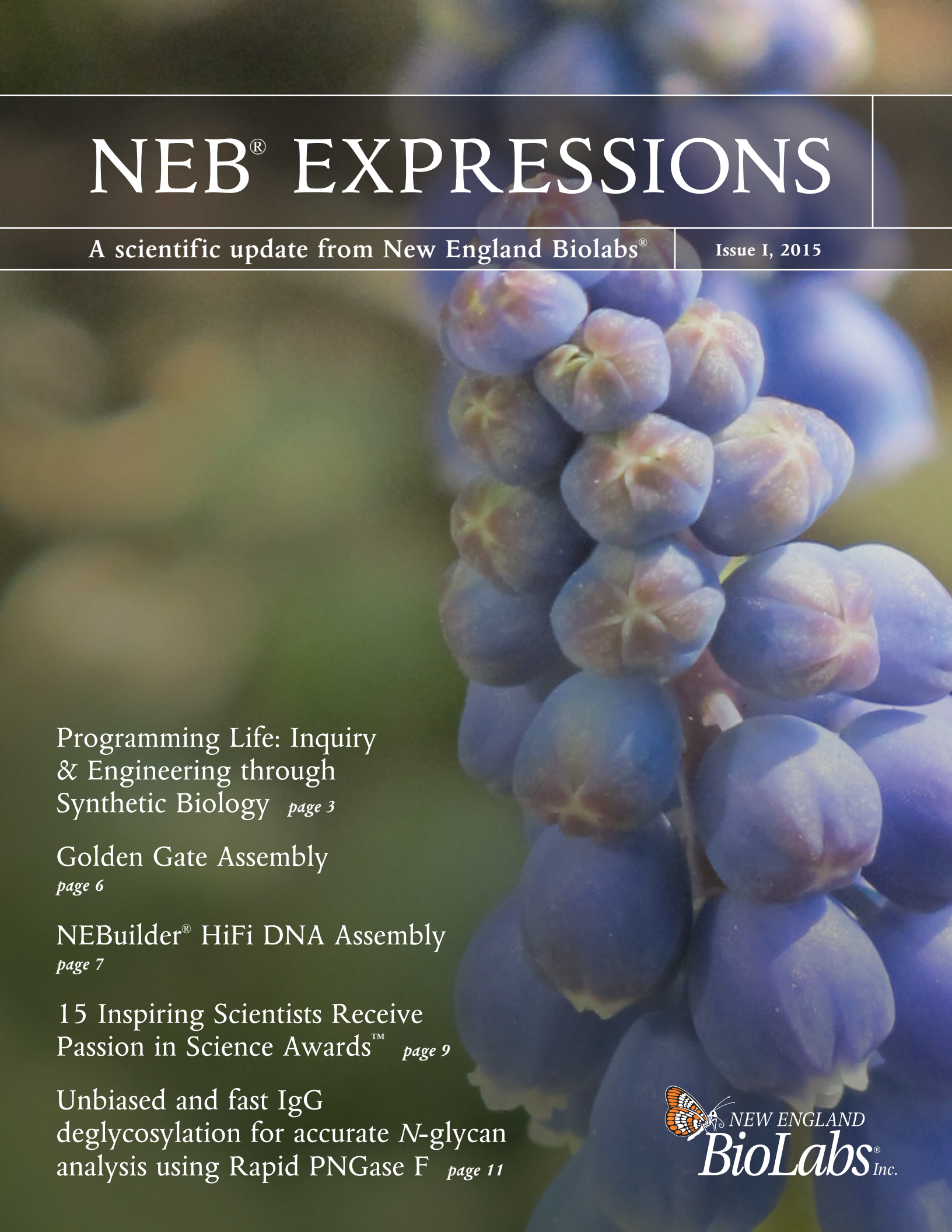
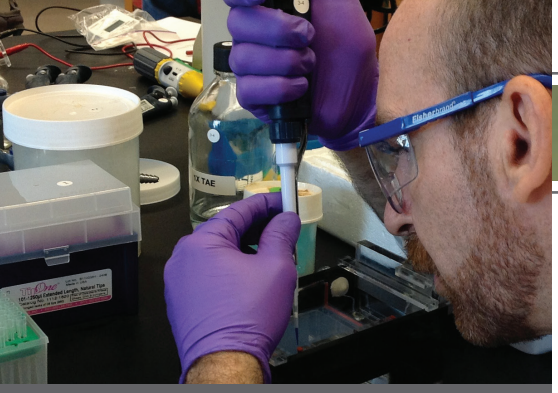
NEB® EXPRESSIONS

A scientific update from New England Biolabs®

Issue I, 2015



NEW ENGLAND BioLabs® Inc.



30TH ANNUAL Molecular Biology Summer Workshop

topics/techniques:

- gene cloning and library construction
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- PCR and quantitative RT-PCR
- genomics and bioinformatics
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Dr. Steven A. Williams, Director

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Contents

Issue I, 2015

COVER PHOTO
Common Grape Hyacinth (*Muscari neglectum*) growing on the NEB campus.

Programming Life: Inquiry & Engineering Through Synthetic Biology

The report of the first chimeric DNA molecule in 1968 (1) ushered in a new age for experimental biology and biotechnology. The ability to propagate DNA obtained, in principle, from virtually any organism within the cytoplasm of *Escherichia coli* (2) set the stage for sequencing of genes and genomes. This advance enabled researchers not only to connect a mutant phenotype with the corresponding genotype, but also paved the way for the industrial production of medically important proteins such as insulin. The *in vitro* construction of recombinant DNA thus became a cornerstone method in the functional and biochemical characterization of genes and proteins.

The five decades following the birth of molecular cloning have witnessed an incredible scaling-up of molecular biology due, in large part, to the development of high-throughput technologies in nucleic acid sequencing and macromolecular analysis. But long absent from the resulting explosion of information has been the ability to rationally recreate, in the laboratory, the regulatory complexity of the very gene networks forming the basis cellular behavior. In short, we know a great deal about the "code of life" but are only now beginning to be able to program with it. This aspiration has, in part, given birth to the rapidly developing field of synthetic biology, which aims to unite the rigor of engineering with the design and construction of recombinant nucleic acids, with which to study and understand the behavior of genetic circuits as well as utilize them for technological ends.

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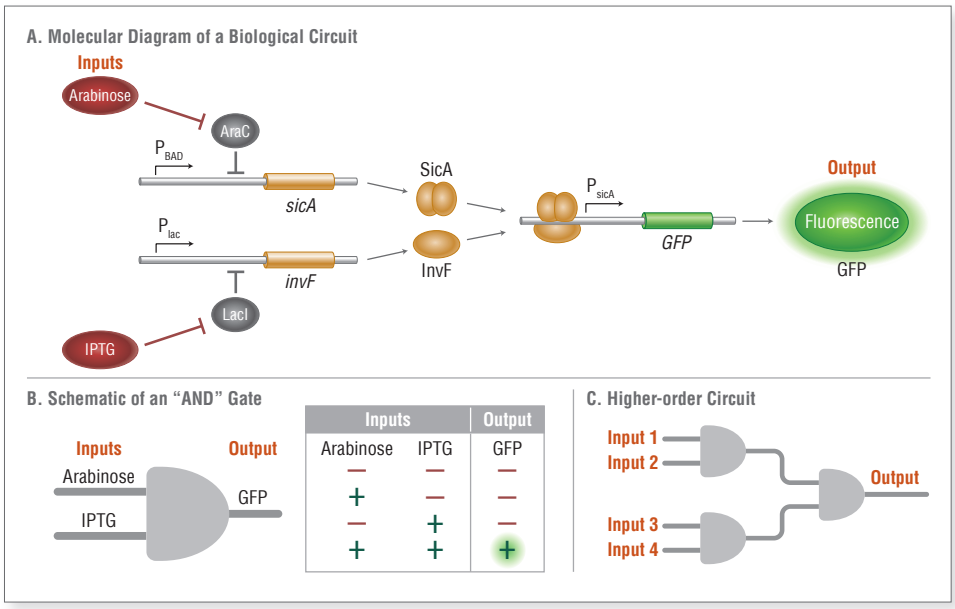
and Jennifer Redig, Ph.D., BiteSize Bio

What is Synthetic Biology?

Though a comprehensive definition of synthetic biology is elusive, one may characterize it as a "build to understand" approach to biology (3). A quote by the famous theoretical physicist Richard Feynman epitomizes a theme characteristic of the field – "What I cannot create, I do not understand." How does this sentiment relate to recombinant DNA? Imagine beginning with a repertoire of well-characterized DNA "parts" encoding biological functions such as receptors, promoters, activators, repressors, terminators and reporter genes (or other outputs) – and attempting to rearrange them into configurations designed to direct a biological system (typically, a cellular "chassis") to accomplish a desired task. Think a pollution detecting *E. coli* cell that expresses green fluorescent protein (GFP) in the presence of arsenic and then self-destructs after a given period of time, or an engineered implantable human cell line that undergoes a preset number of cell divisions and then secretes insulin at precisely regulated levels in response to extracellular glucose concentrations.

Such re-purposed cells would be described, in synthetic biology parlance, as "genetic devices." These devices are designed for multi-step behaviors, and relative to earlier examples of genetic engineering, their design is necessarily complex. How cells can be programmed for such functions is neither intuitive nor obvious. Here, synthetic biology has made a radical departure from previous forms of genetic engineering by borrowing engineering concepts from control theory and digital computing as a framework upon which to design genetic circuits for programming cellular behaviors. A genetic implementation of one such "simple" computational operation, the Boolean AND gate, is shown in Figure 1. Higher order combinations of multiple kinds of genetically encoded Boolean operations, and other types of synthetic gene circuits, have been constructed to perform a variety of simple computational tasks, including edge-detection, cell to cell communication, and counting of signal inputs (4).

Figure 1. Cellular computation.



Synthetic biology draws some of its inspiration from the engineering disciplines of control theory and digital circuit-design. In the illustrated example (A), an assemblage of biological components ideally functions to convert two chemical inputs (IPTG and arabinose) into an output: the expression of the fluorescent reporter protein GFP. Two promoters (P_{BAD} and P_{lac} are each constitutively repressed until induced by their cognate chemical signals (arabinose and IPTG, respectively). Each operon expresses half of a two-part transcriptional activator (the SicA and InvF gene products) which together activate the transcription of the GFP under the control of P_{sicA}. Expression of the reporter only occurs in the presence of both inputs. The DNA circuit can be represented abstractly as a logic gate implementing the Boolean "AND" operation and is shown with the associated truth table (B). Higher order circuits (C) can be created by combinations of modular genetic gates; in this example, three AND gates convert four inputs into a single output. Figure content adapted from Brophy and Voigt (2014).

Going from a circuit schematic to a working genetic device is guided by an engineering paradigm: the design-build-test cycle (Figure 2, p. 4). A key tool in this process is computer-aided mathematical modeling. Unlike their electrical counterparts, genetic circuits operate under conditions that dominate the cellular environment. A model attempting to describe and predict the behavior of a genetic device must accurately incorporate a range of parameters such as diffusion, binding equilibria, networks of protein/DNA interactions, and dynamic reactant concentrations; a variety of deterministic and stochastic approaches have been employed to accomplish this goal (5). As such, the

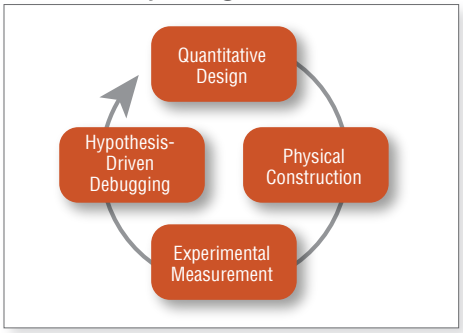
model embodies a sophisticated hypothesis about how the device might work. The genetic device is prototyped (e.g., synthetic DNA is assembled and transformed into the cell) and its behavior evaluated in terms of the model. What is learned during each stage is used to improve the performance of the device in subsequent rounds of the cycle – through changes to the device itself, as well as through refinements to the model.

While synthetic biology shares many of the tools and reagents with hypothesis-driven experimental biology and molecular biology, it follows a fundamentally different approach. Many of the techniques in a molecular biologist's repository (e.g., oligo synthesis, genome editing) may not exist in their current form were it not for synthetic biology. Conversely, synthetic biologists can build upon discoveries made by molecular biologists. *In essence, synthetic biologists assemble genetic components in order to execute an "artificial" function, and in the process of getting it to work, the engineered genetic construct becomes itself an object of study and yields basic principles for application to subsequent designs.*

Chemical engineering *in vivo*

A practical definition of synthetic biology must also include the latest developments in industrial fermentation and metabolic engineering. Even a cursory survey of papers and journals covering synthetic biology shows a significant number of reports describing synthetic biology to synthesize fuels, chemicals and materials. Historically, this technology began as an outgrowth of beer and wine making, after it was discovered that fermentation could also be used to produce economically valuable solvents and organic acids (6). With the advent of greatly expanded sequence databases, inexpensive DNA synthesis, and genome engineering methods, it has become increasingly practical to do chemical synthesis *in vivo*. Designer metabolic pathways utilizing genes encoding enzymes derived from any of the domains of life can inserted into microbes such as *Saccharomyces cerevisiae* or *E. coli*, endowing them with the ability to convert cheap chemical inputs, such as starch- or cellulose-derived sugars, into more commercially valuable chemicals.

Figure 2.
Workflow only an engineer could love.



The synthetic biology workflow is iterative.

The products of synthetic biology applied to industrial fermentation are already in the marketplace and new products are in the works. Large agro-chemical companies such as Cargill® have established plants for the conversion of starch to platform chemicals such as 3-hydroxypropanoate, which can serve as an intermediate for many other commodity chemicals. The engineering of *E. coli* and *Saccharomyces* to produce the anti-malarial precursor artemisinic acid by Amyris® is a landmark achievement in synthetic biology and metabolic engineering. Elements of their engineered biosynthetic pathway have subsequently been repurposed to produce fuels and high value chemicals (7), while Ginkgo Bioworks™ (8) and companies such as Joule® (9) are engineering microbes to mitigate greenhouse gases such as methane and CO_2 and produce valuable products, including biofuels. Industrial biosynthesis is not limited to pharmaceuticals and commodity-scale chemicals. Companies such as Evolva® are working on ways to engineer yeast to produce vanillin, stevia and even the flavor components of saffron (10), while Pronutria® is working to efficiently convert CO_2 to feed and medicinal nutrients (11).

The First Synthetic Gene Circuits

In 2000, the first synthetic circuits were made when Gardner, Cantor and Collins created a genetic toggle switch (12), and Elowitz and Leibler engineered a repressilator, a synthetic genetic regulatory network designed from scratch to produce stable oscillations of gene expression (13). Both of these circuits were model-based, but both also needed experimental fine-tuning to achieve agreement between model and experimental output.

These experiments were quickly followed by "The First International Meeting on Synthetic Biology" or SB1.0, which was held in 2004 at MIT (14). Attended by biologists, chemists, physicists, engineers and computer scientists, the goal of this conference was to bring together those scientists interested in creating and characterizing synthetic biological systems. This meeting, and smaller ones like it, laid down the foundation of a new, emerging discipline.

A Community to Build From

As the synthetic biology discipline grew, it quickly became clear that there needed to be a more efficient way to assemble genetic parts and circuits (4). Without established methods for assembly and testing, researchers were forced to ad hoc experimental designs, wasting time and money by designing, testing and redesigning constructs.

To combat these issues, a public repository, the Registry of Standard Biological Parts (RSBP), was founded at MIT by Tom Knight and Drew Endy. The goal of this repository is to catalog and develop genetic parts into 'BioBricks®' that could be used for the assembly of larger circuits. The BioBrick standard was developed to ensure that parts could be easily shared and used among synthetic biologists by requiring submitted parts to conform to a simplified cloning scheme utilizing four restriction enzymes. However, it became quickly clear that the task of populating the Registry with biological parts, and the work of characterizing them to establish their utility, would dwarf the resources of the relatively small numbers of labs devoted to synthetic biology. Out of this daunting mission, and the need to sustainably train a new generation of synthetic biologists, the International Genetically Engineered Machine (iGEM) competition was born (15).

Training the next generation of bioengineers

Since its inception in 2004, iGEM has evolved into a highly successful vehicle for training and showcasing a new generation of biological engineers using the synthetic biology framework. In 2014, iGEM hosted its 10th annual Jamboree, with over 4,000 participants from across the globe presenting projects that detailed their efforts to model, build and test genetic devices. Students competed in a variety of tracks such as Food, Medicine, Manufacturing and Information Processing. Each team was also asked to demonstrate that they have considered the impact and implications of the technologies they are developing through dialog with relevant stakeholders. Teams were supported by various organizations, including NEB (for more information, visit www.neb.com/igem). To date, more than 28,000 student competitors have participated in this engineering competition.

A rapidly maturing field

Synthetic Biology as a discipline continues to grow rapidly. Recent synthetic biology developments include:

Circuits Get Complex – In the early 2000s, DNA circuits continued to advance. More elements were added (16), and sensing became more diversified (17,18). Additionally, RNA, not just DNA, was used in circuit generation (19–21).

'Synthetic' Used to Investigate 'Native' – Beginning in 2009, designed circuits were used to understand native systems through compare/contrast schemes (22) of engineered versus native systems. Synthetic Biology was not just limited to engineering new biology; it was also used to investigate and understand native biology.

Commercially Valuable Products are Made – In the 2000s, amino acid biosynthesis was used to produce commercially valuable products such as isobutanol (23,24), biodiesel (25) and gasoline (26). These experiments were a logical extension of fermentation biotechnology and highlighted synthetic biology's commercial and environmental promise.

Assembly of a Whole Bacterial Genome in Yeast – In 2008, researchers were able to take advantage of yeast's remarkable ability to recombine overlapping DNA fragments to assemble an entire genome in a single step. This method allowed for speedier assembly of DNA molecules than previous methods (27,28).

New Genome Editing Tools Emerge – Beginning in 2010, zinc finger nucleases gave way to more precise genome editing tools, from TALENS (29) to, in 2013, CRISPR/Cas9 (30) systems. This empowered synthetic and molecular biologists to create and explore as never before. In addition, a catalytically inactive form of Cas9, known as dCas9, has further enhanced the usefulness of the CRISPR/Cas9 system by enabling both activation and repression of transcription in yeast and mammalian cells, allowing modulation of endogenous gene expression (31).

First "Artificial Cell" Engineered – In 2010, Craig Venter and colleagues demonstrated just how far the discipline of synthetic biology had come when they published a paper disclosing the recreation of a *Mycoplasma mycoides* cell controlled by a chemically-synthesized genome (32).

Therapies Engineered – In 2010, Fussenegger and colleagues engineered a synthetic circuit that, when inserted into the genome of a mouse mutant bred to develop hyperuricemia, was able to maintain uric acid homeostasis, essentially correcting an inborn metabolic defect (33). This demonstrated the therapeutic promise of synthetic biology.

Ongoing Challenges

Synthetic Biology is a young field, but it has achieved much in a short time period. However, like all disciplines, it continues to face challenges.

Measurement, Robustness and Predictability – Aspects of synthetic biology still remain an art. Genetic circuits often require much "tweaking" in order to get them to function in the context for which they were designed. Further principles governing the function of genetic circuits will have to be elucidated to improve the interoperability of genetic parts in multiple contexts.

Cells are Not Exactly Digital – Though incredibly powerful as a guiding framework for designing, building, and testing genetic circuits, the digital circuit metaphor has limits. Biological systems differ from electronic ones in fundamental ways, and modeling genetic regulation remains under determined. Synthetic biology researchers continue to incorporate new ideas and theories to describe, model and predict genetic circuit behaviors. A new and promising area utilizes analogies to analog circuitry (34).

Ethics and Safety – Synthetic Biology, and indeed all genetic engineering, has provoked concern over potential misuse, intentional or accidental. There is active discussion regarding potential impacts (35). Built-in forms of biological containment are also an active area of investigation, including the refinement of genetic "kill switches", which ideally would ensure that genetic devices could not survive outside of the laboratory or factory. Government policy has and will continue to weigh in: information on the ethics of synthetic biology can be found in the 2010 Presidential Bioethics Commission report on synthetic biology (36). As with other technologies, a scientifically literate public is a requirement for nuanced and effective dialog.

Future directions

The past sixty years have seen incredible scientific and technological advances based on the ability to compose in DNA. DNA-driven technologies will continue to absorb developments and ways of thinking from diverse fields. Advances in materials sciences, nanotechnology, microfluidics, automated liquid handling, indeed all the applied sciences, will drive new applications using cellular systems and even biological technologies beyond the cell (37,38). The proliferation and use of these technologies will continue to impact our lives. With prudence and foresight, they may prove indispensible to our survival.

See page 6 for references associated with this article.

On the Shoulders of Giants

Helping to Establish the Field of Synthetic Biology

1950s

Custom Oligonucleotides

In the late 1950s, Khorana developed the synthetic approach of blocking/deblocking cycles for the stepwise elongation of oligos. This eventually led to solid phase synthesis and automation.

1960s

Gene Regulation

Jacob & Monod first described a genetic circuit (39), earning a Nobel prize in 1965. Seminal discoveries included the lysis vs. lysogeny developmental switch in bacteriophage lambda (λ) (40,41).

1970s

Recombinant DNA

In 1972, the first published report of recombinant DNA made *in vitro*, and then transformed into *E. coli* (42,43).

1980s

Polymerase chain reaction (PCR)

First uses of PCR in 1983 (44) helped fuel an explosion of scientific applications, from genetic engineering to forensic science.

Early DNA Assembly

In vitro enzymatic approaches using complementary, overlapping oligonucleotides enabled genes to be synthesized directly from sequence (45,46).

1990s

Bioinformatics

Collaboration of biologists and computer scientists led to development of software tools for biologists, including Basic Local Alignment Search Tool (BLAST), as well as other alignment and codon optimization tools (4).

Sequencing Data

Automated DNA sequencers became widespread and more affordable, and the first complete genome of an organism was sequenced (47–49).

2000s

Genome Editing

First reports connecting synthetic DNA to genome editing in the lambda red system in eubacteria. Later developments include the use of engineered zinc finger nucleases, TALENS (29) and CRISPR/Cas9 (30,31).

One-Step DNA Assembly

Development of one-step assembly methods, including Golden Gate (51), USER®, Gibson Assembly® (52) and NEBuilder HiFi DNA Assembly (53).

2010s

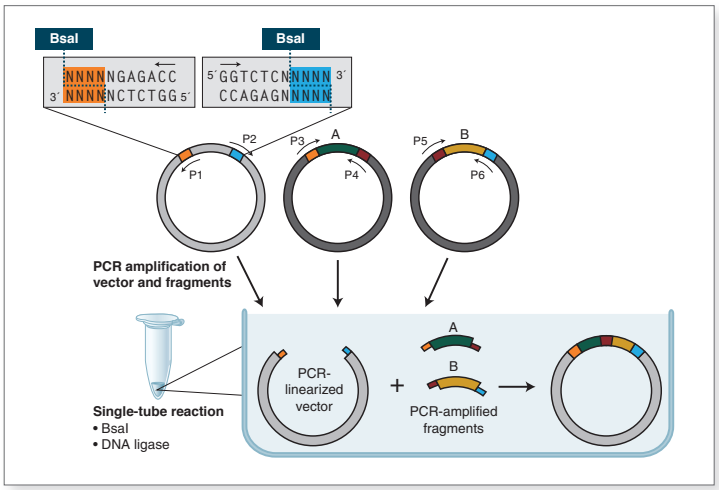
Cost of DNA Synthesis

Cost of synthesizing DNA dropped 1,000-fold in a decade, and speed of production increased (50).

Golden Gate Assembly

The efficient and seamless assembly of DNA fragments, commonly referred to as Golden Gate assembly (1,2), has its origins in 1996 when, for the first time, it was shown that multiple inserts could be assembled into a vector backbone using only the sequential (3) or simultaneous (4) activities of a single type IIS restriction enzyme and T4 DNA ligase. This method can be accomplished using Type IIS restriction enzymes, such as BsaI, and can also be used for the cloning of single inserts. The assembled fragments, or inserts, can either be precloned or in the amplicon form, where the Type IIs recognition site is introduced through primer design and PCR. The overhang sequence is not dictated by the restriction enzyme, and allows the design of appropriate four-base overhang sequences that lead to scarless assembly. The method is efficient and can be completed in one tube in as little as 5 minutes for single inserts, or can utilize cycling steps for multiple inserts. Golden Gate Assembly has been widely used in the construction of custom-specific TALENs for *in vivo* gene editing, among other applications.

Golden Gate Workflow.



In its simplest form, Golden Gate Assembly requires a BsaI recognition site (GGTCTC) added to both ends of a dsDNA fragment distal to the cleavage site, such that the BsaI site is eliminated in the final product.

New England Biolabs supplies reagents for use in Golden Gate Assembly, including restriction enzymes and ligases. Our new **NEB Golden Gate Assembly Mix** utilizes two simultaneous enzymatic activities in a single reaction, specifically digestion with BsaI and ligation with T4 DNA Ligase.

Ordering Information

PRODUCT	NEB #	SIZE
NEW NEB Golden Gate Assembly Mix	E1600S	15 reactions
BsaI	R0535S/L	1,000/5,000 units
BsaI-HF	R3535S/L	1,000/5,000 units
BbsI	R0539S/L	300/1,500 units
BsmBI	R0580S/L	200/1,000 units
T4 DNA Ligase	M0202S/T/L/M	2,000/100,000 units

ADVANTAGES OF GOLDEN GATE ASSEMBLY

- Seamless cloning – no scar remains following assembly
- Can be used to assemble areas of repeats
- Compatible with a broad range of fragment sizes (< 100 bp to > 15 kb)
- Efficient with regions with high GC content

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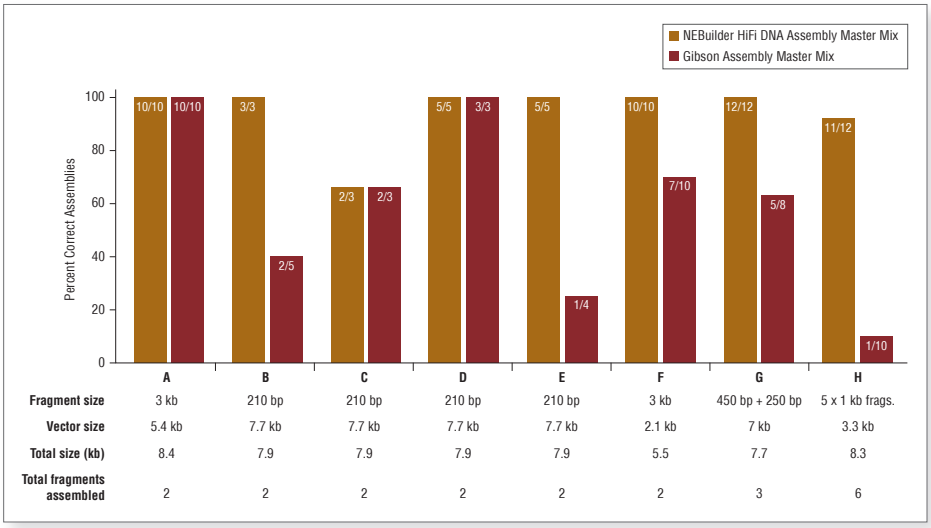
53. www.NEBuilderHiFi.neb.com

NEBuilder HiFi DNA Assembly

NEBuilder HiFi DNA Assembly enables virtually error-free joining of DNA fragments, even those with 5´- and 3´-end mismatches. Available with and without competent *E. coli*, this flexible kit enables simple and fast Seamless Cloning utilizing a new proprietary high-fidelity polymerase. Find out why NEBuilder HiFi is the next generation of DNA assembly and cloning, supporting the advancement of both molecular and synthetic biology.

NEBuilder HiFi DNA Assembly Master Mix offers improved fidelity over Gibson Assembly.



Fidelity of assembled products was compared between NEBuilder HiFi DNA Assembly Master Mix (NEB #E2621) and Gibson Assembly Master Mix (NEB #E2611). Experiments were performed using various fragment and vector sizes following suggested protocols. Experiments B through E varied because sequences of fragments were different. Experiments F and H were performed with fragments containing 3´ end mismatches.

Ordering Information

PRODUCT	NEB #	SIZE
NEBuilder HiFi DNA Assembly Master Mix	E2621S/L/X	10/50/250 rxns
NEBuilder HiFi DNA Assembly Cloning Kit (includes competent cells)	E5520S	10 rxns

OPTIMIZATION TIPS FOR NEBUILDER HIFI DNA ASSEMBLY

Assembly Reaction

- When directly assembling fragments into a cloning vector, the molar concentration of assembly fragments should be 2–3 times higher than the concentration of vector.
- For multiple (4–12) fragment assembly, design 25–30 bp overlap regions between each fragment to enhance assembly efficiency. Use 0.05 pmol of each fragment in the assembly reaction.
- For assembly of 1–3 fragments, 15 minute incubation times are sufficient. For assembly of 4–6 fragments, 60 minute incubation times are recommended. Reaction times less than 15 minutes are generally not recommended.

Primer Design

- For help with primer design, we recommend using NEBuilder Assembly Tool available at **NEBuilder.neb.com**.

Transformation

- The NEBuilder HiFi DNA Assembly Cloning Kit (NEB #E5520) includes NEB 5-alpha Competent *E. coli*. NEB recommends using the competent cells provided with the kit (NEB #C2987) because of their high efficiency. The components of the master mix may inhibit the functionality of competent cells from other companies if not diluted.

ADVANTAGES OF NEBUILDER HIFI

- Save time with simple and fast seamless cloning
- Use one system for both "standard-size" cloning and large gene assembly products (up to 6 fragments)
- DNA can be used immediately for transformation, or as a template for PCR or RCA
- Adapts easily for multiple DNA manipulations, including site-directed mutagenesis

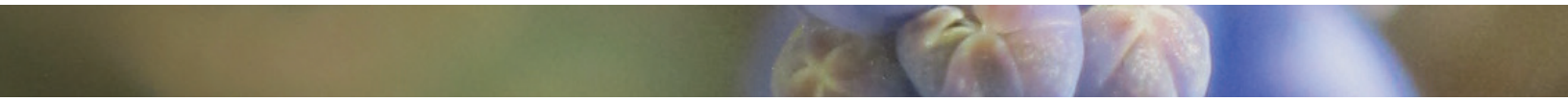
BENEFITS OF NEBUILDER HIFI OVER GIBSON ASSEMBLY MASTER MIX

- Enjoy less screening/re-sequencing of constructs, with virtually error-free, high-fidelity assembly
- Join DNA fragments together more efficiently, even with larger fragments or low DNA inputs
- Use NEBuilder HiFi in successive rounds of assembly, as it removes 5´- and 3´-end mismatches
- Bridge two dsDNA fragments with a synthetic ssDNA oligo for simple and fast construction (e.g., linker insertion or gDNA libraries)
- Switch from other systems easily, as NEBuilder HiFi is compatible with Gibson Assembly-designed (and other) fragments
- No licensing fee requirements from NEB for NEBuilder products

Need help designing primers for DNA assembly?

Try NEBuilder® Assembly Tool at NEBuilder.neb.com

Visit **NEBuilderHiFi.com** to learn more and to request your FREE sample



Synthetic Biology/DNA Assembly Selection Chart

NEB now offers several products that can be used for DNA Assembly. Use this chart to determine which tool would work best to assemble your DNA.

	NEBuilder HiFi DNA Assembly NEB #E2621 NEB #E5520	Gibson Assembly NEB #E5510 NEB #E2611	NEB Golden Gate Assembly Mix NEB #E1600	USER Enzyme NEB #M5505
PROPERTIES				
Removes 5´ or 3´ End Mismatches	★★★	★	N/A	N/A
Assembles with High Fidelity at Junctions	★★★	★★	★★★	★★★
Tolerates Repetitive Sequences at Ends	★	★	★★★	★★★
Generates Fully Ligated Product	★★★	★★★	★★★	NR
Joins dsDNA with Single-stranded Oligo	★★★	★★	NR	NR
Assembles with High Efficiency with Low Amounts of DNA	★★★	★★	★★	★★
Accommodates Flexible Overlap Lengths	★★★	★★★	★	★★
APPLICATIONS				
Simple Cloning (1-2 Fragments)	★★★	★★★	★★★	★★★
4-6 Fragment Assembly	★★★	★★★	★★★	★★★
>6 Fragment Assembly	★★★	★★	★★★	★★★
Template Construction for *In vitro* Transcription	★★★	★★★	★★★	★
Synthetic Whole Genome Assembly	★★★	★	★	★
Multiple Site-directed Mutagenesis	★★★	★★★	★★	★
Library Generation	★★	★★	★★	★★
Pathway Engineering	★★★	★★	★★	★★★
TALENs	★★	★★	★★★	★★
Short Hairpin RNA Cloning (shRNA)	★★★	★★	★	★
gRNA Library Generation	★★★	★★	★	★
Large Fragment (>10 kb) Assembly	★★★	★★★	★★★	★★
Small Fragment (<100 bp) Assembly	★★★	★	★★★	★★★
Use in Successive Rounds Restriction Enzyme Assembly	★★★	★	NR	★

KEY	
★★★	Optimal, recommended product for selected application
★★	Works well for selected application
★	Will perform selected application, but is not recommended
N/A	Not applicable to this application
NR	Not recommended

PURExpress *In Vitro* Protein Synthesis Kit

A rapid method for gene expression analysis, PURExpress is a novel cell-free transcription/translation system reconstituted from the purified components necessary for *E. coli* translation. Express a wide range of proteins free of modification or degradation by simply mixing two tubes, followed by the addition of template DNA. With results available in only a few hours, PURExpress saves valuable laboratory time and is ideal for high throughput applications.

Ordering Information

PRODUCT	NEB #
PURExpress *In Vitro* Protein Synthesis Kit	E6800S/L
PURExpress Δ Ribosome Kit	E3313S
PURExpress Δ (aa, tRNA) Kit	E6840S
PURExpress Δ RF123 Kit	E6850S
PURExpress Disulfide Bond Enhancer	E6820S
E. coli Ribosome	P0763S

Interesting article using PURExpress

Researchers at Harvard University recently developed a method for embedding cell-free expression systems onto paper to study synthetic gene networks.

Pardee, *et al.* (2014) Paper-based synthetic gene networks. *Cell*, http://dx.doi.org/10.1016/j.cell.2014.10.004

To learn more about *PURExpress,* visit **www.neb.com/E6800**

15 Inspiring Scientists Receive Passion in Science Awards™

THE 2014 "PASSION IN SCIENCE" AWARDS, hosted by New England Biolabs in celebration of the company's 40th anniversary, recognized scientists for their inspirational work that crosses into the arts, humanitarian service, environmental stewardship and scientific leadership. Selected from more than 600 candidates worldwide, the Passion in Science awardees provide stirring examples of the impact scientists can make when choosing to help others.

In October 2014, the awardees gathered from around the world for a two-day summit at NEB's campus in Ipswich, Massachusetts, to discuss how scientists can create opportunities to progress their passions in this first-of-its-kind event.

2014 Passion in Science Award winners pictured with their hosts outside the New England Biolabs facility in Ipswich, MA.

Inspiration in Science Award Winners

Laurie Doering – *McMaster University*
Jason Furrer – *University of Missouri*
Whitney Hagins – *Massachusetts Biotechnology Education*
Ite Laird-Offringa – *University of Southern California*
Kalai Mathee – *Florida International University*

Environmental Stewardship Award Winners

Tonni Kurniawan – *Xiamen University*
Andrew Markley – *University of Wisconsin*

Humanitarian Duty Award Winners

Lori Baker – *Baylor University*
Karl Booksh – *University of Delaware*
Peter Hotez – *Sabin Vaccine Institute*
Paul McDonald – *Virginia Tech Carilion Research Institute*

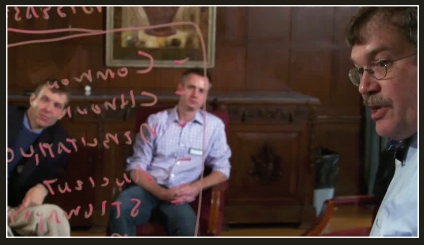
Arts and Creativity Award Winners

Tal Danino – *Massachusetts Institute of Technology*
Louise Hughes – *Oxford Brookes University*
Alia Qatarneh – *Harvard University*
Shelly Xie – *UT Southwestern Medical Center*

*A working session of the arts and creativity award recipients includes medical student **Shelly Xie**, whose evocative sand-art performances depict the heartbreaking stories of people suffering from tropical diseases neglected by modern medicine.*

*Award recipient **Whitney Hagins** tours NEB's greenhouse and wastewater treatment system with fellow scientific leadership winners. Hagins, a BioTeach mentor and program coordinator in Boston, develops hands-on science curriculum and teacher training to support high school science education.*

*Postdoc **Andrew Markley**, pictured here in blue, walks the NEB campus and discusses opportunities to reduce lab waste. Markley founded an initiative to collect expanded polystyrene boxes on his University of Wisconsin, Madison campus and reuse them locally.*



Learn more about our award winners and their inspiring projects in our latest video at www.neb.com/PassionInScience

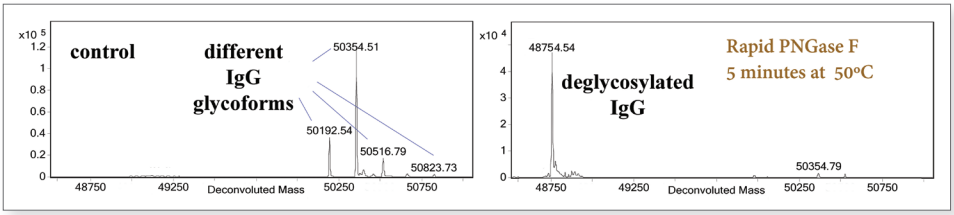
Rapid PNGase F – Complete deglycosylation in minutes

Effective manufacturing of therapeutic proteins requires characterizing their *N*-glycosylation in the shortest time possible. Rapid PNGase F is an improved reagent that allows the complete and rapid deglycosylation of antibodies and immunoglobulin fusion proteins, as well as other glycoproteins. All *N*-glycans are released in five minutes 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.

ADVANTAGES

- Convenient one-step reaction compatible with high throughput applications
- Complete deglycosylation of antibodies and immunoglobulin fusion proteins in minutes
- Release of all *N*-glycans without bias, compatible with downstream chromatography or mass spectrometry analysis
- Recombinant source
- Optimal activity is ensured for 12 months
- Purified to >99% homogeneity

ESI-TOF analysis of an antibody before (left) and after (right) treatment with Rapid PNGase F



Ordering Information

PRODUCT	NEB #	SIZE
Rapid PNGase F	P0710S	50 reactions

Near-infrared Imaging in Living Cells

New England Biolabs now offers a unique cell-permeable near-infrared fluorescent probe that enables live-cell imaging of intracellular proteins using the SNAP-tag® technology.

SNAP-Cell® 647-SiR (SiR-SNAP) is excited at around 650 nm and emits around 670 nm, has a high quantum efficiency in aqueous media, and is stable against photobleaching. The excellent spectroscopic properties of SNAP-Cell 647-SiR, combined with its high permeability, make it ideally suited for super-resolution microscopy of cellular proteins in living cells and *in vivo*.

Recent Publications using SNAP-tag

Jaensch, N. et al. (2014) Stable cell surface expression of GPI-anchored proteins, but not intracellular transport, depends on their fatty acid structure. *Traffic*, 15, 1305-1329.

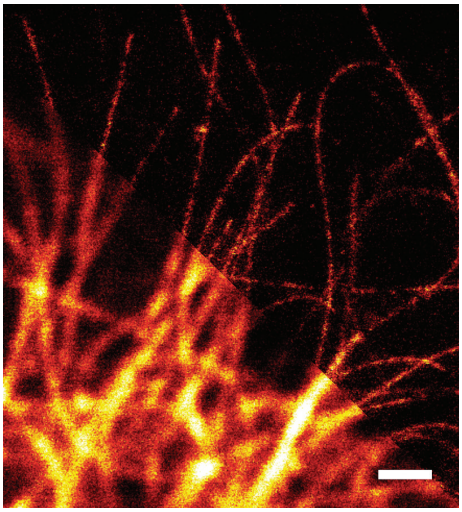
Sun, X. et al. (2014) Probing homodimer formation of epidermal growth factor receptor by selective cross linking. *Euro. J. Med. Chem.* 88, 34-41.

Yang, G. et al. (2014) Genetic targeting of chemical indicators in vivo. *Nature Methods,* doi: 10.138/NMETH.3207.

Lukinavicius, G. et al. (2013) A near-infrared fluorophore for live-cell superresolution microscopy of cellular proteins. *Nature Chemistry,* 5, 132-139.

Ordering Information

PRODUCT	NEB #	SIZE
SNAP-Cell 647-SiR	S9102S	30 nmol



Composite image in confocal (lower left) and super resolution (upper right) showing live U2-OS cells expressing a centrosomal fusion protein, Cep41-SNAP, labeled with SNAP-Cell 647-SiR. Scale bar is 1 µm.

Learn more about the *SNAP-tag technology* at **www.neb.com/SNAPtag**

Unbiased and fast IgG deglycosylation for accurate *N*-glycan analysis using Rapid PNGase F

Paula Magnelli, Ph.D., New England Biolabs, Inc.

Introduction

Because of their usefulness as therapeutic agents, a growing number of monoclonal antibodies are in development for the treatment of cancer, autoimmune conditions and other diseases. The Fc region of IgG carries a conserved *N*-glycan, which is critical for biological activity. Also, some IgGs and IgG fusions have additional *N*-glycans that, together with the conserved Asn297 *N*-glycan, affect recognition, half life and inflammatory reactions.

It has become increasingly important to monitor antibody glycosylation during development and production to obtain the right antibody-glycoforms, while keeping undesired glycans (e.g., Galα1-3Gal epitope) at trace levels. Effective monitoring requires that a complete and accurate *N*-glycan profile be obtained in the shortest time possible, however enzymatic release of *N*-glycans with PNGase F typically takes at least a few hours.

To overcome this limitation, NEB has developed Rapid PNGase F, a new product that allows complete deglycosylation of therapeutic monoclonal antibodies in minutes, and is compatible with downstream LC-MS applications.

Results

Reproducibility and Sensitivity

Rituximab samples were treated for 5 minutes with Rapid PNGase F. Released *N*-glycans were labeled with 2AB, and analyzed by LC-MS. Results show seven replicates analyzed on three different days. The composition of *N*-glycans was highly reproducible from day to day (Fig. 1). There was also negligible variation in the levels of low abundance *N*-glycans (data not shown). All major and minor species previously reported in the literature were found. Relative abundance was within previously reported ranges (1) .

Therapeutic Antibodies with Additional N-glycan Sites

To validate this method with an antibody containing Fab *N*-glycans, a sample of cetuximab (32 µg) was diluted in Rapid PNGase F Buffer, pre-incubated for 2 minutes at 80°C, and treated for 5 minutes at 50°C with Rapid PNGase F. For validation with an IgG-TNF fusion protein, a sample of etanercept (50 µg) was diluted with Rapid PNGase F Buffer, and treated for 5 minutes at 50°C with Rapid PNGase F. Released glycans were analyzed as before.

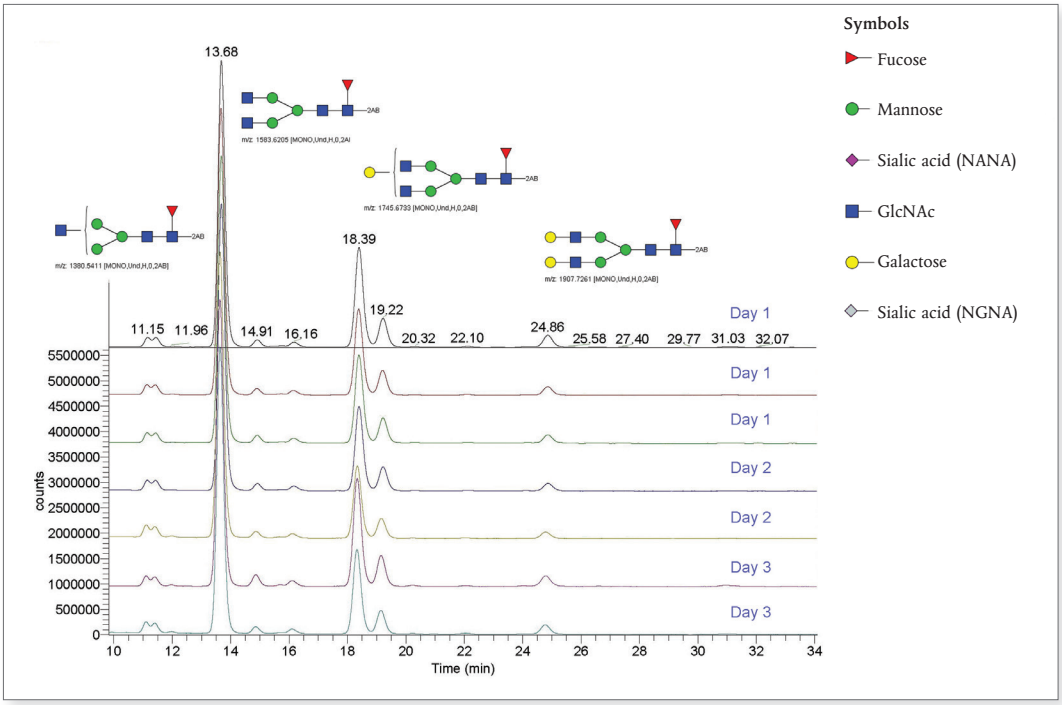
All expected *N*-glycans (from the conserved Fc sites or from other domains) were found (data not shown), in relative abundance as previously reported (2,3).

Concluding Remarks

NEB's Rapid PNGase F can achieve complete and unbiased removal of *N*-glycans from antibodies in minutes. Results obtained using this enzyme were in accordance with published data, demonstrating that sensitivity and accuracy are not compromised by a faster and more convenient glycoprotein characterization workflow. This reaction, which occurs in solution and requires minimal setup, is amenable to high throughput and automation, and is compatible with downstream glycomics analysis by LC/MS.

Visit **www.neb.com/P0710** to download the full application note.

Figure 1. Deglycosylation of Rituximab with Rapid PNGase F



Samples were incubated with Rapid PNGase F for 5 minutes at 50°C. Seven replicates were analyzed on different days, as indicated.

References:
1. Visser J, et al. (2013). *BioDrugs.* 27, 495–507.
2. Ayoub, et al. (2013). *mAbs* 5, 699–710.
3. Houel S, et al. (2014). *Anal. Chem.* 7, 576–584.

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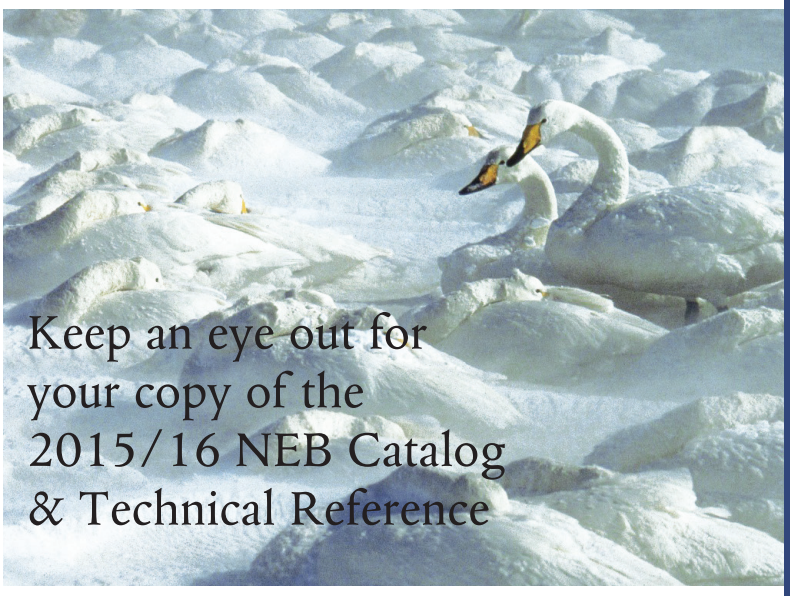

Keep an eye out for your copy of the 2015/16 NEB Catalog & Technical Reference