NEB EXPRESSIONS

A scientific update from New England Biolabs | Summer Edition 2012

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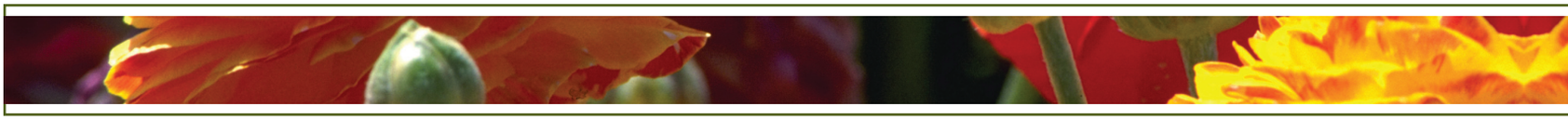

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Upcoming Tradeshows

Visit the NEB booth at the following meetings:

- **ASM (American Society for Microbiology)**
  San Francisco, CA, June 16–19
  http://gm.asm.org/
  Booth 723
- **AACC (American Association for Clinical Chemistry)**
  Los Angeles, CA, July 17–19
  www.aacc.org
  Booth 437
- **NAOSMM (National Association of Scientific Materials Managers)**
  Alberquerque, NM, July 30–August 3
  www.naosmm.org

A Letter from NEB

Dear Researcher,

Good. Better. Best. Self-improvement is an on-going process, even for companies as well established as New England Biolabs. For nearly 40 years, it has been NEB's singular focus to offer state-of-the-science products to elevate and improve your research. Setting a new standard is challenging yet rewarding. It is in this spirit that we introduce *Bst* 2.0 and *Bst* 2.0 WarmStart DNA Polymerases for isothermal DNA amplification. With several improvements over *Bst* DNA Polymerase, including increased speed and specificity, improved salt tolerance, and dUTP incorporation, *Bst* 2.0 sets a new standard for isothermal polymerases. As a measure of increased flexibility, *Bst* 2.0 WarmStart enables room-temperature assay setup without any loss of performance or accuracy. Check out the feature article in this edition of NEB Expressions to learn more about isothermal DNA amplification.

This edition of NEB Expressions also includes a glycobiology-related application note: Glycan Analysis of Murine IgG2a by Enzymatic Digestion with PNGase F and Trypsin, followed by Mass Spectrometric Analysis. Get tips on studying glycosylation patterns, straight from NEB's expert scientists.

Wishing you continued success in your research,

New England Biolabs

Cover photo: Ranunculus

Improved Reagents for Isothermal DNA Amplification

Amplification of a specific nucleic acid sequence or target is a fundamental modern laboratory technique that is used worldwide for both molecular biology research and diagnostic purposes. While the Polymerase Chain Reaction (PCR) has long been the standard sequence-specific amplification technique, recent years have seen a proliferation of non-thermal cycling (isothermal) techniques that offer certain advantages over PCR.

Nathan Tanner, Ph.D., New England Biolabs, Inc.

Introduction:

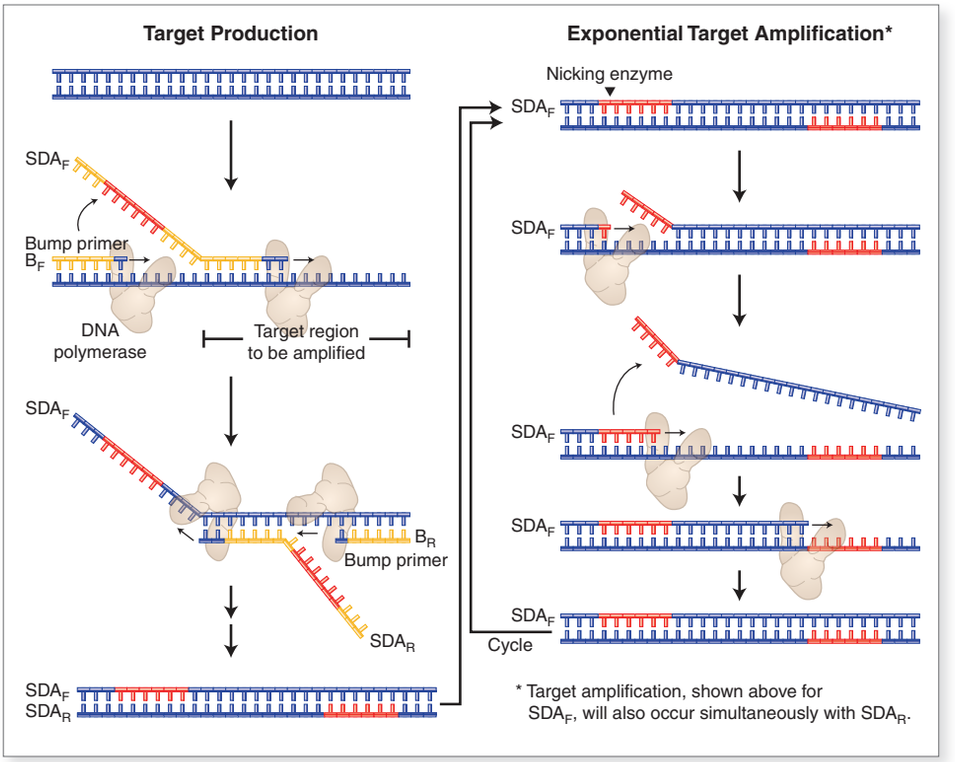
Isothermal techniques, in which amplification reactions are performed at a single temperature, do not require expensive thermal cycling equipment. Reactions can be performed using a water bath, heat block or even ambient temperature. Quantitative detection of amplification is achieved using fluorescence instruments, measuring intercalating dyes, specific probes or cation sensitive dyes. Additionally, isothermal methods provide some unique alternative detection methods. For example, due to the high amounts of DNA synthesized in some techniques (up to 50X PCR yield), precipitation of magnesium pyrophosphate (MgP_2O_7) occurs and can be measured either by simple visual inspection or quantitatively using a turbidimeter (1,2). The potential for simplified instrumentation and range of detection options has spurred the adoption of isothermal amplification methods for field and point-of-care testing, enabling nucleic acid diagnostics without expensive equipment, or even electricity (2-4).

In lieu of a heat denaturation step, most isothermal techniques rely on the strand displacement activity of a DNA polymerase for strand separation of dsDNA. Typically, the large fragment of *Bst* DNA Polymerase (*Bst* DNAP, LF, NEB #M0275) is used, due to its high degree of strand displacement and its optimal temperature range (50–65°C), which facilitates primer annealing. Some isothermal sequence-specific amplification (SSA) methods involve the DNA polymerase alone (e.g., loop-mediated isothermal amplification, LAMP; smart amplification process; SmartAmp), while others use the polymerase in combination with other enzymes (e.g., strand displacement amplification, SDA; nicking enzyme amplification reaction, NEAR; helicase-dependent amplification, HDA; recombinase polymerase amplification, RPA) (5,6). Isothermal techniques are remarkably fast and sensitive, detecting femtograms or very low copy numbers (< 100) of DNA, in as little as five minutes.

While *Bst* DNA Polymerase is quite robust and suitable for most basic applications, it has some limitations when used in isothermal SSA. For example, activity is inhibited at temperatures above 65°C, preventing its use at higher temperatures. In addition, *Bst* DNA Polymerase does not efficiently incorporate dUTP and is severely inhibited when > 50% of dTTP is replaced by dUTP. Non-specific amplification can also be observed at lower temperatures (25°C) (7,8). However, *Bst* DNA Polymerase does exhibit greater tolerance of inhibitors typically found in diagnostic specimens (e.g., blood, humic acid), which is an advantage as compared to PCR polymerases (9). This feature makes *Bst* DNA Polymerase useful for diagnostic testing, although it can be inhibited at low amounts of certain compounds (e.g., 70 mM monovalent salt).

To address some of the limitations described above, NEB has engineered two new DNA polymerases: *Bst* 2.0, which displays improved amplification speed, yield, salt tolerance and thermostability, and *Bst* 2.0 WarmStart, which is designed to enable room temperature reaction setup (for more information, see page 6). Performance improvements, with respect to *Bst* DNAP, LF, can be highlighted using the isothermal diagnostic technique known as strand displacement amplification (SDA). In SDA (Figure 1), internal primers (red), containing 5′ extensions that recognize a target sequence, are extended and then displaced by synthesis that initiates at additional external primers (Bump primers). This initial target generation step results in a short fragment of dsDNA ending with the 5′ extensions of the initial primers. These extensions contain a recognition site for the restriction endonuclease BsoBI, which will cut the target DNA at each end.

Figure 1. Overview of strand displacement amplification (SDA)



However, SDA reactions are performed with a modified deoxyribonucleoside triphosphate, 2´-deoxycytidine-5´-O-(1-thiotriphosphate), or dCTPαS. Incorporation of dCTPαS leaves the top strand of the BsoBI site (C/TCGGG) cleavable, but the bottom strand (GAGCC/C) not cleavable due to created phosphorothioate linkages in the product dsDNA. Thus, as a nick is generated, *Bst* DNAP, LF initiates synthesis and displaces the forward strand, and the amplification reaction will proceed exponentially (10,11).

Results:

Improved reaction performance of *Bst* 2.0

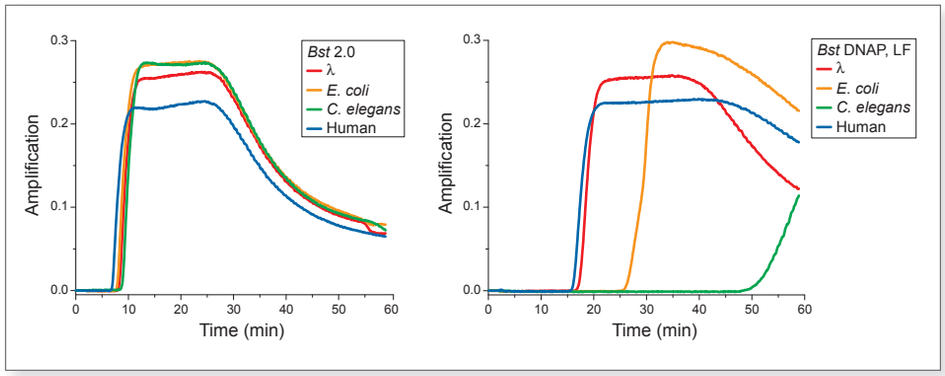
Amplification results from SDA reactions, as measured by turbidity, are presented in Figures 2 and 3. In Figure 2, SDA reactions are graphed as increasing turbidity (amplification) over time. These reactions were performed using various targets, from simple (λ, 48.5 kbp) to increasingly complex (*E. coli, C. elegans* and human) genomic DNA, with equal units of *Bst* DNAP, LF and *Bst* 2.0. In all cases, *Bst* 2.0 provided robust amplification in under 10 minutes, whereas *Bst* DNAP, LF amplification was variable, ranging from 15–45 minutes. Speed is, of course, a major consideration of any amplification technique, and Figure 2 demonstrates the speed improvements conferred in using *Bst* 2.0. However, the consistency of amplification across target and genome is of equal or greater significance, and *Bst* 2.0 displayed little variability in reaction speed with the various targets.

Improvements in speed, sensitivity and salt tolerance are also illustrated in Figure 3. Figure 3A is a standard curve measured across varying amounts of human genomic DNA with BRCA1-specific SDA primers. Linear dose response curves were generated when using either *Bst* DNAP, LF or *Bst* 2.0 highlighting the usability of SDA and these polymerases for reliable target sequence quantification. *Bst* 2.0 reached the detected threshold faster at each concentration of genomic DNA tested, further demonstrating its speed versus *Bst* DNAP, LF.

Bst 2.0 also showed increased resistance to inhibitors, as demonstrated in Figure 3B. *Bst* DNAP, LF was significantly inhibited by salt concentrations above 50 mM, while *Bst* 2.0 proved much more robust, with no inhibition in SDA at salt concentrations up to 150 mM. This property enables a much broader range of reaction conditions, and is especially accommodating of lower purity DNA samples which can contain high amounts of salt.

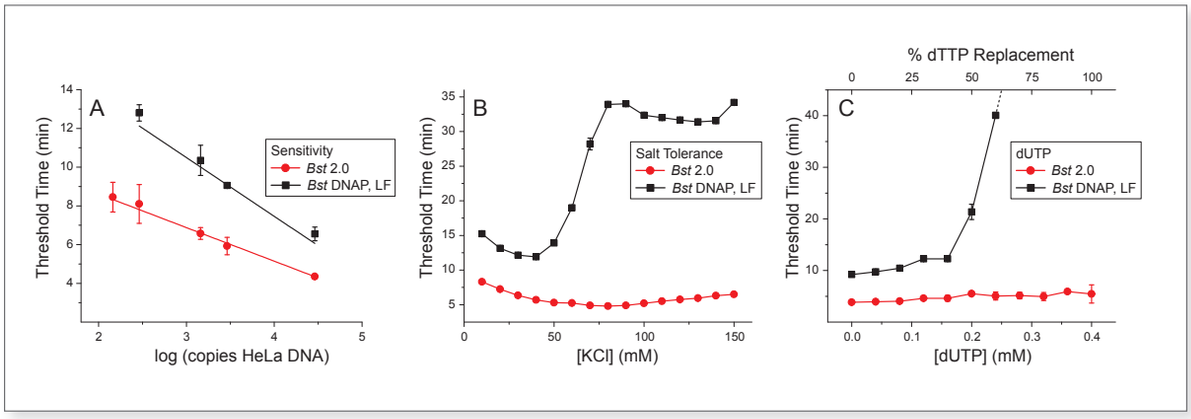
Another consideration for DNA polymerases is incorporation and tolerance of dUTP. The incorporation of dUTP during amplification is commonly used for the prevention of carry-over contamination (7). Figure 3C shows data for dUTP replacement of dTTP in SDA. *Bst* DNAP, LF was completely inhibited above 50% replacement of dTTP with dUTP, while *Bst* 2.0 displayed no inhibition up to 100% replacement. Although various isothermal techniques may be differentially sensitive to dUTP use, *Bst* 2.0 is much more tolerant to dUTP in the reaction than *Bst* DNAP, LF.

Figure 2. *Bst* 2.0 displays improved speed and consistency, as compared to Bst DNA Polymerase, Large Fragment



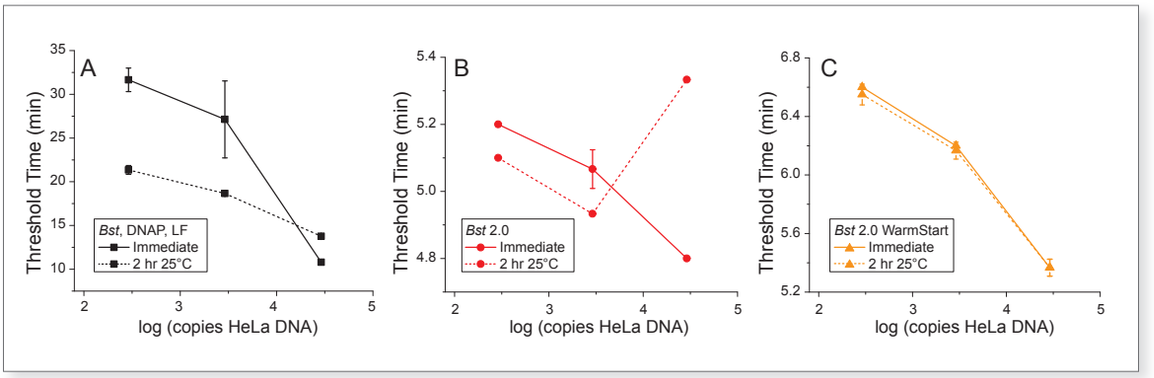
Amplification curves from strand displacement amplification (SDA) reactions using various targets and genomes show consistent, rapid performance using Bst 2.0. All amplicons were detected in less than 10 minutes with Bst 2.0 while Bst DNAP, LF detection required 15–60 minutes. Amplification was followed by real-time turbidity measurements.

Figure 3. *Bst* 2.0 offers increased sensitivity, salt tolerance and tolerance of dUTP



Various SDA reaction parameters show the improved performance of Bst 2.0. Faster, more reliable detection of lower target copy number is shown in Panel A. Panel B shows salt tolerance of the two polymerases in SDA, with significant inhibition of Bst DNAP, LF above 50 mM KCl, while Bst 2.0 provided robust amplification through 150 mM. dUTP incorporation was shown to be inhibitory to Bst DNAP, LF (Panel C), with > 50% dTTP replacement completely inhibiting SDA reactions (dashed line). In contrast, Bst 2.0 performed equally well from 0–100% dUTP in SDA reactions.

Figure 4. Bst 2.0 WarmStart offers consistent, reliable amplification, even after a room temperature pre-incubation step



Setting up an SDA reaction on ice and immediately starting the reaction gave consistently accurate results with all three DNA polymerases tested: Bst DNAP, LF; Bst 2.0; and Bst 2.0 WarmStart (Panels A, B, and C, respectively). However, if the amplification reactions were set up at room temperature and then left at room temperature (pre-incubated) for 2 hours before transferring the reactions to a real-time instrument, both Bst DNAP, LF and Bst 2.0 showed inconsistency and inaccuracy (Panels A and B). Only Bst 2.0 WarmStart gave consistent and accurate results even under the extreme case of a 2 hour preincubation (Panel C).

Increased specificity with *Bst* 2.0 WarmStart

As mentioned above, DNA polymerases often display undesired amplification at low temperature, a result of extension of DNA primers or nonspecific annealing of primers to template. This amplification produces nonspecific bands in PCR, with the potential to give high backgrounds and/or false positives in nucleic acid diagnostics. Any reaction or test must be set up on ice and transferred directly to the detection instrument to avoid spurious amplification and inconsistent results. This property imposes a technical challenge to high throughput diagnostic applications, where consistency and reproducibility are of extreme importance. To address this problem, various approaches have been successfully applied to making "Hot Start" DNA polymerases, which are inactive until the reaction temperature is raised above a certain permissive temperature. Most of these methods, whether chemical modification or antibody-based, work well for PCR, where the temperature is raised to approximately 95°C and the inactivating component is heat labile. However, isothermal reaction temperatures do not typically rise above 65–70°C, thus requiring the temperature sensitive inhibitor to undergo thermal inactivation at a much lower temperature than in PCR. *Bst* 2.0 WarmStart employs a specifically-selected and modified DNA aptamer that tightly binds to *Bst* 2.0 at low temperatures, and is released from the polymerase above 45°C, permitting amplification reactions at temperatures typically used in isothermal amplification techniques. We termed the resulting DNA polymerase-aptamer complex *Bst* 2.0 WarmStart, as temperatures are significantly below those of PCR hot start enzymes. This temperature range was chosen to accommodate isothermal techniques currently using *Bst* DNAP, LF, while enabling room-temperature setup and consistent reaction performance.

Data from SDA using *Bst* DNAP, LF, *Bst* 2.0, and *Bst* 2.0 WarmStart is shown in Figure 4. Three concentrations of target DNA were used, and reactions performed in two sets: one immediately after setup, and another after pre-incubation for two hours at room temperature. For both *Bst* DNAP, LF and *Bst* 2.0, the two conditions produced strikingly dissimilar results, with substantial variability in the detection time at each template concentration (data plots have different scales for the y-axis to accommodate the faster reaction times of *Bst* 2.0). However, reactions with *Bst* 2.0 WarmStart produced identical results with or without a two hour pre-incubation. This property enables room temperature setup (temperatures up to 40°C) and, equally important, provides consistent, reliable results in diagnostic applications.

Conclusion

The performance enhancements obtained with the engineered *Bst* 2.0 and *Bst* 2.0 WarmStart DNA Polymerases bring much-needed flexibility to isothermal amplification techniques. Tolerance of a broader range of reaction conditions enables improvements to the different assay technologies to be realized. Improving the capabilities of the essential reagents will also expand the role of isothermal methods and facilitate additional progress as these techniques become an even more significant part of molecular diagnostics and molecular biology research.

References

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Bst 2.0 and *Bst* 2.0 WarmStart DNA Polymerases

Bst 2.0 DNA Polymerase is an *in silico* designed homologue of *Bacillus stearothermophilus* DNA Polymerase I, Large Fragment (*Bst* DNA Polymerase, Large Fragment). *Bst* 2.0 DNA Polymerase possesses 5´→3´ DNA polymerase activity and strong strand-displacement activity, but lacks 5´→3´ exonuclease activity. *Bst* 2.0 DNA Polymerase displays improved amplification speed, yield, salt tolerance and thermostability compared to wild-type *Bst* DNA Polymerase, Large Fragment.

Bst 2.0 is tolerant of inhibitors, such as salt, and is able to incorporate dUTP during amplification. *Bst* 2.0 is active at salt concentrations up to 150 mM allowing flexibility in buffer choice. This is potentially useful in techniques that require multiple enzymes, such as Helicase Dependent Amplification or Strand Displacement Amplification. Furthermore, the ability to incorporate dUTP makes *Bst* 2.0 useful in methodologies for prevention of carryover contamination.

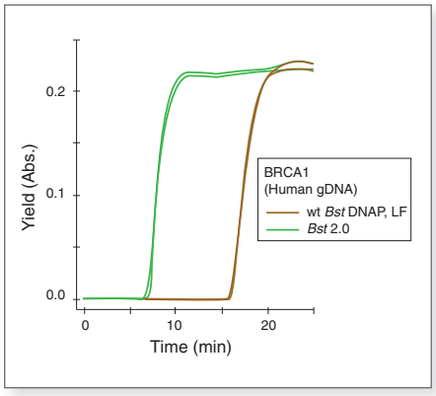
The WarmStart feature of *Bst* 2.0 WarmStart DNA Polymerase is unique among isothermal polymerases. Like "Hot Start" PCR polymerases, this feature prevents activity at temperatures below the optimal reaction temperature. This enables room temperature reaction set up and increases the reproducibility of the results.

In contrast to chemical modifications or antibodies commonly used with hot start PCR polymerases, NEB's *Bst* 2.0 WarmStart DNA Polymerase utilizes aptamer technology. Aptamers are extensively modified, unique oligonucleotides which bind to the polymerase through non-covalent interactions, inhibiting activity at non-permissive temperatures (< 50°C). Additionally, no separate activation step is required for *Bst* 2.0 WarmStart DNA Polymerase.

Advantages

- Fast polymerization
- Robust reactions with a broad range of conditions and primer sets
- Flexible reaction conditions, including a higher salt tolerance than *Bst* DNA Polymerase, LF
- Optimal reaction performance from 60-72°C
- Minimal effect of substitution of dTTP with dUTP
- Highly pure product with minimal lot-to-lot variation
- WarmStart feature enables room temperature reaction set-up

Bst 2.0 DNA Polymerase improves amplification speed



Strand Displacement Amplification (SDA) of BRCA1 from HeLa genomic DNA under optimal SDA conditions for each enzyme. Data shows that the reaction reaches threshold faster with Bst 2.0 than with wild-type (wt) Bst DNA Polymerase, Large Fragment.

Ordering Information

PRODUCT	NEB #	SIZE
Bst 2.0 DNA Polymerase	M0537S/L/M*	1,600/8,000/8,000 units
Bst 2.0 WarmStart DNA Polymerase	M0538S/L/M*	1,600/8,000/8,000 units
COMPANION PRODUCT		
Isothermal Amplification Buffer Pack	B0537S	6.0 ml

*M = for high (15X) concentration

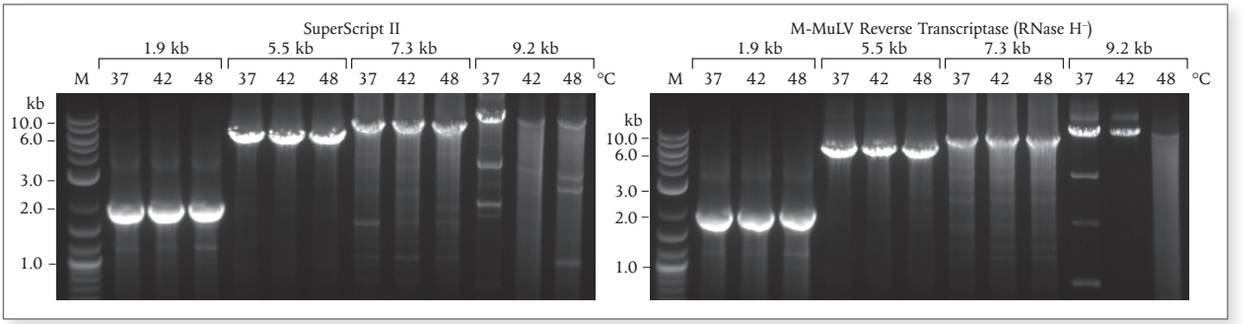
M-MuLV Reverse Transcriptase (RNase H⁻): NEB's alternative to SuperScript II

M-MuLV Reverse Transcriptase (RNase H⁻) is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild-type M-MuLV. The enzyme is active up to 50°C, providing higher specificity, higher yield of cDNA and more full-length cDNA product, up to 12 kb in length.

Advantages

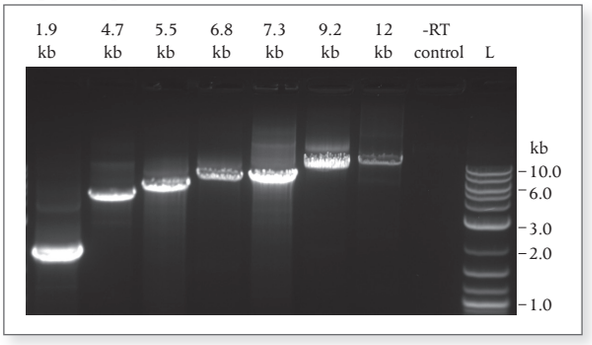
- High cDNA yield
- Superior performance for longer templates
- Increased thermostability
- Value pricing

M-MuLV Reverse Transcriptase (RNase H⁻) performs as well as other RNase H⁻ Reverse Transcriptases



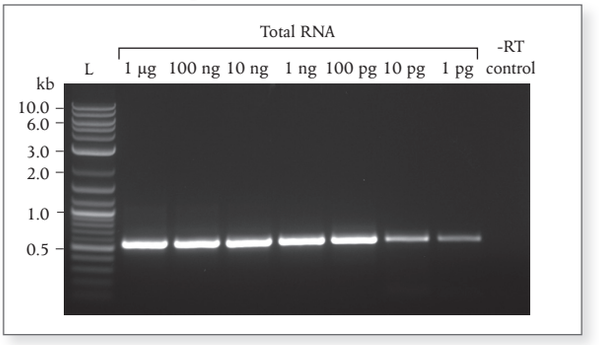
Jurkat total RNA (1 µg) was used in a 20 µl first strand cDNA synthesis. Mixtures of all reaction components, except for reverse transcriptase, were held at different temperatures for 3 minutes. 200 units of SuperScript II or of NEB's M-MuLV Reverse Transcriptase (RNase H⁻) was added and incubated at the indicated temperature for 50 minutes, followed by heat inactivation for 5 minutes at 80°C. 1 µl of cDNA was used in a 25 µl PCR using LongAmp® Hot Start Taq 2X Master Mix (NEB #M0533) for 35–40 cycles. Ladder L is the Quick-Load® 2-Log DNA Ladder (NEB #N0469).

Robust cDNA synthesis is achieved even with longer templates



Jurkat total RNA (1 µg) was used in a 20 µl first strand cDNA synthesis with 200 units of NEB M-MuLV Reverse Transcriptase (RNase H⁻). Reactions were incubated at 42°C for 50 minutes, followed by heat inactivation for 5 minutes at 80°C. 1 µl of cDNA was used in a 25 µl PCR using LongAmp Hot Start Taq 2X Master Mix (NEB #M0533) for 35–40 cycles. Sizes are indicated above gel. Ladder L is the Quick-Load 2-Log DNA Ladder (NEB #N0469).

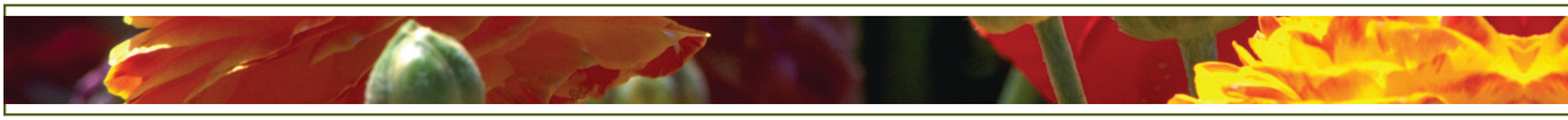
Generate high quality cDNA even with very low amounts of starting RNA



Decreasing amounts of Jurkat total RNA (1 µg – 1 pg) were used in 20 µl first strand cDNA synthesis with 200 units of NEB M-MuLV Reverse Transcriptase (RNase H⁻). Reactions were incubated at 42°C for 50 minutes, followed by heat inactivation for 5 minutes at 80°C. 1 µl of cDNA was used in a 25 µl PCR using LongAmp Hot Start Taq 2X Master Mix (NEB #M0533) for 40 cycles. The target is a 0.6 kb fragment of GAPDH. Ladder L is the Quick-Load 2-Log DNA Ladder (NEB #N0469).

Ordering Information

PRODUCT	NEB #	SIZE
M-MuLV Reverse Transcriptase (RNase H⁻)	M0368S/L/X	4,000/10,000/40,000 units



Application Note

Glycan Analysis of Murine IgG2a by Enzymatic Digestion with PNGase F and Trypsin, followed by Mass Spectrometric Analysis

Jack Benner, Ph.D., New England Biolabs, Inc. and
Ellen Guthrie, Ph.D., New England Biolabs, Inc.

Introduction:

Proteomics, the systematic study of proteins in biological systems, has expanded the knowledge of protein expression, modification, interaction and function. However, in eukaryotic cells, the majority of proteins are post-translationally modified (1). A common post-translational modification, essential for cell viability, is the attachment of glycans. Glycosylation defines the adhesive properties of glycoconjugates and it is largely through glycan-protein interactions that cell-cell and cell-pathogen contact occurs.

Glycosylation is also important in the production of therapeutic proteins as it can significantly affect the potency of a biological drug. Producing a homogenously glycosylated protein is very difficult and often impractical. For this reason, development and manufacturing processes are highly monitored to minimize glycosylation variability. Therefore, the ability to determine the presence or absence of a glycan at a particular site is critical to the production of therapeutic proteins. A combination of enzymes (PNGase F and Trypsin) in tandem with mass spectrometry can be used to release the N-glycans present on glycoproteins and determine the sites of N-glycosylation on the protein.

Immunoglobulin Gs (IgGs) are antibody molecules that are composed of four peptide chains – two heavy chains and two light chains (Figure 1). There are four IgG subclasses (IgG1, 2a, 2b, and 3) in mice. The heavy chains are known to be glycosylated. The glycans present on the heavy chains of IgG are attached to asparagine residues (N-linked). N-linked glycans are produced by the secretory pathway (ER and Golgi). Synthesis of N-glycans begins with the transfer of a common oligosaccharide to a nascent polypeptide in the ER. Some N-glycans remain unmodifided ("high mannose"), while others are initially trimmed and then extended as the glycoprotein matures in the Golgi ("complex").

Here, we describe the enzymatic removal of N-linked glycans using PNGase F from a model glycoprotein, murine monoclonal IgG type 2a, expressed in a mouse hybridoma cell line. We also demonstrate the use of trypsin to identify the site of glycosylation. Two mass spectrometers (MS) are used in this protocol: an Agilent™ 6210 Time-of-Flight (TOF) MS for analysis of the intact protein and a Thermo® LTQ-Orbitrap XL MS for analysis of the trypsin-digested murine IgG.

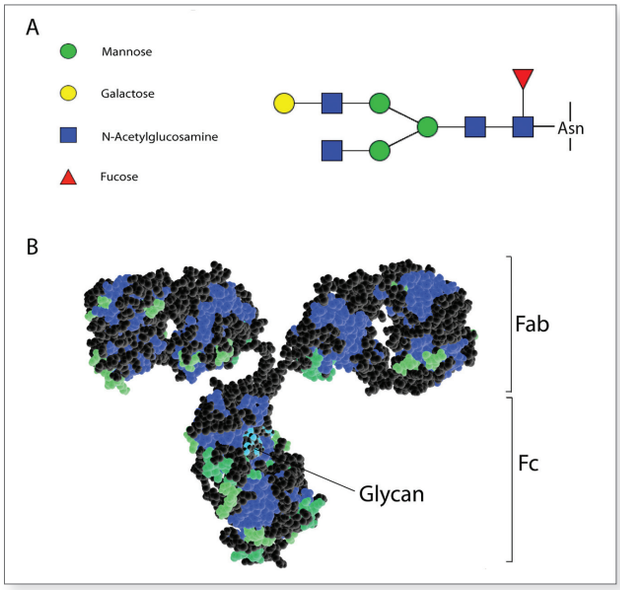
Results:

Mass Spectrometric Analysis

Samples of IgG treated with PNGase F were prepared and analyzed by reverse phase liquid chromatography (LC) and electrospray ionization time of flight mass spectrometry (ESI-TOF MS). The acquired spectra were extracted and the protein spectra were deconvoluted.

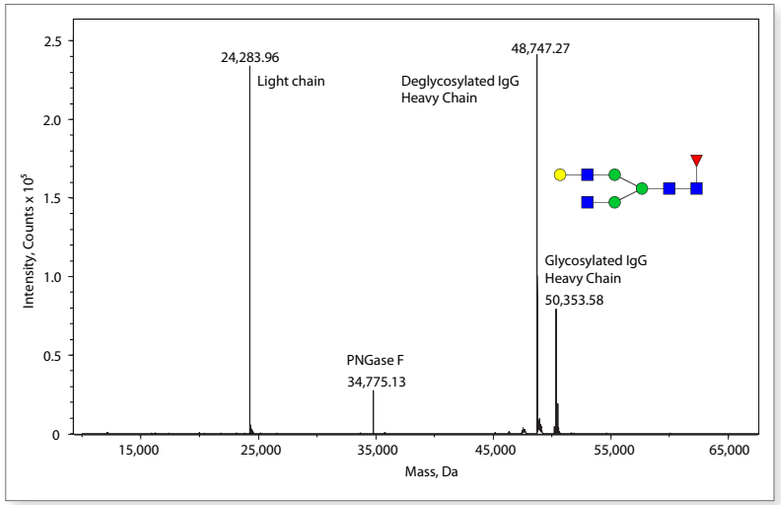
The spectra shown in Figure 2 is from a PNGase F digestion done under native conditions. The two heavy chain species observed are the deglycosylated heavy chain, 48,747.27 Da, and a small amount of remaining glycosylated species, 50,353.58 Da. The most likely structure of the remaining glycan is also shown in Figure 2.

Figure 1: Structure and glycosylation of a murine IgG



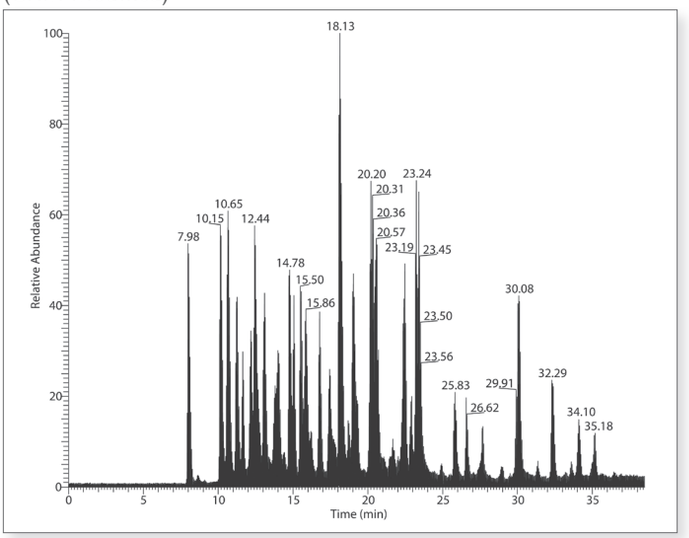
(A) Schematic representation of the fully substituted IgG heavy-chain glycan. (B) Structural model of murine IgG. In the IgG, beta-sheets are colored blue, loops are colored black and the helices are colored green. The brackets indicate the antigen-binding Fab portion and the Fc effector portion of IgG. The arrow indicates the two conserved glycans (aqua) attached to Asn-180 of the heavy chains. The model was generated using JMOL 12.2.23 from a model deposited in the Protein Data Bank by L. Harris (University of California, Riverside).

Figure 2: Murine IgG-treated with PNGase F



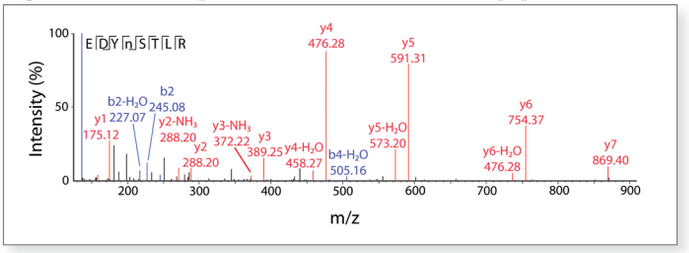
Murine IgG-treated with PNGase F was subjected to chromatography, nanoESI and TOF MS. Spectra were deconvoluted and the major peaks were identified.

Figure 3: Total Ion Current of Trypsin-digested BSA MS Standards (CAM Modified)



Total Ion Current of Trypsin-digested BSA MS Standards (CAM Modified). 100 fmol of MS Standard was analyzed using the LTQ-Orbitrap.

Figure 4: MS/MS spectrum of PNGase F treated peptide



MS/MS spectrum of PNGase F treated peptide

Trypsin Peptide MS and MS/MS Data Acquisition

Each Trypsin digested sample was injected using a Proxeon (Thermo Fisher) EASY-nLC trapped on a 2 cm x 100 mm C18 column (Thermo Fisher) and separated on a C18 reverse phase column (75 µm x 100 mm, Thermo Fisher).

Multiple charged peptide ions were automatically chosen during a 30,000 amu resolution scan in the Orbitrap and fragmented by HCD in the C trap of a LTQ-Orbitrap XL-ETD mass spectrometer (Thermo Fisher) with a Nano-Electrospray ionization source (Thermo Fisher) (Figure 4). A peptide was injected (100 fmol) to test the LC and MS system (Figure 3).

The MS and MS/MS fragmentation data were analyzed using Proteome Discoverer 1.2 (Thermo Fisher) using both Sequest and the Mascot version 2.27 (2). For both analyses, data were searched using the SwissProt FASTA database. For these analyses, theoretical peptides generated by a tryptic digest with a maximum of two missed cleavages were considered, and the precursor and product mass tolerances were set to ± 10 ppm and ± 0.01 Da, respectively. Variable modifications of asparagine were allowed for, including the conversion of asparagine to aspartic acid that occurs when PNGase F removes the glycan. Data was validated using a reverse database decoy search to a false discovery rate of 0.5%.

Summary:

A search of the data collected from the PNGase F/Trypsin-digested sample produced the above result of a peptide with the characteristic N-X-S/T (shown in Figure 4), where the N residue has been modified to become an aspartic acid residue (a mass change of +0.98 amu). The peptide observed to contain the modification was EDYNSTLR from the heavy chain of the murine IgG. These data are consistent with previously observed glycosylation site of murine IgG.

To download the full application note, including protocols, visit www.nebglycosidase.com.

References:

1. Spiro, R.G. (2002) *Glycobiology* 12, 43R-56R.
2. Perkins, D.N., et al. (1999) *Electrophoresis,* 20, 3551-3567.

Featured Products used in this Application Note

PNGase F

N-Glycosidase F is an amidase that cleaves the innermost GlcNAc and asparagine residues from N-linked glycoproteins.

Modified Trypsin (TPCK-treated)

TCPK treatment eliminates chymotryptic activity, making this trypsin ideal for proteome analysis by MS.

Trypsin-digested BSA MS Standard (CAM-modified)

A complex mixture of peptides produced by trypsin digestion of CAM-modified BSA.

Ordering Information

PRODUCT	NEB #	SIZE
PNGase F	P0704S/L	15,000/75,000 units
Modified Trypsin (TPCK-treated)	P8101S	100 µg
Trypsin-digested BSA MS Standard (CAM-modified)	P8108S	500 pmol

To learn more about our selection of proteases, endoglycosidases and exoglycosidases for research, download our new glycobiology brochure at **www.nebglycosidase.com**.

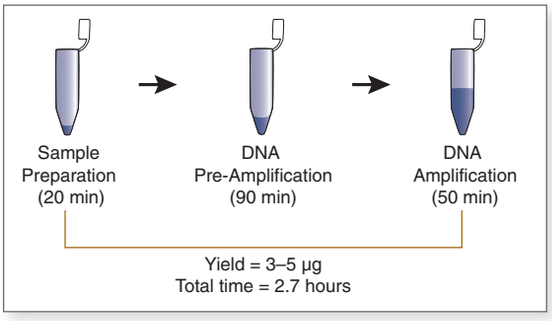
Single Cell WGA Kit

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 gDNA from single cells approximately 1 million-fold to produce 3–5 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 microarray.

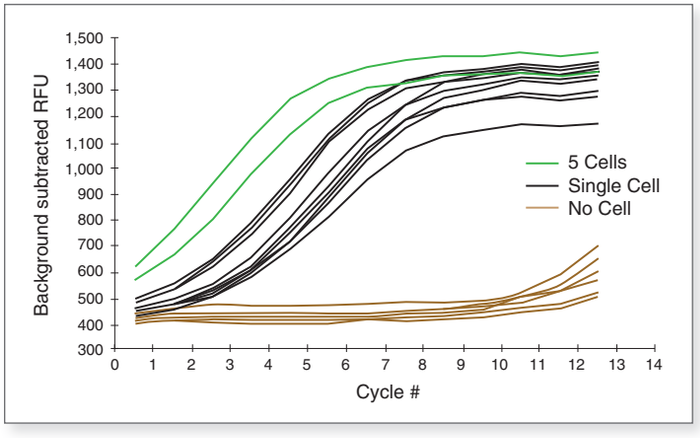
Advantages

- 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 registered 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

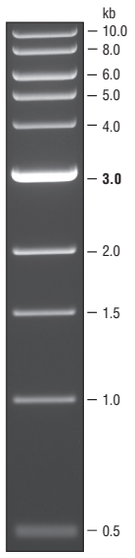
DNA Ladders

DNA ladders from New England Biolabs take the guesswork out of your DNA sample analysis. Select the ladder to suit your experimental needs and let our sharp, evenly-spaced bands help you achieve accuracy in your analysis. For your convenience, supplemental loading dye is provided. In addition, our most popular ladders are available in a Quick-Load (contains blue dye to track migration) and a TriDye™ format (contains three dyes).

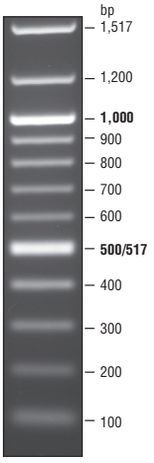
Advantages

- Stable at room temperature
- Sharp uniform bands with easy-to-identify reference bands
- Can be used for sample quantitation
- Exceptional value

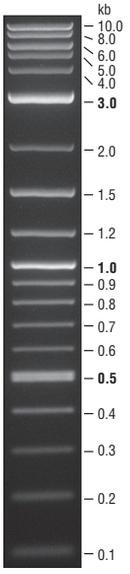
A Selection of DNA Ladders from New England Biolabs



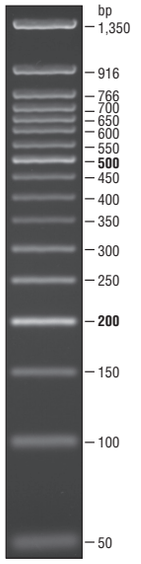
1 kb DNA Ladder*
0.8% TAE agarose gel.
NEB #N3232S/L



100 bp DNA Ladder*
1.3% TAE agarose gel.
NEB #N3231S/L

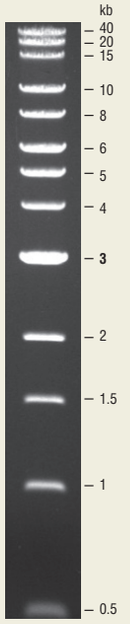


2-Log DNA Ladder*
1.0% TBE agarose gel.
NEB #N3200S/L

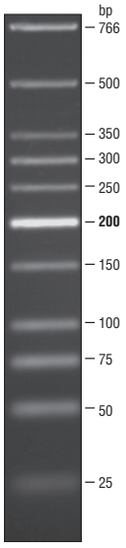


50 bp DNA Ladder**
1.8% TBE agarose gel.
NEB #N3236S/L

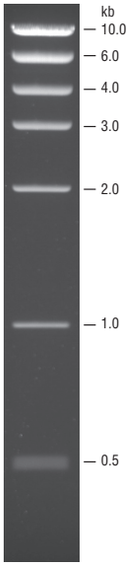
NEW



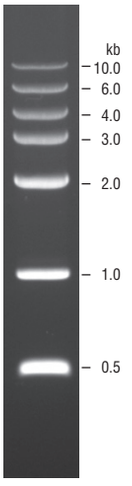
Quick-Load 1 kb Extend DNA Ladder
0.6% TBE agarose gel. Mass values are for 0.5 µg/lane.
NEB #N3239S



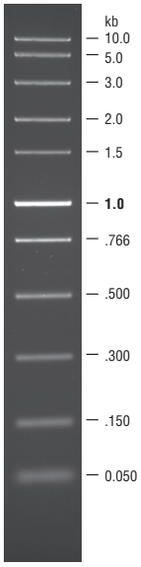
Low Molecular Weight DNA Ladder**
1.8% TBE agarose gel.
NEB #N3233S/L



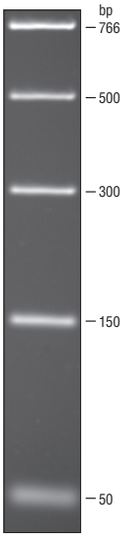
Mass DNA Ladder
1% TBE agarose gel.
NEB #N3237S



Reverse Mass DNA Ladder
0.8% TAE agarose gel. Mass values are for 0.75 µg/lane.
NEB #N3240S



Fast DNA Ladder
1.2% TBE agarose gel.
NEB #N3238S



PCR Marker
1.8% TBE agarose gel.
NEB #N3234S/L

* *Available in Quick-Load and TriDye formats*
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