Issue II **2016**

NEB expressions

a scientific update

in this issue

Monarch Nucleic Acid Purification Kits –
optimized for performance with low environmental impact

page 8

NEW ENGLAND BioLabs®

be INSPIRED | drive DISCOVERY | stay GENUINE

1 – New England Biolabs, Ipswich MA 2 – NIBRT GlycoScience Group, National Institute for Bioprocessing Research and Training, Dublin, Ireland and BTI, A*STAR, Singapore

Glycomics:
A rapidly evolving field with a sweet future

Glycobiology is entered in the Oxford English Dictionary as "f. GLYCO- + BIOLOGY n.: coined by Prof. Raymond Dwek in 1982" and is defined as the branch of science concerned with the role of sugars in biological processes. Glycobiology addresses the assembly, structure and biology of chains of sugars (termed 'glycans'). Glycans are widely distributed in nature and have physical, chemical, and biological properties that make them important players in areas such as biofuels, food, materials science, biotechnology, and pharmaceuticals. Glycans are also among the most important molecules in cell biology. Together with nucleic acids, proteins, and lipids, glycans are one of the four basic building blocks from which all cells are comprised (1).

INTRODUCTION

Glycans play many critical roles in both the normal function of cells and in disease. They assist in the folding of many proteins, aid in protein trafficking, mediate cell adhesion, differentiate blood groups, modulate the immune system, are implicated in many signaling pathways, and provide a protective extracellular matrix for many types of cells. Glycans are also implicated in the process of infectivity for many pathogenic bacteria (2) and most viruses (3), including those that cause the common cold, influenza, and HIV/AIDS.

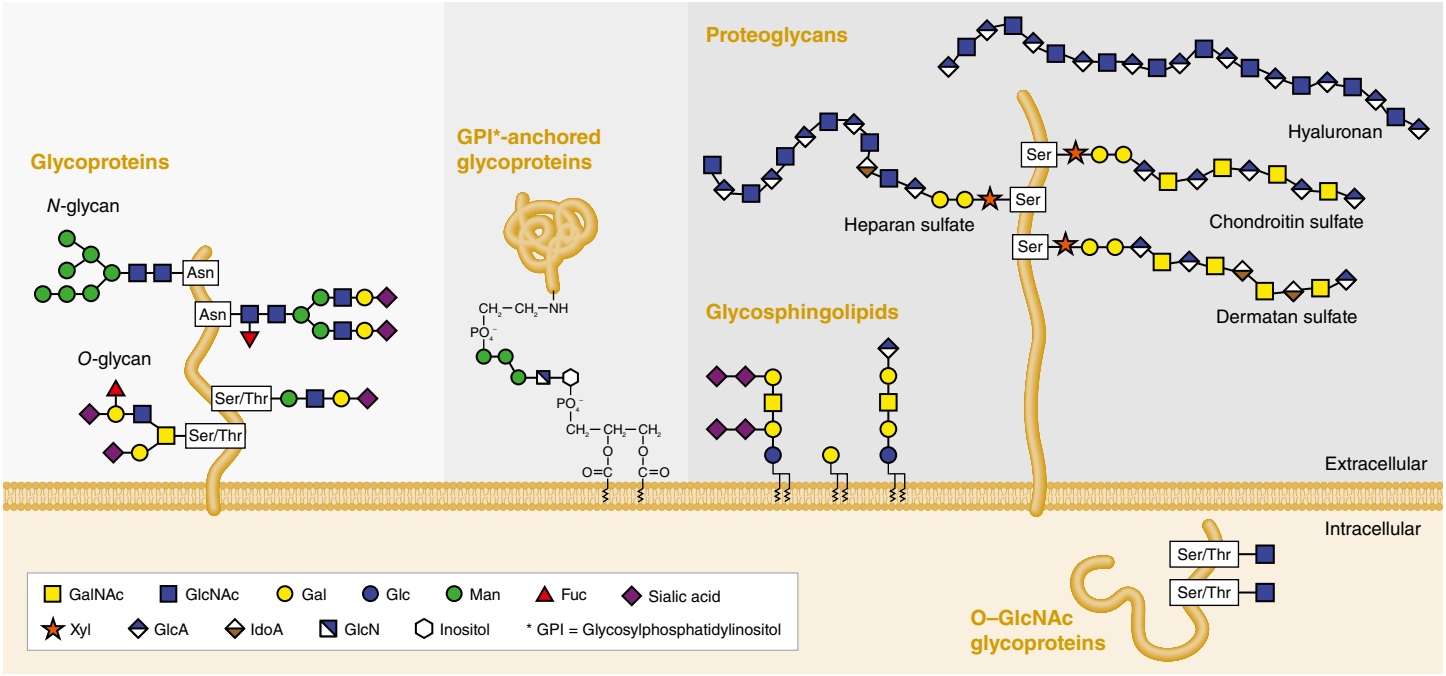
Individual glycans are assembled from monosaccharides that are linked together via glycosidic bonds, and can be covalently bound to various proteins and lipids (termed 'glycoconjugates' in this context). Several classes of glycoconjugates are synthesized by mammalian cells (Figure 1) and populate the membranes of the secretory pathway, the cell surface, and the extracellular matrix. Glycans that are appended to certain serine/threonine residues (*O*-glycans) or certain asparagine residues (*N*-glycans) of secretory proteins are the most abundant post-translational modifications of proteins. It is estimated that >50% of mammalian proteins possess appended glycans (4) and the surface of each mammalian cell may contain as many as 10 million *N*- or *O*-linked glycans attached to proteins (5). In addition, *N*- and *O*-linked glycans are present on nearly all proteins that are secreted from cells. Thus, glycoproteins are present in all mammalian body fluids.

Glycan structure is inherently complex, a fact that relates to the way glycans are synthesized. Unlike DNA or proteins that are built from copying or interpreting a genetic template, glycans are assembled through the action of complex biosynthetic pathways. The flexibility built into this system allows cells to alter their glycan structures to respond rapidly to changes in their environment without needing to alter their genomes. It is estimated that the human genome encodes over 900 proteins involved in various aspects of glycan assembly or recognition (6). Of these, more than 200 human genes encode glycosyltransferases, enzymes that specifically add new sugars to glycans. The expression of many of these proteins can vary amongst different cell types and tissues giving rise to significant glycan structural variation. (...)

Read the complete Feature Article online at:
www.neb-online.eu/glycomics-article

FIGURE 1:
A schematic representation of the mammalian glycome

Different classes of glycans are appended to proteins and lipids. These glycoconjugates populate the surface of mammalian cells. Additionally, *O*-linked GlcNAc is found on many cytoplasmic and nuclear proteins. The most common post-translational modifications of extracellular proteins are *N*- and *O*-linked glycans that are attached to certain asparagine or serine/threonine residues, respectively. In glycobiology nomenclature, various sugars are graphically represented by different geometric shapes and colors. Abbreviations in the key: GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; Gal, galactose; Glc, glucose; Man, mannose; Fuc, fucose; Xyl, xylose; GlcA, glucuronic acid; IdoA, iduronic acid; GlcN, glucosamine.

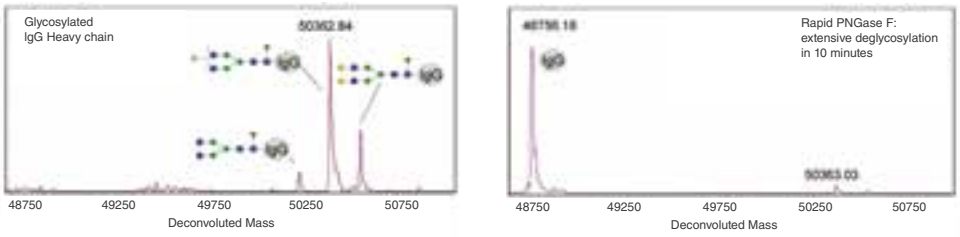


Rapid PNGase F

Deglycosylation in minutes for *N*-glycan analysis

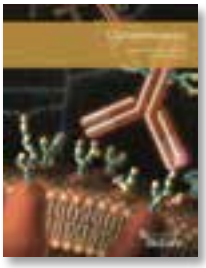
Rapid PNGase F enables complete and rapid deglycosylation of antibodies and immunoglobulin fusion proteins, as well as other glycoproteins, in minutes. All N-glycans are released rapidly and without bias, and are ready to be prepared for downstream chromatography or mass spectrometry analysis. Rapid PNGase F creates an optimized workflow, reducing processing time without compromising sensitivity or reproducibility.

ESI-TOF analysis of an antibody before and after treatment with Rapid PNGase F



Interested in learning more?

Request your free Brochure "Glycoproteomics" from your local distributor and download related application notes incl. "Proteomics: Fast and Efficient Antibody Deglycosylation using Rapid PNGase F" at:
http://www.neb-online.eu/glyco-appnotes



advantages

- Complete deglycosylation of antibodies and immunoglobulin fusion proteins in minutes
- Release of all *N*-glycans rapidly and without bias, ready for downstream chromatography or mass spectrometry analysis
- Recombinant source
- Optimal activity is ensured for 12 months
- Purified to >99% homogeneity, as determined by SDS-PAGE

ORDERING INFORMATION:

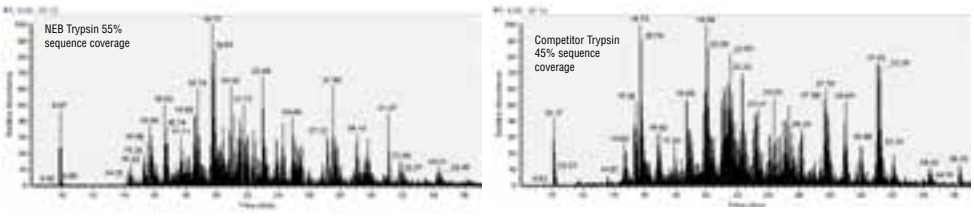
PRODUCT	NEB #	SIZE
Rapid PNGase F	P0710S	50 rxns
Rapid PNGase F (non-reducing format)	P0711S	50 rxns
COMPANION PRODUCT		
Rapid PNGase F Antibody Standard	P6043S	250 µg

Trypsin-ultra, Mass Spectrometry Grade

Excellent Performance at Value Price

Trypsin-ultra, Mass Spectrometry Grade is a serine endopeptidase. It selectively cleaves peptide bonds C-terminal to lysine and arginine residues.

NEB's Trypsin-ultra has improved peptide coverage using BSA as a standard protein sample



The protein standard BSA was digested with NEB Trypsin and a leading competitor Trypsin and analyzed via 1d-LC-MS/MS with following protocol:

- BSA was denatured, reduced and digested with NEB Trypsin or competitor Trypsin
- The peptide mixture from each digestion was directly injected onto a nano C18 trapping column and desalted
- The peptide mixture was resolved onto a C18 nanocolumn and nanosprayed into a LTQ-Orbitrap for a 1hr LC-MS/MS run
- MS/MS spectra were searched against a bovine database with SEQUEST

The figure illustrates extracted base peak chromatograms for NEB Trypsin-ultra (left) and competitor Trypsin (right)

advantages

- Acetylated to prevent autolysis
- TPCK treatment eliminates chymotryptic activity
- Ideal for proteome analysis even ultra-complex proteomes (tested on human microbiome samples)
- Universal digestion buffer containing $CaCl_2$ provided
- Can be used for both in-gel and solution digest applications
- Value pricing

ORDERING INFORMATION:

PRODUCT	NEB #	SIZE
Trypsin-ultra, Mass Spectrometry Grade	P8101S	100 µg
COMPANION PRODUCT		
Endoproteinase GluC	P8100S	50 µg
Endoproteinase AspN	P8104S	50 µg
Trypsin-digested BSA MS Standard (CAM-modified)	P8108S	500 pmol

First successful PCR experiment performed in space

Genes in Space competition's winning experiment conducted aboard the International Space Station using Q5 HiFi DNA Polymerase*

**a modified version*

Nicole Nichols, Ph.D., New England Biolabs, Inc.

On April 19, 2016, the first PCRs in space were conducted onboard the International Space Station (ISS). The reactions were designed by New York high school student Anna-Sophia Boguraev, and contained a modified Q5® master mix developed to specifically amplify bisulfite-treated DNA, a critical part of Boguraev's winning Genes In Space (GIS) proposal to study epigenetic changes in DNA during spaceflight.

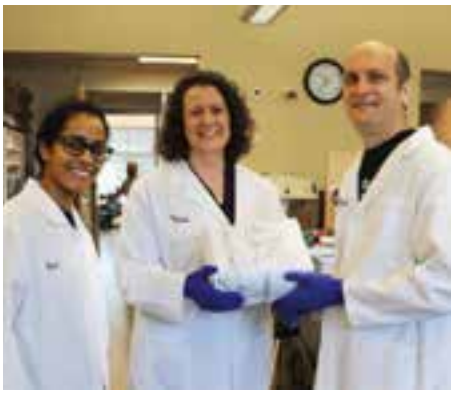
Enabling these experiments is a thermocycler called miniPCR™, a Kickstarter-funded instrument from co-founders Sebastian Kraves and Ezequiel Alvarez-Saavedra. The miniPCR is portable and can be controlled by a laptop or cell phone, making it perfect for teaching labs, field work, and now, the ISS.

GIS, a contest conceived of by Boeing and Amplyus and supported by Math for America, the Center for the Advancement of Science in Space (CASIS), FedEx and New England Biolabs, aims to support young innovators in bridging the physical and biological sciences. Boguraev's 2015 winning entry built upon previous work identifying immune system alterations during spaceflight. She proposed a study to examine epigenetic changes as a possible underlying cause of these alterations and worked with the Giraldez lab at Yale University to design a proof-of-concept test system using bisulfite-treated zebrafish DNA that could be completed on the ISS. Control reactions (which included either ***the modified Q5 or Taq DNA polymerases,*** a pUC-based plasmid, and DNA primers in a variety of reaction volumes) were also designed and sent up to the ISS. These reactions were designed not only to help troubleshoot Boguraev's test reactions, but also to empirically determine microgravity-related technicalities like the amount of liquid that would stay at the bottom of a typical PCR strip tube (to remain in contact with the heating element of the thermocycler).

The reactions and miniPCR machine were launched into space aboard the SpaceX CRS-8 Dragon Resupply Mission on April 8th from the Kennedy Space Center in Cape Canaveral, FL with Boguraev, her family and members of the miniPCR, Boeing, and NEB teams in attendance.

About 4 weeks later, the completed reactions were sent back to earth and brought to NEB for analysis. Anna-Sophia (shown, top right) along with members of her family and many of the teams that supported GIS were all on hand to learn whether the reactions had worked or not. Happily, the control samples and Anna-Sophia's test reactions all worked well – they now represent the first successful PCRs in space! As for the miniPCR, it will stay behind to enable future experiments, including those designed by the next GIS winning team, who were chosen at the ISS R&D conference in San Diego, CA this July.

More than 30 years after it's invention by Kary Mullis of the Cetus Corporation, it's difficult to find a molecular biologist that hasn't performed a PCR. And now, thanks to the Genes In Space contest, it may soon be difficult to find an astronaut who hasn't run a PCR, either.



Photos from top to bottom: 1- Anna-Sophia Boguraev holding PCR samples returned from space. 2 - PCR reactions being run on the ISS (photo kindly provided by NASA). 3 - Mudhda Narasimhan (Amplyus), Nicole Nichols (NEB) and Zeke Alvarez-Saavedra (Amplyus) holding sample wrapped in Anna-Sophia's artwork.

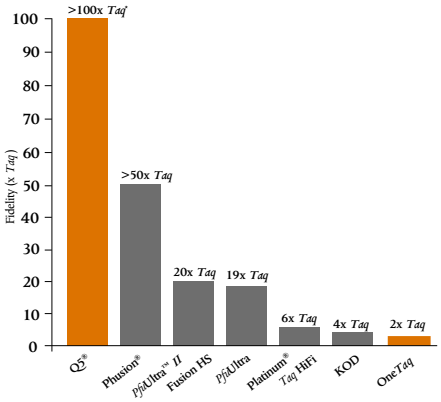
To learn more about the competition, visit
www.genesinspace.org

NEB TV
What´s trending in science

Watch **NEB TV Episode 10** to learn more about the first PCR in space:
http://www.neb-online.eu/neb-tv-10

Q5 High-Fidelity DNA Polymerase: Ultra-fidelity PCR in space & on earth!

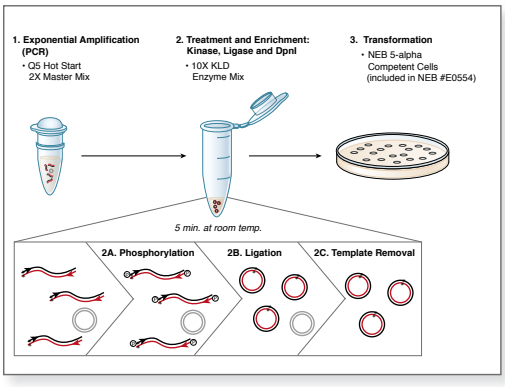
Q5 High-Fidelity DNA Polymerase is an ultra-fidelity PCR polymerase. With a fidelity >100-fold higher compared to *Taq* DNA Polymerase and 12-fold higher than *Pyrococcus furiosus* (Pfu) DNA Polymerase, Q5 High-Fidelity DNA Polymerase is ideal for cloning (virtually "error-free").
It also can be used for long or difficult amplicons, as it is fused to a processivity-enhancing protein domain that supports robust DNA amplification.
Q5 High-Fidelity DNA Polymerase is the first choice for all high-fidelity PCRs on earth as well as in space!



Highest fidelity DNA amplification available. Fidelity of various PCR Polymerases as compared to Taq DNA Polymerase.

Q5 Site-Directed Mutagenesis Kit

The Q5 Site-Directed Mutagenesis Kit enables rapid, site-specific mutagenesis of double-stranded plasmid DNA in less than 2 hours. The kit utilizes Q5 Hot Start High-Fidelity DNA Polymerase, along with custom mutagenic primers to create substitutions, deletions and insertions in a wide variety of plasmids. Transformation into high-efficiency NEB 5-alpha Competent *E. coli* cells ensures robust results with plasmids up to, at least, 14 kb in length.



This kit is designed for rapid and efficient incorporation of insertions, deletions and substitutions into double-stranded plasmid DNA:

1. Step: Exponential amplification using standard primers and a master mix fomulation of Q5 Hot Start High-Fidelity DNA Polymerase.
2. Step: Incubation with a unique enzyme mix containing a kinase, a ligase and DpnI.
3. Step: High-efficiency transformation into chemically-competent cells (included in NEB #E0554).

ORDERING INFORMATION

PRODUCT	NEB #	SIZE
Q5 High-Fidelity DNA Polymerase	M0491 S/L	100/500 units
Q5 Hot Start High-Fidelity DNA Polymerase	M0493 S/L	100/500 units
Q5 Site-Directed Mutagenesis Kit (***incl.*** Competent Cells)	E0554S	10 rxn
Q5 Site-Directed Mutagenesis Kit (***Without*** Competent Cells)	E0552S	10 rxn

advantages

- **Fidelity** – the highest fidelity amplification available (> 100x higher than *Taq* and 2x higher than Phusion)
- **Robustness** – high specificity and yield with minimal optimization
- **Coverage** – superior performance for a broad range of amplicons (from high AT to high GC)
- **Speed** – short extension times
- **Amplicon length** – robust amplifications up to 20 kb for simple templates, and 10 kb for complex

advantages

- Generation of mutations, insertions or deletions in plasmid DNA
- Non-overlapping primer design ensures robust performance for desired mutations from a wide range of templates
- Low error rate of Q5 High-Fidelity DNA Polymerase reduces screening time
- Easy-to-use master mix format available with or without competent cells

Speed up your experimental design with our primer design tool at
NEBaseChanger.neb.com

NEW PRODUCT

A Novel Combined Target Enrichment & NGS Library Prep Technology

ILLUMINA® COMPATIBLE

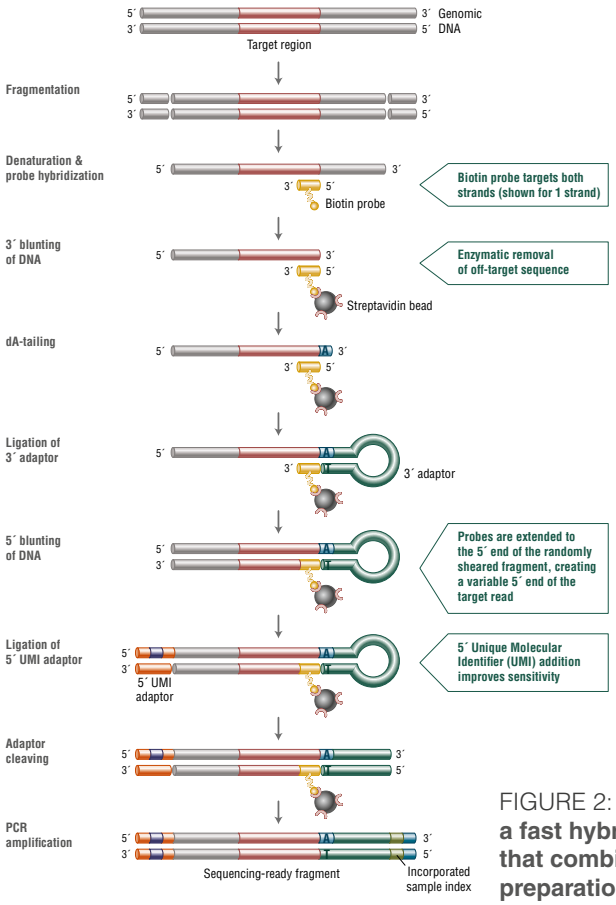
Introducing the NEBNext Direct™ Cancer HotSpot Panel

NEBNext Direct enables deep sequencing on the Illumina® platform of genomic regions of interest for the discovery and identification of low frequency variants from challenging sample types. Employing a novel approach to target enrichment, the NEBNext Direct Cancer Hotspot Panel allows highly specific hybridization-based capture of 190 common cancer targets from 50 genes. The NEBNext Direct technology offers significant advantages over both traditional in-solution hybridization and multiplex PCR protocols. Target enrichment is combined with library preparation, reducing processing time and minimizing sample loss. NEBNext Direct is also ideal for automation.

FIGURE 1: **Targets include regions covering 37 kb from the following cancer-related genes, including >18,000 cosmic features:***

ABL1	BRAF	EGFR	FGFR1	GNAQ	IDH2	KRAS	NPM1	PTPN11	SMO
AKT1	CDH1	ERBB2	FGFR2	GNAS	JAK2	MET	NRAS	RB1	SRC
ALK	CDKN2A	ERBB4	FGFR3	HNF1A	JAK3	MLH1	PDGFRA	RET	STK11
APC	CSF1R	EZH2	FLT3	HRAS	KDR	MPL	PIK3CA	SMAD4	TP53
ATM	CTNNB1	FBXW7	GNA11	IDH1	KIT	NOTCH1	PTEN	SMARCB1	VHL

** For research use only; not intended for diagnostic use.*



In the NEBNext Direct target enrichment approach (Figure 2), fragmented DNA is rapidly hybridized to biotinylated oligonucleotide baits that define the 3′ end of each target of interest. The bait-target hybrids are bound to streptavidin beads and any 3′ off-target sequence is removed enzymatically.

This combination of a short hybridization time with the enzymatic removal of 3′ off-target sequence enables greater sequencing efficiency relative to conventional hybridization-based enrichment methods.

The trimmed targets are then converted into Illumina-compatible libraries that include unique molecular identifiers (UMIs) and a sample barcode. Sequence-ready libraries are generated within one day. The procedure is compatible with most automated liquid handling instruments.

FIGURE 2: **NEBNext Direct employs a fast hybridization-based workflow that combines capture with library preparation**

advantages

- No upfront library prep: enriches targets and converts into a **sequence-ready library in 1 day**
- Generate a **higher percentage of your sequencing reads** that align to your targets
- Eliminate the need to over-sequence, **reducing cost per sample**
- Obtain **uniform sequencing of all targets**, regardless of GC content
- Generate high quality libraries with **limited input amounts and degraded DNA samples**, including FFPE and ctDNA
- Distinguish molecular duplicates, reducing false positive variants and **improving sensitivity**

ORDERING INFORMATION:

PRODUCT	NEB #	SIZE
NEBNext Direct Cancer HotSpot Panel	E7000S/L/XL	8/24/96 rxns

Interested in learning more?
Visit **NEBNextDirect.com**
or contact your local distributor!

Target with precision.

feedback

"NEBNext Direct enrichment technology is by far the fastest and most automation friendly protocol available today. I can have samples on the sequencer in 6 hours starting from genomic DNA. The technology produces very high on target percentages (>90%) for even very small panels, and in combination with molecular barcoding produces low duplication rates. From an optimization perspective, NEBNext Direct enrichment allows me to assign individual captured fragments to a probe unambiguously, thus giving the opportunity for optimizing the coverage distribution of any target."

– **Eric C. Olivares**, Founder, SEQanswers.com

"Using the NEBNext Direct kit, we were able to detect all known single nucleotide variants and indels in DNA extracted from fresh frozen or FFPE tissue derived from glioma biopsies. We could also clearly see amplification in genes like EGFR or PDGFR. The workflow is really easy and fast and can be rapidly implemented in a lab."

– **Yannick Marie**, Sequencing Core Facility Manager, Brain and Spine Institute (ICM)

"The kit and its technology are easy to use and easy to automate, allowing us to get up and running quickly. The protocol itself is fast and efficient to obtain deep coverage of targets, giving homogeneous results for FFPE and frozen tumors, therefore opening doors for customized panels."

– **Francis Rousseau, Ph.D.**, Director of Genomics for IntegraGen SA

PRODUCT TIP:

NEBNext® Ultra™ II DNA Library Prep!

NOW ALSO AVAILABLE WITH **SPRIselect® BEADS**

Even more from less.

- Get more of what you need, with the **highest library yields**
- Generate high quality libraries, even when you have only limited amounts of DNA, with **inputs as low as 500 pg**
- Improved library complexity with **fewer PCR cycles**
- Prepare libraries from all of your samples, including **GC-rich** and **FFPE samples**
- Save time with **streamlined workflows, reduced hands-on time, and automation compatibility**
- Enjoy the **flexibility of kit or module format** products

ORDERING INFORMATION:

PRODUCT	NEB #	SIZE
NEBNext Ultra II DNA Library Prep Kit for Illumina	E7645S/L	24/96 reactions
NEBNext Ultra II End Repair/dA-tailing Module	E7546S/L	24/96 reactions
NEBNext Ultra II Ligation Module	E7595S/L	24/96 reactions
NEBNext Ultra II Q5® Master Mix	M0544S/L	50/250 units

Monarch™

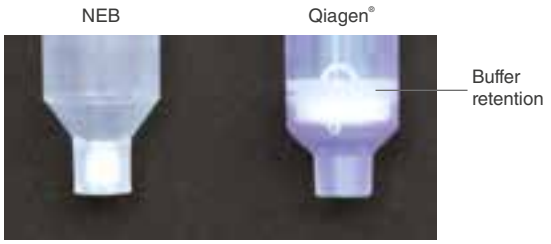
Nucleic Acid Purification Kits

It's time for change.

Monarch Nucleic Acid Purification Kits from NEB

It's time to transform your DNA purification experience. NEB's Monarch Nucleic Acid Purification Kits are optimized for maximum performance and minimal environmental impact. Our unique thin-walled column design uses less plastic, prevents buffer retention, eliminates the risk of carryover contamination, and enables elution in smaller volumes. The result: high performing DNA purification for your downstream applications.

Designed for performance



OPTIMIZE YOUR RESULTS WITH OUR UNIQUE COLUMN DESIGN

- Improved recovery of concentrated, pure DNA
- Low volume elutions, resulting in highly-concentrated DNA
- No buffer retention, eliminating the risk of carryover contamination

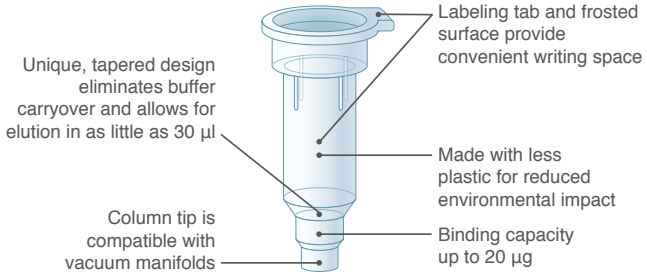
ENHANCE YOUR DNA PURIFICATION EXPERIENCE

- Fast, user-friendly protocols
- Columns designed for easy labeling and handling
- Improved buffer system for robust performance

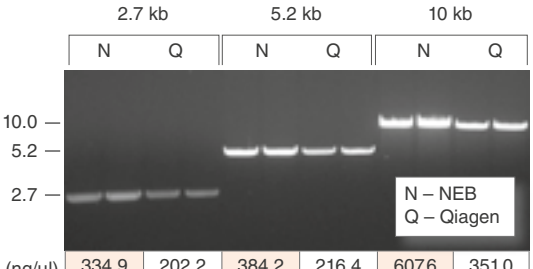
FEEL GOOD ABOUT CHOOSING MONARCH

- Significantly less plastic in every kit*
- Custom-designed, thin-walled columns and collection tubes
- Responsibly sourced and recyclable packaging
- Packaging and protocol cards printed with water and soy-based inks
- Reusable kit boxes
- Buffers and columns available separately

Optimized design of Monarch Miniprep Columns



Monarch Plasmid Miniprep Kits consistently produce more concentrated plasmid DNA with equivalent yield, purity and functionality as compared to the leading supplier



	2.7 kb		5.2 kb		10 kb	
	N	Q	N	Q	N	Q
Conc. (ng/µl)	334.9	202.2	384.2	216.4	607.6	351.0
Total yield (µg)	10.0	10.1	11.5	10.8	18.2	17.6
$A_{260/280}$	1.9	1.9	1.9	1.9	1.9	1.9
$A_{260/230}$	2.4	2.3	2.4	2.4	2.3	2.4

* *Visit NEBMonarchPackaging.com for details.*

See what your fellow European scientists are saying: >90% are satisfied with the performance!

In the last NEB Expressions edition we asked you to provide us feedback on your experience with the new Monarch Nucleic Acid Purification Kits.

The overwhelming feedback was extremely positive: Over 90% of all entrants were "satisfied" or "extremely satisfied" with the overall performance of the Monarch kits. Visit ***www.nebmonarch.eu*** to learn more.

NEB thanks all entrants for their feedback and wishes continued succes for their future scientific careers.

> *Easy to understand and use, small package, no waste, straight to the point.*

– **Tatiana F.**, University of Ulm, GERMANY

> *I did enjoy everything, from the packaging, to the efficiency and the yield, the quality of the protocol...*

– **Dr. Cedric S.**, R&D, OZ Biosciences, FRANCE

> *It is efficient and fast!*

– **Dr. Francesca M.**, University of Milan, ITALY

> *Reduction of used plastic, nice colour scheme of buffers, easy to remember protocol, few handling steps, good quality product.*

– **Dr. Christian S.**, ENS, FRANCE

> *I like special position of resin and low volumes to elute DNA.*

– **Dr. Shahid Muhammad I.**, University of Vienna, AUSTRIA

Trust your peers – Order your favorite Monarch Kit today to experience similar positive results!

ORDERING INFORMATION:

PRODUCT	NEB #	SIZE
Monarch Plasmid Miniprep Kit	T1010S/L	50/250 preps
Monarch Gel Extraction Kit Kit	T1020S/L	50/250 preps
Monarch PCR & DNA Cleanup Kit (5 µg)	T1030S/L	50/250 preps

Interested in learning more?
visit **NEBMonarch.eu**

SPECIAL CAMPAIGN – Offer closes Jan 31th, 2017

Get a free 9x9 Vial Storage Box!

Order:

- at least **1× L-Pack of any Monarch Kit**
- or **order all 3 Monarch Kits** as S-Pack
- cite your **Promo-Code "Box"** when ordering and
- receive a **free 81-place vial storage box** for your precious -20°C sample storage with your P.O.!*

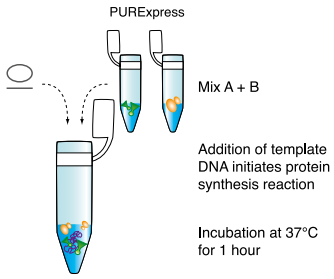
**Limited supply; One storage box per order; Offer closes Jan 31th, 2017 or as long as stocks last! Ask your local distributor for details.*

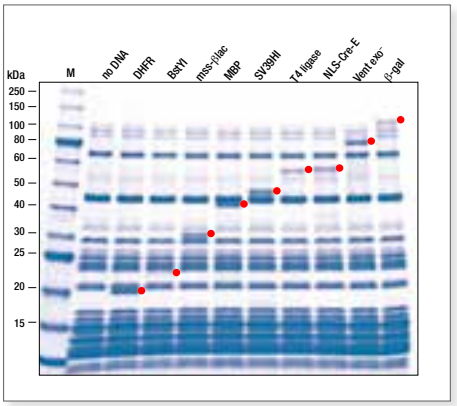
PURExpress *In Vitro* Protein Synthesis Kits – from Gene to Protein in <2 hrs!

A rapid method for gene expression analysis, PURExpress is a cell-free transcription/translation system reconstituted from the purified components necessary for *E. coli* translation. The nuclease-free and protease-free nature of the PURExpress platform preserves the integrity of DNA and RNA templates/complexes and results in proteins that are free of modification and degradation. Transcription and translation are carried out in a one-step reaction, and require the mixing of only two tubes. With results available in a few hours, PURExpress saves valuable laboratory time and is ideal for high throughput technologies.

PURExpress allows for fastest, reliable and convenient protein expression, even from ORFs that encode toxic proteins

Protein synthesis using PURExpress





25 µl reactions containing 250 ng template DNA were incubated at 37°C for 2 hours. 2.5 µl of each reaction was analyzed by SDS-PAGE using a 10–20% Tris-glycine gel. Note that proteins can be purified using reverse affinity chromatography (reagents not supplied). The red dot indicates the protein of interest. Marker M is the Protein Ladder (NEB #P7703).

advantages

- Easy-to-use - requires the mixing of two tubes followed by the addition of template ORF-DNA, either circular or linear template
- Fast - reaction is complete in approximately two hours
- Efficient - purified components and lack of endogenous proteases or nucleases eliminates sample degradation

ORDERING INFORMATION:

PRODUCT	NEB #	SIZE
PURExpress In Vitro Protein Synthesis Kit	E6800S/L	10 /100 rxns
PURExpress Δ Ribosome Kit	E3313S	10 rxns
PURExpress Δ (aa, tRNA) Kit	E6840S	10 rxns
PURExpress Δ RF123 Kit	E6850S	10 rxns
COMPANION Products		
PURExpress Disulfide Bond Enhancer	E6820S	50 rxns
E. coli Ribosome	P0763S	1 mg

Protein Markers from NEB
– built for perfect migration & "ready-to-load"

Want to try first? Then request your free sample from your local distributor
Limited to 1 sample per workgroup. As long as stocks last. Ask your distributor for availability.

advantages

- Suitable for a wide range of proteins (10 - 250 kDa)
- "ready-to-load" – no heating prior to gel loading required
- Uniform band intensities
- Convenient band spacing
- Easy-to-identify reference bands

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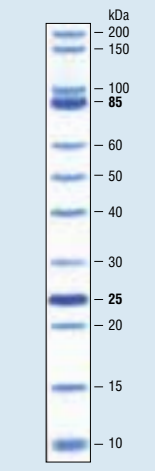
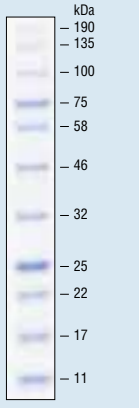
ORDERING INFORMATION:

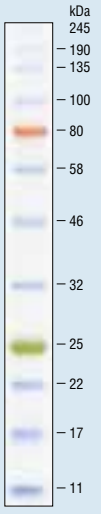
PRODUCT	NEB #	SIZE
Unstained Protein Standard, Broad Range (10-200 kDa)	P7704S/L	150 / 750 lanes
Blue Prestained Protein Standard, Broad Range (11-190 kDa)	P7706S/L	100 / 500 lanes
Color Prestained Protein Standard, Broad Range (11–245 kDa)	P7712S/L	100 / 500 lanes



Unstained Protein Standard, Broad Range (10–200 kDa)
10–20% Tris-glycine SDS-PAGE
NEB #P7704



Blue Prestained Protein Standard, Broad Range (11–190 kDa)
10–20% Tris-glycine SDS-PAGE
NEB #P7706



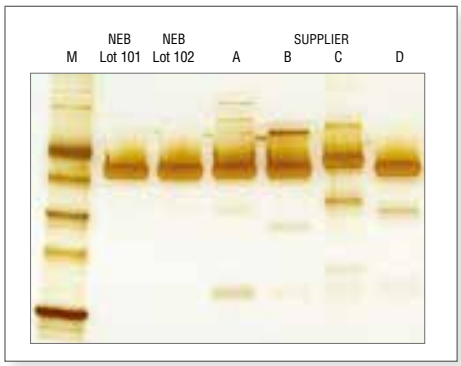
Color Prestained Protein Standard, Broad Range (11–245 kDa)
10–20% Tris-glycine SDS-PAGE
NEB #P7712

Choose NEB DNA Ligases: for quality, performance & convenience

With over 40 years of experience in the development and production of enzymes for molecular biology, NEB offers the most extensive selection of high-quality, and performance- optimized DNA ligases and ligase master mixes.

NEB ligases and ligase master mixes are manufactured to the highest level of purity in the industry, and then rigorously tested for optimal performance. NEB's T4 DNA Ligase has been referenced in peer-reviewed publications for decades, and is renowned for its consistent quality.

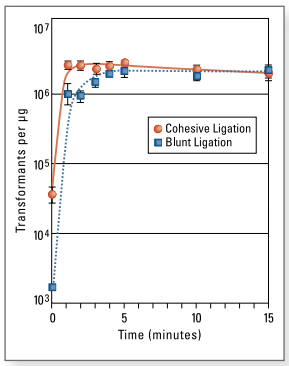
Benefit from industry leading purity: NEB's T4 DNA Ligase



Equivalent amounts of protein were loaded and silver stained using SilverXpress™. M = Protein Marker.

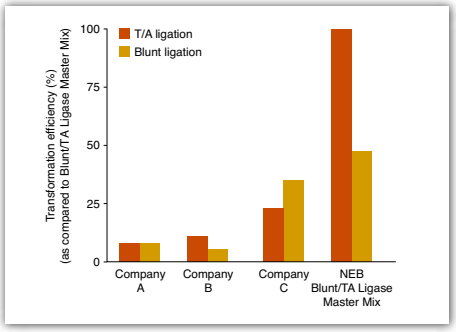
For details on industry leading quality, see www.neb.com/ligasequality.

Fast & versatile: NEB's Quick Ligation Kit



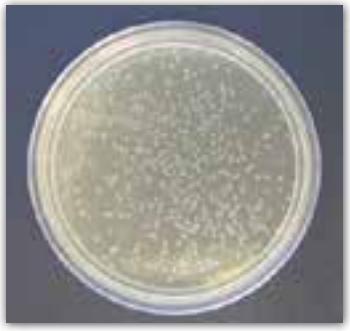
The Quick Ligation Kit enables ligation of cohesive end or blunt end DNA fragments in 5 minutes at room temperature . (25°C)

Outperforms the competition: Blunt/TA Ligase Master Mix



Duplicate ligation reactions of blunt or T/A vector/insert pairs were set up according to the master mix vendors' suggestions. Equal amounts of ligated DNA were used to transform NEB 10-beta Competent E. coli (NEB #C3019).

Robust Ligation with No Incubation Step: Instant Sticky-end Ligase Master Mix



Sticky-ends ligations were set up using the Instant Sticky-end Ligase Master Mix. Without any additional incubation time, 2 µl were immediately withdrawn and used to transform a 50 µl aliquot of NEB 10-beta Competent E. coli (NEB #C3019).

NEBioCalculator®

For help with calculating molar ratios for your ligation reaction try **NEBioCalculator.neb.com**.

advantages

- Industry standard for purity
- Highest reliability
- Fast ligations
- Robust ligation efficiency
- Convenient master mix formats

Select the Ligase product that works best for you

	T4 DNA LIGASE	QUICK LIGATION™ KIT	BLUNT/TA LIGASE MASTER MIX	INSTANT STICKY-END LIGASE MASTER MIX
DNA APPLICATIONS				
Ligation of sticky ends	★★	★★★	★★	★★★
Ligation of blunt ends	★★	★★★	★★★	★
T/A cloning	★★	★★	★★★	★
Electroporation	★★			

★★★ Optimal, recommended ligase for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended

ORDERING INFORMATION:

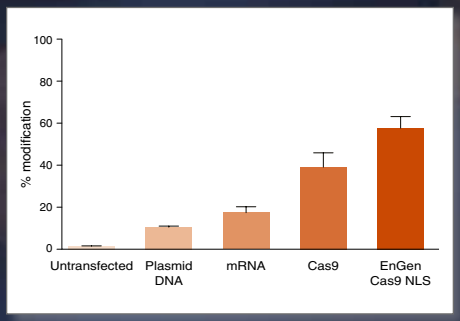
PRODUCT	NEB #	SIZE
T4 DNA Ligase	M0202S/L	20.000 u/100.000 u
T4 DNA Ligase (high conc.)	M0202T/M	20.000 u/100.000 u
Quick Ligation Kit	M2200S/L	30 rxn (20 µl vol)/ 150 rxn (20 µl vol)
Blunt/TA Ligase Master Mix	M0367S/L	50 rxn (10 µl vol)/ 250 rxn (10 µl vol)
Instant Sticky-end Ligase Master Mix	M0370S/L	50 rxn (10 µl vol)/ 250 rxn (10 µl vol)

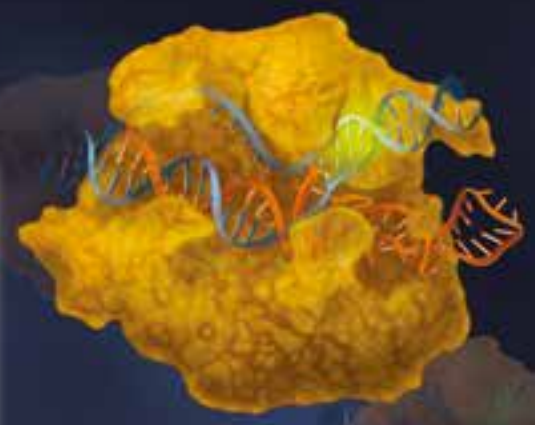
Genome Editing with NEB EnGen Products

From introduction of Cas9 and single guide RNA (sgRNA) on plasmids, to direct introduction of Cas9 ribonucleoprotein (RNP) and detection of edits using next generation sequencing or enzymatic mutation detection, NEB provides reagents that simplify and shorten genome editing workflows.

EnGen Cas9 NLS, *S. pyogenes*

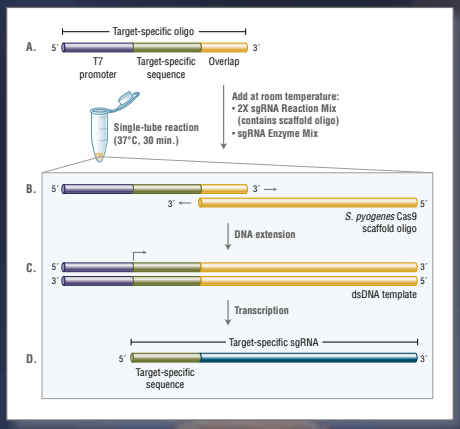
Highest efficiency strategy for genome engineering with CRISPR/Cas9





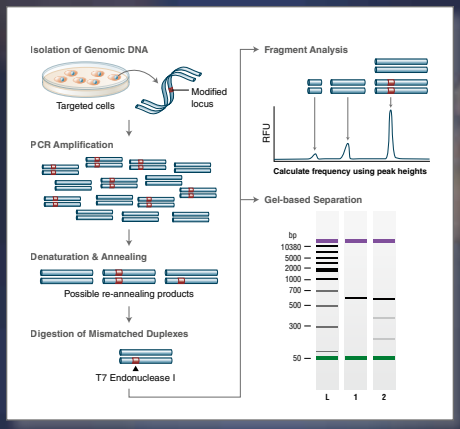
EnGen sgRNA Synthesis Kit, *S. pyogenes*

Simplifies the generation of sgRNAs in an hour or less



EnGen Mutation Detection Kit

Provides reagents for detection of on-target genome editing events



ORDERING INFORMATION

PRODUCT	NEB #	SIZE	PRICE
EnGen™ Cas9 NLS, *S. pyogenes*	M0646 T/M	400 / 2000 pmol	**158 € / 632 €**
EnGen™ sgRNA Synthesis Kit, *S. pyogenes*	E3322 S	20 rxns	**420 €**
EnGen™ Mutation Detection Kit	E3321 X	25 rxns	**210 €**

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