Issue II **2016**

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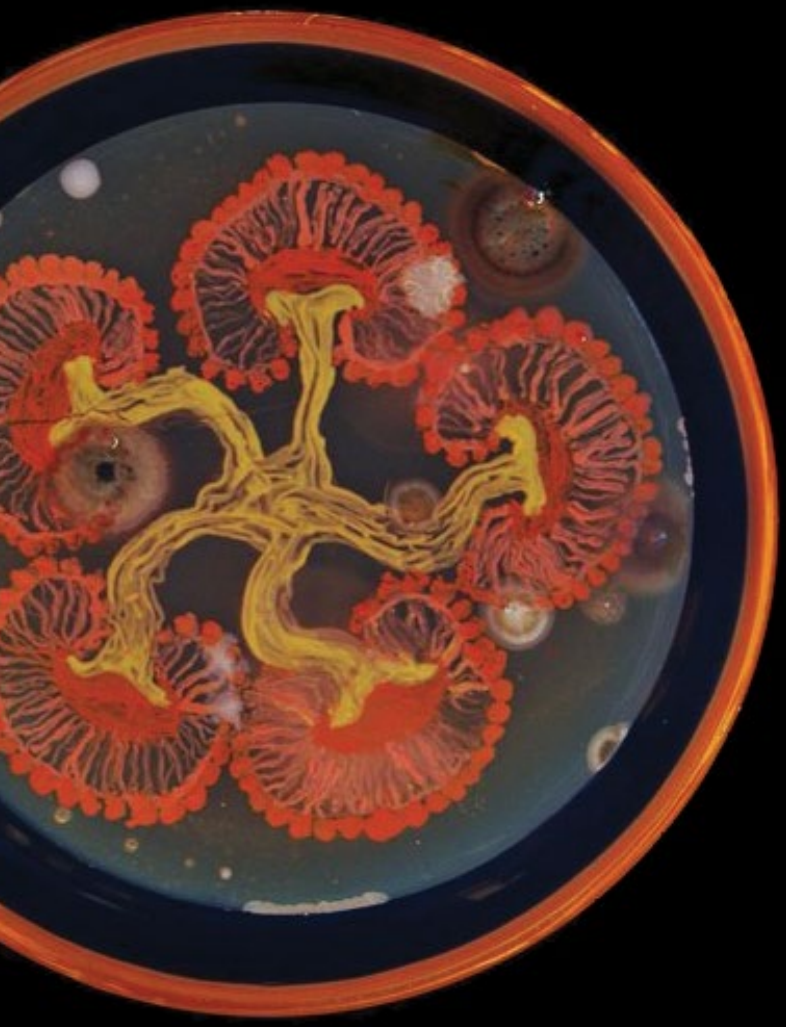
a scientific update



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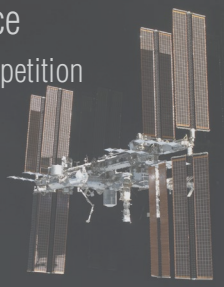

FEATURE ARTICLE by Christopher H. Taron[1] and Pauline M. Rudd[2]

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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. For example, over 140 structural variants of *N*-glycans alone have been identified on glycoproteins present in human serum (7). In addition, for any individual mammalian glycoprotein, numerous different glycans can be attached and processed. A glycoform is a single copy of a glycoprotein with a defined glycan present at each glycosylation site. Thus, proteins exist as collections of glycoforms and the sugars that are present depend on the cell, the protein, and the 3D structure around the site of their attachment.

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.

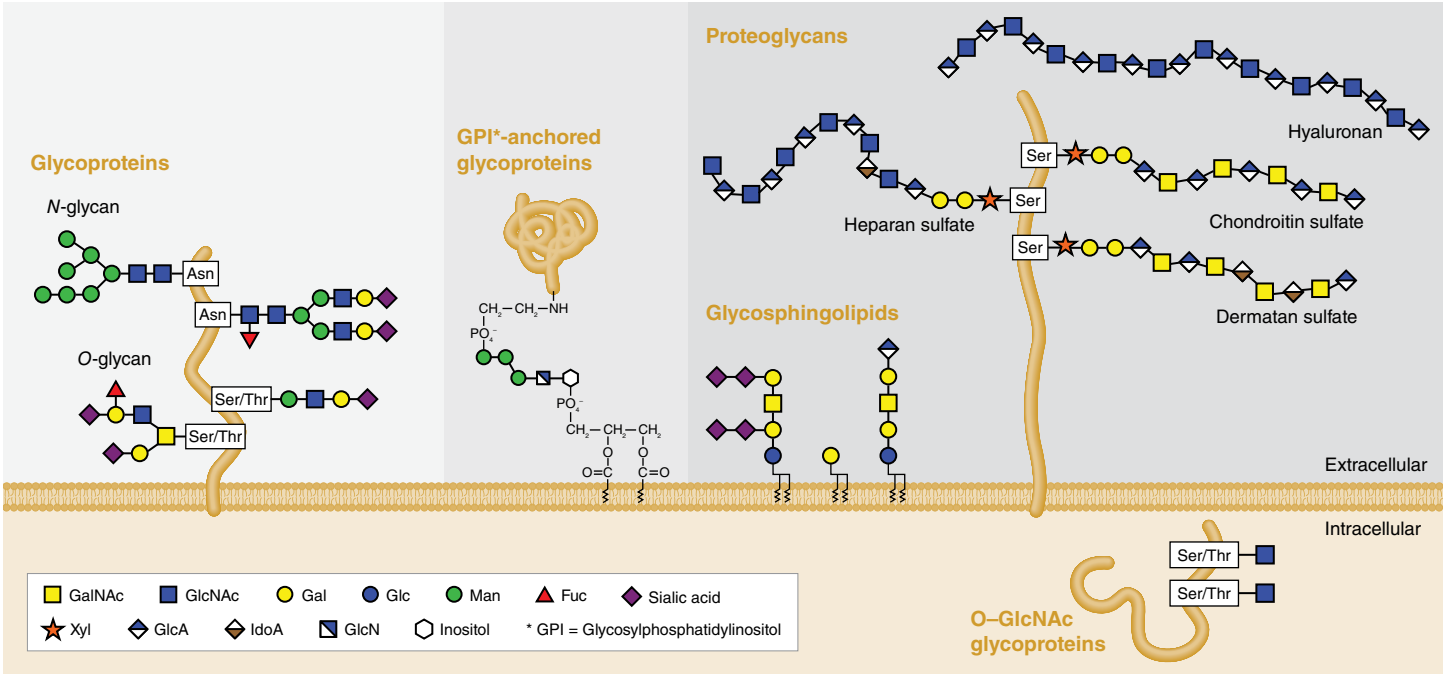
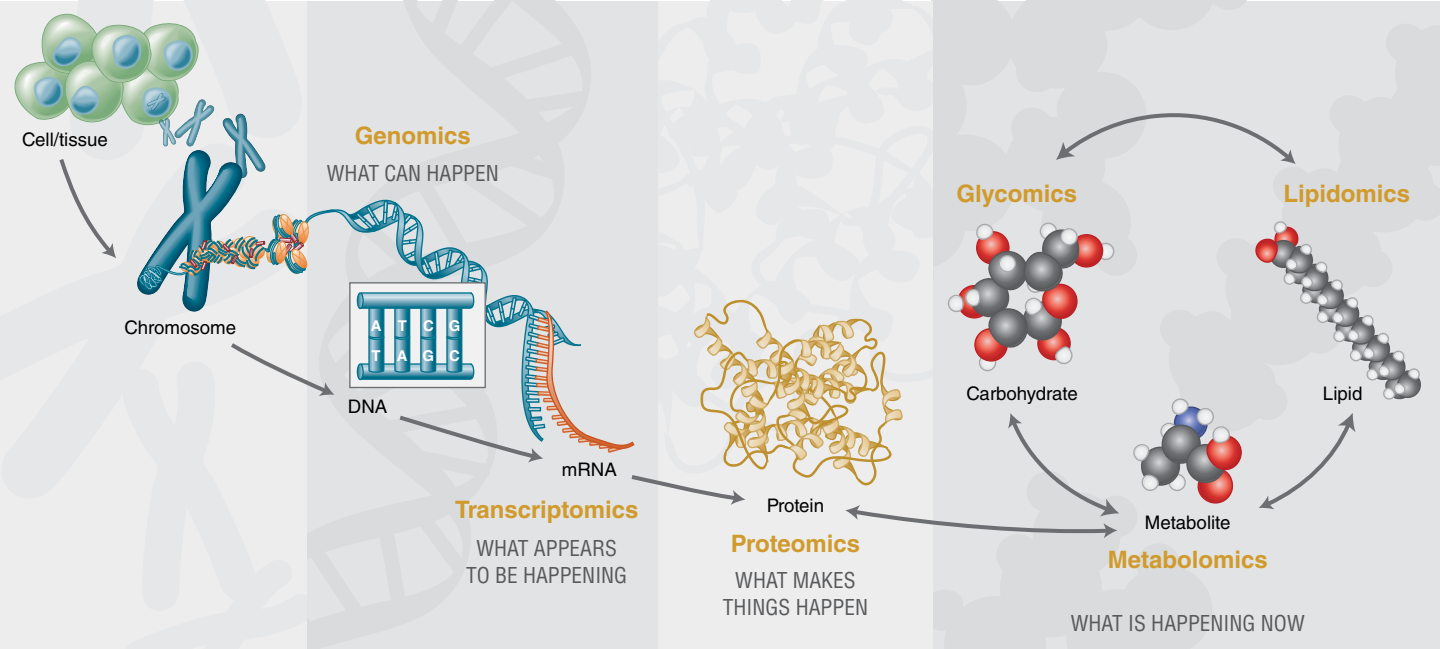


FIGURE 2:
The relationship of major 'omics' technologies

Different omics methods seek to unlock information that is present within cellular molecules with a view toward understanding how cells function. For example, genomics analyzes the genetic blueprint of a cell and informs what could happen in a cell based on the genes that are present or absent. Transcriptomics examines genes that are actively expressed and is a good measure of what appears to be happening in cells. The proteome is the machinery that makes things happen by synthesizing cellular components. Glycans, metabolites and lipids are not directly the products of template driven synthesis, but are instead synthesized by the proteome via biosynthetic pathways. As such, they are the end products of a cell's information flow, and are excellent indicators of what a cell is presently doing. When considered together, these omics fields provide a holistic view of cell biology and cellular function.



In this article, we give an overview of the emergence of high-throughput glycomics and its potential to improve our understanding of the roles glycans play in cell biology. We will also summarize the rapidly growing field of mammalian serum glycomics and discuss its potential as a new reservoir of biomarkers of health and disease, and its diagnostic potential.

TECHNICAL ADVANCES IN GLYCOMICS

Over the past two decades, the cellular and molecular biology fields have been transformed by 'omics' technologies that permit analysis of large pools of biological molecules *en masse*. The advent of deep sequencing technologies has dramatically changed the analysis of nucleic acids and has revolutionized the fields of genomics and transcriptomics. Additionally, improvements in mass spectrometry sensitivity and speed has strongly impacted the depth to which proteomes can be explored. However, nucleic acids and proteins do not alone provide a complete view of cellular function. Enabled by similar recent analytical advances, the past decade has also seen rapid growth of glycomics, lipidomics and metabolomics, fields that explore cellular compounds that are not synthesized by genetic template-driven processes, but instead are products of biosynthetic pathways created by a cell's proteome. These newer fields, in conjunction with the more mature fields of genomics, proteomics and transcriptomics, are now generating a more holistic view of cellular function during both normal cell physiology and in disease (Figure 2).

Glycomics addresses the comprehensive high-throughput structural analysis of glycans produced by an organism or its components (*e.g.*, cells, organs, body fluids, etc). The number of published glycomics studies has been steadily increasing over the past decade. This growth has been fueled by technical advancement in nearly every aspect of glycan analysis (see ref. 8 for a recent technical review). New enzymatic tools have emerged that permit faster and/or more complete liberation of glycans from proteins (9) and glycolipids (10). Highly pure recombinant versions of the field's preferred exoglycosidases are now available, and the first array consisting of pre-mixed combinations of these enzymes optimized for high-throughput glycan sequencing applications has been devised by New England Biolabs. New fluorescent dyes have been developed with advantages for more quickly labeling glycans and/or increased sensitivity in mass spectrometry applications (9). Improvements have been made to solid-phase extraction techniques and faster chromatographic separation of glycan samples using ultra-high performance liquid chromatography systems or capillary electrophoresis. Bioinformatics resources such as GlycoBase (11, 12), a database that correlates known glycan structures to their liquid chromatography mobilities, have been developed to improve structural interpretation of glycan analysis data. Finally, high-throughput analytical workflows that incorporate many of these innovations have been devised to expand the applicability of glycomics to large sample sets (10, 13, 14). Armed with these new analytical tools, researchers have begun unlocking the exciting potential of the mammalian glycome.

MAMMALIAN SERUM GLYCOMICS

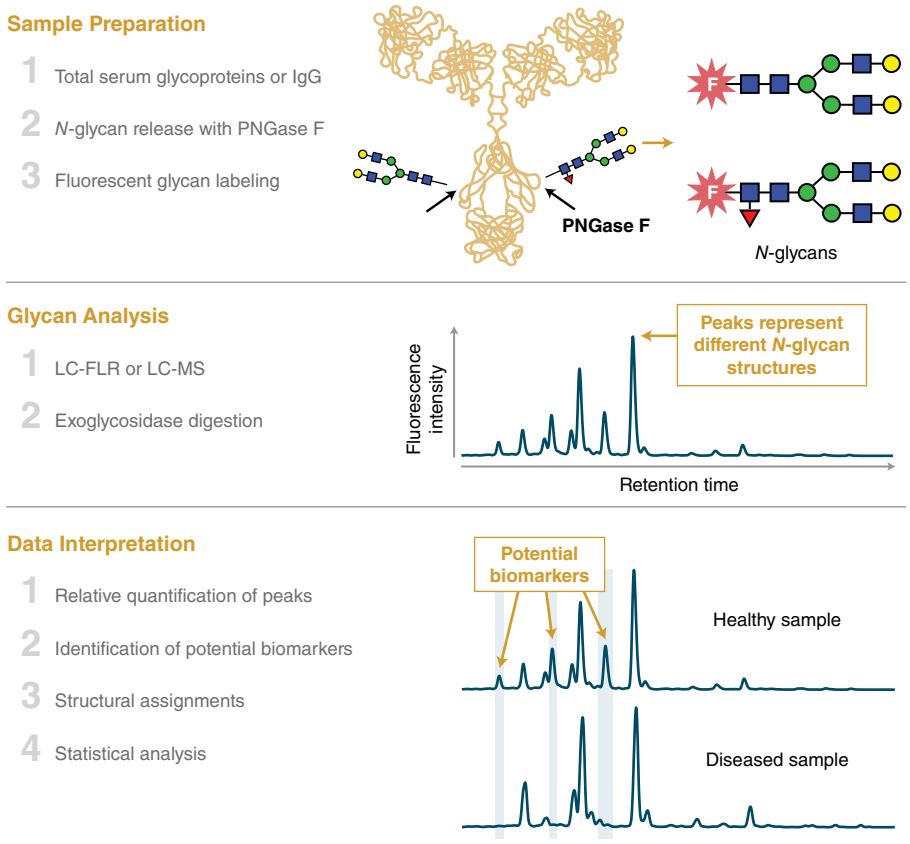
One area of the glycomics field that has seen remarkable progress over the past decade is the characterization of the mammalian blood serum glycome using *N*-glycan profiling. In this field, *N*-glycans are isolated *en masse* from glycoproteins that circulate in the mammalian bloodstream (or commonly from IgG purified from serum), and are subjected to chromatography and structural profiling (Figure 3, page 4). Serum *N*-glycan profiles from different cohorts of individuals (*e.g.*, healthy versus disease samples) can be compared to identify changes in the abundance of individual glycan species.

This methodology has been transforming our understanding of the serum glycome and how it changes in response to both normal physiological processes like aging, or the onset of various diseases such as cancer. Features of the serum and IgG glycomes have already been shown to be associated with genome, transcriptome, and metabolome changes in individuals, and point the way to a more detailed understanding of disease pathways (15–19).

continued on page 4...

FIGURE 3:
A general workflow for serum glycan profiling

Serum glycan profiling workflows are commonly comprised of three basic steps: sample preparation, chromatography/mass spectrometry (MS), and data interpretation. During sample preparation, *N*-glycans from serum glycoproteins or isolated IgG are removed by treatment with PNGase F and fluorescently labeled at their reducing end (FLR). Labeled glycans are then separated by liquid chromatography with fluorescence detection to generate a profile of glycan peaks. Glycan chromatography is often in-line with MS or MS/MS detection. Optionally, treatment with exoglycosidases (enzymes that remove specific monosaccharides) can be used to alter peak mobilities and help interpret their structures (not shown). The area of each profiled peak is determined and compared, for example, between replicates of healthy and disease samples to identify potential biomarkers. Structures can be assigned to peaks of interest by comparison of their mobilities and masses to those of known structures in glycan databases. Finally, the statistical significance of potential biomarkers is assessed mathematically using data from large sample populations.



In the coming years, there is amazing potential for the serum glycome to be exploited as a predictor of health and disease, and to have a role in personalized medicine.

Serum *N*-glycan profiling has already identified glycan biomarkers associated with the aging process. Studies have shown that our glycomes change in predictable ways as we grow older or when our bodies undergo major physiological transitions, such as adolescence or menopause in women (20). Serum glycan biomarkers have also been used to estimate both chronological age (one's actual age) and biological age (a measure of how well a body is performing relative to its chronological age) (21). Because we all age at different rates due to differences in physiology and lifestyle choices, it is possible that serum glycans may someday be used to help manage each individual's personal health risks associated with the onset of age-related illnesses. Finally, glycan biomarkers have been used to estimate human age from *N*-glycan profiles obtained from dried bloodstains (22), suggesting that analysis of blood *N*-glycans may also find application in forensic medicine.

The serum *N*-glycome can also predictably change in response to disease. Correlations between changes in the composition and/or abundance of circulating *N*-glycans and many diseases have been observed. A non-exhaustive list includes numerous cancers (*e.g.*, breast, gastric, liver, lung, pancreatic, ovarian and prostate), rheumatoid arthritis, and schizophrenia (23–25). Additionally, compositional analysis of glycans has been used to identify glycan markers that correlate to good and poor prognoses in cohorts of prostate cancer patients (25) and breast cancer patients (19), suggesting that glycan profiling could someday have potential in patient stratification and disease staging. Altered *N*-glycan profiles have also been observed in serum from mammals affected by infectious diseases such endometritis (a postpartum bacterial infection in cattle; 26), *Leishmania* parasite infection in humans (27), and *Dirofilaria immitis* (heart worm) infection in dogs (28).

SERUM GLYCANS AS DIAGNOSTICS

There is hope that serum glycans will help change the way in which many diseases are diagnosed and/or monitored during treatment. In the field of genomics, there has been much discussion around the promising concept of "liquid biopsy" (a blood test) for the diagnosis of cancer without the need for invasive tissue biopsy (see ref. 29 for a recent review). In this approach, tumor cells or tumor cell DNA circulating in the bloodstream of patients with certain cancers can be isolated and subjected to genomic sequencing to identify mutations that give information as to how a tumor is changing. Serum glycomics has the potential to further expand upon the promise of the liquid biopsy concept. Both analyses seek to detect tumors earlier, characterize the type or stage of a cancer, or determine how a cancer is responding to treatment, directly from small amounts of patient's blood instead of invasive tumor biopsy.

One hope is that serum glycan biomarkers might live up to the published data and be more valuable as earlier indicators of disease than current methods. This stems from several observations about the blood glycome. First, in contrast to tumor-derived nucleic acids, glycans are very abundant molecules in serum. For example, glycan profiles can be obtained from as little as 5 microliters of serum. Secondly, changes in serum glycan structures may be more immediate indicators of a changing physiology. For example, some changes in the serum glycome composition could arise from the increasing presence of many aberrantly glycosylated proteins secreted by tumor cells. Alternatively, changing glycosylation patterns on circulating immune system proteins may indicate a systemic response to a tumor or infectious agent's presence (i.e., the types of changes currently being observed using IgG *N*-glycan profiling). In the latter case, the serum IgG glycome may act as an early sensor that a problem is developing or changing.

Some glycan biomarker studies support the notion of earlier detection of disease and physiological changes. For example, diagnostic glycan biomarkers of bovine endometritis are detectable in the maternal bloodstream as early as seven days after birth of a calf (prior to the onset of clinical symptoms) compared to diagnosis upon presentation of physical signs of infection at 2-5 weeks postpartum (26). Similarly, bovine pregnancy can be predicted from a glycan biomarker present in a cow's milk some 2-4 weeks earlier than the standard method of detection of pregnancy by rectal ultrasound (26).

The field of serum glycan biomarker discovery has made tremendous progress, however, it remains to be determined if it will inspire a new generation of clinical diagnostics. It is conceivable that serum glycan profiling could provide an orthogonal blood-based analysis to DNA sequencing, or other diagnostic methods, to increase the statistical confidence around early tumor identification or

progression. This could help inform the best choices for treatment. Such "liquid biopsy" concepts will require significantly more research in the coming years to determine their clinical feasibility.

PERSPECTIVE AND CONCLUSIONS

The past decade has seen tremendous progress in the field of glycomics. Enabled by numerous technical advances in analytical sensitivity and throughput, researchers are now starting to unlock the secrets of the mammalian glycome. Glycomics is now being used in combination with genomics, epigenomics, proteomics, lipidomics and metabolomics to provide a more holistic view of how cellular pathways function and how they change in response to disease. In the field of human serum glycomics, there has been an explosion in strong associations made between changes in glycan structure and various diseases, disease stages and traits, changes in circulating tumor cells, and age-related changes to our physiology. These correlations are revealing critical new insights into how diseases develop.

While much progress has been made, there is still a mountain to climb. The coming years will continue to see improvements to glycan analytical techniques with emphasis on analytical automation and miniaturization. Additionally, bioinformatics will play an increasing role in improving the interpretation of glycomics data. To date, the lessons learned about the human serum glycome have come largely from the analysis of *N*-glycans. The field will strive to expand serum glycomics into other classes of circulating glycans such as glycosphingolipid head group glycans (10) and *O*-glycans. Finally, it is anticipated that the first glycan-based diagnostic tests will start to emerge.

It is clear that it is an exciting time for glycomics. The field sits in a position to write an important chapter in our understanding of cell biology and positively affect the direction of human health.

Acknowledgments
The authors thank Prof. Stephen Carrington and Prof. Mark Crowe of University College Dublin, Ireland for sharing unpublished information on glycan biomarkers in cattle. The authors also thank Andrew Bertera, Alicia Bielik, Rebecca Duke, Mehul Ganatra, Ellen Guthrie, Jeremy Foster, Paula Magnelli, Beth McLeod, Stephen Shi, Barbara Taron, and Saulius Vainauskas for comments on the article.

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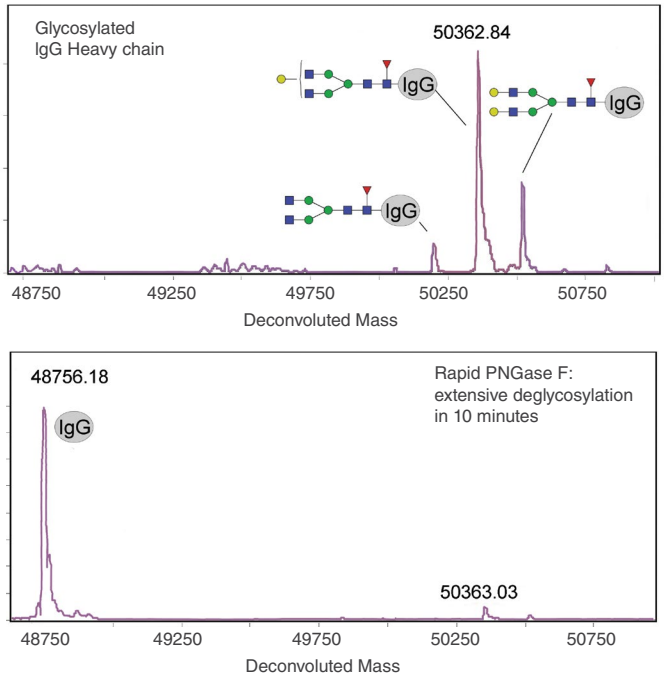
Deglycosylation in minutes for *N*-glycan analysis

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



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
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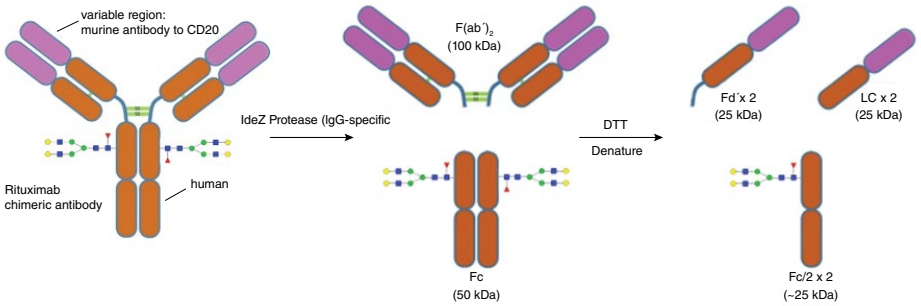
Digestion of IgG with IdeZ Protease (IgG-specific), followed by denaturation



details

DETAILED SPECIFICITY:

human IgG1, IgG3, IgG4: CPAPELLG▼GPSVF
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murine IgG2a: CPAPNLLG▼GPSVF
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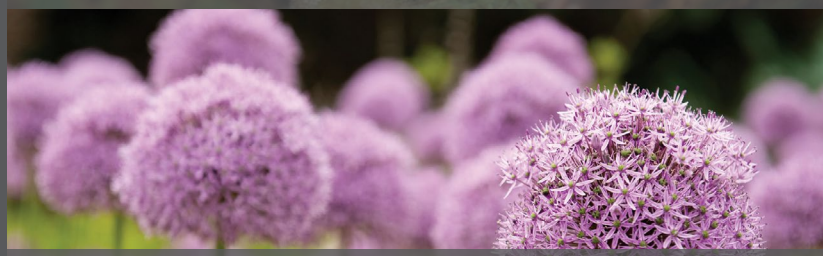

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Using a novel approach to target enrichment, the NEBNext Direct Cancer Hotspot Panel enables highly specific hybridization-based capture of 190 common cancer targets from 50 genes. The NEBNext Direct technology offers significant advantages over both traditional in-solution hybridization and multiplex PCR protocols. Target enrichment is combined with library preparation, reducing processing time and minimizing sample loss. Ideal for automation, 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.

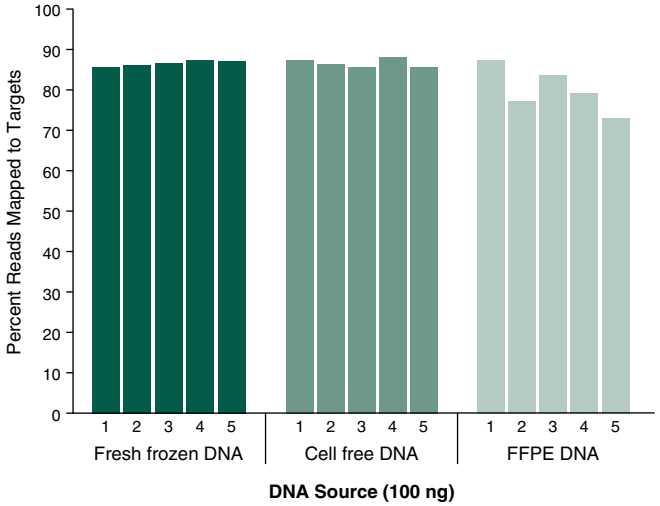
FIGURE 1: **Targets include regions covering 37 kb from the following cancer-related genes, including >18,000 cosmic features:***

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.*

FIGURE 2: **The NEBNext Direct Cancer HotSpot Panel delivers a high percentage of sequence reads mapping to targets, even with challenging sample types**

- Graph shows the percentage of aligned sequence reads that map to the targets
- 100 ng of DNA was used for each library preparation
- Reads were generated on an Illumina® MiSeq® with 2x75 bp reads, 8 bp sample ID, and 12 bp unique molecule ID
- Alignments were performed with BWA-MEM and PCR duplicates were filtered using the unique molecule IDs



benefits

- 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
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- Distinguish molecular duplicates, reducing false positive variants and **improving sensitivity**

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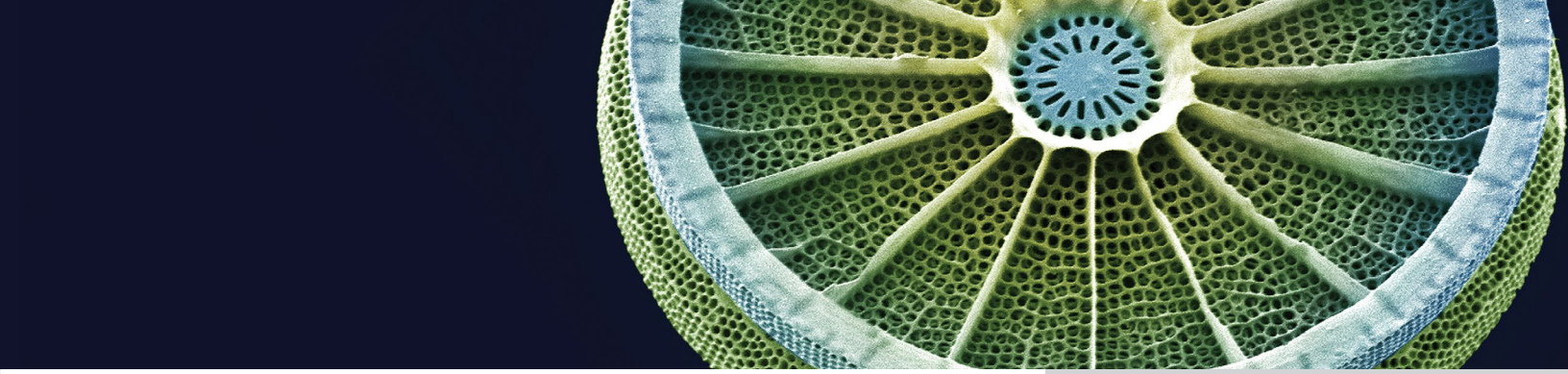
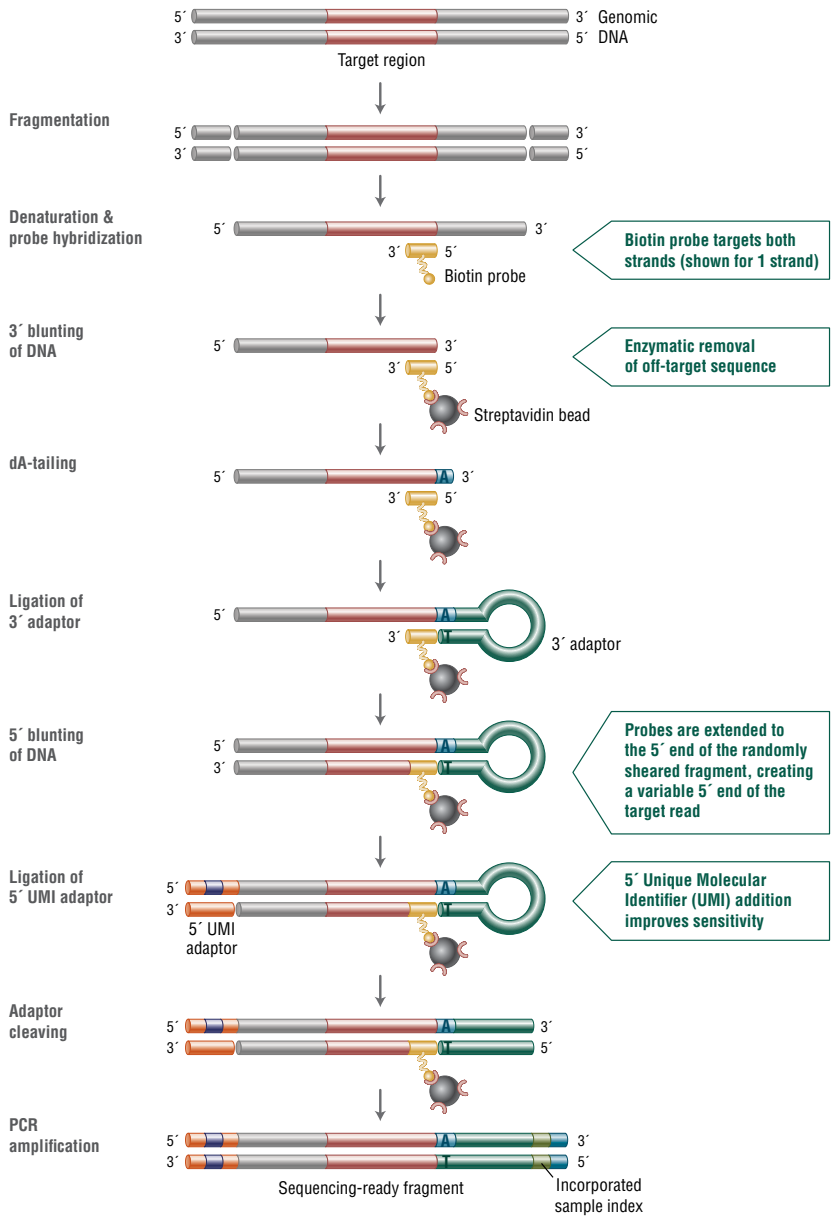


In the NEBNext Direct target enrichment approach (Figure 3), fragmented DNA is rapidly hybridized to biotinylated oligonucleotide baits that define the 3´ end of each target of interest. The bait-target hybrids are bound to streptavidin beads and any 3´ off-target sequence is removed enzymatically. This 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 3: **NEBNext Direct employs a fast hybridization-based workflow that combines capture with library preparation**



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

ORDERING INFORMATION:

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

Proteomics: Fast and Efficient Antibody Deglycosylation using Rapid PNGase F

Paula Magnelli and Coleen McClung, New England Biolabs, Inc.

INTRODUCTION

Innovations in process development and manufacturing of therapeutic monoclonal antibodies have been critical for their clinical and economic success. Along with these advances, methods for quality control are constantly evolving to guarantee the safety and effectiveness of these drugs. During development and production, mass spectrometry (MS) "top-down" methods (e.g., analysis of intact or reduced proteins) complemented by "bottom-up" approaches (e.g. peptide and glycopeptide mapping) are used to verify structural attributes of monoclonal antibodies.

Removal of *N*-glycans with PNGase F simplifies MS analysis. However, complete deglycosylation of IgG typically requires lengthy protein denaturation and PNGase F incubation steps, because for a given site (e.g., Fc conserved glycosylation) some *N*-glycans are easier to remove than others. Moreover, non-conserved *N*-glycan sites (e.g., Fab) can be particularly resistant to PNGase F, and are only fully removed after several hours of incubation. To address these limitations, NEB has developed Rapid PNGase F (NEB #P0710). This novel product completely removes *N*-glycans from antibodies in just minutes and is compatible with high throughput proteomics applications.

We demonstrate that all targets were deglycosylated extensively and without bias in less than 10 minutes. Moreover, Rapid PNGase F efficiently removes *N*-glycans from antibodies with additional glycosylation sites.

RESULTS

Incomplete *N*-glycan removal is a concern, because some species might be removed faster than others, resulting in a biased composition. Following a traditional protocol, overnight treatment is often required to achieve complete conversion of IgGs to their deglycosylated form (not shown). In contrast, glycan removal is complete with a 5 minute incubation using Rapid PNGase F (Figures 1,2).

CONCLUDING REMARKS

NEB's Rapid PNGase F reagent can achieve complete and unbiased removal of *N*-glycans from antibodies, a requisite for accurate measurement of critical quality attributes by mass spectrometry. This reaction, which occurs in solution and requires minimal setup, is remarkably fast, amenable to high throughput and automation, and is compatible with downstream proteomics analysis by LC/ESI-MS.

NEB's Rapid PNGase F portfolio has expanded to include Rapid PNGase F (non-reducing format) (NEB #P0711). For more information, see page 5.

Interested in learning more?

Visit **www.neb.com/P0710** to **download the full application note**, which includes data and tables not shown.

FIGURE 1: **Deglycosylation with Rapid PNGase F**

Antibodies treated for 5 minutes with Rapid PNGase F (RP), in comparison with an untreated control (C), and with a standard denaturing reaction with SDS (std). Mouse IgG2, rituximab, and etanercept were efficiently deglycosylated in 5 minutes. Cetuximab (which carries Fab N-glycans) required a 2 step protocol (RP2): compare with the partial shift down observed with a one step (RP1).

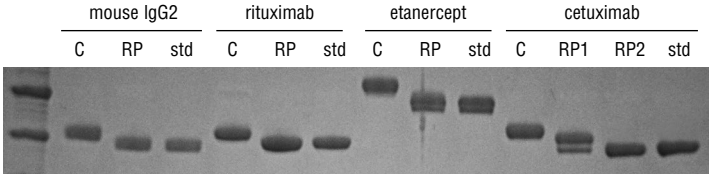
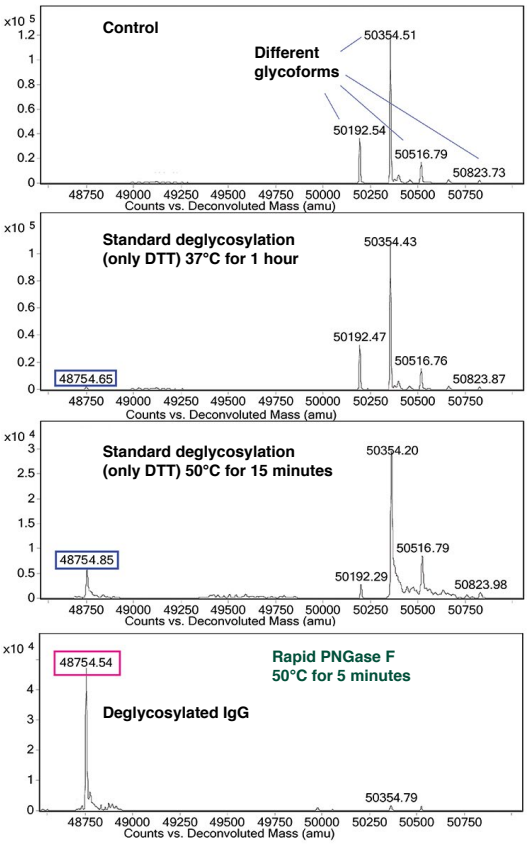


FIGURE 2: **The ESI-TOF deconvoluted spectra of a mouse IgG2 sample before (control) and after several deglycosylation treatments**



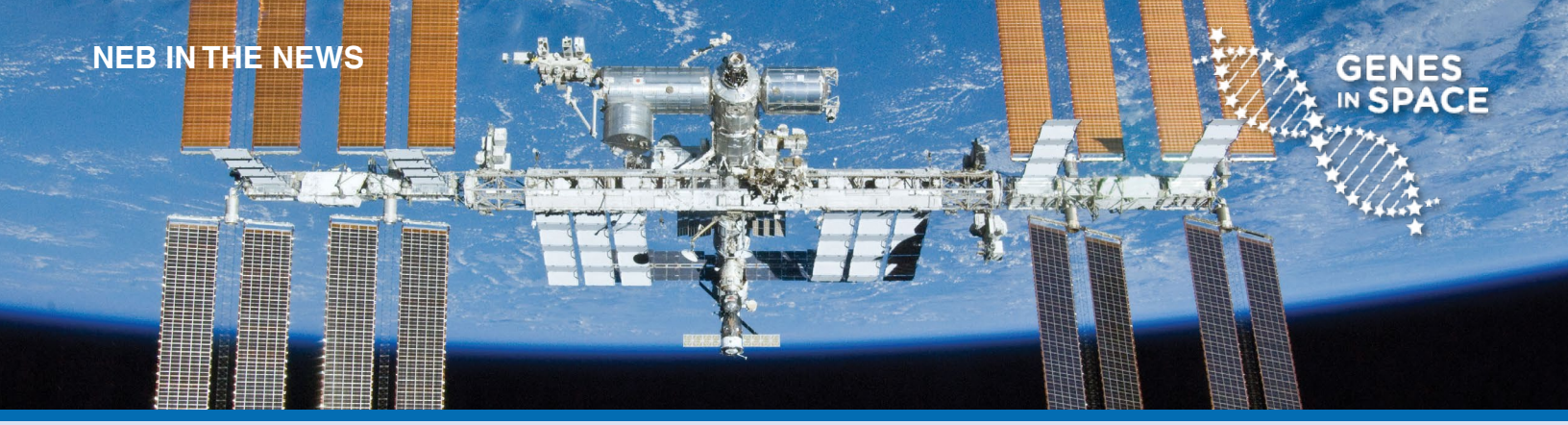
A standard (only DTT) reaction incubated with PNGase F for 1 hour yields virtually no deglycosylated product (deconvoluted mass 48754).

After incubation for 15 min at 50°C, more product is observed. However, the glycosylated precursors (deconvoluted mass 50192, 50354, 50516, and 50823) are still the most abundant peaks.

A sample treated with Rapid PNGase F for 5 min at 50°C has virtually no glycosylated species left. The peaks from the original material are transformed into a deglycosylated single peak at 48754.

ORDERING INFORMATION:

PRODUCT	NEB #	SIZE
Rapid PNGase F	P0710S	50 rxns
PNGase F (Glycerol-free), Recombinant	P0709S/L	15,000/75,000 units
Rapid PNGase F (non-reducing format)	P0711S	50 rxns
Rapid PNGase F Antibody Standard	P6043S	250 µg
IdeZ Protease (IgG-specific)	P0770S	4,000 units
COMPANION PRODUCTS		
Blue Loading Buffer Pack	B7703S	8 ml
Unstained Protein Standard, Broad Range (10-200 kDa)	P7704S/L	150/750 gel lanes



First successful PCR experiment performed in space

Genes in Space competition's winning experiment conducted aboard the International Space Station and analyzed at New England Biolabs

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.

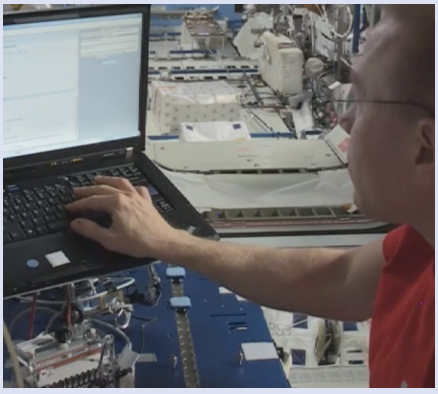
GIS, a contest conceived of by Boeing and Amplyus and supported by Math for America, the Center for the Advancement of Science in Space (CASIS), FedEx and New England Biolabs, aims to support young innovators in bridging the physical and biological sciences. Boguraev's 2015 winning entry built upon previous 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).

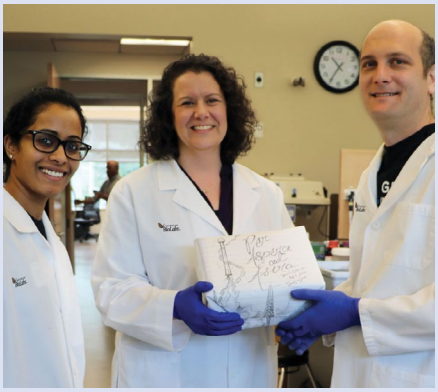
The reactions and miniPCR machine were launched into space aboard the SpaceX CRS-8 Dragon Resupply Mission on April 8th from the Kennedy Space Center in Cape Canaveral, FL with Boguraev, her family and members of the miniPCR, Boeing, and NEB teams in attendance.

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 will be 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.

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





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.

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