Fall 2007, vol. 2.3

NEB expressions

a scientific update from New England Biolabs

Welcome to the fall edition of NEB Expressions. This issue features various protein expression systems available from NEB, and a selection chart based on application. The topic of CpG methylation is dicussed, including its effect on restriction digests and selected reagents for studying methylation patterns. The feature section highlights the environmental theme of this year's catalog, "The Water Planet."

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

inside:

NEW ENGLAND BioLabs® Inc.
the leader in enzyme technology

The Water Planet

as highlighted in the 2007–08 NEB catalog

For over 30 years, the NEB catalog has been a resource for scientists around the world. Each edition of the catalog contains minireviews that address various environmental topics. As scientists, we hope these articles raise awareness of the impact we have on our environment and our need to maintain stewardship of the earth.

Past minireviews have included:

1996–97	Endangered Coastal Environments
1998–99	Marine Protected Areas
2000–01	Nature's Role in Sustaining the Biosphere
2002–03	Biodiversity
2005–06	Non-Governmental Organizations (NGOs)

The theme of the 2007–08 catalog is "The Water Planet", and it features a collection of essays that highlight this abundant, important and precious natural resource.

Raindrops filter naturally through moss capsules, or sporangia, on the forest floor.
© Stephen Dalton/Minden Pictures

Many interesting topics are presented, including plans to drill through four kilometers of ice into Lake Vostok, which is thought to contain an untouched microbial ecosystem. The future of our drinking water is also addressed through advances in desalination technology and the unprecedented decision by a major US city to pursue a natural water filtration system. Excerpts from a selection of minireviews are found on page 2.

Attention New Graduate Students

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

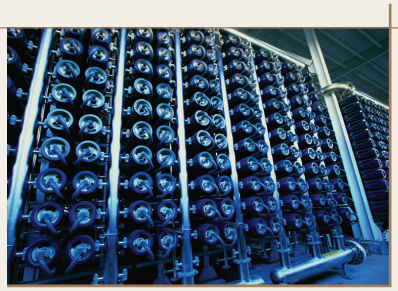
New Student Starter Pack Includes:

- 2007–08 NEB Catalog and Technical Reference
- Product Samples
- Time-Saver™ Restriction Enzyme Information
- Technical Reference Cards
- NEB Buffer Chart Poster
- Floatie and D-Cap It Tube Opener
- Post-it®

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 your local distributor.

Highlights from "The Water Planet"

Excerpts from several of "The Water Planet" minireviews are found below; articles in their entirety can be found in the 2007-08 Catalog & Technical Reference, and on our website, www.neb.com, under "NEB Highlights".



Reverse osmosis cartridge filters as seen in Tampa Bay Seawater Desalination Plant, Tampa, Florida.
© David Sailors/Corbis

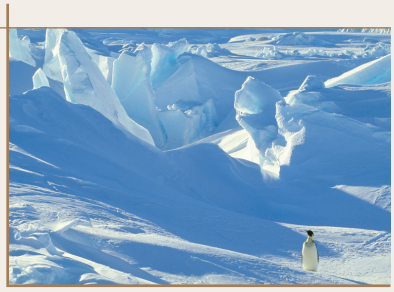
Water from the Sea

Although the earth is called the "water planet", 97 percent of that water is too salty for human consumption, as well as unfit for agriculture except for the most salt tolerant crops. Desalination– the extraction of freshwater from seawater or other salty or brackish sources– is common in the desert nations of the Middle East, which rely on this approach for more than 70 percent of their drinking water. In other regions, where water is more plentiful, desalination has been comparatively rare. That picture, however, is now changing. With a third of the world's population lacking adequate water supplies and underground aquifers rapidly being drained, more and more countries believe the solution to their water woes may lie, at least in part, in the oceans.

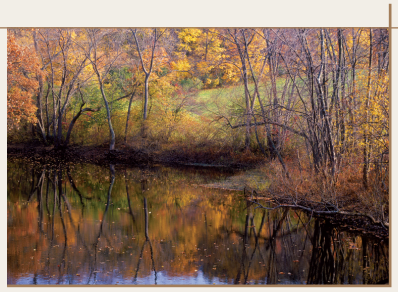
(continued on page 150 of the catalog)

Life in the Deep Freeze

If all goes as planned, a Russian research group will soon drill into Lake Vostok– the largest of Antarctica's underground lakes, buried beneath nearly four kilometers of ice. Drilling toward Vostok began in 1989, proceeding with fits and starts, and it was suspended from 1998 to 2005 as scientists weighed the risks of environmental contamination. Apart from the technical difficulties of operating a drill in the ultra harsh conditions found in Antarctica, the major stumbling block has been... *(continued on page 112 of the catalog)*



Emperor penguin (Aptenodytes forsteri) on icefield, Weddell Sea, Antarctica.
© Frans Lanting/Minden Pictures



Reflection of the landscape on the Ipswich River, Massachusetts.
© Edward Monnelly

The Not-So-Mighty Ipswich

In May 2006, after a week of heavy rains that dumped more than a foot of water in parts of northeastern Massachusetts, the Ipswich River experienced record floods. The normally tranquil waterway became a raging torrent, overrunning its' banks and inundating streets, homes and businesses. By August, however, flows on the river had dropped to very low levels, putting fish and other aquatic life in jeopardy.

(continued on page 254 of the catalog)

Iceland: A Nation Powered by Water

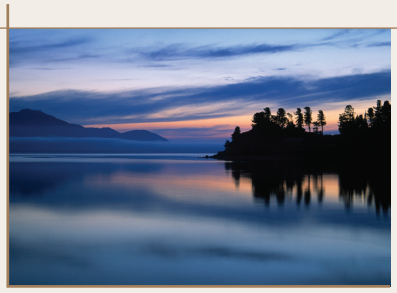
Gulfoss, or Golden Falls, on Iceland's White River is one of the most powerful waterfalls in Europe. On this land, water doesn't just drop from above; heated to the boiling point by volcanic magma below, it can burst through the earth's surface and shoot hundreds of feet into the air. In addition to reveling in the natural splendor of their waterfalls and geysers, the citizens of Iceland have also put their moving water to good use. Iceland could become the first country to have a fossil free energy economy completely powered by water in various guises... *(continued on page 366 of the catalog)*

Gullfoss waterfall on the glacial river Hvita cascades 105-feet, Iceland.
© Frans Lanting/Minden Pictures

Giant Clams (Tridacna squamosa), Phoenix Islands, South Pacific.
© Paul Nicklen/National Geographic

One Last Haven of Ocean Wilderness

A series of biodiversity surveys headed by marine biologist Gregory Stone have shown the Phoenix Islands, an eight island archipelego in the central Pacific, to be one of the last bastions of ocean wilderness, containing some of the most pristine coral reefs left on the planet. The New England Aquarium has joined forces with Conservation International and the Republic of Kiribati to create the Phoenix Islands Protected Area, the third largest marine protected area (MPA) in the world. These parties have worked out a unique arrangement to preserve this marine wilderness... *(continued on page 210 of the catalog)*



Silhouette of trees by twilight, Lake Baikal, Russia.
© Konstantin Mikhailov/Foto Natura/Minden Pictures

Lake Baikal: The Pearl of Siberia

At first glance, it seems a most unlikely setting. Whoever expected to find a rich clustering of life forms – a veritable "biodiversity hotspot" – in a freshwater lake in Siberia? How could an oasis, sometimes compared to the Galapagos Islands, be found on one of the coldest, most unforgiving locales on Earth? But Lake Baikal, Siberia's "blue pearl" or "Sacred Sea", is unlike any other lake on the planet.

(continued on page 192 of the catalog)

The water minireviews in the 2007-08 catalog are written by Steve Nadis, a writer based in Cambridge, MA.

Access to safe drinking water is the top development priority in the third world, but rural communities are often overlooked. © Erik Decamp

Agua Para La Vida

As part of our commitment to the water planet, NEB has offered support to Agua Para La Vida (APLV).

Since 1987, APLV has been helping rural Nicaraguan communities build their own drinking water and sanitation systems. Nicaragua is the poorest Central American country and its rural population, like that of many other third world nations, suffers from contaminated water supplies. Current estimates are that 50% of rural Nicaraguans do not have access to safe drinking water. Although much of Nicaragua is either hilly or mountainous and relatively rich in small springs, the impoverished Nicaraguan government lacks the resources to institute an effective rural program to construct drinking water systems. The bulk of infant disease and death is due directly or indirectly to water-born bacteria, viruses and parasites.

As the only active organization providing assistance to rural communities for drinking water systems, APLV works with communities that have demonstrated a commitment to plan, organize and complete a drinking water project. APLV provides technical assistance and materials, but the community does the work. Together, APLV and the community find an appropriate spring, test it for contaminants, cap it with concrete, bury a pipe from the spring to the community in a trench 4 feet deep, build a storage tank, and design and build a distribution system to bring the water to tap-stands in the community.

Once host to 9,000 different species of trees and plants, Nicaragua has lost approximately fifty percent of its forest cover since 1950. This deforestation, a result of land-clearing, logging, burning and cattle-grazing, has had a profound effect on the quality and quantity of water available for drinking. In addition to providing clean water, sanitation and health education, APLV works closely with communities to protect local watersheds.

If you are interested in learning more about Agua Para La Vida's work in Nicaragua, please visit their website, http://www.aplv.org

An Unconventional Approach to Wastewater Treatment

Andrew Posner, JTED, Inc.

NEB's headquarters in Ipswich, MA was designed to minimize its environmental impact. As part of the plan to build a more environmentally sound facility, NEB chose an innovative method to treat wastewater, rather than use conventional sewerage. All of NEB's wastewater is treated on-site using a Solar Aquatics® Wastewater Treatment System that is capable of treating up to 27,500 gallons per day. Housed in a beautiful greenhouse abound with tropical plants, the system utilizes and accelerates the processes found in streams and wetlands to purify the water to tertiary standards.

The water treatment process combines proven wastewater treatment practices with the biological components of ecologies found in nature. Water first enters an in-ground blending tank, where it is aerated and bioaugmented with microorganisms that have been recycled from the clarification process described below. The aerobic biological breakdown of organic matter begins here; compounds such as fats, starches and proteins are converted to carbon dioxide and simpler matter that can be utilized by organisms downstream.

The water continues into the greenhouse where it enters several six foot tall translucent tanks. These tanks host a variety of vegetation which provide a habitat for bacteria, zooplankton, snails, and other organisms, while algae grow on the tank walls. This ecosystem works in unison to metabolize nutrients and further break down and remove wastes. Autotrophic bacteria nitrify ammonia by oxidizing it into nitrate while algae and plants directly metabolize nitrate, ammonia and soluble orthophosphates. The bacteria are consumed by rotifers while snails, zooplankton, and worms begin the process of sludge digestion.

Next is the clarification process, where the suspended biological solids are allowed to settle. The biologically rich sediment that forms in the clarifier is recycled back to bioaugment the incoming wastewater stream in the blending tank. Water leaving the clarifier is clear in appearance but still contains microbes and certain nutrients (including nitrates). After clarification, any remaining solids are removed by a sand filter.

Tropical plants growing inside the greenhouse of the Solar Aquatics Wastewater Treatment have a key role in the water treatment process.

Following filtration, water enters the self-contained subsurface flow wetlands, where denitrification is achieved in anoxic conditions. Nitrate is reduced by facultative heterotrophic bacteria to nitrogen gas, hydroxide, and water in the presence of an electron donor. Certain pathogenic bacteria are destroyed by the action of wetland plants and further nutrient removal is achieved. To keep the wetland biology flourishing, approximately 50 percent of the water that flows to the end is returned back to the beginning of the wetland for bioaugmentation.

The final treatment step is disinfection. Ultraviolet light is used to kill or denature bacteria and viruses safely without the use of chemicals such as chlorine. The tertiary quality water meets stringent requirements, making it clean enough for water reuse or groundwater recharge. NEB discharges this high quality water for the purpose of groundwater recharge; a great option given its locality to the Ipswich River*.

** The Ipswich River, with 45 tributaries, has a 155 square mile watershed and was named one of the 10 most endangered rivers in America due to repeated low-flow and no-flow periods.*

We're looking for your input...

If you have any suggestions for the environmental theme of the upcoming 2009–10 Catalog & Technical Reference, please e-mail them to catalogtheme@neb.com. We look forward to hearing your ideas.

> Expression Systems Update

Gene Expression & Protein Purification

Protein expression and purification can be very challenging because there is no single approach that is appropriate for every protein or downstream application. Successful expression can be affected by each individual protein's solubility, toxicity, need for post-translational modifications or its intrinsic ability to fold. Additionally, the application for which the protein is ultimately to be used may dictate how the protein needs to be expressed or purified. It is therefore important to have a flexible set of tools and methods that can be applied to each unique expression and purification project.

NEB offers systems for *E. coli,* yeast and mammalian expression. Each technology offers different advantages enabling you to choose the strategy that best suits your protein expression and purification needs. Various applications along with the recommended NEB kit are highlighted below.

Application	Kit	Advantages
High Yield Expression	pMAL™ Protein Fusion and Purification System	Substantial yields (up to 100 mg/L) in more than 75% cases tested; uses the strong P_{tac} promoter.
	K. lactis Protein Expression Kit	Uses the strong *LAC4* promoter; multiple integrations of plasmid may result in higher yield.
	IMPACT™ Kit	Uses the T7 promoter for higher expression and tight control.
Enhanced Solubility	pMAL™ Protein Fusion and Purification System	Fusion to MBP enhances solubility of proteins in *E. coli.**
	K. lactis Protein Expression Kit	Utilizes *K. lactis* eukaryotic folding pathway.
Affinity Tag Chromatography	IMPACT™ Kit	Utilizes an intein-CBD tag on either the N- or C- terminus.
	pMAL™ Protein Fusion and Purification System	Fusions to MBP allows for purification on amylose resin.
Post-translational Modification	*K. lactis* Protein Expression Kit	Secretion of both N- and O- glycosylated proteins.
	RheoSwitch® Mammalian Inducible Expression System	
Periplasmic Expression	pMAL™ Protein Fusion and Purification System	Periplasmic expression enhances folding of proteins with disulfide bonds.
Secreted Expression	*K. lactis* Protein Expression Kit	Eliminates cell lysis, simplifying purification.
	RheoSwitch® Mammalian Inducible Expression System	
Characterization of Eukaryotic Proteins in Homologous Systems	RheoSwitch® Mammalian Inducible Expression System	Synthetic inducer and engineered receptor eliminate non-specific side effects.
Toxic Genes	*K. lactis* Protein Expression Kit	Utilizes a *LAC4* promoter that has been modified to lack background expression in *E. coli.*
	RheoSwitch® Mammalian Inducible Expression System	Precise regulation of expression.
	IMPACT™ Kit	Can express the toxic gene in two fragments and ligate the proteins together.
Protein Labeling or Ligation	IMPACT™ Kit	Elutes proteins with reactive ends (N-terminal cysteine and/or C-terminal thioester). The C-terminal thioester can be labeled after purification.
No Additional Amino Acid Residues	IMPACT™ Kit	Native protein sequence is fused adjacent to site of cleavage.

*Kapust and Waugh (1999) *Protein Science*, 8, 1668–1674.

> Superior Control

RheoSwitch® Mammalian Inducible Expression System

The RheoSwitch® Mammalian Inducible Expression System represents the next generation of inducible gene expression systems. Precise regulation is achieved through the highly specific interaction of a synthetic inducer and a chimeric bipartite nuclear receptor. Applications for this system include inducible protein expression, expression of toxic genes and overexpression/mutant rescue studies.

Advantages

- Precise regulation of expression
- Suitable for expression of toxic genes and post-translationally modified proteins
- Greater than 10,000 fold induction with inducer (RSL1)
- Negligible basal expression
- Synthetic inducer and engineered receptor eliminate non-specific side effects
- No special culture medium requirements

Kit includes:

pNEBR-R1 Regulator Plasmid, pNEBX1-GLuc Control Plasmid and pNEBR-X1Hygro Vector

RheoSwitch Ligand RSL1

Gaussia Luciferase Assay Kit (Buffer & Substrate)

RheoSwitch R-X1 Sequencing Primer

RheoSwitch Mammalian Inducible Expression System
#E3000S

References:
1. Chong, S. et al. (1997) *Gene*, 192, 277–281.
2. Chong, S. et al. (1998) *Nucl. Acids Res.*, 26, 5109–5115.
3. Evans, T.C. et al. (1998) *Protein Sci.*, 7, 2256–2264.

> Improved Kit for One-step Purification

IMPACT™ Kit

Intein Mediated Purification with an Affinity Chitin-binding Tag (IMPACT™) is a unique technology that utilizes the inducible self-cleavage activity of a protein splicing element (termed intein) to separate the target protein from an affinity tag, enabling the purification of recombinant proteins in a single chromatographic step (1–3). This kit also distinguishes itself from other fusion systems by its ability to separate the recombinant protein from the affinity tag without the use of a protease. This kit replaces the IMPACT-CN and IMPACT-TWIN systems.

Advantages

- No proteases required to remove affinity tag
- Expression of target protein with no additional amino acids
- Fusion to either the C- or N-terminus of the target protein
- Isolation of proteins with or without an N-terminal methionine
- Ideal for the ligation and labeling of recombinant proteins

Kit includes:

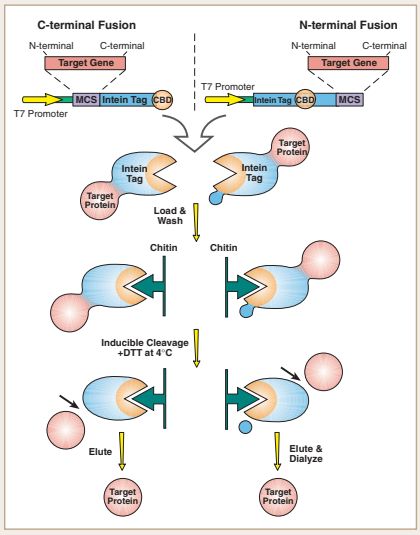
pTXB1 and pTYB11 vectors
pMXB10 control vector
Chitin Beads
E. coli Strain ER2566
Anti-CBD Serum (rabbit)
1,4-Dithiothreitol (DTT)
Blue Loading Buffer

IMPACT Kit
#E6901S

Special Offer

Purchase the IMPACT Kit with T7 Express Competent *E. coli* (20 single-use transformation tubes) at a discount.

IMPACT Kit with T7 Express Cells
#E0543S



Schematic of the IMPACT System

> Yeast Expression with Attractive Commercial Sublicensing

K. lactis Protein Expression Kit

The *K. lactis* Protein Expression Kit provides a simple method to clone and express your gene of interest in the yeast *Kluyveromyces lactis*. This system offers many advantages over bacterial systems and eliminates the methanol containing medium and antibiotic requirements of *Pichia pastoris*.

Advantages

- High yield protein expression
- Rapid high cell density growth
- Methanol-free growth media
- Plasmid integration enhances stability
- Acetamide selection enriches for multiple copy integrants, enhancing yield
- Expression of toxic genes
- Access to eukaryotic protein folding and glycosylation
- Simultaneous expression of multiple proteins

Kit includes:

pKLAC1 vector and pKLAC1-*mal*E control plasmid
Integration Primer Set
Yeast Medium Pack
SacII and NEBuffer 4
K. lactis GG799 Competent Cells and Transformation Reagent

K. lactis Protein Expression Kit
#E1000S

> High Yield Expression; Improved Vectors for More Efficient Purification

pMAL™ Protein Fusion & Purification System

For high yield expression system in *E. coli*, the pMAL™ Protein Fusion & Purification System utilizes an MBP-fusion protein which is then purified by affinity chromatography. This system now includes new and improved pMAL vectors, engineered for tighter binding to amylose.

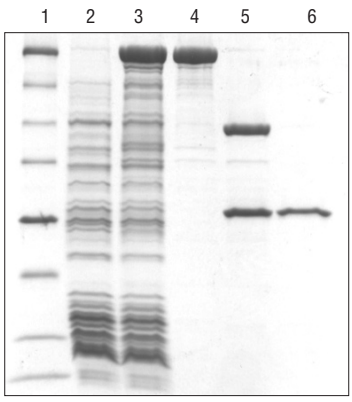
Advantages

- High yield
- Fusion to MBP enhances solubility
- No detergents or denaturants required for elution
- Choice of vectors for cytoplasmic or periplasmic expression

pMAL Protein Fusion and Purification System
#E8000S

Kit includes:

pMAL–c4X and pMAL–p4X vectors
Amylose Resin
Factor Xa
Anti-MBP Antiserum
MBP2* (marker for SDS-PAGE)
MBP2*-paramyosin-ΔSal (control protein)
E. coli Host TB1



SDS-PAGE analysis fractions from the purification of MBP-paramyosin-ΔSal. *Lane 1: Protein Marker, Broad Range (NEB #P7702). Lane 2: uninduced cells. Lane 3: induced cells. Lane 4: purified protein eluted from amylose column with maltose. Lane 5: purified protein after Factor Xa cleavage. Lane 6: paramyosin fragment in flow-through from second amylose column.*

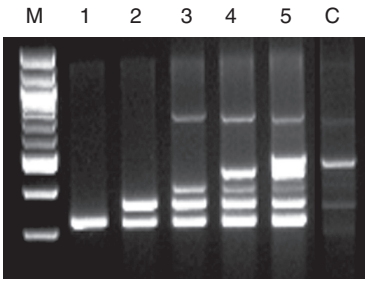
New ET SSB (Single-Stranded Binding Protein)

enhance the specificity and speed of your PCR reactions

ET SSB (Extreme Thermostable Single-Stranded Binding Protein) enhances PCR reactions by stabilizing single-stranded DNA, thus preventing reannealing. Isolated from a hyperthermophilic microorganism, ET SSB remains fully active after incubation at 95°C for 60 minutes. Due to its extreme thermostability, ET SSB can be used in applications that require extremely high temperature conditions, such as thermocycling, nucleic acid amplification and sequencing.

Advantages

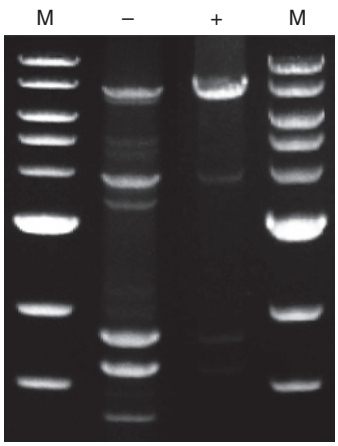
- Increases polymerase yield
- Increases specificity and processivity
- Active in any polymerase buffer
- Ideal for challenging reactions, including multiplex PCR, long range and GC rich amplification



Improve multiplex PCR reactions with ET SSB. *By adding ET SSB, specific amplification was accomplished in PCR using one to five primer pairs in increasing order (lanes 1-5). Lane C is the control reaction using 5 primer pairs, and no ET SSB. Lane M: 100 bp DNA Ladder (NEB #N3231).*

ET SSB
#M2401S 50 µg

Developed and produced by Biohelix Corporation, a New England Biolabs affiliated company.



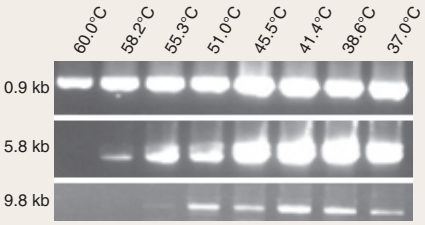
ET SSB enhances long range amplification. *A PCR reaction using primers specific for a 7.4 kb PCR product shows that the presence of ET SSB enhances amplification of the 7.4 kb target, and reduces amplification of non-specific bands. Marker M is the 1 kb DNA Ladder (NEB #N3232).*

New AMV Reverse Transcriptase

higher temperature optimum than other reverse transcriptases

Advantages

- Synthesize cDNA from ssRNA for cloning or quantitation
- Greater than 95% pure by SDS-PAGE
- Suitable for RNA Sequencing and RT-PCR



AMV has a higher optimum temperature.
Approximately 2 units of AMV were included in 20 µl first-strand cDNA synthesis reactions using 1 µg human spleen total RNA in the presence of $dT_{23}VN$ primer. Reactions were carried out for 1 hour at different temperatures. Different fragment sizes of p532 guanidine exchange factor were amplified with 35 PCR cycles using 1/10 of the cDNA products.

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

for high (2.5X) concentration
#M0277T 500 units

NEB FAQ Spotlight

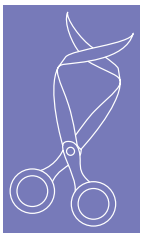
designing PCR primers for downstream cloning reactions

Q I am designing primers to amplify a PCR fragment, and would like to incorporate BclI restriction sites close to the end. Where can I find the number of flanking nucleotides required for efficient cleavage using this enzyme?

A The table on page 331 of the 2007-08 NEB Catalog entitled "Cleavage Close to the End of DNA Fragments (linearized vector)" can be used as a guide to help determine the number of bases necessary for efficient cleavage of PCR products. This table is also available in the Technical Reference section of our website. In general, when engineering sites close to the ends of a primer, 4 to 6 extra bases should be added to ensure efficient cleavage. These extra bases should be carefully chosen to avoid the formation of palindromes, primer-dimers and the priming to secondary sites on the template.

Q Why was the chart "Cleavage Close to the Ends of DNA Fragments (oligonucleotides)" removed from your catalog?

A Originally, there were two tables listed in the catalog entitled "Cleavage Close to the Ends". One used a series of short, double-stranded oligonucleotides as substrate, while the other used linearized vector. The table which utilized oligonucleotides was removed from the catalog several years ago, since this data was often misinterpreted as being predictive of efficiency of cleavage of sites close to the ends of PCR products. Data in this table is valid only for the specialized protocol detailed for studying the efficiency of restriction enzyme cleavage of short oligonucleotides. This chart is still available in the Technical Reference section of our website.



CpG (^{m}CG) Methylation

identifying CpG Methylation and its effect on digestion

DNA methyltransferases (MTases) transfer a methyl group from S-adenosylmethionine to either adenine or cytosine residues. CpG MTases, found in higher eukaryotes (e.g., Dnmt1), transfer a methyl group to the C5 position of cytosine residues. Patterns of CpG methylation are heritable, tissue specific, and correlate with gene expression. Consequently CpG methylation has been postulated to play a role in differentiation and gene expression (1).

CpG methylation should be considered when digesting mammalian and plant genomic DNA. Cleavage with restriction endonucleases that contain the sequence CG in their recognition site, can be blocked or impaired when the cytosine residue is methylated. Restriction sites can also be blocked if an overlapping site is present. In this case, part of the CpG recognition site is generated by the restriction enzyme sequence, and is either followed or preceeded by the remaining sequence. Both situations should be considered when designing restriction enzyme digests. Since most bacterial DNA (including *E. coli*) is not CpG methylated, inhibition of enzyme activity is not an issue for most DNA prepared from normal *E. coli* strains.

A chart detailing methylation sensitivity for all NEB restriction enzymes, part of which is shown below, is found on pages 314–316 of the 2007–08 NEB Catalog & Technical Reference, as well as the website.

Enzyme	Sequence	Dam	Dcm	CpG
AatII	GACGTC	●	●	■
AccI	GTMKAC	●	●	□ ol
Acc65I	GGTACC	●	□ scol	□ scol
AciI	CCGC	●	●	■
AclI	AACGTT	●	●	■
AcuI	CTGAAG(16/14)	●	●	●
AfeI	AGCGCT	●	●	■

Legend

●	Not Sensitive
■	Blocked
□ ol	Blocked by Overlapping
□ scol	Blocked by Some Combinations of Overlapping
◆	Impaired
◇ ol	Impaired by Overlapping
◇ scol	Impaired by Some Combinations of Overlapping

Additional Reagents for CpG Methylation Studies

New Control DNA

Altered epigenetic patterns are a hallmark of cancer. Many genes are silenced in cancerous cells due to newly acquired de novo methylation of CpG islands (2). Currently, there are several methods to detect methylated CpGs. Methylation-specific PCR (MSP) is a technology used for the sensitive detection of gene methylation in the genome (3). Since this is an extremely sensitive assay, the use of control DNA is necessary.

NEB offers several genomic DNAs that can be used as pattern controls for MSP, Bisulfite sequencing, Methylation-sensitive Single-Nucleotide Primer Extension (Ms-SNuPE), Combined Bisulfite Restriction Analysis (COBRA), Bisulfite treatment and PCR-Single-Strand Conformation Polymorphism Analysis (Bisulfite-PCR-SSCP/BiPS).

These genomic DNAs can also be used in PCR, SNP Analysis, Southern Blotting and Genomic DNA library construction.

Catalog #	Name*	Size
N4001S	Jurkat Genomic DNA	15 µg
N4002S	CpG Methylated Jurkat Genomic DNA	15 µg
N4003S	5-Azadc Treated Jurkat Genomic DNA	15 µg
N4004S	NIH 3T3 Mouse Genomic DNA	15 µg
N4005S	CpG Methylated NIH 3T3 Mouse Genomic DNA	15 µg
N4006S	HeLa Genomic DNA	15 µg
N4007S	CpG Methylated HeLa Genomic DNA	15 µg

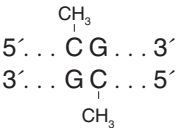
** Genomic DNAs have been isolated from tissue culture cells and will retain their partial methylation patterns. "CpG Methylated" DNAs have been fully modified in vitro using CpG methyltransferase.*

CpG Methyltransferase (M.SssI)

RR NEB2 37° SAM Yes

The CpG Methyltransferase, M.SssI, methylates all cytosine residues (C5) within the double-stranded dinucleotide recognition sequence 5´...CG...3´.

#M0226S 100 units
#M0226L 500 units



McrBC

RR NEB2 BSA 37° Yes

McrBC is a tool for determining the methylation state of CpG dinucleotides. McrBC will act upon a pair of Pu^{m}CG sequence elements, thereby detecting a high proportion of methylated CpGs, but will not recognize HpaII/MspI sites (CCGG) in which the internal cytosine is methylated.

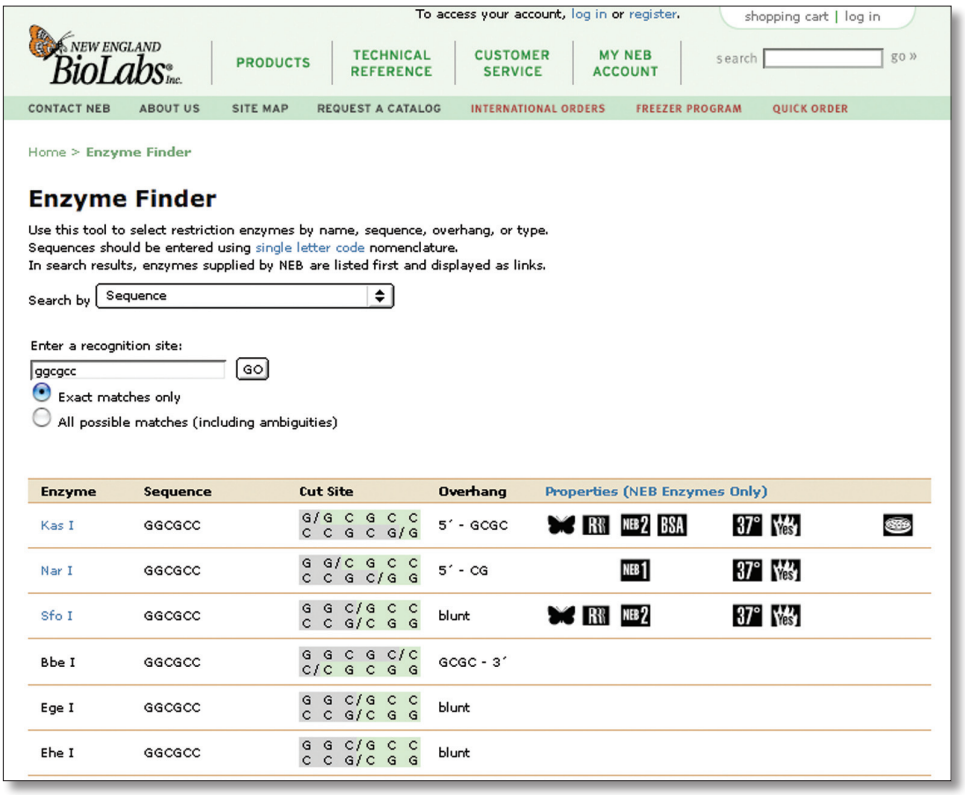
#M0272S 500 units
#M0272L 2,500 units

5´. . .Pu^{m}C ($N_{40\text{-}3000}$) Pu^{m}C. . . 3´

References:
1. Siegfried, Z. and Cedar, H. (1997) *Curr. Biol.*, 7, 305–307.
2. Bird, A. P. (1986) *Nature*, 321, 209-213.
3. Herman, J. G. et al. (1996) *Proc. Natl. Acad. Sci. USA*, 18, 9821–9826.

Enzyme Finder

The latest version of Enzyme Finder, accessible from our homepage, can be used to search for restriction enzymes by name, sequence, overhang or type. Search results include all enzyme matches, with properties for NEB enzymes listed.



Enzyme Finder:
www.neb.com/nebecomm/EnzymeFinder.asp

Other web-based programs available from NEB:

- Double Digest Finder – selects optimal conditions for a double digest
- NEBcutter – finds large ORF's, restriction sites and generates custom digests for selected data
- REBASE – contains comprehensive information for all known restriction endonucleases
- Freezer Program Locator – contains extensive information on NEB freezer programs

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