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FEATURE ARTICLE

Improving Enzymatic DNA Fragmentation for Next Generation Sequencing Library Construction

by Lynne Apone, Ph.D., Eileen Dimalanta, Ph.D., and Fiona Stewart, Ph.D., New England Biolabs, Inc.

INTRODUCTION

The Human Genome Project (HGP), which was officially completed in 2003, was considered to be one of the world's largest collaborative projects of its time (1). This involved many research groups worldwide and had the lofty goal of deciphering all 3 billion bases of the human genome. The project cost almost $4 billion dollars and took 13 years to complete with the available technology. Over a decade later, advancements in next generation sequencing (NGS) technologies have enabled sequencing of a human genome to become routine, taking less than two days, and at a tiny fraction of the cost of the original HGP.

The ability to quickly and inexpensively sequence whole genomes has truly revolutionized genomics research. Where once single genes or families of genes were studied, now whole genomes, exomes, transcriptomes and epigenomes are interrogated. With recent advances, such as the ability to multiplex and sequence many samples at once, NGS has transitioned from a basic research tool into the clinic, where it impacts discovery, diagnostics and treatment of disease.

Advances in genomics driven by NGS, as well as advances in the technology itself, continue at an amazing pace and move us closer to the realization of personalized medicine, where clinical decisions are tailored to an individual's genome. However, if this pace is to continue, advances in all aspects of the technology must also continue. This includes early steps of the sequencing workflow, specifically in the preparation of samples, before they are sequenced.

To date, there are no sequencing platforms that can sequence intact DNA. Therefore, prior to sequencing, DNA molecules must be fragmented, or broken, into smaller pieces. These DNA fragments are then converted into libraries, by different methods depending on the sequencing platform to be used (Figure 1). In all cases, the libraries generated consist of the fragments of the unknown DNA to be sequenced, flanked by pieces of known DNA (adaptors), which are specific to each sequencing platform.

FIGURE 1:
Traditional library preparation workflow

DNA FRAGMENTATION APPROACHES

One of the major bottlenecks to sample prep is the first step: DNA fragmentation.

The size of the DNA fragments generated depend on the sequencing platform being used, and can range from several hundred base pairs for short read sequencing technologies (e.g., Illumina®, Ion Torrent™) to >10 kb pieces for long read sequencing technologies (e.g., Pacific Biosciences® and Oxford Nanopore Technologies®). Methods for fragmenting DNA are broadly split into two basic categories: mechanical and enzyme-based. Mechanical shearing methods include acoustic shearing, hydrodynamic shearing and nebulization, while enzyme-based methods include transposons, restriction enzymes and nicking enzymes. Although many different options exist to fragment DNA, final fragment size, amount of starting material, upfront capital investment, and scalability must be considered when choosing a fragmentation method. Critically, in order to be useful for NGS, the method used must shear the DNA sufficiently randomly, so that the library being sequenced is fully representative of the original sample.

Mechanical Shearing

Options for mechanical fragmentation of DNA range from small plastic nebulizer devices to sophisticated electronic instruments. The most commonly used technique utilizes **focused acoustic shearing** devices, such as the instruments made by Covaris®. This involves focused transmission of high-frequency, short wavelength acoustic energy on the DNA sample. The size of the DNA fragments generated (150 – 5,000 bp) is controlled by changing both the intensity and the duration of the acoustic waves, and the protocols used are the same regardless of the amount or GC content of the DNA. Cost, challenges of scalability and sample loss (often caused by sample transfer after shearing), are some of the reasons that users of this method seek alternatives, especially as throughput increases.

If larger DNA fragments are required, **hydrodynamic shearing** can be used. In this method, hydrodynamic shear forces are applied by pushing DNA through the small orifice of a syringe. Size is controlled by altering the speed at which the DNA is pushed through the syringe. Centrifugation can also be used to create hydrodynamic force, by pulling the DNA sample through a hole with a defined size. Here, the rate of centrifugation determines the degree of DNA fragmentation. DNA fragments generated with hydrodynamic shear forces are typically in the

DNA fragmentation landscape prior to NEBNext Ultra II FS

Focused acoustic shearing

\+
- Historical "gold standard"
- Simple control of fragment size
- Same protocol regardless of GC content or input amount

–
- Significant upfront instrument cost
- Significant disposables cost and waste generation (tubes/plates)
- Time-consuming
- Challenging to scale
- Inflexible reaction volumes
- Prone to DNA damage

Enzymatic fragmentation

\+
- Scalable
- Flexible reaction volumes
- Ease of use
- Minimized DNA damage

–
- Prone to sequence bias
- May be sensitive to contaminants in the DNA sample
- Significant optimization may be required
- Transposases can have very specific input requirements

range of 1-75 kb, but require large DNA input amounts (> 1 µg) and throughput is low.

Nebulization is another method used to mechanically fragment DNA. Nebulization uses compressed air to force DNA through a small hole in a nebulizer unit and DNA fragment size is determined by the pressure used. Although this method is inexpensive and fragment size is somewhat tunable (typically 700 – 5000 bp in size), microgram quantities of DNA are required for starting material, and the method is most suitable for small numbers of samples.

Enzymatic Fragmentation

Enzyme-based fragmentation of DNA is an attractive alternative to mechanical shearing methods, as it does not require upfront capital expense, is amenable to quickly processing many samples at the same time, and reduces sample loss. Historically, the main concern with this method has been sequence bias, as many enzymes that cleave DNA have recognition sequences or sequence preferences.

Transposases fragment DNA by cleaving and inserting a short double-stranded oligonucleotide to the ends of the newly cleaved molecule. The inserted oligonucleotide must contain a sequence that is specific to the particular transposase being used. While this method is fast and has low input requirements, the known sequence bias associated with transposases make them incompatible with some applications.

The great majority of **restriction enzymes** have very specific recognition and cleavage sites, and therefore are not suitable for the random cleavage required for most NGS applications. However, restriction enzymes do have utility in a workflow called RAD-Seq (Restriction-site Associated DNA Sequencing). Here, the sequence bias from the restriction enzyme cut site is exploited to target certain regions for sequencing.

Lastly, **non-specific nicking enzymes** can be used to fragment DNA. These enzymes have less sequence bias than transposases or restriction enzyme-based methods, and generate fragments of different sizes (generally 50 bp-1 kb) in a time-dependent manner: the longer the reaction time, the smaller the fragments. Historically, these enzymes have required significant reaction optimization. They have been sensitive to the buffering conditions of the DNA sample, and required different reaction conditions for different DNA input amounts and for varying GC content. While appealing in theory, this has made enzymatic fragmentation methods more challenging to implement, especially in laboratories where a variety of sample types and amounts are used.

Emerging drawbacks to the gold standard

Of all the techniques described, acoustic shearing has traditionally been the method of choice for short read sequencing technologies, such as Illumina; its popularity a result of robust shearing with low bias. However, this method requires a significant financial investment in equipment, which can be prohibitive for many researchers. In addition, throughput is low and sample loss high, creating bottlenecks and limitations for users of the technology. Interestingly, recent studies have also shown that oxidative damage can occur during mechanical shearing processes (2,3).

FIGURE 2:
NEBNext Ultra II FS Kit workflow

IMPROVING DNA FRAGMENTATION FOR NGS LIBRARY CONSTRUCTION

To address the challenges associated with existing fragmentation approaches, NEB has developed a fragmentation system, the NEBNext® Ultra™ II FS DNA Library Prep Kit (NEB #E7805, E6177), in which a unique enzymatic fragmentation reagent is fully integrated into library preparation to generate low bias, high quality NGS libraries, with a simple, scalable workflow (for more information see page 6).

In order to reduce the NGS sample prep bottleneck, improvements in both performance and ease of use were necessary. In this work, we have focused on the DNA fragmentation step. Our new DNA fragmentation reagent is combined with end repair and dA-tailing reagents, and subsequent adaptor ligation is also carried out in the same vial (Figure 2). For low input samples, PCR amplification is performed prior to sequencing.

Importantly, enzymatic shearing of DNA with this method does not introduce bias into the library, and this method is suitable for input DNA with a full range of GC content. Reduced sample loss and increased efficiencies of the workflow enable use of lower input amounts, with a range of 100 pg – 0.5 µg, and insert sizes of 100 bp to 1 kb can easily be generated.

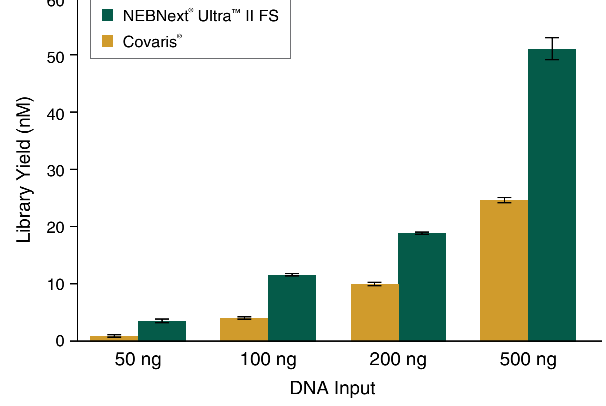
PERFORMANCE

Increased Library Yields

The use of enzymatic fragmentation can result in higher library yields as compared to mechanical shearing workflows, as the latter results in sample loss and DNA damage. Achieving sufficient library yields for high quality sequencing from very low input amounts can be especially challenging with mechanical shearing of DNA, a situation compounded by the preference to amplify libraries using as few PCR cycles as possible. Integration of our unique fragmentation reagent with end repair and dA-tailing, removing sample cleanup prior to ligation and eliminating multiple transfer steps all help to minimize sample loss. When combined with the high reaction efficiences of each step in the workflow and lack of DNA damage cuased by mechanical shearing, NEBNext Ultra II FS generates higher yields than library preparation using mechanical shearing methods. High library yields can be achieved with input amounts as low as 100 pg of human genomic DNA with amplification, or as low as 50 ng for PCR-free workflows (Figure 3, page 3).

FIGURE 3:
NEBNext Ultra II FS DNA produces higher yields of PCR-free libraries

Libraries were prepared from Human NA19240 genomic DNA using the input amounts shown, without amplification. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. "Covaris" libraries were prepared by shearing each input amount in 1X TE Buffer to ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Library yields were determined by qPCR using the NEBNext Library Quant Kit for Illumina (NEB #E7630). Error bars indicate standard deviation for an average of 2 libraries.



Improvements in Library Quality

As mentioned above, a historical concern regarding the use of enzymatic fragmentation methods was the potential for introduction of bias into a sequencing library, and ultimately into the sequencing data. As shown, this new fragmentation reagent provides consistent uniformity of GC coverage at the full range of input amounts (Figure 4) and over a broad range of GC composition (Figure 5).

In addition, oxidative damage markers typically associated with mechanical-shearing methods (2,3) are observed at significantly lower frequency in libraries made using this new fragmentation system (Figure 6, page 5). Importantly, a greater difference between the observance of these markers is seen with lower input amounts. This highlights the higher quality of libraries constructed with this new method compared to Covaris-sheared DNA libraries, especially at low input amounts.

EASE OF USE

Robustness of DNA Fragmentation

Consistent and reliable fragmentation is critical for a new method to be adopted. We optimized this new fragmentation system to be insensitive to variables such as input amounts, GC content, and DNA buffer conditions. In practice, these details are often unknown for a sample, requiring clean up and quantification prior to traditional enzymatic DNA fragmentation methods. Even when all of the variables are known, traditional enzymatic methods require different fragmentation parameters for each type of sample and DNA input amount. This new fragmentation system addresses all of these issues by requiring just a single-fragmentation protocol for the full range of input amounts (100 pg – 0.5 µg) (Figure 7, page 5) and for the full range of GC content (Figure 5). Also, input DNA can be in water, Tris, 0.1X TE or 1X TE (Figure 8, page 5). Fragmentation using the new system is time dependent, and final library sizes ranging from 100 bp – 1 kb can be generated by simply changing the fragmentation time.

CONCLUSION

The continued expansion of the use of next generation sequencing depends in significant part on overcoming the limitations and bottlenecks associated with high-quality library preparation, including the initial DNA fragmentation step. While acoustic shearing has for some time been the method of choice for NGS, limitations in terms of instrumentation, throughput and sample damage necessitate sourcing an alternative solution for many users. This new method for enzymatic DNA fragmentation and library

FIGURE 4:
NEBNext Ultra II FS DNA provides uniform GC coverage with human DNA over a broad range of input amounts

Libraries were prepared from Human NA19240 genomic DNA using the input amounts and number of PCR cycles shown, and a 20-minute fragmentation time was used. Libraries were sequenced (2 x 76 bp) on an Illumina® MiSeq®. Reads were mapped to the hg19 reference genome using Bowtie 2.2.4 and GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library.

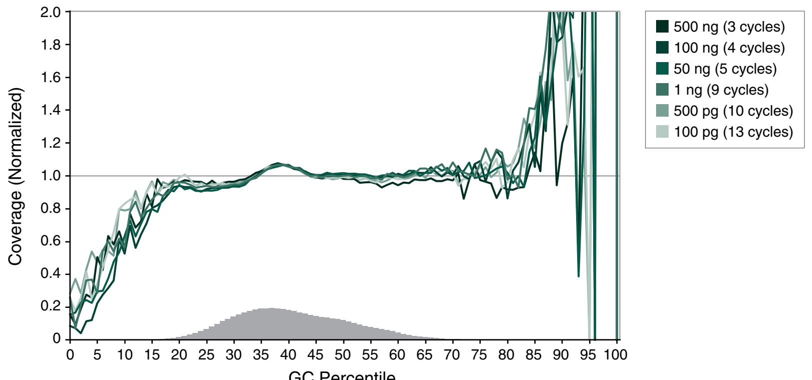
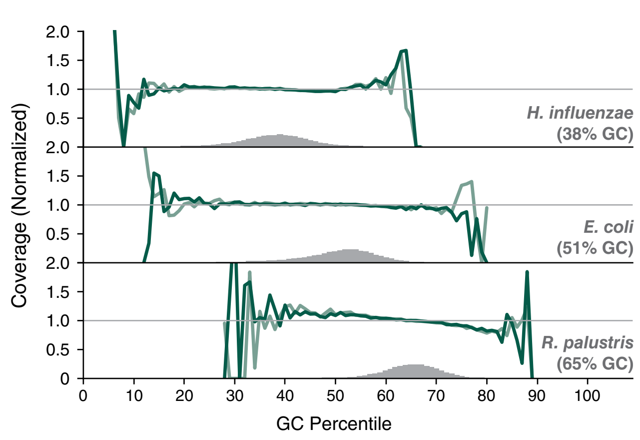


FIGURE 5:
NEBNext Ultra II FS DNA provides uniform GC coverage for microbial DNA over a broad range of GC composition

Libraries were prepared using 1 ng of a mix of genomic DNA samples from *Haemophilus influenzae, Escherichia coli* (K-12 MG1655) and *Rhodopseudomonas palustris*, with 9 PCR cycles, and sequenced on an Illumina MiSeq. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. "Covaris" libraries were prepared by shearing 1 ng of DNA in 1X TE Buffer to an insert size of ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Reads were mapped using Bowtie 2.2.4 and GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library.



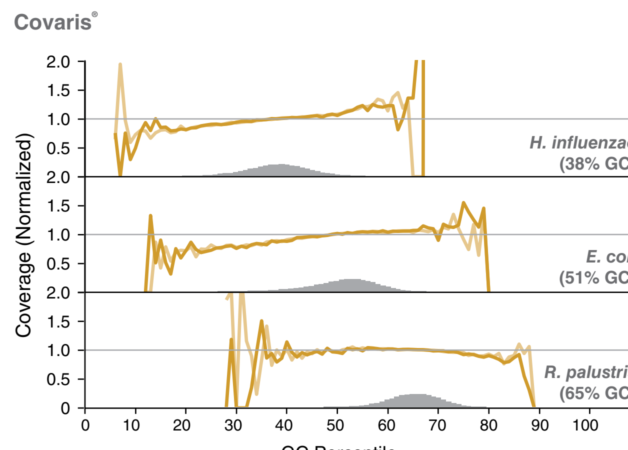
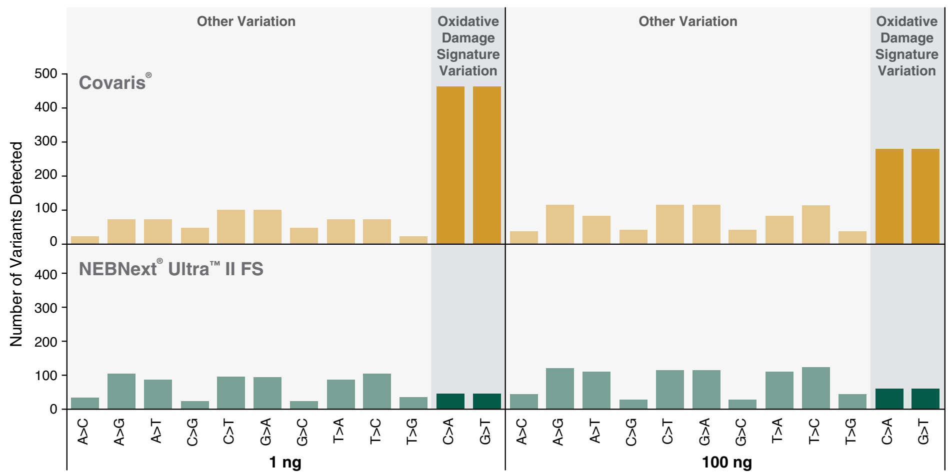


FIGURE 6:
NEBNext Ultra II FS DNA libraries show reduced markers of oxidative damage compared to libraries produced by mechanical shearing

Libraries were prepared from 1 ng and 100 ng Human NA19240 genomic DNA, using 9 and 4 PCR cycles, respectively. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. "Covaris" libraries were prepared by shearing each input amount in 1X TE Buffer to ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Libraries were sequenced on an Illumina® HiSeq® 2500 (2 x 75 bp). 723M reads were randomly sampled (seqtk) and aligned to the GRCh38 full reference genome using bwa (0.7.15). Adaptors were trimmed prior to alignment using trimadap (r9). Duplicates were marked using samblaster (0.1.24). Variants were called on chromosome 1 using freebayes (1.0.2.29) with frequency based options requiring at least 10 reads per site. More variants are observed for C>A and G>T transversions compared with all other variants for PCR-amplified Covaris libraries. These variants indicative of oxidative damage are not pronounced in NEBNext Ultra II FS libraries.



preparation addresses these issues, further streamlines the process and improves the quality of NGS libraries. The broadening of the input amount range to as low as 100 picograms enables access to high-quality sequencing of samples not achievable by previous methods, and the reliability and ease of use of the method not only allows automation, but also successful adoption by users with a wide range of laboratory skills.

References:

1. https://www.battelle.org/docs/default-source/misc/battelle-2011-misc-economic-impact-human-genome-project.pdf
2. M. Costello et al. (2013) *Nucleic Acids Research*, 41, e67.
3. L. Chen, et al. (2017) *Science*, 17, 355: 752-756.

FIGURE 7:
NEBNext Ultra II FS DNA provides consistent fragmentation regardless of input amount

Libraries were prepared from Human NA19240 genomic DNA using the input amounts shown. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. Library size was assessed using the Agilent® Bioanalyzer®. Low input (1 ng and below) libraries were loaded on the Bioanalyzer without a dilution. High input libraries were loaded with a 1:5 dilution in 0.1X TE.

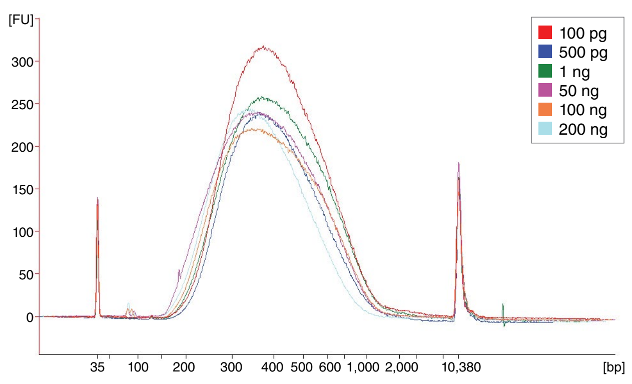
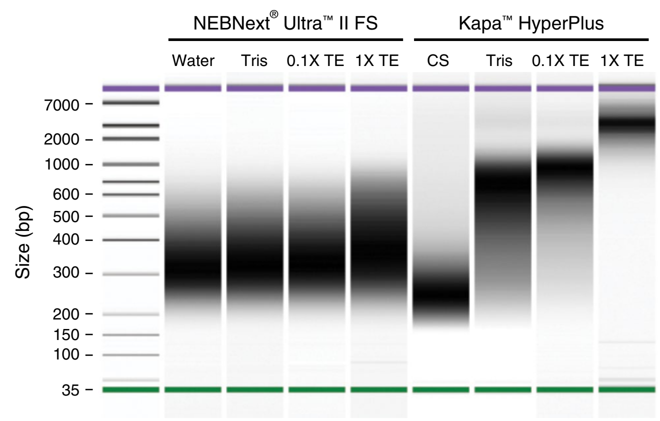


FIGURE 8:
NEBNext Ultra II FS DNA provides consistent fragmentation of DNA in water, Tris or TE

Libraries were made using 100 ng Human NA19240 genomic DNA using the NEBNext Ultra II FS kit or the Kapa HyperPlus Kit. Fragmentation conditions targeting ~200 bp inserts were used, which would generate ~320 bp libraries. For the NEBNext Ultra II FS kit, input DNA was in H2O, Tris, 0.1X TE or 1X TE . For the Kapa HyperPlus kit, libraries were made using the recommended dilution of the supplied Conditioning Solution (CS), or using DNA in Tris, 0.1X TE or 1X TE, in the absence of either Conditioning Solution or 3X bead clean up. Library size distribution was assessed using the Agilent Bioanalyzer. Fragmentation is consistent for the NEBNext Ultra II FS kit for DNA in H_2O, Tris, 0.1X TE or 1X TE.



You'll be thrilled to pieces.

Novel Enzymatic Fragmentation System

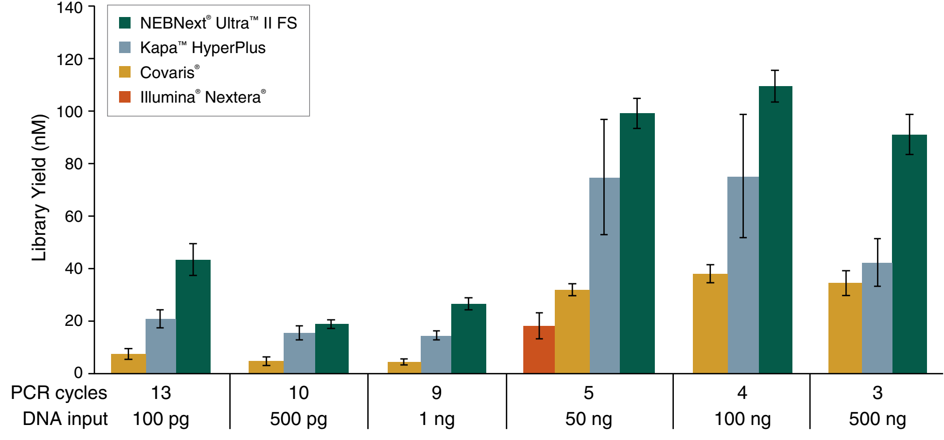
Do you need a faster or more reliable solution for DNA fragmentation and library construction? Our new NEBNext Ultra II FS DNA Library Prep Kit meets the dual challenges of constructing high quality next gen sequencing libraries from ever-decreasing input amounts, and simple scalability. Further, this kit provides a fragmentation and library prep solution that can handle different input amounts and sample types, quickly and reliably.

The Ultra II FS kit includes a new fragmentation reagent, which is also combined with end repair and dA-tailing reagents, enabling these steps to be performed in the same tube, with no clean-up steps or sample loss. The same fragmentation protocol is used for any input amount (100 pg–500 ng), or GC content.

You'll be thrilled to pieces with the result – a reliable, flexible, high-quality library prep that is fast and scalable.

FIGURE 1:
NEBNext Ultra II FS DNA produces the highest yields, from a range of input amounts

Libraries were prepared from Human NA19240 genomic DNA using the input amounts and numbers of PCR cycles shown. For NEBNext Ultra II FS, a 20-minute fragmentation time was used. For Kapa™ HyperPlus libraries, input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute fragmentation time was used. Illumina® recommends 50 ng input for Nextera®, and not an input range; therefore, only 50 ng was used in this experiment. "Covaris®" libraries were prepared by shearing each input amount in 1X TE Buffer to an insert size of ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Error bars indicate standard deviation for an average of 3–6 replicates performed by 2 independent users.



View more performance data by downloading our technical notes at
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High-yield, Scalable Library Preparation with the NEBNext Ultra II FS DNA Library Prep Kit

Next generation DNA library construction (LC) for high-throughput genomics – *Data presented by Peter Ellis, Senior Staff Scientist at the Wellcome Trust Sanger Institute*

Advantages

- Perform fragmentation, end repair and dA-tailing with a **single enzyme mix**
- Experience **reliable fragmentation with a single protocol**, regardless of DNA input amount or GC content
- Prepare high quality libraries from a **wide range of input amounts**: 100 pg–500 ng
- Use with DNA in standard buffers (e.g., TE, Tris-HCl) and water
- Save time with a **streamlined workflow**: ~ 2.5 hours, with < 15 minutes hands-on time
- Experience **reliable fragmentation**, even with very low input amounts
- Generate **high yields** with increased reaction efficiencies and minimized sample loss
- Vary incubation time to generate a **wide range of insert sizes**

For more information and to request a sample, visit

NEBNextUltraII.com

NEBNext® Ultra™ II FS DNA Library Prep Kit

FIGURE 2:
NEBNext Ultra II FS DNA provides uniform GC coverage for microbial genomic DNA over a broad range of GC composition

Libraries were prepared using 1 ng of a mix of genomic DNA samples from *Haemophilus influenzae, Escherichia coli* (K-12 MG1655), *Rhodopseudomonas palustris* and the library prep kits shown with 9 PCR cycles for consistency across samples, and sequenced on an Illumina MiSeq. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. For Kapa HyperPlus libraries, input DNA was cleaned up with 3X beads prior to library construction, as recommended, followed by a 25-minute fragmentation time. "Covaris" libraries were prepared by shearing 1 ng of DNA in 1X TE Buffer to an insert size of ~200 bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Reads were mapped using Bowtie 2.2.4 and GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library.

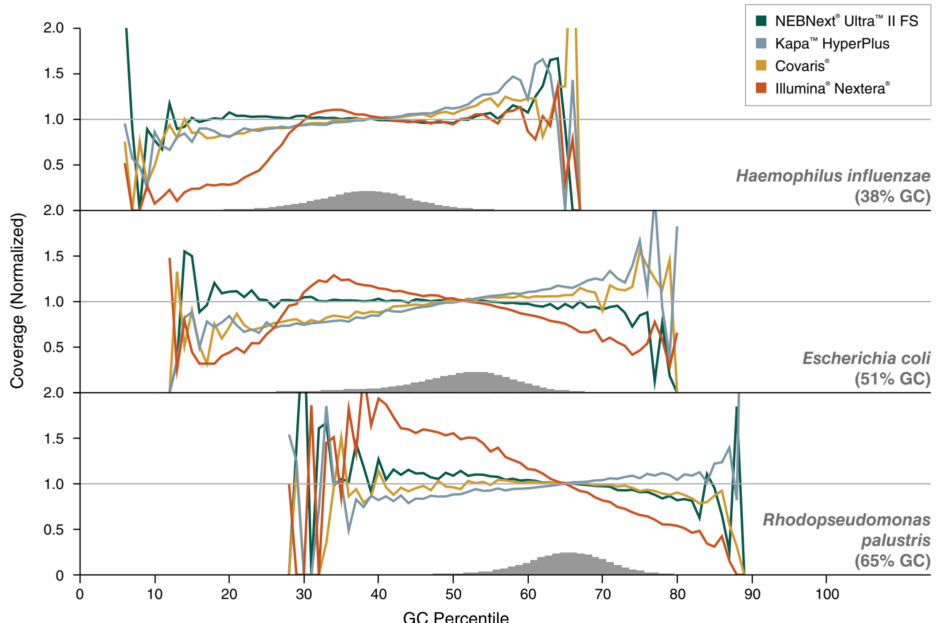


TABLE 1:
NEBNext Ultra II FS DNA Library Prep Workflow

	Fragmentation/ End Repair/ dA-Tailing	Adaptor Ligation	Clean Up/ Size Selection	Amplification	Clean Up	Total Workflow
Hands-On	2 min.	1 min.	5 min.	0–1 min.	0–5 min.	**8–14 min.**
Total	37–62 min.	16–31 min.	27–37 min.	0–34 min.	0–27 min.	**1.3–3.2 hr.**

OTHER PRODUCTS YOU MAY BE INTERESTED IN	NEB #	SIZE
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1, 2, 3, 4)	E7335, E7500, E7710, E7730S/L	24/96 rxns
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns

Available products:

- **NEBNext Ultra II FS DNA Library Prep Kit for Illumina**
  Includes optimized mixes for DNA library preparation (enzymatic fragmentation/end repair/dA-tailing, adaptor ligation and PCR enrichment steps) for sequencing on the Illumina platform. This kit includes a new DNA fragmentation reagent, which is also combined with end repair and dA-tailing reagents, enabling these steps to be performed in the same tube, with no clean-ups or sample loss.
  NEB #E7805S/L 24/96 rxns

- **NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads**
  Includes optimized mixes for DNA library preparation (enzymatic fragmentation/end repair/dA-tailing, adaptor ligation and PCR enrichment steps) plus SPRIselect® beads for size selection and cleanup. This kit includes a new DNA fragmentation reagent, which is also combined with end repair and dA-tailing reagents, enabling these steps to be performed in the same tube, with no clean-ups or sample loss.
  NEB #E6177S/L 24/96 rxns

- **NEBNext Ultra II FS DNA Module**
  This module is part of the NEBNext Ultra II FS workflow, and includes optimized mixes for DNA library preparation (enzymatic fragmentation/ end repair/dA-tailing, adaptor ligation and PCR enrichment steps).
  NEB #E7810S/L 24/96 rxns

Redesigning glycosidase manufacturing quality for pharmaceutical and clinical applications

by Christopher H. Taron, Ph.D., Alicia M. Bielik, Ph.D., Ellen P. Guthrie, Ph.D., and Xiaofeng Shi, Ph.D., New England Biolabs, Inc.

INTRODUCTION

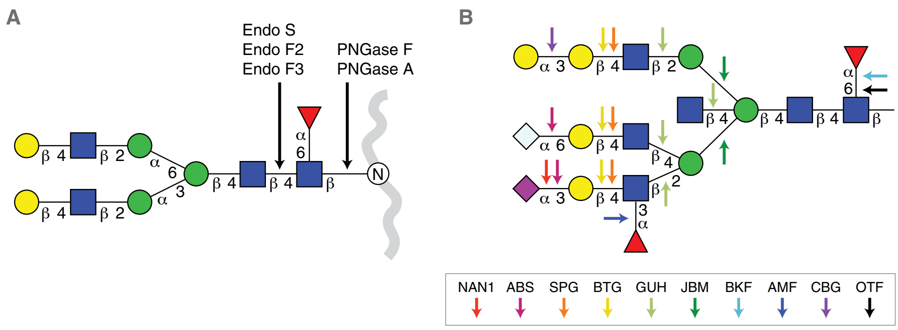
The growing importance of protein glycosylation in both pharmaceutical (1) and clinical science (2) is placing new demands on the quality of reagents used for glycan analysis and manipulation. Over the past 20 years, the list of enzymes and enzymatic specificities available for glycan analysis has steadily grown. Several enzymes have become mainstays in the published literature for applications such as glycan sequencing or N-glycan release from proteins ahead of downstream analytical techniques (Figure 1). Many of these enzymes are transitioning from academic research into workflows that characterize glycans on biotherapeutics and in glycan-based clinical tests (4,5). However, many of these enzymes are not being manufactured and quality tested in a manner suitable for use in regulated industries. It is therefore critical that the reagent industry adopt more rigorous quality standards for manufacturing carbohydrate hydrolases for use in clinical and pharmaceutical analytical applications.

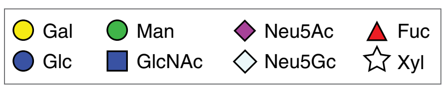
EXOGLYCOSIDASES ILLUSTRATE THE NEED FOR MANUFACTURING QUALITY IMPROVEMENTS

Exoglycosidases sequentially remove monosaccharides from the non-reducing end of glycans (5). They typically have specificity for a particular type of sugar, its anomeric configuration (α or β), and its position of attachment to an adjacent sugar in an oligosaccharide. They are useful tools because they can modify the composition of glycans either *in vitro* or *in situ* (i.e., on the surface of a cell). However, their most common use is in glycan sequencing (6-10). In this application, several exoglycosidases with differing specificities are used in series to decipher the structure of a glycan (Figure 1B; Table 1). Used in conjunction with various analytical methods, like LC-MS or CE-LIF, enzymatic sequencing can provide an orthogonal confirmation of a glycan's structure. This is particularly applicable to the structure characterization of N-glycans derived from biologic drugs to meet with regulatory requirements.

FIGURE 1:
Common endo- (A) and exoglycosidases (B) that cleave N-linked glycans

Symbols follow accepted SNFG nomenclature to denote the individual sugars that comprise glycans (3). α and β represent the anomeric configuration, and the numbers represent the position of attachment. Enzymes corresponding to the colored arrows in panel (B) are listed in Table 1, below.





Abbreviations: Neu5Ac, N-acetylneuraminic acid; Neu5Gc, N-glycolylneuraminic acid; Gal, galactose; GlcNAc, N-acetylglucosamine; Man, mannose; Fuc, fucose.

TABLE 1:
Common analytical exoglycosidases

COMMON ENZYME ABBREVIATION	NEB ENZYME NAME	SPECIFICITY	ORIGIN	SUPPLIER & FORMAT		
				NEB	PROZYME®	SIGMA
NAN1	α2-3 Neuraminidase S	α2-3 Neu5Ac	*Streptococcus pneumoniae*	R	R	R
ABS	α2-3,6,8,9 Neuraminidase A	α2-3,6,8,9 Neu5Ac/ Neu5Gc	*Arthrobacter ureafaciens*	R	R	R
SPG	β1-4 Galactosidase S	β1-4 galactose	*Streptococcus pneumoniae*	R	N	R
BTG	β1-3,4 Galactosidase	β1-3,4 galactose	*Bovine (testes)*	R	R	R
GUH	β-*N*-Acetylglucosaminidase S	β *N*-acetylglucosamine	*Streptococcus pneumoniae*	R	N	N
JBM	α1-2,3,6 Mannosidase	α1-2,3,6 mannose	*Jack beans*	R	N	N
BKF	α1-2,3,4,6 Fucosidase	α1-2,3,4,6 fucose	*Bovine (kidney)*	R	N	N
AMF	α1-3,4 Fucosidase	α1-3,4 fucose	*Almond meal*	R	–	–
CBG	α1-3,4,6 Galactosidase	α1-3,4,6 galactose	*Coffee beans*	R	N	N
OTF	α1-2,4,6 Fucosidase O	α1-2,4,6 fucose	*Omnitrophica bacterium*	R	–	–

R = recombinant, N = native source

Exoglycosidases have been used to elucidate glycan structure for over 30 years (6-10). Over this time, several glycosidases have emerged as the field's preferred enzymes for glycan sequencing due to their desirable specificities (Table 1). However, commercial sources of these enzymes have often lacked supply chain consistency and have had poor overall product quality.

For many glycosidases, quality issues begin from the use of outdated manufacturing processes. For example, many commercial glycosidases are non-recombinant and are still isolated as native proteins from sources, such as ground plant seed meals (jack beans, almonds, coffee beans) or animal tissues (bovine testes, bovine kidney). These sources are notorious for containing numerous exoglycosidase specificities, contaminating proteases, and often multiple isoforms of the desired enzyme. For example, proteomics analyses of a commercial preparation of native bovine α-fucosidase (BKF) showed that it is a mixture of two different fucosidases (FUCA1 and FUCA2), two similar enzymes with different specificities. The physical similarity and potential interaction between these two enzymes may complicate their ability to be separated in a native purifi-

cation process. Other examples illustrate that existing commercial supplies of many enzymes vary dramatically in their physical purity (Figure 2) or contain contaminating glycosidase side activities that would severely complicate their use in analytical workflows (Figure 3).

REDESIGNING GLYCOSIDASE MANUFACTURING

New England Biolabs® (NEB®) is committed to improving the manufacturing quality of analytical glycosidases with the goal of establishing their routine use in biopharma and clinical diagnostics markets specifically. NEB's objectives are two-fold:

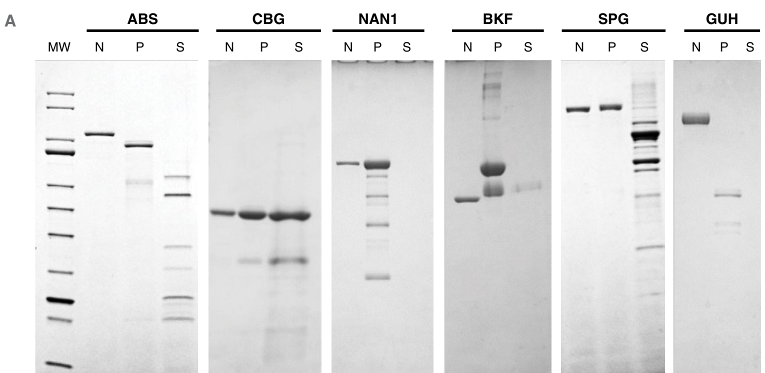
1. To improve the manufacturing process design and consistency for analytical glycosidases
2. To establish the most rigorous assessment of final product quality in the industry

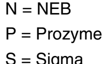
To improve overall consistency of its manufacturing process, NEB established new bioprocessing methods for glycosidase production. First, the use of highly variable native enzyme sources, such as seed meals, animal organs, or pathogenic bacteria, was eliminated. Genes were cloned and overexpressed in bacteria or yeast for all NEB analytical glycosidases. For certain enzymes (i.e., jack bean α-mannosidase and almond meal α-fucosidase), this effort involved the discovery of previously unknown plant genes. The use of engineered microbial hosts permitted the design of consistent and scalable fermentation and purification methods. Additionally, NEB has eliminated the direct use of animal-derived components (i.e., in growth medium, chromatography resins or buffers) in each manufacturing process. Finally, as part of the product's development, NEB strives to produce three independent production lots for each enzyme to demonstrate process consistency and permit batch-to-batch comparisons.

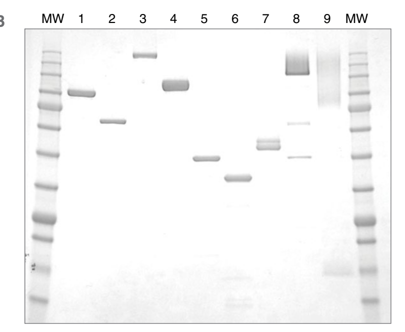
To improve final product quality, NEB has implemented the industry's most rigorous program of product quality testing and continual improvement. NEB glycosidases are purified to >95% homogeneity, as assessed by SDS-PAGE and ESI-TOF mass spectrometry. Final products are tested to rule out contaminating endo- and exo-glycosidase activities using 18 independent quality assays (Table 2). Another important advance has been NEB's use of natural carbohydrate substrates in place of synthetic colorimetric sugar analogs (i.e., p-nitrophenyl (pNP) derivatized sugars) in both quality assurance and unit definition assays. Colorimetric sugar analogs are not hydrolyzed by all exoglycosidases (11). Thus, quality control assays that use such substrates to detect unwanted exoglycosidase contaminants do not fully ensure their absence (see Figure 3B for an example). NEB maintains a large collection of fluorescently-labeled carbohydrate substrates isolated from natural sources, and each glycosidase is subjected to

FIGURE 2:
Purity and quality of commercially available glycosidases

(A) Comparison of commercial preparations of common analytical glycosidases visualized by SDS-PAGE separation followed by staining. Three letter enzyme abbreviations correspond to Table 1. The empty lane for Sigma-Aldrich® NAN1 represents separation of 20 µL of commercial stock without the observation of any protein. (B) SDS-PAGE separation of NEB commercial preparations. AMF, JBM, and BTG are each glycosylated proteins produced recombinantly in yeast.







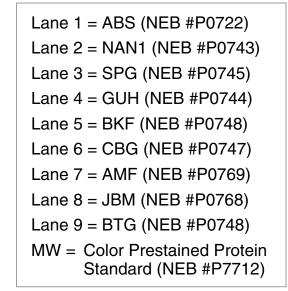
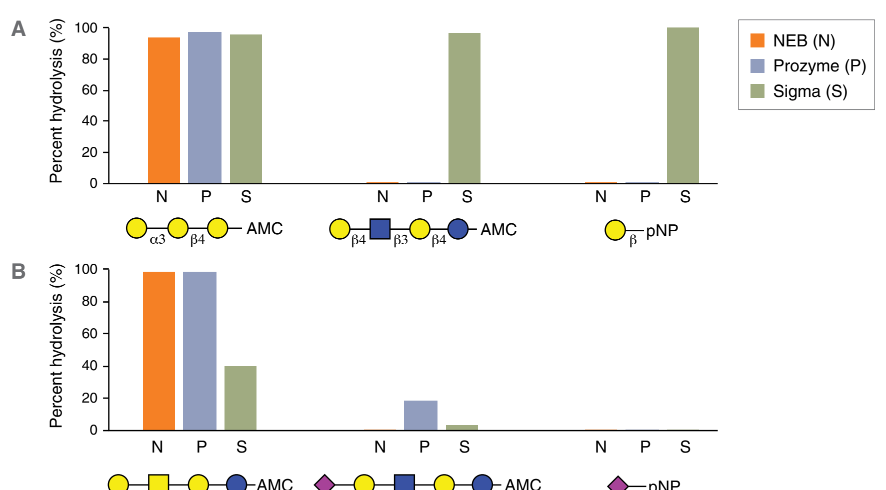


FIGURE 3:
Contaminating activities detected in some commercially available exoglycosidases

(A) The presence or absence of contaminating β-galactosidase activity in commercial preparations of Coffee Bean α-galactosidase (CBG). (B) The presence or absence of contaminating α-neuraminidase activity in commercial preparations of *S. pneumoniae* β-galactosidase (SPG). Notably, this contaminant is not observed using the substrate analog pNP-α-Neu5Ac, underscoring the importance of using oligosaccharide substrates for quality control assessments. Abbreviations: Neu5Ac, N-acetylneuraminic acid; Gal, galactose; GlcNAc, N-acetylglucosamine; Glc, glucose; AMC, 7-amino-4-methylcoumarin (fluorophore); pNP, p-nitrophenyl (chromophore). Reactions were carried out according to manufacturer's recommendations. In each assay, % hydrolysis was calculated by comparison to a control reaction using an enzyme that fully hydrolyzes each substrate.

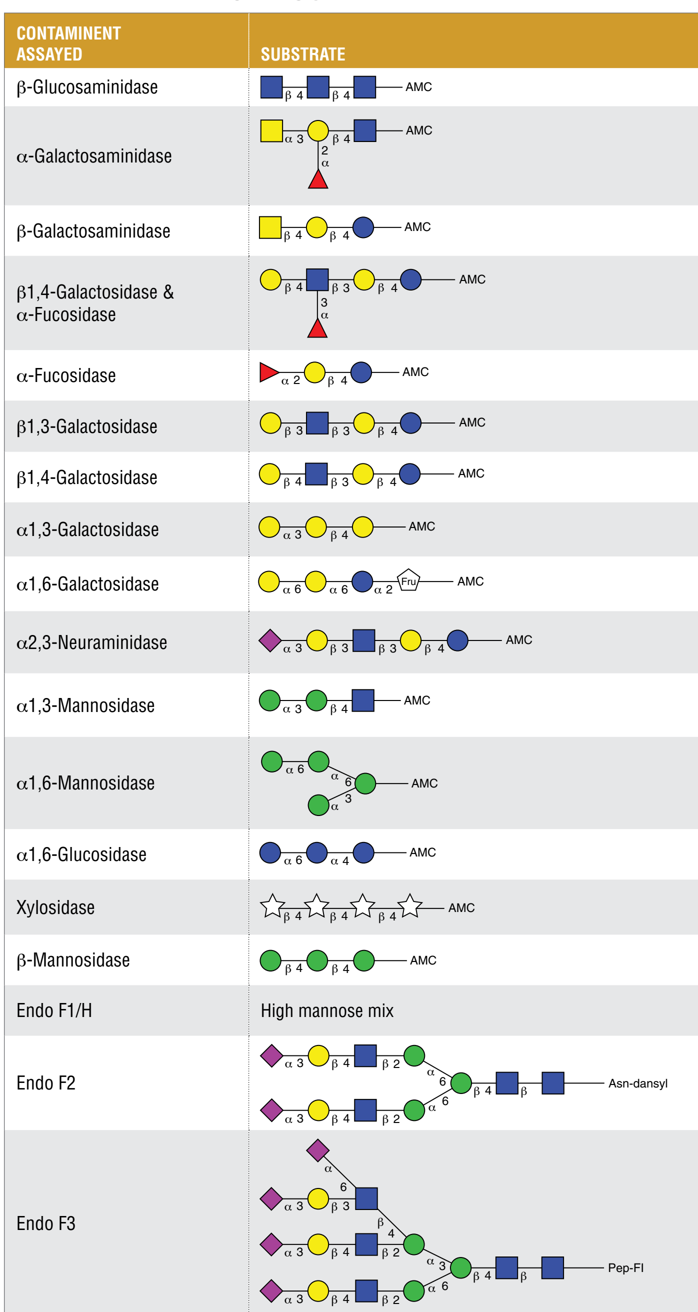


continued on page 10...

multiple quality tests using these substrates to ensure the absence of unwanted activities in the final product.

Finally, important to the needs of biopharma, NEB manufactures under both ISO 9001 and ISO 13485 quality management systems. These international standards validate that all NEB glycosidases are manufactured with the highest level of traceability and quality control. As some molecular diagnostics are regulated as medical devices, glycosidases manufactured to the ISO 13485 standard may have particular relevance in the emerging field of glycan-based molecular diagnostics. Furthermore, NEB is expanding to include cGMP manufacturing capabilities. This manufacturing option has potential to extend the applicability of NEB glycosidases into glycoengineering where in-process alterations to glycan compositions of biologic drugs or biosimilars could be achieved.

TABLE 2:
NEB's extensive quality control assays detect a wide variety of contaminating exoglycosidases



CONTAMINENT ASSAYED	SUBSTRATE
β-Glucosaminidase	β4 β4 AMC
α-Galactosaminidase	α3 β4 2α AMC
β-Galactosaminidase	β4 β4 AMC
β1,4-Galactosidase & α-Fucosidase	β4 β3 β4 3α AMC
α-Fucosidase	α2 β4 AMC
β1,3-Galactosidase	β3 β3 β4 AMC
β1,4-Galactosidase	β4 β3 β4 AMC
α1,3-Galactosidase	α3 β4 AMC
α1,6-Galactosidase	α6 α6 α2 Fru AMC
α2,3-Neuraminidase	α3 β3 β3 β4 AMC
α1,3-Mannosidase	α3 β4 AMC
α1,6-Mannosidase	α6 α6 α3 AMC
α1,6-Glucosidase	α6 α4 AMC
Xylosidase	β4 β4 β4 AMC
β-Mannosidase	β4 β4 AMC
Endo F1/H	High mannose mix
Endo F2	α3 β4 β2 α6 α3 β4 β2 α6 β4 β Asn-dansyl
Endo F3	α6 α3 β3 β4 α3 β4 β2 α3 α3 β4 β2 α6 β4 β Pep-Fl

CASE STUDY: PNGASE F MANUFACTURING

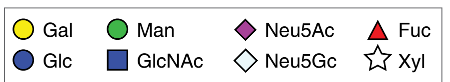
Peptide-N-Glycosidase F (PNGase F) is a broad specificity amidase that liberates high mannose, hybrid and complex N-linked glycans from glycoproteins. It is the most commonly used enzyme for N-glycan removal from glycoproteins. PNGase F digestion is a critical first step in most workflows that permits the analysis of released N-glycans, or deglycosylated proteins or peptides. PNGase F has become increasingly important in the biopharma industry, where defining the structure of N-glycans on therapeutic glycoproteins (e.g., antibodies, fusion proteins) is imperative for defining their performance, quality control, and validating the structural equivalence of biosimilars. Additionally, PNGase F, is increasingly being used in high-throughput analyses of novel glycan-based markers of disease and the development of clinical tests. The changing landscape of glycan analysis has placed new demands on PNGase F with respect to its suitability for regulated environments, and for high-throughput workflows.

NEB's recombinant PNGase F (Cat #P0708/P0709) is manufactured using the ethos and quality management systems described previously. PNGase F is produced to ≥95% purity, as assessed by SDS-PAGE and intact ESI-MS (Figure 4). The enzyme is subjected to rigorous quality testing to assure the absence of proteases and contaminating exo- and endoglycosidase activities. Over 18 independent quality control assays with labeled glycan substrates are performed on each enzyme lot (Table 2). Finally, three independent production lots are continually maintained to ensure consistency and reproducibility in the manufacturing process (Figure 4A). The result is the most rigorously quality-tested PNGase F on the market.

CONCLUSION

The importance of protein glycosylation in pharmaceutical and clinical science has necessitated quality improvements to the enzymatic reagents used for glycan analysis in these fields.

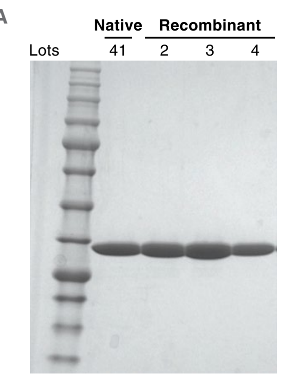
Many commercial preparations of key analytical glycosidases have significant quality issues and are poorly suited for use in regulated industries. NEB has addressed this need through the development of the industry's most comprehensive and rigorous approach to glycosidase manufacturing and quality testing. With these quality improvements, NEB glycosidases are poised to address both current and future needs of pharmaceutical and clinical glycan analysis.

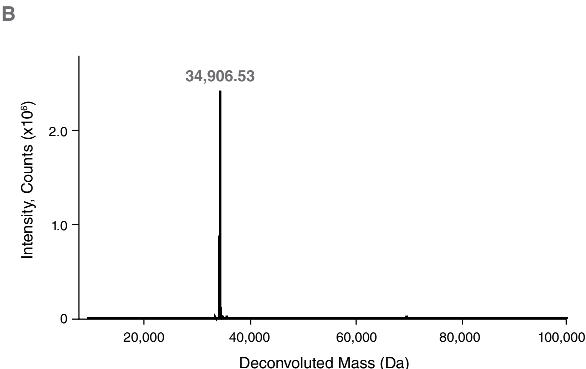


Abbreviations: Neu5Ac, N-acetylneuraminic acid; Neu5Gc, N-glycolylneuraminic acid; Gal, galactose; GlcNAc, N-acetylglucosamine; Man, mannose; Fuc, fucose.

FIGURE 4:
SDS-PAGE Analysis (A) and ESI-TOF Analysis (B) of NEB PNGase F (Glycerol-free), Recombinant (MW: 34,906.53 Daltons)

SDS PAGE analysis; Lane 1: Protein Ladder (NEB #P7703); Lane 2: PNGase F (Glycerol-free) Lot 41, (NEB #P0705); Lane 3: PNGase F (Glycerol-free), recombinant Lot 2 (NEB #P0709); Lane 4: PNGase F (Glycerol-free), recombinant Lot 3 (NEB #P0709); Lane 5: PNGase F (Glycerol-free), recombinant Lot 4 (NEB #P0709). Mass determination was performed using an Agilent® 6210 TOF LC/MS.





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Acknowledgements:
The authors thank Andy Bertera and Kiran Gulati for helpful comments.

FEATURED PRODUCT

PNGase F – Designed for your application

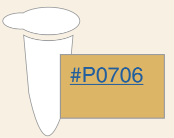
PNGase F is the most effective enzymatic method for removing almost all N-linked oligosaccharides from glycoproteins. PNGase F digestion deaminates the asparagine residue to aspartic acid, and leaves the oligosaccharide intact for further analysis.

The growing importance of protein glycosylation in both pharmaceutical and clinical science, as well as basic research, is placing new demands on the quality of reagents for glycan analysis. NEB offers a selection of PNGase F reagents, all highly pure, to support a variety of applications.

PNGase F formulations include:

Glycerol-free
#P0705
#P0709
For downstream HPLC and mass spectrometry

Fast deglycosylation
#P0710
#P0711
For rapid and complete glycan or protein analysis (non-reducing format)

Tagged-enzyme

#P0706
For easy removal from your reaction

Custom formulations

Upon Request
Lyophilized or immobilized enzymes are available upon request

Not sure which PNGase F will work best for you?

The selection chart below recommends which PNGase F product will work best for your application:

	RAPID PNGASE F (#P0710)	PNGASE F Std.* (#P0704)	PNGASE F GF** (#P0705)	REMOVE-IT® PNGASE F (#P0706)	PNGASE F, RECOMBINANT Std.* (#P0708)	PNGASE F, RECOMBINANT GF** (#P0709)
N-glycan removal from glycoproteins		•	•	•	•	•
Optional removal of the enzyme from the reaction				•		
Glycoprotein analysis, regulatory monitoring	•***			•	•	•
High-throughput antibody analysis, regulatory monitoring	•					
Glycomics	•	•	•	•	•	•
Proteomics	•		•	•		•

* Std. = Standard formulation ** GF = Glycerol-free formulation
*** Rapid PNGase F works for many other glycoproteins (not antibody-related), but this needs to be empirically tested for each glycoprotein.

Interested in learning more?
Visit www.neb.com/PNGaseF

Learn about glycobiology and its importance in clinical and diagnostic applications at **NEB TV**.

Download select **application notes** and **publications** featuring PNGase F

Enzymes for Innovation

The NEB catalog highlights a wide variety of enzyme functionalities found in nature or engineered for specific purposes. However, in molecular biology, new tools can often lead to new discoveries. Taking advantage of the enzymology expertise at NEB, we are expanding our offering of novel enzymes with interesting and unique activities for manipulating DNA, RNA, proteins and glycans, even if specific applications for them have yet to be discovered. Our hope is that by engaging researchers' imaginations, our "Enzymes for Innovation" initiative will enable the development of new molecular techniques that so often lead to new discoveries.

Enzymes for Innovation include:

NAME	NEB #	ACTIVITY
EcoGII Methyltransferase	M0603	EcoGII Methyltransferase is a non-specific methyltransferase that modifies adenine residues (N6) in any sequence context.
Endoglycoceramidase I (EGCase I)	P0773	Ceramide; Gal, Glc, GalNAc, NeuAc
Nb.BssSI	R0681	Generates a nick in the 3´ strand at the following recognition site: CACGAG (none/-1).
RtcB Ligase	M0458	Joins single-stranded RNA with a 3´-phosphate or 2´,3´-cyclic phosphate to another RNA with a 5´ -hydroxyl.
TelN Protelomerase	M0651S	Cuts dsDNA at a TelN recognition sequence (56 bp) and leaves covalently closed ends at the site of cleavage.
Thermostable FEN1	M0645	Catalyzes the cleavage of 5´ DNA flaps from branched double-stranded DNA substrates.

FAQs

Q: What are Enzymes for Innovation?

A: Enzymes for Innovation (EFI) is an initiative by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. These enzymes have interesting properties and unique specificities, but their application is unknown and they are often not commercially available elsewhere.

Q: What quality controls are performed to validate Enzymes for Innovation?

A: Products designated as Enzymes for Innovation pass stringent quality controls, including removal of contaminants and characterization of enzyme stability.

Q: Can I submit an Enzyme for Innovation idea?

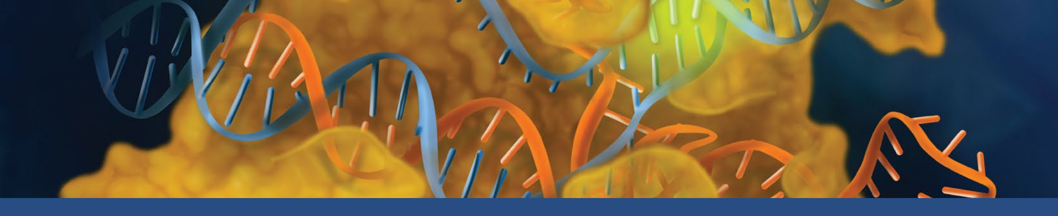
A: Yes! Please email EnzymesForInnovation@neb.com with ideas for new enzymes.

(Please note enzymes should modify either DNA, RNA or protein, or be useful in glycobiology applications.)

Q: Can I purchase large amounts of an existing Enzyme for Innovation?

A: Yes, please contact our custom solutions group at custom@neb.com.

Learn more and view our video at **www.neb.com/EnzymesForInnovation**



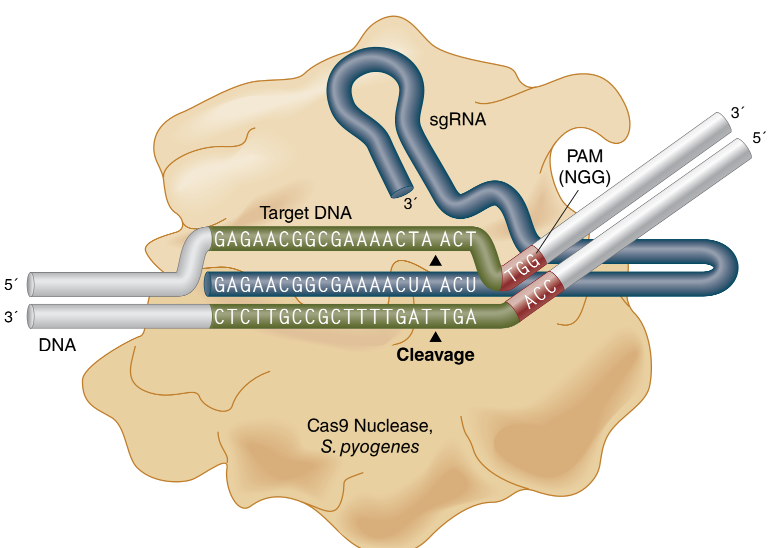
NEW PRODUCTS

Programmable CRISPR/Cas9 Nucleases

NEB now offers EnGen® Spy Cas9 Nickase and EnGen Spy dCas9 (SNAP-tag®) with nuclear localization sequences for genome editing studies.

Cas nucleases have been adapted for use in genome engineering, because they can be easily programmed for target specificity by supplying gRNAs of any sequence. In cells and animals, genome targeting is performed by expressing nucleases and gRNA from DNA constructs (plasmid or virus), supplying RNA encoding Cas nuclease and gRNA, or by introducing RNA-programmed Cas nuclease directly.

Schematic representation of Cas9 Nuclease, *S. pyogenes* (Spy) sequence recognition and DNA cleavage



Available products:

- **EnGen Spy Cas9 Nickase** is a variant of Cas9 nuclease differing by a point mutation (D10A) in the RuvC nuclease domain, which enables it to nick, but not cleave, DNA. Double-stranded breaks can be generated with reduced off-target cleavage by targeting two sites with EnGen Cas9 Nickase, NLS in close proximity.

  NEB #M0650S/T 70/400 pmol

- **EnGen Spy dCas9 (SNAP-tag)** is an inactive mutant of Cas9 nuclease that retains programmable DNA binding activity. The N-terminal SNAP-tag allows for covalent attachment of fluorophores, biotin, and a number of other conjugates useful for visualization and target enrichment.

  NEB #M0652S/T 70/400 pmol

- **EnGen Cas9 NLS, *S. pyogenes***, is an RNA-guided endonuclease that catalyzes site-specific cleavage of double-stranded DNA. The location of the break is within the target sequence 3 bases from the NGG PAM (Protospacer Adjacent Motif).

  NEB #M0646T/M 400/2,000 pmol

Do you use DNA ladders in your daily lab work?

We thought so! We're confident that you will love our Quick-Load® purple DNA ladders, with their sharp bands, ready-to-use format, and competitive pricing. In addition, our purple ladders have no UV shadow, so you'll never miss a band.

For a limited time*, try our Quick-Load Purple 100 bp DNA Ladder and our Quick-Load Purple 1 kb DNA Ladder for free. Request your sample pack today at www.neb.com/DNAladders

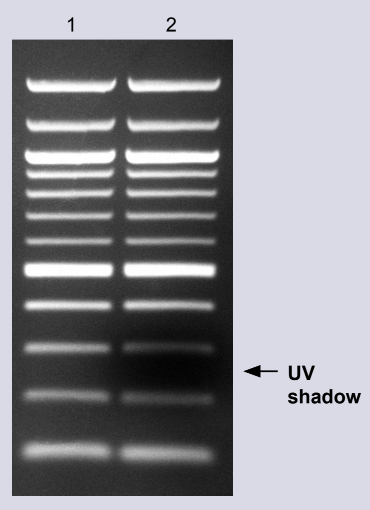
ORDERING INFORMATION

PRODUCT	NEB #	SIZE
Quick-Load Purple 100 bp DNA Ladder	N0551S	1.25 ml
Quick-Load Purple 1 kb DNA Ladder	N0552S	1.25 ml

**Offer valid in the US only, while supplies last. Limit one per customer.*

UV shadow comparison

The new Gel Loading Dye, Purple (6X) (NEB #B7024S) (Lane 1) included in the Quick-Load Purple 100 bp DNA Ladder does not cast a UV shadow over the underlying bands, unlike the Gel Loading Dye, Blue (6X) (Lane 2).



The Do's and Don'ts of plasmid minipreps

Are you planning to perform some plasmid minipreps?

Don't stress! Here are some basic things to keep in mind in order to get clean, plasmid DNA, ready for use in downstream applications.

DON'T use too many cells

The Monarch Miniprep Kit can process 1–5 ml of cell culture, but it is important that the culture not exceed 15 O.D. units. In most cases, 1–3 ml of culture is sufficient. If the recommended amount of cells is exceeded, the amount of lysis buffer recommended in our Monarch® Plasmid Miniprep Kit protocol may not be able to efficiently lyse all the cells. Also, excess cell debris resulting from lysis of too many cells can clog the column. If you need to use more cells than recommended, consider splitting the sample in half and using two columns.

DO lyse your cells completely

In order to release ALL of the plasmid DNA, ALL of the cells need to be lysed. To do this, make sure the cells are resuspended completely, without any clumps, and incubate the cells for the recommended amount of time.

DON'T vortex your cells after lysis

Vortexing can cause shearing of host chromosomal DNA, resulting in gDNA contamination. If you do experience gDNA in your prep, you can treat your plasmid with Exonucleases V (Rec BCD) (NEB #M0345) which will digest linear DNA only. For more details, see page 11.

DON'T skip or shorten the RNase A digestion step

Lucky for you, Monarch Neutralization Buffer comes with RNase A already added (other kits require you to add it, and an extra step is easy to forget!). The neutralization step is very important, as this is the time when RNase A digests the contaminating RNA. It is also important to follow the incubation recommendations for this step to ensure complete RNA removal. For best results, or if >1 ml of cell culture is used, increase the spin time after neutralization to 5 minutes.

DO use both wash buffers as directed

Both Monarch wash buffers should be used in the volumes recommended to ensure removal of cell debris, endotoxin and salts.

DON'T mix up your buffers

The buffers need to be added in a particular order, since each one carries out a different function in the purification workflow. Using them out of order can cause your minipreps to fail. Monarch miniprep buffers are color coded for your convenience.

DON'T let the tip of the column touch the flow-through in the collection tube after washing

Ethanol can carry over from the collection tube to the column tip. Ethanol in your eluate can interfere with downstream applications and cause your sample to float out of the gel well. If you suspect that the tip has touched the flow-through, another spin should do the trick.

DO heat the elution buffer when purifying large plasmids (>10 kb)

Large DNA binds more tightly to the silica matrix. Heating the elution buffer to 50°C before applying to the column helps to more efficiently release the DNA from the matrix.

NEB's **Monarch® Plasmid Miniprep Kit** (NEB #T1010) rapidly and reliably purifies high quality DNA using a fast workflow and a colored buffer system to easily monitor completion. Our unique column design enables low-volume elution and prevents buffer retention.

For a complete list of nucleic acid purification products, including buffers and columns sold separately, visit **NEBmonarch.com**.

ORDERING INFORMATION

PRODUCT	NEB #	SIZE
Monarch Plasmid Miniprep Kit	T1010S/L	50/250 preps
Monarch Plasmid Miniprep Columns	T1017L	100 columns

Using Exonuclease V (RecBCD) to eliminate residual genomic DNA when purifying low copy plasmids with the Monarch® Plasmid Miniprep Kit

Peichung Hsieh, Ph.D., New England Biolabs, Inc.

Introduction

The use of low and/or single-copy plasmids to clone large pieces of DNA (up to 200 kb) or to drive expression of slow folding or toxic proteins in *E.coli* is a commonly used strategy. Purification of low-copy plasmids or bacterial artificial chromosomes (BACs) presents some challenges that are not evident when working with higher copy number plasmids, such as pUC19. The ratio between bacterial gDNA and plasmid DNA is higher, thereby reducing the yield of the desired plasmid produced by typical plasmid miniprep protocols. Additionally, elevated levels of host gDNA are often co-purified, reducing the accuracy of quantitation by UV absorbance or dsDNA-specific dyes. Neither method can distinguish the contribution from gDNA to the overall nucleic acid content. Co-purification of host gDNA also affects the appearance of the sample when resolving by gel electrophoresis and adds unwanted contaminating DNA for any amplification-based application.

Exonuclease V (RecBCD, NEB #M0345) is an exonuclease that degrades both linear ss- and dsDNA, while keeping the circular DNA intact. Treatment of miniprep DNA samples of low copy plasmids with this exonuclease degrades the contaminating gDNA, restoring purity and ease of use in downstream applications.

Protocol

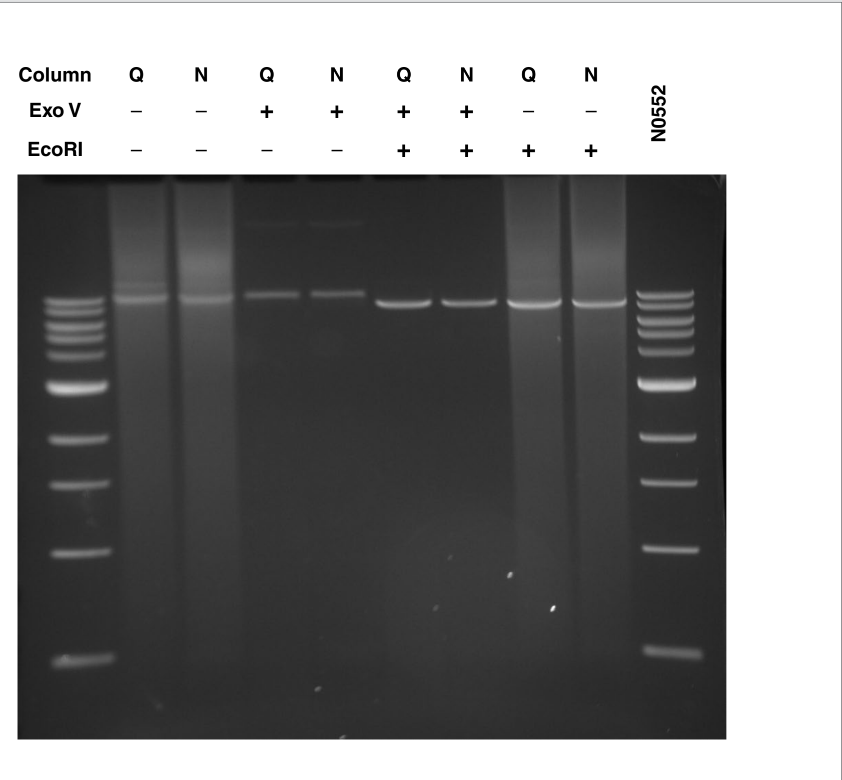
1. Transform an *endA*- strain (e.g., NEB 10-beta, NEB #C3019) with the BAC plasmid DNA and plate outgrowth onto a media plate with appropriate antibiotic. Incubate overnight at 30°C. BACs with Cam^R require reduced stringency selection. Chloramphenicol levels should be maintained between 10-15 µg/ml on the selective plate.
   Note: Strains with an F' plasmid are not compatible with BACs or miniF plasmids.
2. Pick a colony, inoculate 10 ml LB + antibiotic, and incubate overnight at 30°C (200-250 RPM).
3. Check OD_{600} (usually it will be around 4 O.D./ml of cells).
4. Harvest 3 ml of the overnight culture and purify the plasmid DNA using the Monarch Plasmid Miniprep kit (NEB #T1010) following the recommended protocol.
5. In the final elution step, elute the DNA with 30 µl of Monarch DNA Elution Buffer (pre-heated to 50°C).
6. To the eluted DNA, add 4 µl of NEBuffer 4 (10X), 4 µl of 10 mM ATP, and 2 µl of Exonuclease V (RecBCD). Mix reaction and incubate at 37°C for 1 hr.
7. Heat-inactivate the Exonuclease V by incubating at 70°C for 30 min. The plasmid DNA is now ready for restriction enzyme digestion, PCR or transformation.
   Note: Typically 30-60 ng of single-copy plasmid can be purified from 3 ml of an overnight E.coli culture with (OD_{600} = 4 O.D./ml)

Results:

Three milliliters of an overnight culture of NEB-10 beta competent *E. coli* cells transformed with pBAC were processed using the Monarch Plasmid DNA Kit and an equivalent Miniprep kit from another vendor. After isolating the DNA, samples were treated with Exonuclease V (RecBCD) and then digested with EcoRI. Samples were run on an agarose gel to assess the quality of the isolated DNA, and whether or not the Exonuclease V-treated DNA still was able to be digested to completion. The Exonuclease V-treated samples showed no gDNA contamination (#3-6) while the untreated samples exhibited a significant amount of gDNA as seen by the smear observed in those samples (#1,2,7,8).

These results indicate that Endonuclease V can be used to efficiently degrade contaminating gDNA from plasmid purification steps, including those of low copy number.

FIGURE 1:
pBAC samples exhibit no bacterial gDNA contamination after treatment with Exonuclease V (RecBCD).
Miniprep plasmid DNA samples isolated with the Monarch Plasmid Miniprep Kit (N) and a similar kit from a competitor (Q) were either treated (+) or not treated (-) with Exonuclease V, and then digested with EcoRI. The samples treated with Exonuclease V showed no contaminating gDNA and they were correctly cut with EcoRI.



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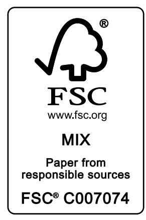

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