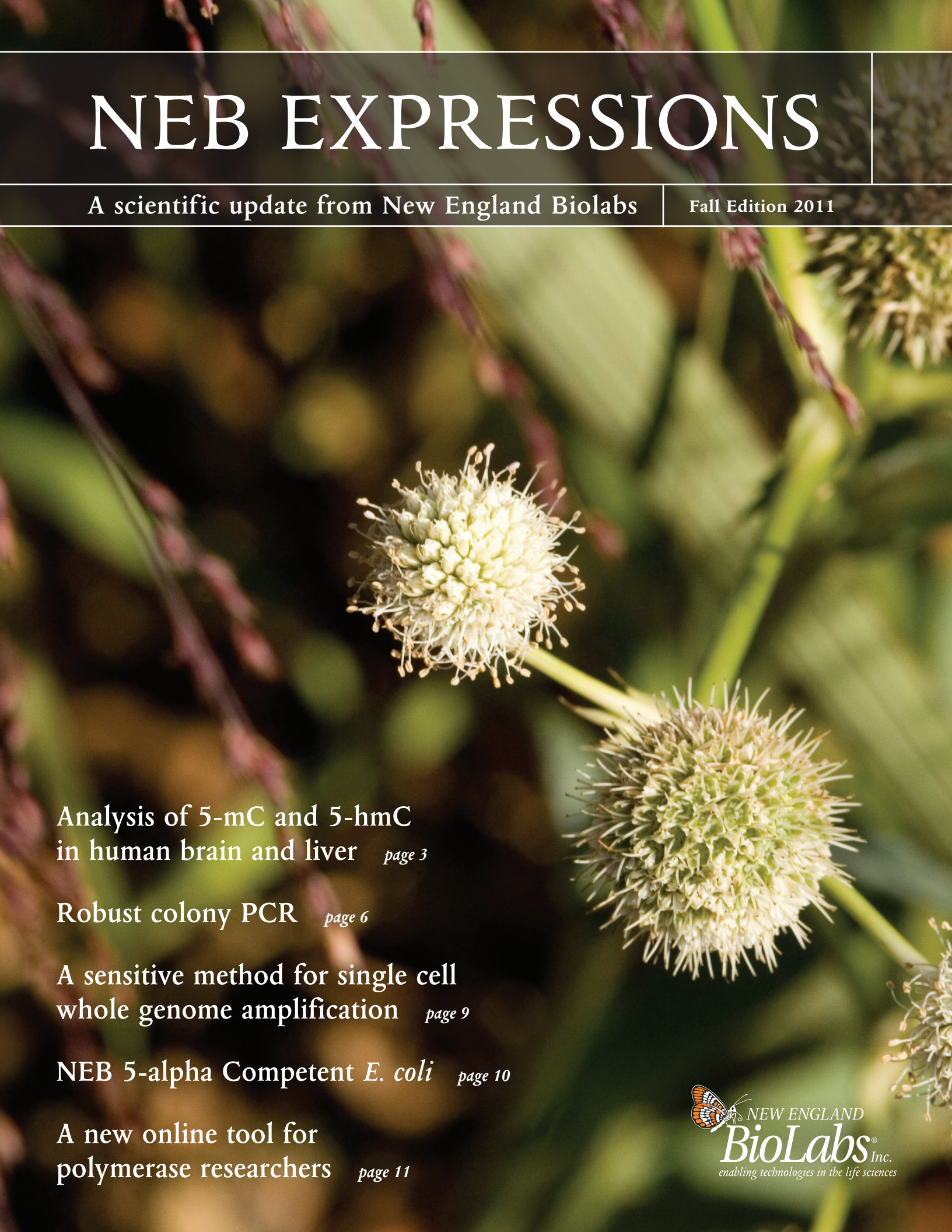
NEB EXPRESSIONS

A scientific update from New England Biolabs | Fall Edition 2011



NEW ENGLAND BioLabs® Inc.
enabling technologies in the life sciences

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Upcoming Tradeshows

Visit the NEB booth at the following meetings:

- **Society for Glycobiology Annual Meeting**
  November 9–11, 2011
  Seattle, WA
  Booth #4
  http://www.glycobiology.org/

- **Society for Neuroscience**
  November 12–16, 2011
  Washington, DC
  Booth #2338
  http://www.sfn.org/am2011/

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A Letter from NEB

Dear Researcher,

As scientists, we are driven to understand change. Whether biological, physical or technological, it is in our genes to ask questions, hypothesize solutions and make new discoveries that evoke change. At New England Biolabs, we are committed to providing specialized reagents that facilitate discovery and the understanding of change. Epigenetics, a rapidly growing area of research that involves the study of heritable changes in the genome, is an important area of interest for NEB scientists. By engaging in epigenetics research projects and collaborating with other scientists, we have been able to better understand our customers' needs and develop the appropriate tools to aid their research. This understanding has led to the development of a suite of EpiMark™ validated products that simplify DNA and histone modification studies. In support of these efforts we have also sponsored the development of E3, an online forum for epigenetics researchers to connect and discuss pertinent topics.

This edition of NEB expressions also highlights applications that utilize our growing line of PCR polymerases, specifically colony PCR and single cell whole genome amplification. As part of NEB's support of open access to scientific information, we are pleased to introduce Polbase, the first free and open database focused specifically on polymerases. More information on Polbase can be found on page 8.

Wishing you continued success in your research,

New England Biolabs

Cover photo: White flowers of Eryngium yuccifolium (Rattlesnake Master) on the New England Biolabs campus. Photographed by Kim Indresano.

DNA Ligases SPECIAL OFFER

Purchase NEB 5-alpha with NEB's Quick Ligation Kit and receive the item of lesser value for FREE.

For more information, visit **www.neb.com/DNAligases**

Offer valid in the US only, through November 30, 2011.

Analysis of 5-methylcytosine and 5-hydroxymethylcytosine Levels in Human Brain and Liver DNA Samples using the EpiMark™ 5-hmC and 5-mC Analysis Kit

Shannon Morey Kinney, Ph.D., Hang Gyeong Chin and Sriharsa Pradhan, Ph.D., New England Biolabs, Inc.

Introduction

In the mammalian genome, enzymatic DNA methylation is the predominant epigenetic modification. DNA methylation is dynamic, heritable, and reversible, providing important determinants for an array of epigenetic states regulating phenotype and gene expression patterns (1). Cytosine residues undergo modification at the carbon-5 position by DNA (cytosine-5) methyltransferases (2) to form 5-methylcytosine (5-mC). In the genome, variable percentages of 5-mC are enzymatically oxidized to 5-hydroxymethylcytosine (5-hmC) by a family of Ten Eleven Translocation (TET1/2/3) enzymes. 5-mC is predominately found in CpG dinucleotide sequences, although small percentages are also found in non-CpG (CpHpG and CpHpH) context (3). The functional role of CpG methylation in mammalian genome is structural, possibly involving disruption of transcription factor binding and/or recruitment of transcriptional repressors such as methyl CpG binding proteins (MeCPs). Compared to 5-mC, 5-hmC may influence chromatin structure and local transcriptional activity by repelling 5-mC binding proteins or recruiting 5-hmC specific proteins. Indeed, in a previous report it was demonstrated that methyl-binding protein MeCP2 does not recognize or bind to 5-hmC (4). More recent reports using several other methyl binding proteins, including MBD1, MBD2, and MBD4, support this hypothesis (5). Because 5-hmC is present in the mammalian genome, specifically in gene bodies (intragenic regions) and enriched at promoters, but largely absent from non-gene regions in DNA, suggests its biological functions differ from 5-mC (6). The presence of 5-hmC in gene bodies is positively correlated with gene expression levels. Therefore, the importance of studying 5-mC and 5-hmC separately for their biological roles is of scientific interest.

The EpiMark 5-hmC and 5-mC Analysis Kit offers a robust method for the quantitative determination of 5-hmC in CpG context, embedded in CCGG sites in the mammalian genome. This enzymatic approach utilizes MspI sensitivity to glucosylated 5-hmC in a simple three step protocol (Figure 1, page 4).

It is known that certain areas of human brain and liver DNA have densely methylated CpG sites (7). Understanding the amount of 5-mC and 5-hmC percentage in specific tissues will help to elucidate the role of 5-hmC in epigenetic regulation. In this application note, a CpG site from four different genes (EGFR, PRKAA2, RPL11P5, and VANGL1) was interrogated using the Epimark Kit in both liver and brain tissue DNA, in order to determine the percentage of 5-mC and 5-hmC at specific loci.

continued on page 4...

Ordering Information

PRODUCT	NEB #	SIZE
EpiMark™ 5-hmC and 5-mC Analysis Kit	E3317S	20 reactions
COMPANION PRODUCT		
T4 Phage β-glucosyltransferase	M0357S/L	500/2,500 units

References:

1. Kim, J.K., Samaranayake, M. and Pradhan, S. (2009) *Cell Mol. Life Sci.* 66, 596–612.
2. Kinney, S.R. and Pradhan, S. (2011) *Prog. Mol. Biol. Transl. Sci.* 101, 311–333.
3. Lister, R., et al. (2009) *Nature,* 462, 315–322.
4. Valinluck, V., et al. (2004) *Nucleic Acids Res.* 32, 4100–4108.
5. Jin, S. G., Kadam, S., and Pfeifer, G. P. (2010) *Nucleic Acids Res.* 38, e125
6. Stroud, H., et al. (2011) *Genome Biol.* 12, R54.
7. Kinney, S.M., et al. (2011) *J. Biol. Chem.* 286, 24685–24693.

Protocol

1. Assemble glucosylation reaction on ice in nuclease free, 0.5 ml microcentrifuge tubes (x, X, y, and Y are dependent on DNA concentration):

TUBE	CONTROL	EXPERIMENTAL
H_2O	X µl	Y µl
10X NEBuffer 4	15 µl	10 µl
gDNA	x (4.5 µg)	y (3 µg)
EpiMark BGT	0 µl	1 µl
EpiMark UDP-glc	1.5 µl	1 µl
Total	150 µl	100 µl

Mix the reaction gently by tapping or pipetting. Briefly centrifuge the reaction mixture and incubate at 37°C for 12 to 18 hrs.

2. Assemble restriction enzyme digestion reactions on ice in nuclease-free, 0.5 ml microcentrifuge tubes:

TUBE	1	2	3	4	5
H_2O	–	–	–	–	1 µl
Mix from tube C	50 µl	50 µl	–	–	50 µl
Mix from tube E	–	–	50 µl	50 µl	–
EpiMark MspI (100U/µl)	1 µl	–	1 µl	–	–
EpiMark HpaII (50U/µl)	–	1 µl	–	1 µl	–
Total	51 µl	51 µl	51 µl	51 µl	51 µl

Mix the reaction gently by tapping or pipetting. Briefly centrifuge the reaction mixture and incubate at 37°C for 1 hr. Digested samples can be diluted up to 2-fold for use with PCR.

3. Perform qPCR in triplicate (volumes are for a single 10 µl reaction):

TUBE	1	2	3	4	5
H_2O	3.2 µl	3.2 µl	3.2 µl	3.2 µl	3.2 µl
2X SYBR Green MM	5 µl	5 µl	5 µl	5 µl	5 µl
Primer 1 (400 nM final conc.)	0.4 µl	0.4 µl	0.4 µl	0.4 µl	0.4 µl
Primer 2 (400 nM final conc.)	0.4 µl	0.4 µl	0.4 µl	0.4 µl	0.4 µl
Sample	1 µl	1 µl	1 µl	1 µl	1 µl
Total	10 µl	10 µl	10 µl	10 µl	10 µl

Fig 1: EpiMark 5-hmC and 5-mC Analysis Kit Workflow

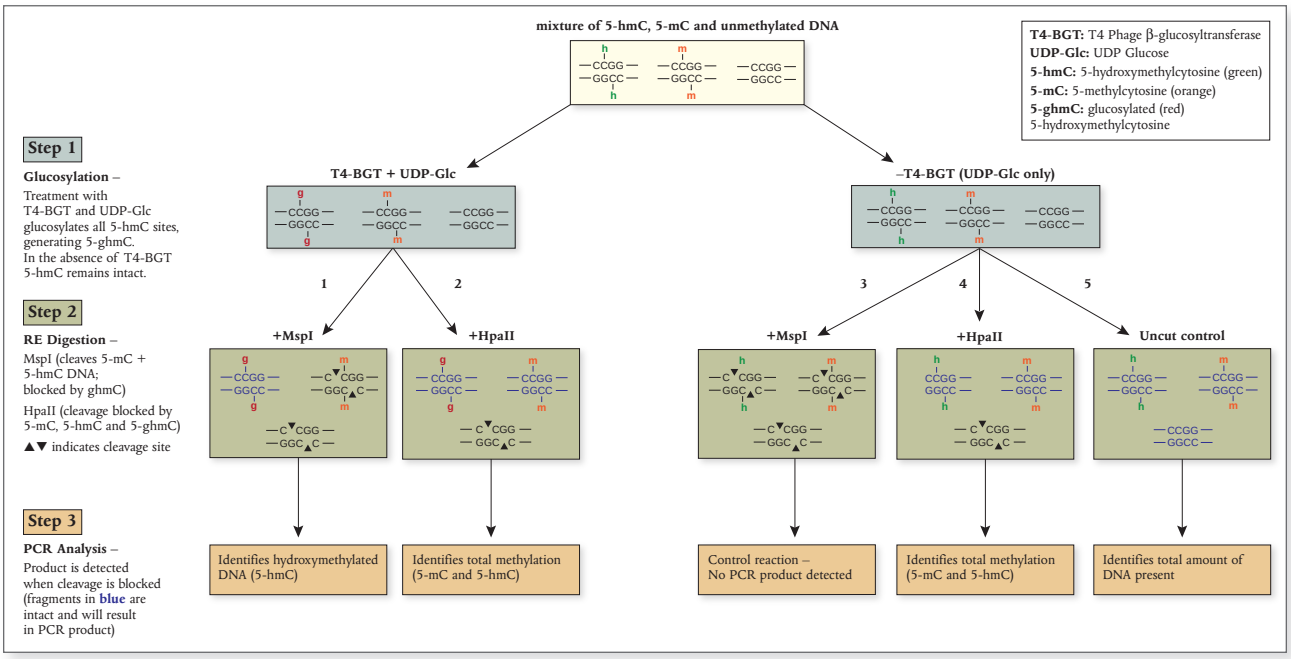
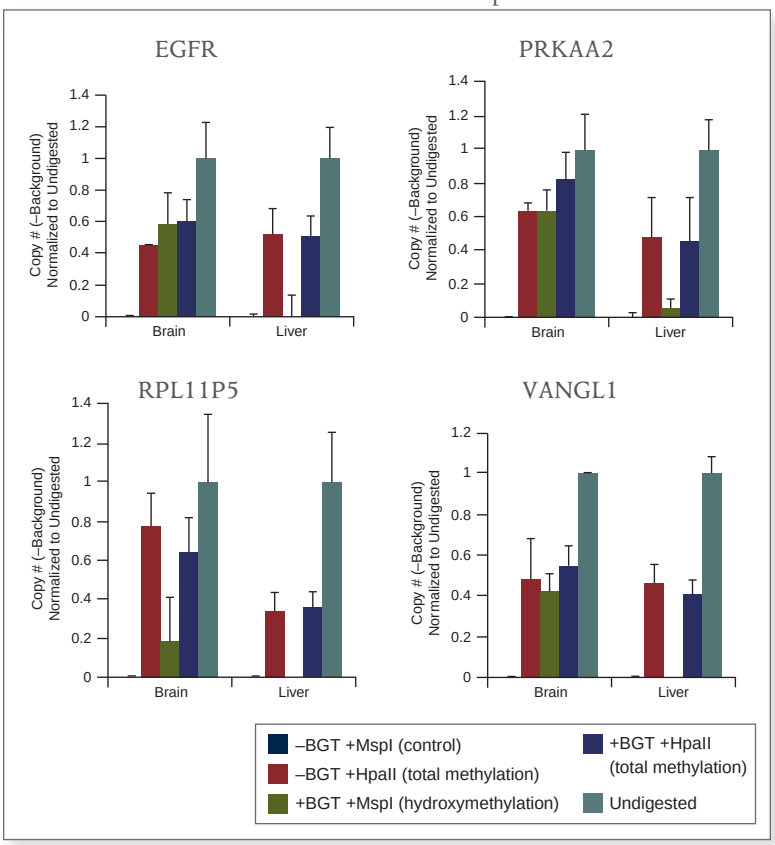


Fig 2: Analysis of specific genes in human brain and liver samples shows a variation in the amounts of 5-hmC and 5-mC present.



Reactions were set up according to protocol on page 3 and analyzed by qPCR.

Results

The densely methylated CpG sites (EGFR, PRKAA2, RPL11P5 and VANGL [all >60% methylated]) were chosen after bisulphite DNA sequencing of human brain DNA. Quantitative PCR analysis of 5-mC and 5-hmC were consistent with the results obtained with bisulphite sequencing (data not shown). The results showed that total methylation was similar for the genes examined in both brain and liver tissues. However, there were variations in the amount of 5-hmC present. Specifically, brain DNA was consistently hydroxymethylated from 20-60% at each interrogated locus, whereas liver DNA contained almost no 5-hmC at the same sites.

Summary

The EpiMark 5-hmC and 5-mC Analysis Kit was used to interrogate and quantify 5-mC and 5-hmC in human brain and liver DNA at four different genetic loci. Similar to 5-mC, 5-hmC also displays tissue specific patterns that may be involved in regulating gene expression in different tissues. After glucosylation and restriction enzyme digestion, the sample DNAs were used for qPCR with gene specific primers. Direct measurement of the percent cytosine, 5-mC and 5-hmC was possible with small quantities of input DNA. These tools can be utilized with any genomic DNA that contains 5-mC or 5-hmC modified bases for both detection and quantification, when coupled with qPCR. Furthermore, one could utilize standard PCR and gel analysis of PCR products to determine the presence or absence of 5-mC or 5-hmC at CCGG loci.

The Big Picture: 5-hmC Content is Differentiation Dependent in Adult Tissues

Posted on E3 on September 16, 2011 by Nicole Kelesoglu

They say beauty is in the eye of the beholder. For me, well done immunolocalization images demonstrate scientific artistry. Last week, I was pleased to find this paper, "Global 5-hydroxymethylcytosine content is significantly reduced in tissue stem/progenitor cell compartments and in human cancers" (1), posted on the Dr. Denise O'Keefe lab blog (E3 member).

This research showed that fully differentiated adult tissues had the highest levels of 5-hmC, while stem cells had very low levels. There was uniform loss of 5-hmC in cancer tissues, matched to normal adult tissues. Reductions in 5-hmC can occur independently of 5-mC in solid tumors. Global loss of 5-hmC could be an early event of carcinogenesis.

The work came from Dr. Yegnasubramanian's lab group at John Hopkins University (Maryland, USA). He was kind enough to answer a few of my basic questions.

1. Was your lab surprised by the adult tissue 5-hmC results?

Yes, we were quite surprised and intrigued by the results. Some very elegant recent studies had shown that 5-hmC levels were high in embryonic stem cells and very early in embryogenesis and appeared to decrease in subsequent differentiation (e.g., ref 2). We initially suspected that a similar trend may hold in stem/progenitors cells later in embryogenesis and in adult tissues as well. Interestingly, we found just the opposite…

2. You have shown that 5-hmC is lost independently from 5-mC in cancer cells. Do you believe that the primary way this happens would be through oncogenic metabolites, rather than mutation of Tet genes?

The mechanism by which 5-hmC is lost in cancer genomes for the majority of cancer types is largely unknown. Recent reports have shown that inactivating mutations in Tet enzymes could certainly be a primary mode of 5-hmC loss in those cancers, such as a subset of AML, that harbor such mutations (e.g., ref 3). Similarly, recent work has shown that oncogenic metabolites produced by gain-of-function mutations in IDH enzymes could potentially lead to inhibition of Tet enzymes, which can be inhibited by these metabolites (e.g., ref 4). However, the majority of cancers of the types we examined are not known to harbor such mutations, and the mechanism by which these cancers have low 5-hmC in the genome is essentially unknown.

3. Would you say that IHC of 5-hmC could potentially be more useful than 5-mC in clinical testing? How would the biomarker information be richer ?

This is difficult to speculate on without further testing. However, for the cancers that we examined, the degree of 5-hmC loss is certainly more pronounced than 5-mC loss. I suspect that there will also be some cancers, such as seminomas, where there is already profound loss of 5-mC (e.g., ref 5), and such cancers will likely also show significant loss of 5-hmC. The clinical utility of either IHC stain in different cancer types needs to be explored more thoroughly.

We are all keenly following the progress on identifying functional roles of 5-hmC – especially in the context of oncology. Thanks again to Dr. Yegnasubramanian's lab for giving us the "big picture" on 5-hmC.

This is a blog posting from E3, a scientific social network for epigenetics experts sponsored by New England Biolabs.

To read and comment about epigenetics topics such as this, we encourage you to visit the E3 blog at www.epiexperts.com.

Reference:
1. Haffner M.C., et al. (2011) *Oncotarget*, 2, 627–637.
2. Ito, S., et al. (2010) *Nature*, 466, 1129–1133.
3. Ko, M., et al (2010) *Nature*, 468, 839–843.
4. Figueroa, M.E. (2010) *Cancer Cell*, 18, 553–567.
5. Netto, G.J., et al. (2008) *Mod. Pathol.* 21, 1337–1344.

Application Note

Robust Colony PCR from Multiple *E. coli* Strains using One*Taq*™ Quick-Load® Master Mixes

Yan Xu, Ph.D., New England Biolabs, Inc.

Introduction

Colony PCR is a commonly used method to quickly screen for plasmids containing a desired insert directly from bacterial colonies. This method eliminates the need to culture individual colonies and prepare plasmid DNA before analysis. However, the presence of bacterial cell contents and culture media in colony PCR reactions often results in polymerase inhibition. A robust polymerase is required to perform colony PCR with high efficiency in many different bacterial strains.

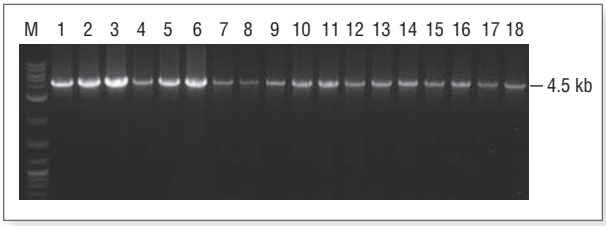
One*Taq*™ DNA Polymerase, an optimized blend of *Taq* and Deep Vent$_R$™ DNA polymerases, has been formulated for robust yields with minimal optimization. This robustness makes One*Taq* ideal for use in demanding applications, such as colony PCR. (For more information on One*Taq* DNA Polymerase, please see page 7.)

Furthermore, the One*Taq* Quick-Load Master Mix product format increases the ease-of-use for colony PCR. The master mix formulation contains dNTPs, $MgCl_2$, buffer components and stabilizers, as well as two commonly used tracking dyes for DNA gels. On a 1% agarose gel in 1X TBE, Xylene Cyanol FF migrates at ~4 kb and Tartrazine migrates at ~10 bp. Both dyes are present in concentrations that do not mask any co-migrating DNA bands.

Results

Transformation of a plasmid containing a 4.5 kb insert into eighteen different competent cell lines was performed, followed by colony PCR using either the One*Taq* or One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer. Similar results were obtained using One*Taq* and One*Taq* Hot Start (One*Taq* data not shown), and the 4.5 kb insert was successfully amplified in each case.

Colony PCR of a 4.5 kb insert using One*Taq* Hot Start Quick-Load 2X Master Mix and 18 different *E. coli* strains



Reactions were set up according to the protocol and analyzed by agarose electrophoresis. Marker M is the 1 kb DNA Ladder (NEB #N3232S/L)

Lane	Name	NEB #	Lane	Name	NEB #	Lane	Name	NEB #
1	NEB 10-beta	C3019	7	Lemo21(DE3)	C2528	13	T7 Express lysY	C3010
2	NEB 5-alpha	C2987	8	NiCo21(DE3)	C2529	14	T7 Express	C2566
3	NEB 5-alpha F´Iq	C2992	9	NEB Express I^q	C3037	15	T7 Express Crystal	C3022
4	dam-/dcm-	C2925	10	NEB Express	C2523	16	SHuffle® Express	C3028
5	NEB Turbo	C2984	11	T7 Express I^q	C3016	17	SHuffle® T7 Express lysY	C3030
6	BL21(DE3)	C2527	12	T7 Express lysY/I^q	C3013	18	SHuffle® T7 Express	C3029

Summary

One*Taq* and One*Taq* Hot Start Quick-Load Master Mixes provide reliable performance in colony PCR, and are compatible with multiple *E. coli* strains. Reliable performance has been seen with amplicons up to 10 kb (data not shown). The Quick-Load format offers additional convenience by enabling direct loading of the PCR reaction onto an agarose gel for analysis. Lastly, the Hot Start formulation provides additional functionality by reducing interference from primer-dimers and secondary amplification products.

General Protocol

1. Transform ligation mix or other plasmid-containing reaction mixture into the desired bacterial strain, and incubate agar plates overnight at the appropriate temperature.
2. Set up 50 µl reactions as follows:

One*Taq* Master Mix	25 µl
PCR primer	200 nM
H_2O	to 50 µl

Note: *If OneTaq Hot Start Quick-Load 2X Master Mix is used, reactions can be set up at room temperature. If OneTaq Quick-Load 2X Master Mix is used, reactions should be set up on ice.*

3. Use a sterile toothpick to pick up individual colonies and dip into each reaction tube.
4. As soon as the solution looks cloudy, remove the toothpick. To create a stock of each individual colony either:
   a.) Dip the toothpick into 3 ml growth media with appropriate antibiotics and culture overnight.
   or
   b.) Streak the toothpick onto another agar plate containing the appropriate antibiotics and grow overnight.
5. Transfer reactions to a PCR cycler, and perform PCR following the guidelines below for cycling conditions:

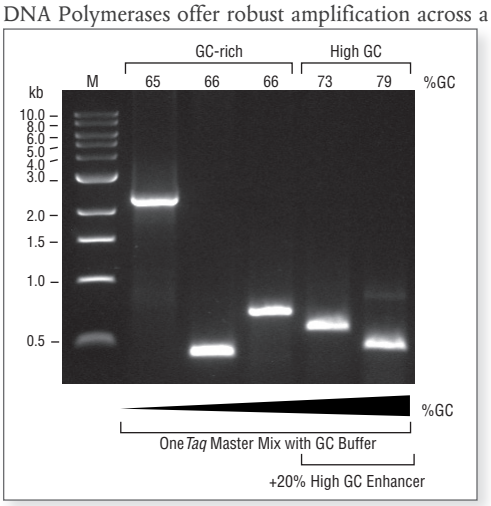
Initial denaturation:	
94°C	2 minutes
30 cycles:	
94°C	15–30 seconds
45–68°C	15–60 seconds
68°C	1 minute per kb
Final hold:	
68°C	5–10 minutes
4°C	hold

6. Load 4-6 µl of each PCR reaction directly onto an agarose gel, alongside an appropriate DNA ladder.

Note: *The full application Note can be downloaded at www.neb.com/OneTaq*

One*Taq*™ DNA Polymerase – The ONE Polymerase for your Endpoint PCR Needs

An optimized blend of *Taq* and Deep Vent$_R$® DNA polymerases, One*Taq*™ and One*Taq* Hot Start DNA Polymerases offer robust amplification across a wide range of templates. The 3′–5′ exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robustness of *Taq*, and the hot start formulation combines convenience with decreased interference from primer-dimers and secondary amplification products. Available in convenient product formats, including master mixes, One*Taq* shows exceptional performance against other commercially available polymerases, especially when amplifying difficult or GC-rich templates.



Amplification of a selection of sequences with varying GC content from human genomic DNA using OneTaq 2X Master Mix with GC Buffer. GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).

Advantages

- Exceptional performance in endpoint PCR across a wide range of templates
- Robust yields with minimal optimization
- Convenient product formats (stand-alone enzyme, master mixes, and Quick-Load® formats)
- Hot start version allows room temperature reaction setup and does not require a separate activation step
- Compatible with standard *Taq* protocols

Ordering Information

PRODUCT	NEB #	SIZE
One*Taq*™ DNA Polymerase	M0480S/L/X	200/1,000/5,000 units
One*Taq*™ 2X Master Mix with Standard Buffer	M0482S/L	100/500 rxns (50 µl vol)
One*Taq*™ 2X Master Mix with GC Buffer	M0483S/L	100/500 rxns (50 µl vol)
One*Taq*™ Quick-Load® 2X Master Mix with Standard Buffer	M0486S/L	100/500 rxns (50 µl vol)
One*Taq*™ Quick-Load® 2X Master Mix with GC Buffer	M0487S/L	100/500 rxns (50 µl vol)
One*Taq*™ Hot Start DNA Polymerase	M0481S/L/X	200/1,000/5,000 units
One*Taq*™ Hot Start 2X Master Mix with Standard Buffer	M0484S/L	100/500 rxns (50 µl vol)
One*Taq*™ Hot Start 2X Master Mix with GC Buffer	M0485S/L	100/500 rxns (50 µl vol)
One*Taq*™ Hot Start Quick-Load® 2X Master Mix with Standard Buffer	M0488S/L	100/500 rxns (50 µl vol)
One*Taq*™ Hot Start Quick-Load® 2X Master Mix with GC Buffer	M0489S/L	100/500 rxns (50 µl vol)

Proof that it was Worth the Wait

"The OneTaq polymerase worked wonders! I tried multiple conditions with other commercial polymerases for my PCR and resulted in low yield. When I tried OneTaq polymerase, the result was an instant gratification!"

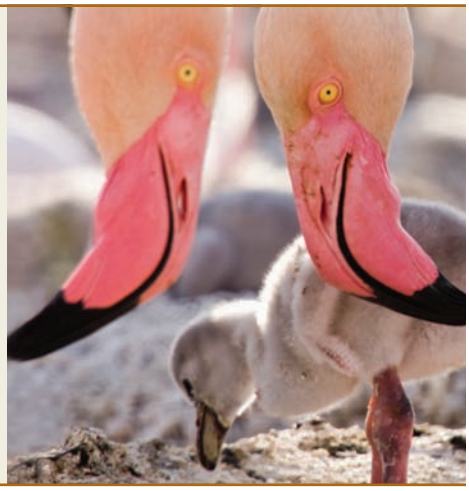
– Hua Wang, Graduate Student, University of Oxford

"I have been trying to PCR a promoter onto an oligo to make an shRNA. The inherent secondary structure in the primer made this amplification particularly difficult. The GC Enhancer gave significantly better yield than anything else I have tried."

– Breanne Karanikolas, Postdoctoral Fellow, UC Irvine

"I was able to get equally robust amplification from genomic DNA at all Tm's tested in gradient PCR with a new set of primers!"

– Chiranthani Sumanasekera, Ph.D., Postdoctoral Fellow
Vanderbilt University School of Medicine



Polbase: A New Online Tool for Polymerase Researchers

Bradley Langhorst, Ph.D. New England Biolabs, Inc.

New England Biolabs is pleased to introduce Polbase, a free and open database covering DNA polymerase biochemistry, genetics and structural biology. Inspired by REBASE (the Restriction Enzyme Database, also developed and maintained by NEB), Polbase compiles the information that exists in the body of polymerase literature, making it easy for researchers to use and interrogate.

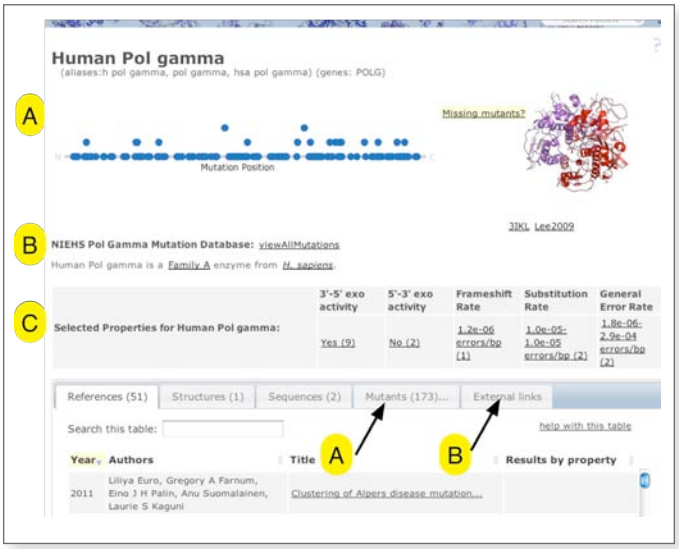


Figure 1: Each polymerase in the database has a landing page containing summary information. This page includes an interactive map of mutants (A) which shows the relationship between mutants and their parent enzymes. External links are also included where relevant (B). Selected properties are summarized as ranges with links to the primary information source (C).

What information can be found in Polbase?

Polbase includes individual landing pages for each indexed polymerase (Figure 1). Detailed information is extracted from the primary literature, including PubMed, the Protein Databank (PDB) and other protein resources. Polbase builds on the general protein structure information available in PDB, but also includes polymerase-specific details (e.g., polymerase family, whether the structure includes template DNA or incoming nucleotides). Biochemical results listed (e.g., kinetic parameters, error rates, etc.) are linked to their original sources and presented with experimental details to aid interpretation. Landing pages also allow for comparisons of properties between polymerases or between a polymerase and its mutants.

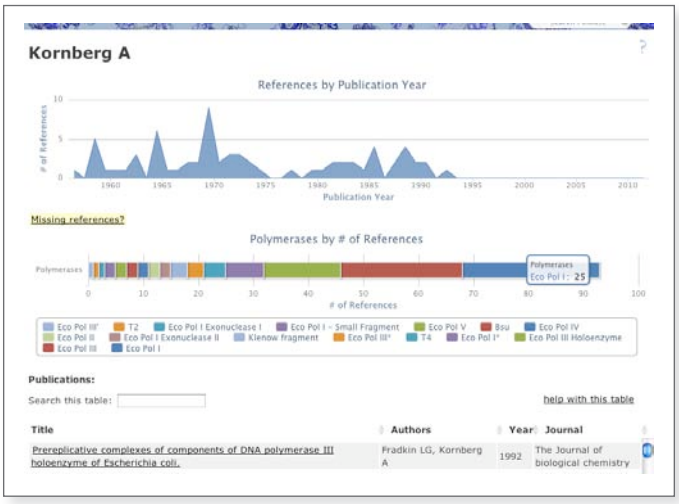


Figure 2: The author page contains a listing of polymerases studied and publications. This page allows authors to quickly assess Polbase's coverage of their publications.

Currently over 7,000 references, representing more than 15,000 authors, are indexed in Polbase. Each reference has a dedicated page that presents topics, polymerases, abstracts and an author list. Separate author pages (Figure 2) contain their publication history and an overview of polymerases studied.

Polbase utilizes a layered searching feature, making it possible to find relevant results using simple search terms (e.g., exonuclease) that might produce undesired results in databases with a broader focus. Furthermore, information is presented in dynamic tables, allowing users to sort and filter by specific polymerase properties (e.g., 3′→5′ exonuclease activity, error rates, etc). Users can also sort and filter references by author name, publication date, journals and other bibliographic information.

How is Polbase populated?

As a collaborative database, Polbase depends on authors to help with the population and verification of the content. As new references are imported, corresponding authors are contacted by email and asked to complete and confirm the information that automated tools have extracted from their papers. Polbase uses this process to ensure that it accurately represents the work reported in a paper, without taking an editorial position on the applicability of those results. All results are linked to their source reference, so more detailed information can be accessed directly. By adding information about polymerase and mutants studied, topics, and details about results, authors essentially enable Polbase's powerful sorting and searching features.

Currently, over 800 polymerases and mutants from 105 organisms, and all polymerase families are documented. As Polbase continues to grow, contributions of references, mutants and feature suggestions are welcomed. In the spirit of NEB's long-standing commitment to open sharing of scientific information through publications and other databases (e.g., REBASE, InBase, etc), Polbase is available to the public as a free and open resource at **http://polbase.neb.com**.

Featured Product

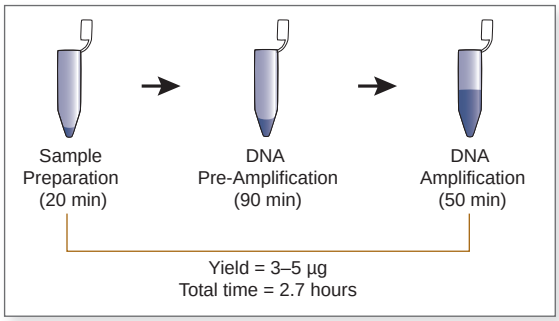
Single Cell WGA Kit

The Single Cell WGA Kit enables reproducible whole genome amplification (WGA) of DNA from single cells and other low DNA input amount samples, with superior specificity, sensitivity and reproducibility. With a simple single-tube protocol, this kit efficiently amplifies total DNA from single cells approximately 1 million-fold to produce 2–5 µg of amplified DNA in under 3 hours, with reproducible locus and allele representation. The resulting DNA is suitable for subsequent analysis by multiple methods, including qPCR and microarrays.

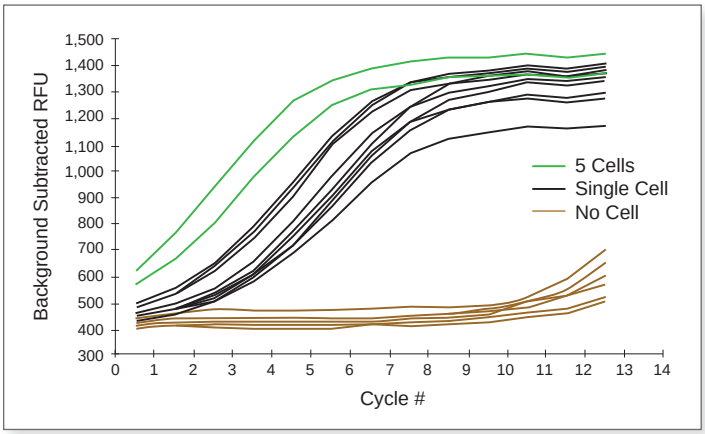
Advantages

- Single-copy sensitivity
- High specificity
- Low background
- Superior reproducibility
- Simple, fast protocol (under 3 hours)

Whole genome amplification of single cell DNA is complete in less than 3 hours



Example of Background Subtracted RFU amplification curves for replicate single-cell and control (no-cell) WGA reactions that were monitored on a Bio-Rad iCycler iQ™.



Data provided by Rubicon Genomics, Inc.
iQ is a trademark of BioRad Laboratories, Inc.

Ordering Information

PRODUCT	NEB #	SIZE
Single Cell WGA Kit	E2620S/L	12/50 rxns
COMPANION PRODUCTS		
Gel Loading Dye, Blue (6X)	B7021S	6.0 ml
Gel Loading Dye, Orange (6X)	B7022S	6.0 ml
Quick-Load® 100 bp DNA Ladder	N0467S/L	125/375 lanes

For licensing information, visit http://www.neb.com/nebecomm/products/productE2620.asp

Building a strong foundation

NEB 5-alpha Competent *E. coli* – Built for efficiency

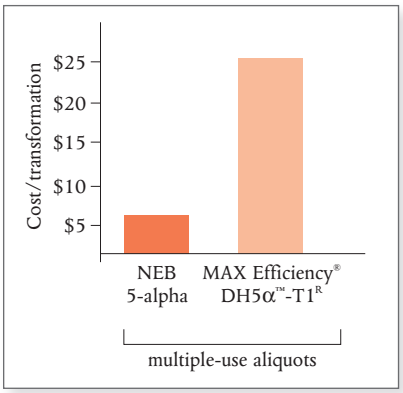
Ensure successful transformations with NEB's competent *E. coli* for cloning. Choose from several high efficiency competent cell strains in a variety of formats and deliver performance, convenience and value to your cloning experiments.

A versatile *E. coli* strain, NEB 5-alpha is a derivative of DH5α™. It has the same genetic features of this popular cloning strain, and offers high transformation efficiencies, convenient formats and value pricing. Whether you are doing routine cloning, subcloning or looking for a high efficiency (electro-competent) format, NEB 5-alpha is the ideal strain for you.

Advantages

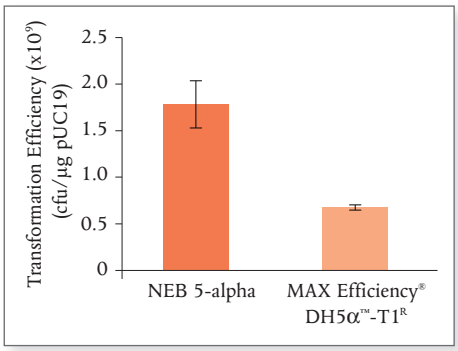
- High transformation efficiencies
- Value pricing and no dry ice charges
- Convenient product formats, including single-use tubes

Take advantage of the low cost per transformation.



Calculations were based on list price and recommended transformation volumes.

Benefit from high transformation efficiencies with NEB 5-alpha.



The transformation efficiencies of NEB 5-alpha and MAX Efficiency DH5α-T1R were compared using manufacturers' recommended protocols. Values shown are the average of triplicate experiments.

Ordering Information

PRODUCT	NEB #	SIZE
NEB 5-alpha Competent *E. coli* (High Efficiency)	C2987H/I	20 x 0.05 ml / 6 x 0.2 ml

DH5α is a trademark of Invitrogen.

Optimization tips for transformation reactions

- Incubation with shaking or rotation results in 2-fold higher transformation efficiency
- Warm, dry plates are easier to spread and allow for the most rapid colony formation
- DNA should be purified and resuspended in water or TE buffer
- Cells are best thawed on ice, any warming above 0°C decreases efficiency
- Incubate DNA with cells on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes this step is shortened.
- The 5 minute protocol results in 10% efficiency as compared to the high efficiency protocol
- Outgrowth at 37°C for 1 hour is best for cell recovery and expression of antibiotic resistance. Expect a 2-fold loss in transformation efficiency for every 15 minutes this step is shortened.
- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss in transformation efficiency
- DNA contaminants such as detergents, phenol, ethanol, isopropanol, PEG and DNA binding proteins interfere with transformation and further DNA purification may be required
- Additional transformation tips can be found on the product page and in the technical reference section of the catalog and our website, **www.neb5alpha.com**.

for your cloning experiments.

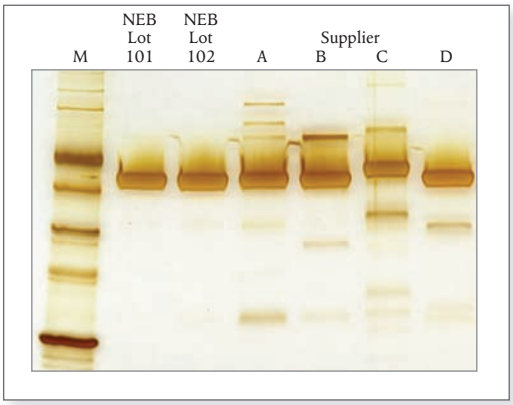
T4 DNA Ligases – Built for performance

Why compromise any aspect of performance in your cloning experiments? DNA ligases from NEB set the industry standard for function and purity, and are available at exceptional value. Choose from T4 DNA Ligase or the Quick Ligation Kit to meet the demands of a variety of reaction conditions. With continuous product development and extensive quality controls, DNA ligases from NEB ensure success in your ligation reactions.

Advantages

- Highly pure enzyme with no lot-to-lot variation
- Flexible reaction setup
- Active in a variety of reaction buffers

Experience extreme purity with
NEB's T4 DNA Ligase



Equivalent amounts of protein were loaded and analyzed by SDS-Page and silver stained using SilverXpress®. Marker M is NEB's Broad Range Protein Marker (NEB #P7702).

Special Offer

Purchase NEB 5-alpha Competent Cells (High Efficiency) with NEB's Quick Ligation Kit and receive the item of lesser value for FREE.

For more information, visit **www.neb.com/DNAligases**

Offer valid in the US only, October 17 through November 30, 2011.

Ordering Information

PRODUCT	NEB #
T4 DNA Ligase	M0202S/L/M/T
Quick Ligation Kit	M2200S/L
BUNDLE PRODUCT INFORMATION	
Quick Ligation Kit (150 rxn) & NEB 5-alpha (6 x 0.2 ml) Bundle	E8609L
Quick Ligation Kit (150 rxn) & NEB 5-alpha (20 x 0.05 ml) Bundle	E7285L
Quick Ligation Kit (30 rxn) & NEB 5-alpha (6 x 0.2 ml) Bundle	E5340S
Quick Ligation Kit (30 rxn) & NEB 5-alpha (20 x 0.05 ml) Bundle	E1111S

Note: *Products contained in bundle require different storage temperatures and should be stored at the appropriate temperatures upon receipt.*

SilverXpress is a registered trademark of Life Technologies, Inc.

Troubleshooting tips for ligation reactions

- Add controls, including vector alone, insert alone, and uncut vector
- Vary the molar ratio from 1:1 to 1:5 vector:insert
- Insert or plasmid should have a 5´ phosphate
- Use fresh buffer as the ATP or DTT may degrade over time
- Total DNA concentration should be less than 10 ng/µl
- When excising a fragment from a gel, minimize UV exposure and use longwave UV (360 nm) to reduce damage to the ends of the DNA
- Additional ligation tips can be found on the product page in the technical reference section of our catalog and website, **www.neb.com/DNA ligases**

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enabling technologies in the life sciences

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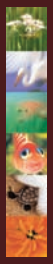
NEB Tools App is now available for the Android™ platform

For more information, visit
https://market.android.com/details?id=com.whiz.nebtools

Android is a trademark of Google, Inc.



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