnist for the Cambridge Chronicle, Nadis has also written for Nature, Science, Scientific American, Audubon, National Wildlife, and other journals. In addition, he has written or contributed to about two dozen books. When he's not working or spending time with his family, he likes to play volleyball and handball.

A Watershed Decision

In the early 1990s, New York City was forced to consider the fate of its water supply, which provides the area's 9 million inhabitants with 1.3 billion gallons a day. The issue came to a head as development brought more people and, with them, more pollutants into the upstate watershed region that had long provided New York residents with "the champagne of tap water." The U.S. Environmental Protection Agency presented two options: construct a new water filtration plant that would cost $6 to $8 billion to build and an additional $300 to $400 million per year to operate, or adopt a comprehensive strategy for protecting the watershed that feeds the Catskill and Delaware reservoirs.

In a move that may be unprecedented for a large, modern metropolis, the city chose the latter course, opting to stake its future on nature.

This minireview is continued on p.222 of the 2007•08 NEB Catalog & Technical Reference.

New Restriction Endonucleases from NEB

NEB maintains an aggressive research program in the discovery, cloning and overexpression of restriction endonucleases. This allows us to offer the largest selection of these essential reagents. Four of our newest enzymes are listed below. For a more up to date list of restriction endonucleases, please see our website, www.neb.com.

CviQI

CviQI is a recombinant neoschizomer of RsaI with a lower optimum incubation temperature.

#R0639S	2,000 units
#R0639L	10,000 units

5′...G▼TAC...3′
3′...CAT▲G...5′

Nb.BsmI

Nb.BsmI is a nicking endonuclease that cleaves only one strand of DNA on a double-stranded DNA substrate.

#R0706S	1,000 units
#R0706L	5,000 units

5′...GAATGCN...3′
3′...CTTAC▲GN...5′

HpyAV

HpyAV is a recombinant Type IIs restriction endonuclease with a unique recognition sequence only available from NEB.

#R0621S	100 units
#R0621L	500 units

5′...CCTTC$(N)_6$▼...3′
3′...GGAAG$(N)_5$▲...5′

NmeAIII

NmeAIII requires two copies of its recognition sequence for cleavage to occur. It produces a stable partial digestion pattern even with excess enzyme. The cleavage point may shift one base pair depending on the DNA sequence context between the recognition site and the position of cleavage. For a given sequence, generally one site will predominate. For details, see www.neb.com.

#R0711S	250 units
#R0711L	1,250 units

5′...GCCGAG$(N)_{20\text{-}21}$▼...3′
3′...CGGCTC$(N)_{18\text{-}19}$▲...5′

RR = Recombinant ⏱ = Time-Saver Qualified

In addition, seventeen enzymes have undergone unit size increases, resulting in even greater value. For more information, see the 2007•08 Catalog & Technical Reference or www.neb.com.

NEB Japan Opens for Business

It is with great pleasure that we announce the opening of our new Tokyo based subsidiary, New England Biolabs Japan, Inc (NEBJ). This is an exciting time for NEB in Japan as we look to further enhance our service and build even stronger relationships with the scientific community. For further information please go to www.nebj.jp or contact the office at info@neb-japan.com.

2007•08 NEB Catalog & Technical Reference

reference appendix update

The 2007•08 Reference Appendix contains up-to-date technical information for restriction enzyme digests, cloning, PCR, expression and general molecular biology applications. New features include selection charts, technical tips and information about our web-based tools. The comprehensive restriction enzyme section now includes tips for optimizing reactions, Timer-Saver information, activity in PCR buffers and a consolidated chart that includes activity in NEBuffers, heat inactivation, incubation temperature and diluent.

Enzyme	Supplied NEBuffer	% Activity in NEBuffers 1	2	3	4	Heat Inactivation (temperature)	Incubation Temperature	Diluent
AvrII	2	100	100	50	100	No	37°	B
BaeI	2 + BSA + SAM	50	100	50	75	65°	25°	A
BamHI	3 + BSA	75	100	[dd]100	100	No	37°	A
BanI	4	50	100	50	100	65°	37°	A
BanII	4	100	10	50	100	65°	37°	A
BbsI	2	100	100	25	75	65°	37°	B
BbvI	2	100	100	25	75			
BbvCI	4	50	100	10	100			
BccI	1 + BSA	100	50	10	50			
BceAI	3 + BSA	100	100	100	100			
BcgI	3 + SAM	NR	NR	[dd]100	NR			

Buffer System Improvements

In an effort to simplify our buffer system, BamHI, Sau3AI and BceAI are now being supplied with one of our NEBuffers. All of these enzymes have been carefully purified and characterized so there is no loss of activity in its newly supplied buffer. If you are still using a unique buffer that was previously supplied with some of our enzymes, please refer to our website, www.neb.com, for double digest information with unique buffers.

50 ml Magnetic Separation Rack
(NEB #S1507) Designed to be used for small-scale separations using magnetic beads.

New Products for RNA Research

In the last few years the view of the importance of RNA in the cell has dramatically changed. Small RNAs are now known to have a major role in the post-transcriptional regulation of gene expression (1). Though RNAi was initially discovered in nematodes (2) and plants (3), RNA mediated regulation is widely found in eukaryotic organisms. Small RNAs play critical roles in regulating gene expression in development, cancer biology, anti-viral defense and chromatin modification. Short interfering RNAs, siRNAs, are widely used as reagents for selective gene knockdown. The role of small RNAs is an active area of research with new RNA classes still being discovered (4). New England Biolabs is committed to developing new reagents for the isolation, amplification, copying and cloning of RNA molecules. We have also developed enzymatically generated potent siRNA mixes for selective gene knockdown studies and transfection reagents to transfer the RNAs into the cell.

Find these products in the RNAi & RNA Enzymes chapter of the 2007•08 NEB Catalog & Technical Reference (p.176–191).

References:
(1) Zaratiegui, M. et al. (2007) *Cell*, 128, 763–776.
(2) Fire, A. et al. (1998) *Nature*, 391, 806–811.
(3) Baulcombe, D. (2004) *Nature*, 431, 356–363.
(4) Ruby, J.G. et al. (2006) *Cell*, 127, 1193–1207.

> RNA Synthesis

E. coli Poly(A) Polymerase

template independent addition of AMP from ATP to the 3´ end of ssRNA

Applications

- Label 3´ end of RNA
- Prepare a priming site for cDNA synthesis using oligo-(dT)
- Enhance translation of RNA transfected into eukaryotic cells
- Label RNA with cordycepin or ATP
- Generate poly(A) tail for cDNA synthesis, cloning or affinity purification

E. coli Poly(A) Polymerase RR
#M0276S 100 units
#M0276L 500 units

phi6 RNA Polymerase (RdRP)

phage RNA dependent RNA polymerase

Applications

- Copy ssRNA into dsRNA in a highly processive manner
- Synthesize dsRNA for RNAi experiments
- Synthesize dsRNA *in vitro*

phi6 RNA Polymerase (RdRP) RR
#M0255S 60 units
#M0255L 300 units

> cDNA Synthesis

AMV Reverse Transcriptase

RNA-directed DNA polymerase

Advantages

- Synthesize cDNA from single-stranded RNA for cloning or quanitation
- Greater than 95% pure by SDS-gel electrophoresis free of detectable levels of RNase, endonuclease and exonuclease activities (Highly purified enzyme is required for synthesis of full-length DNA)
- Higher temperature optimum than reverse transcriptases from mammalian viruses
- Suitable for RNA Sequencing and RT-PCR

AMV Reverse Transcriptase
#M0277S 200 units
#M0277L 1,000 units

for high (2.5X) concentration
#M0277T 500 units

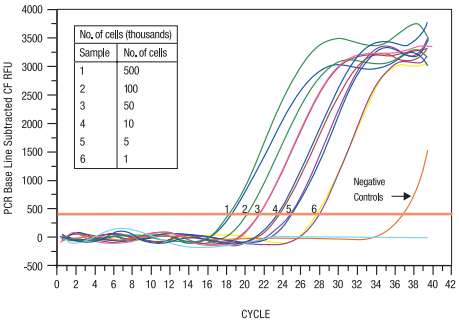
> Isolation

Magnetic mRNA Isolation Kit

isolate intact poly(A)+ RNA from cells and tissue

Advantages

- Automated high-throughput applications
- Eliminates need for phenol or other organic solvents
- Eliminates need to precipitate poly(A)$^+$ transcripts in eluent
- Complete isolation in less than one hour
- Negligible gDNA contamination
- Reusable Oligo d(T)$_{25}$ paramagnetic beads



Consistency and wide isolation range are demonstrated by poly(A)$^+$ RNA isolation from duplicate samples of decreasing numbers of HEPG2 cells by direct lysis/binding in microtiter plate followed by mRNA isolation with the poly (A) magnetic method. 1/10th of isolated mRNA is converted to oligo (dT) primed cDNA using ProtoScript First Strand cDNA Synthesis Kit (NEB #E6500) and qPCR done with validated primers for the peptidylpropyl isomerase, a low-abundance housekeeping gene.

Magnetic mRNA Isolation Kit
#S1550S 25 isolations

> Analysis

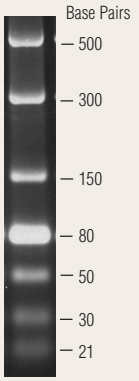
RNA Ladders and Markers

dsRNA Ladder
#N0363S 25 µg

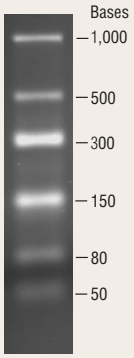
Low Range ssRNA Ladder
#N0364S 25 µg

siRNA Ladder
#N2101S 4.5 µg

microRNA Ladder
#N2102S 6 µg

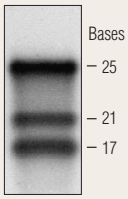


dsRNA Ladder
1.5 µg/lane
2% TBE agarose gel



Low Range ssRNA Ladder
1 µg/lane
2% TBE agarose gel

siRNA Marker
(dsRNA)
45 ng/lane
20% TBE polyacrylamide gel



microRNA Marker
(ssRNA)
60 ng/lane
12% polyacrylamide-urea gel

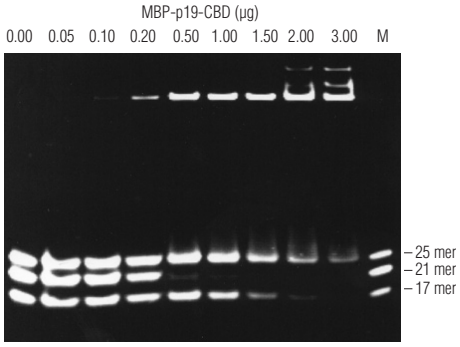
> Isolation

p19 siRNA Binding Protein

for the isolation of siRNA

Applications

- Bind RNA in a size dependent and sequence independent manner
- High affinity purification of siRNA with chitin magnetic beads
- Suppresses RNA interference in plants



Size specific binding of siRNA by p19. *Three dsRNAs of 17, 21 and 25 bases, 90 ng total, were mixed with increasing amount of MBP-p19-CBD fusion protein from 0 to 3 µg in a 20 µl reaction. Binding reaction was incubated at room temperature for 2 hours. The 20% polyacrylamide gel was stained after electro phoresis with ethidium bromide. Marker M is the siRNA Marker (NEB #N2101).*

p19 siRNA Binding Protein RR
#M0310S 1,000 units
#M0310L 5,000 units

> Ligation and Cloning

T4 RNA Ligase 2, truncated

ligation of RNA and/or DNA

Advantages

- Requires 5´ pre-adenylated RNA or DNA for ligation (reduces background ligation)
- Joins a single stranded adenylated primer to small RNAs for cloning
- Selectively ligates an adenylated oligo in an RNA:DNA hybrid
- miRNA cloning

T4 RNA Ligase 2, truncated RR
#M0242S 100 units
#M0242L 500 units

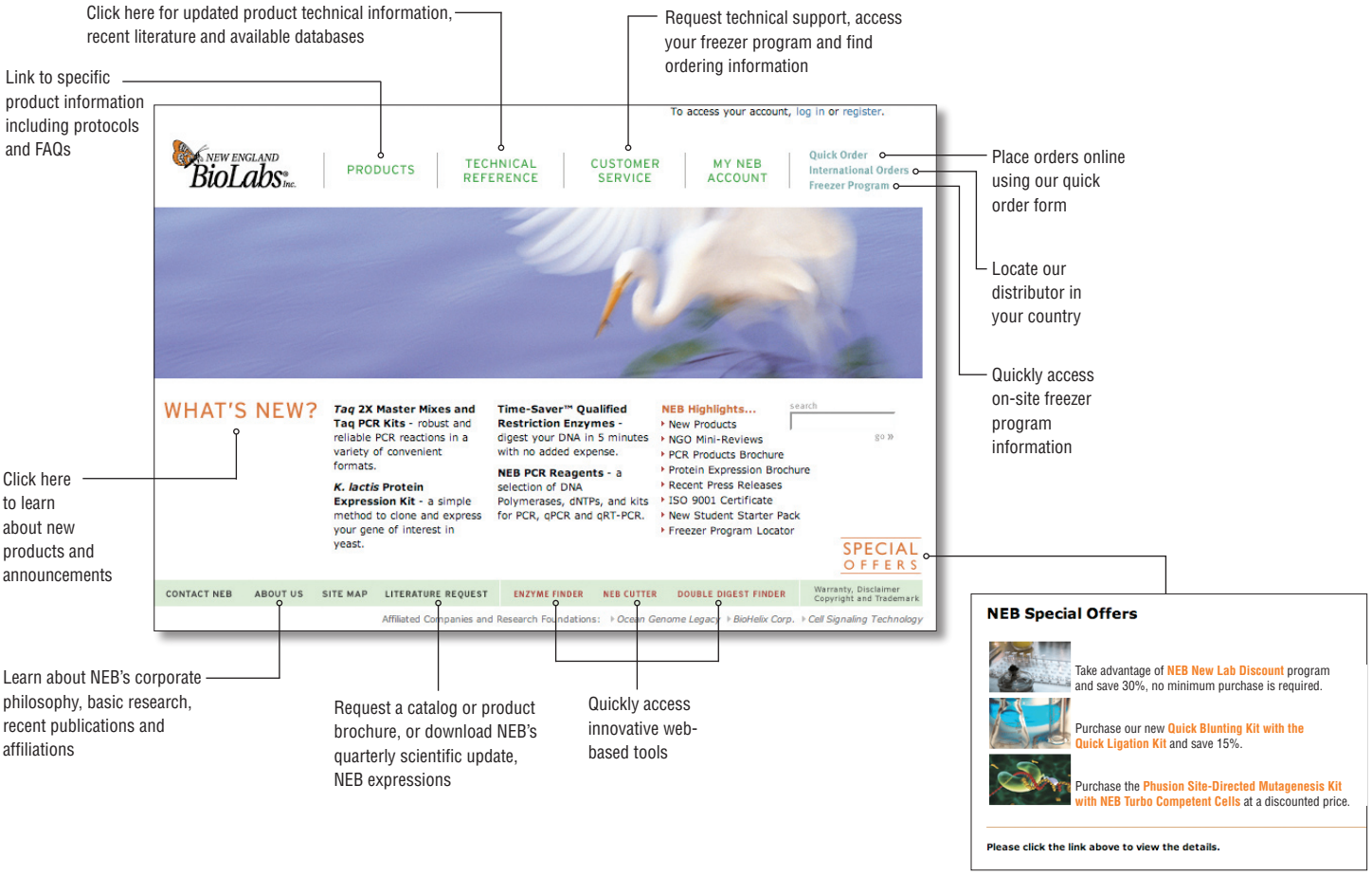
22nd Annual Molecular Biology Workshop

This two-week summer intensive is held at Smith College in Northampton, MA. The course emphasizes hands-on molecular biology laboratory work and covers a wide variety of topics and techniques, including:

- Gene cloning
- Gene expression analysis
- PCR and qRT-PCR
- Genomics
- Bioinformatics
- DNA sequencing and fingerprinting
- RNAi and siRNA
- Microarrays

No previous experience in molecular biology is required or expected.
For additional information, course dates and to fill out an application, visit the Summer Workshop website: http://www.science.smith.edu/neb

www.neb.com



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