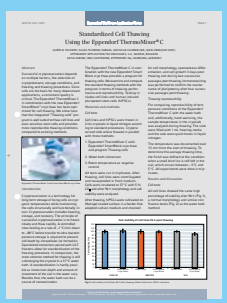
BIO NEWS

No. 55 – 2021

eppendorf

Sustainability in the Lab Concerns Us All

> Expand your bioprocess with ease
> Accelerate your research with Centrifuge 5910 Ri
> A new era for pipetting



Application Notes

Standardized cell thawing using the Eppendorf ThermoMixer® C · PCR optimization for single-molecule experiments using the Mastercycler® X50 2D-Gradient · etc.

Welcome

to a new BioNews issue. The topic of sustainability has been gaining enormous momentum over the past years, in the private as well as the scientific sphere. Our leading article informs you about measures already implemented at Eppendorf, our holistic approach as well as our future commitment to this multifaceted and challenging topic (p. 4–5).

A highlight this year is the launch of our new Centrifuge 5910 Ri, which enables you to accelerate your research and achieve results more quickly. During the development of the touch user interface, we focused on extremely simple operation, which was verified through multiple customer tests (p. 6–7).

Long, long ago! In 1958, a patent application was filed for a "device for the fast and exact pipetting of small liquid volumes", and 60 years ago, Eppendorf brought it to market as the first industrially manufactured piston-stroke pipette – the beginning of a great success story (p. 10). And even in today's digital age, we are doing our best to equip our users for the demands of the laboratory of the future. The newly launched VisioNize® pipette manager is our first step towards comprehensive digital liquid handling (p. 11).

Also in this issue are the new Eppendorf Conical Tube SnapTec® 50 (p. 8), solutions for expanding your bioprocessing systems (p. 9), and much more. Last, but not least: as always, four compact Application Notes and our popular prize competition.

Your Eppendorf BioNews Team

PS: Would you like to share your thoughts with us regarding BioNews? Do you have ideas or requests? If so, please send us an e-mail to bionews@eppendorf.de

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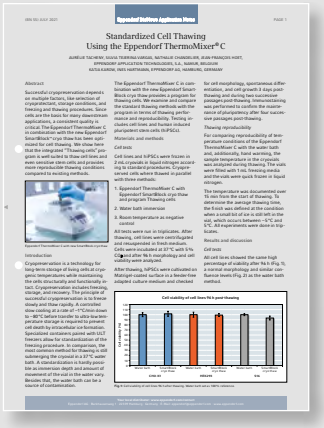
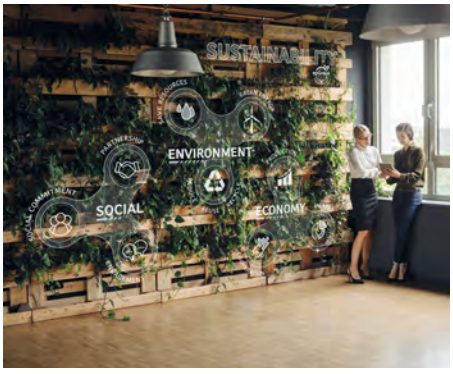
Eppendorf BioNews Application Notes


Standardized Cell Thawing
Using the Eppendorf ThermoMixer® C

JAN-HENDRIK BEBERMEIER, EPPENDORF AG

Sustainability in the Lab Concerns Us All

Whenever the topic of sustainability comes up in the scientific community, there are many issues to consider. Energy-guzzling ULT freezers, containers with biological, chemical, or radioactive waste, large bags of used plastic tips and tubes are just some examples. In addition, lab employees are facing packaging materials, noisy instruments or devices with unergonomic handling, 24/7 science jobs, fixed-term job contracts, and limited budgets. The topic of sustainability in the laboratory has many facets.



Sustainability is becoming more and more important in public life. Wherever we interact in our personal area, there is a growing number of sustainability topics. The same holds true for our interaction with you, our customers in the labs worldwide.

Whereas just a few years ago the topic of sustainability was mainly driven by dedicated employees in academic institutes and research-based companies, there has been a noticeable change for some time now. Purchasing departments as well as procurement systems request information and we see a growing interest in Eppendorf and our approach to sustainability.

Retrospect

In 2009, our centrifuge team started a green sustainability project. We found that a new centrifuge provided a far higher energy saving than expected. To obtain reliable data, standardized runs were developed, and measurements were performed. We were proud of the results. But the concept came too early: energy efficiency in the laboratory was not yet the focus at that time.

Change is here

Meanwhile, more and more customers expect sustainability facts about our products. For our freezer team, this has been daily business for years as lower power consumption and green cooling liquids are of high interest. In respect to consumables, the new disposable racks for pipette tips enable material savings of up to 35 %. Internal projects for further improvements and recycling of materials have started.

How far can we go?

Despite all needs for improved sustainability, there is a special situation in the lab: the safety of the employees, combined with safety for the samples, is the focus, embraced by sustainable progress. Single-use tubes and tips are mandatory in many labs due to purity and risk of samples; however, this generates a lot of plastic waste.

New disposable racks for pipette tips enable material savings of up to 35 %

CryoCube® ULT freezer, with touchscreen interface, green cooling liquids, and air-cooling

Despite good ideas, this plastic waste still cannot be recycled efficiently, e.g. due to regulatory requirements for biological, chemical, or radioactive hazards.

The safety of valuable samples is always our top priority. For example, the set temperature for centrifuging sensitive RNA samples must be maintained accurately throughout the process to ensure data reliability and reproducibility. Slow recovery of an ULT freezer after door opening may save energy but you are putting 50,000 high-value samples at risk.

The use of new, innovative technologies can optimize both resource consumption during production and power consumption during use. Even small improvements optimize the longevity of devices and thus contribute to greater sustainability.

Beyond green

The green idea may be the most familiar, but in fact sustainability encompasses many environmental, social, and economic factors.

At Eppendorf, we consider the entire supply chain and define our impact on sustainability in areas such as climate change and the use of natural resources, social compliance, and human well-being, as well as with regard to data security and our role as responsible citizens in society.

This consideration begins before the product; it encompasses Eppendorf as an employer as well as a customer vis-à-vis our suppliers. Thus, our own Code of Conduct sets the bar for both employees and our supply chain. Our social behavior includes treating each other with respect, respecting the intellectual property of others, sticking to facts, and making ourselves reliable partners for our counterparts. These expectations are clearly defined and subject to continuous improvement and learning.

The optimization of our product design for improved ergonomic handling began in the 1970s. Today, the PhysioCare Concept® implemented in all Eppendorf products supports the well-being of our users.

Sustainability: an on-going journey

Science is based on facts. Statements such as "Our product is more sustainable compared to others" are questioned by researchers in many ways. Analyzing and improving sustainability issues requires knowledge and intensive work. None of the sustainability challenges can be solved quickly or easily. But they all require manufacturers and customers to listen to each other and work together.

This work is never over, but an on-going journey in which we communicate in constant dialogue with our stakeholders. We are researching new technologies, alternative materials and concepts. Each of these changes has the potential to contribute to progress in sustainability.

More information at
www.eppendorf.com/sustainability

News

Who Will Win in 2021?

For the 2021 International Freezer Challenge competition, the International Institute for Sustainable Laboratories (I2SL) and My Green Lab have partnered again to reward the best concept to improve cold storage.

freezer challenge.

Users of ULT freezers were invited to compete with colleagues from around the globe. Participants can earn points by taking actions from Good Management Practices, Temperature Tuning, and other areas, as well as for sharing information about best practices.

For Eppendorf, sustainability is paramount. Consequently, we are again a proud sponsor of this Challenge.

Freezer Challenge accomplishments 2019 and 2020

2019: 41 organizations with 400 labs worldwide; savings of ~2.4 million kWh/year, equivalent to reducing carbon emissions by ~1,700 tons.

2020: 88 organizations with 218 labs worldwide; savings of ~3.2 million kWh/year, equivalent to reducing carbon emissions by ~2,260 tons.

And 2021?

The results for 2021 had not yet been determined at the time of going to press. The 2021 Challenge ended on July 1, 2021. You can find out which teams won and how much energy was saved from October 2021 at
https://www.freezerchallenge.org/

NICOLE SEELIGMÜLLER, EPPENDORF AG

Accelerate Your Research: Centrifuge 5910 Ri

Large benchtop centrifuges are often shared by a great number of users, each with different applications and requirements. As a result, rotors, buckets, or adapters have to be exchanged, and parameters have to be reset or checked before each run. With the new Eppendorf Touch-Interface Centrifuge 5910 Ri, these time-consuming and error-prone tasks are finally a thing of the past. It was developed to simplify your daily centrifugation tasks so that you can concentrate on what really matters – your results.

Centrifugation – more than just "spinning"

You are most likely familiar with this – or similar – situations in the lab: in the course of a series of experiments, many tasks are carried out in parallel, and one challenge is that laboratory equipment such as multipurpose centrifuges must be shared with other colleagues. As a result, the rotor, buckets, or adapters may have to be exchanged and new run parameters set for one's own vessel types.

Stressful situations as well as inexperience may open the door for inadvertent mistakes when it comes to correctly selecting rpm or *rcf*, adjusting braking ramps or employing the "at set rpm" function. Errors of this kind often result in the need for repeating the run, and in the worst case, the sample may be lost.

Development focused on the user

During the development of the user interface of the Centrifuge 5910 Ri, attention was paid to extremely simple operation, which has been tested by multiple customer tests. In a final customer test, we also determined the user-friendliness by means of the System Usability Scale (SUS): the final score of 93 is significantly higher than the average SUS score of 68 (https://measuringu.com/sus).

Everyday use is thus as simple as it can be. On the Home screen, the user finds several options to operate the instrument: the unique favorites function, as well as the program function and a user management are the most important ones.

Using the favorites, the four most frequently-used values for the parameters time, speed, and temperature can be saved and selected with a click. A change of the parameters is thus completed within three clicks, which greatly reduces the risk of error.

The program function offers an even faster usage: to create a program, the desired parameters, including settings for braking ramps and "at set rpm", are selected and saved under the desired program name, for example: "Pelleting of *E. coli*". By calling up the program, parameter setting is done in seconds, inadvertent errors are excluded, and good reproducibility of the results is given. Even users who have never worked with the instrument before will be able to operate the interface immediately and intuitively.

Undefeated versatility

Our test customers were not only impressed by the above-average user-friendliness of the instrument; in addition to its appealing design, many customers remarked on the very quiet operation and

Program function of the touch user interface of Centrifuge 5910 Ri

the very large selection of 10 different rotors, as well as the unique universal concept for the main swing-bucket rotor. The latter allows centrifugation of conical tubes, plates, and 250 mL bottles without the need for exchanging buckets or adapters. Thus, the Centrifuge 5910 Ri considerably simplifies your daily laboratory routine.

Fit for the future

Last but not least, Centrifuge 5910 Ri supports you on your way to the paperless lab of the future. Documentation of all runs performed, including any parameter changes during the run, can be exported as PDF or CSV file via USB and documented in the eLabJournal® software by Eppendorf, a fully integrated solution for the management of data, samples, and protocols in your laboratory. This way, you can easily optimize your laboratory workflow by documenting and searching research data, tracking sample collections, managing protocols or SOPs, and centralizing the ordering of laboratory supplies.

Access to the electronic lab book is web-based, so you can view your data at any time, from any location and any device, using the app. Do you work in a regulated environment? With eLabJournal, your laboratory is able to follow ISO regulatory guidelines and work in compliance with Good Laboratory Practice (GLP).

Experiments may be signed and countersigned electronically in accordance with FDA 21 CFR part 11, protecting them from any modification.

Management of data, samples, and protocols with eLabJournal

More information on the Centrifuge 5910 Ri is available at: **www.eppendorf.com/accelerate-your-research**

More information on eLabJournal is available at: **www.eLabJournal.com**

Centrifuge 5910 Ri in action:

Tip

Proud Owner of an Eppendorf Product?

Eppendorf is a matter of the heart. And matters of the heart stay with you – for a lifetime. So do our products.

Have you recently bought an Eppendorf product? By taking just a few minutes to register your products, you can improve your customer experience with Eppendorf. It's as simple as it sounds. You can either register your products via website or the Eppendorf App and enjoy exclusive benefits.

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Check out the website for participating products **www.eppendorf.com/productregistration**

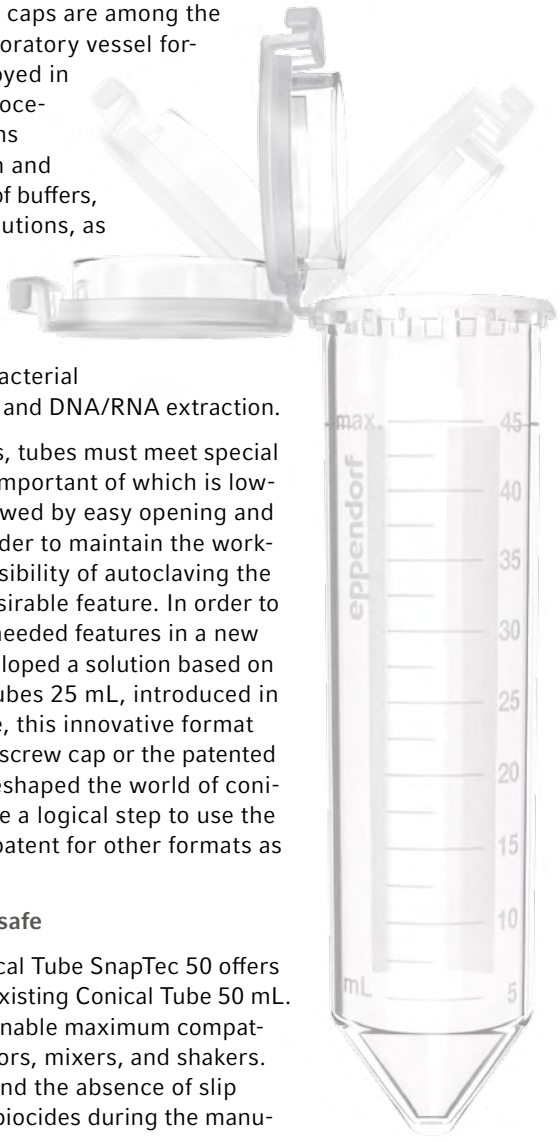
*Information on participating products and countries, incl. the terms and conditions of the raffle available at www.eppendorf.com/productregistration.
All promotions end on December 31, 2021.

BRIGITTE KLOSE, EPPENDORF AG

"Done in a Snap": SnapTec® Makes the Difference

In this article we would like to introduce a new member of the Eppendorf Conical Tubes family. The new Eppendorf Conical Tube SnapTec 50 mL features the same patented* SnapTec cap as the Eppendorf Conical Tubes 25 mL which were launched in 2019. The new tube variant has the same dimensions as a 50 mL screw cap conical tube and is ideally suited for safe, optimized, and faster handling of sample solutions up to 45 mL.

Conical tubes with screw caps are among the most commonly used laboratory vessel formats, and they are employed in a variety of laboratory procedures. Typical applications include sample collection and preparation, preparation of buffers, aliquots, and working solutions, as well as centrifugation, long-term sample storage and sample transport, predominantly for the purpose of cell and bacterial culture, as well as protein and DNA/RNA extraction.



In these application areas, tubes must meet special requirements, the most important of which is low-contamination work followed by easy opening and closing of the tubes in order to maintain the workflow. In addition, the possibility of autoclaving the tubes would also be a desirable feature. In order to combine all these much-needed features in a new tube, Eppendorf has developed a solution based on the Eppendorf Conical Tubes 25 mL, introduced in 2019. Within a short time, this innovative format (optionally available with screw cap or the patented SnapTec snap cap) has reshaped the world of conical tubes. It was therefore a logical step to use the benefits of our SnapTec patent for other formats as well.

Compatible, innovative, safe

The new Eppendorf Conical Tube SnapTec 50 offers essential benefits of our existing Conical Tube 50 mL. The precise dimensions enable maximum compatibility with centrifuge rotors, mixers, and shakers. Premium raw materials and the absence of slip agents, plasticizers, and biocides during the manufacturing process ensure highest sample integrity, high *g*-Safe® centrifugation stability, and optimal sample and pellet visibility.

The SnapTec cap is the decisive, innovative detail. It allows opening and closing of the tube with one hand to ensure a smooth workflow, especially in routine and multi-step protocols. The lid is securely connected to the tube, and it therefore never comes into contact with the laboratory bench. The risk of cross-contamination is reduced, and accidental confusion with other lids is avoided. Furthermore, the ability to autoclave the SnapTec 50 tube opens up new application possibilities.

"45 in 50"

Even though, for technical reasons, the guaranteed nominal volume of the Eppendorf Conical Tube SnapTec 50 is "only" 45 mL, this covers more than 75 % of all main applications. The tube is available in Eppendorf Quality™, PCR clean and Sterile (free from pyrogens, DNase, RNase, human and bacterial DNA).

More information at
www.eppendorf.com/SnapTec50

*US Patent 8,540,948

Standardized Cell Thawing Using the Eppendorf ThermoMixer® C

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Abstract

Successful cryopreservation depends on multiple factors, like selection of cryoprotectant, storage conditions, and freezing and thawing procedures. Since cells are the basis for many downstream applications, a consistent quality is critical. The Eppendorf ThermoMixer C in combination with the new Eppendorf SmartBlock™ cryo thaw has been optimized for cell thawing. We show here that the integrated "Thawing cells" program is well suited to thaw cell lines and even sensitive stem cells and provides more reproducible thawing conditions compared to existing methods.

Eppendorf ThermoMixer C with new SmartBlock cryo thaw

Introduction

Cryopreservation is a technology for long-term storage of living cells at cryogenic temperatures while maintaining the cells structurally and functionally intact. Cryopreservation includes freezing, storage, and recovery. The principle of successful cryopreservation is to freeze slowly and thaw rapidly. A controlled slow cooling at a rate of –1 °C/min down to –80 °C before transfer to ultra-low temperature storage is required to prevent cell death by intracellular ice formation. Specialized containers paired with ULT freezers allow for standardization of the freezing procedure. In comparison, the most common method for thawing is still submerging the cryovial in a 37 °C water bath. A standardization is hardly possible as immersion depth and amount of movement of the vial in the water vary. Besides that, the water bath can be a source of contamination.

The Eppendorf ThermoMixer C in combination with the new Eppendorf SmartBlock cryo thaw provides a program for thawing cells. We examine and compare the standard thawing methods with the program in terms of thawing performance and reproducibility. Testing includes cell lines and human induced pluripotent stem cells (hiPSCs).

Materials and methods

Cell tests

Cell lines and hiPSCs were frozen in 2 mL cryovials in liquid nitrogen according to standard procedures. Cryopreserved cells where thawed in parallel with three methods:

1. Eppendorf ThermoMixer C with Eppendorf SmartBlock cryo thaw and program Thawing cells
2. Water bath immersion
3. Room temperature as negative control

All tests were run in triplicates. After thawing, cell lines were centrifugated and resuspended in fresh medium. Cells were incubated at 37 °C with 5 % CO_2 and after 96 h morphology and cell viability were analyzed.

After thawing, hiPSCs were cultivated on Matrigel-coated surface in a feeder-free adapted culture medium and checked for cell morphology, spontaneous differentiation, and cell growth 3 days post-thawing and during two successive passages post-thawing. Immunostaining was performed to confirm the maintenance of pluripotency after four successive passages post-thawing.

Thawing reproducibility

For comparing reproducibility of temperature conditions of the Eppendorf ThermoMixer C with the water bath and, additionally, hand warming, the sample temperature in the cryovials was analyzed during thawing. The vials were filled with 1 mL freezing media and the vials were quick frozen in liquid nitrogen.

The temperature was documented over 15 min from the start of thawing. To determine the average thawing time, the finish was defined at the condition when a small bit of ice is still left in the vial, which occurs between –5 °C and 5 °C. All experiments were done in triplicates.

Results and discussion

Cell tests

All cell lines showed the same high percentage of viability after 96 h (Fig. 1), a normal morphology and similar confluence levels (Fig. 2) as the water bath method.

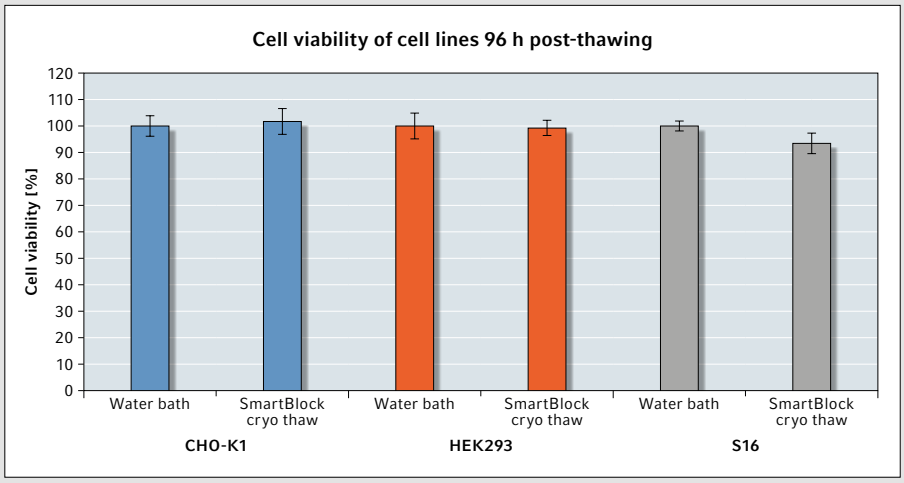

Fig. 1: Cell viability of cell lines 96 h after thawing. Water bath set as 100 % reference.

Standardized Cell Thawing Using the Eppendorf ThermoMixer® C

The stem cells showed the typical and expected hiPSC morphology after 24 h. No abnormalities in shape or densities could be observed. 72 h post-thawing, cells thawed with the Eppendorf ThermoMixer C formed a confluent monolayer as well as the cells thawed with the water bath. No spontaneous differentiation or spontaneous detachment was observed during all conditions. Immunostaining results after four successive passages confirmed the maintenance of pluripotency (for figures for stem cells please refer to the original Application Note*). All cells thawed with the Eppendorf ThermoMixer C showed similar fast recoveries, cell viability, and growth patterns as cells thawed with the water bath. The results confirm that the Thawing cells program is well suited to thaw cells.

Thawing reproducibility

The in-vial temperature profile of the samples thawed with the Thawing cells program showed the most consistent and reproducible temperature conditions, whereas thawing with the water bath and hand thawing were far less reproducible. Different handling procedures within the water bath, immersing the vial with or without moving, or using a floater, had an additional negative impact on thawing reproducibility. Hand thawing also resulted in less reproducible profiles, as hand size and hand temperature vary between persons. All methods resulted in thawing times ≤5 min with the water bath being the fastest, Hand 2 being the slowest.

The Eppendorf SmartBlock cryo thaw provides the most reproducible thawing conditions and still thaws quickly in <5 min. The Thawing cells program is pre-set for 3 min and 3–4 min worked for all cells tested (Fig. 3).

Conclusion

The results show that the Eppendorf ThermoMixer C is well suited to thaw cells with the same high retrieval rate as a water bath. The handling is easy with the pre-set program and with the interchangeable Eppendorf SmartBlock system the device allows integration in flexible workflows.

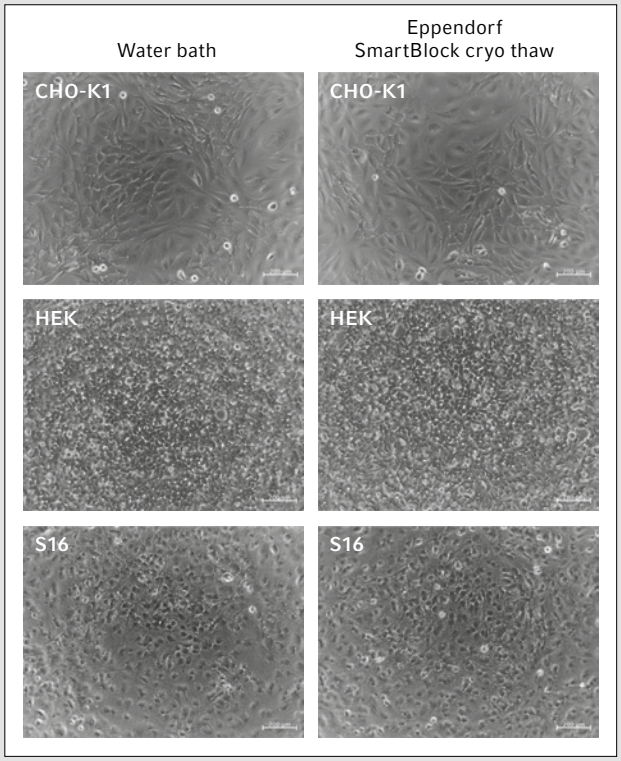

Fig. 2: Microscopic analysis of cell lines 96 h after thawing. Cells show normal morphology and similar confluences (magnification 100x).

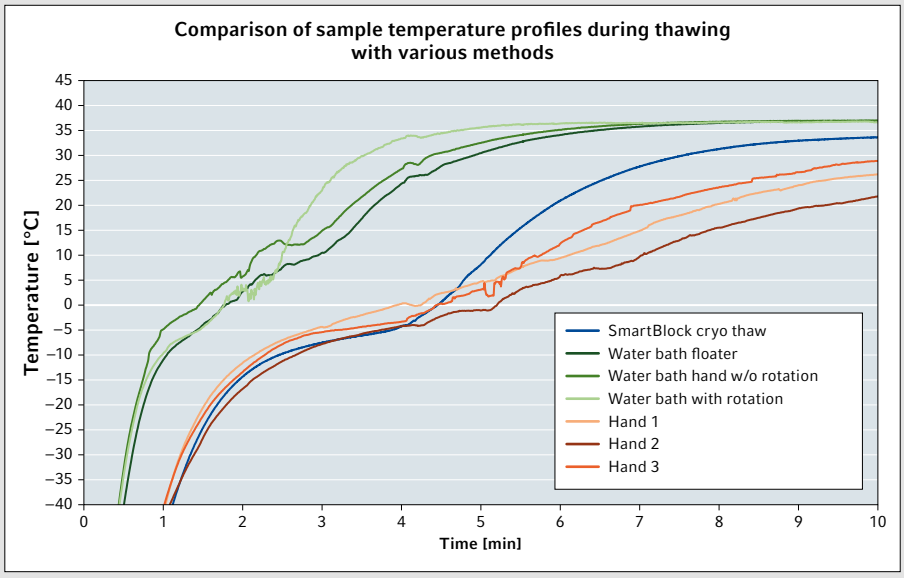

Fig. 3: Comparison of sample temperature profiles with water bath, ThermoMixer C, and hand warming. Each curve represents a mean of three vials.

*The full Application Note 437 can be downloaded at: http://eppendorf.global/IDT

Minimizing Shear Stress when Using Liquid Handling Systems

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Abstract

Shear stress is a factor which can heavily influence analysis results, e.g. in cell culture or when working with genomic DNA. Within this study we have explored the impact of pipetting with different manufacturers standard and wide-bore tips on the integrity of HEK 293 cells. We found the cell proliferation to be slightly decreased after five successive pipetting steps using an electronic Eppendorf Xplorer® plus pipette set to maximum speed. Thereby the difference between standard and wide-bore tips was found to be small, only.

Introduction

Shear stress develops when a liquid is moved, for example, within a tube. Due to its adhesion forces, the liquid velocity at the tube wall is zero, but it is highest in the middle of the tube. This velocity gradient leads to a stress for the liquid as well as for the functional units (nucleic acids, cells, organisms) it is moving: the so-called shear stress.

The creation of shear stress as well as its impact depends on different factors:

a) the sensitivity of the functional unit, for example, a cell. Some classical factors influencing the cell's sensitivity to shear stress are cell type, cell size, and pretreatment;

b) the medium as it influences the shear stress by its viscosity, density, and the cell density;

c) the liquid handling method as it defines the duration of the exposure to shear stress and/or the frequency of exposure to shear stress (e.g. number of pipetting steps);

d) finally, the liquid handling system influences the shear stress especially by the flow velocity and its tip's orifice diameter and shape.

For persons pipetting liquids which contain functional units it is important to consider the possible influence of the shear stress on these functional units. Genomic DNA, for example, is known to break under shear stress. And if stem cells become exposed to shear stress, it may initiate their spontaneous differentiation into a variety of cell types representing all three germ layers.

Although the influence of shear stress on different functional units is widely proven, only few information exists helping researchers to estimate the shear stress caused by manual liquid handling systems. Within a broad study we addressed this topic and determined the reaction of different cell types transferred by different liquid handling systems. In the following we focus on pipetting.

Material and methods

HEK 293 cells (1 mL cell suspension) were pipetted in five successive pipetting steps using an electronic 50–1000 µL Xplorer plus pipette set to maximum piston speed. The influence of shearing was determined by cell proliferation (in serum-free EMEM medium) and increase of DNA release. The tip orifice estimation was performed using testing pins. The results for only one competitor are shown here. For further information please refer to Application Note 442.*

Results and discussion

At a given piston speed, different pipette tip orifice diameters lead to different flow velocities. As the orifice diameter of the tested pipette tips ranged from 0.68 mm (standard tip) to 1.89 mm (wide-bore tip) the mean flow velocity ranged from 3.1 m/s (standard tip) to 0.4 m/s (wide-bore tip). Due to the existence of a velocity gradient over the orifice radius the factual flow velocity at certain locations is higher. But for an estimation of the liquid's velocity the mean flow velocity can be used.

Table 1: Mean orifice diameter, volume flow, and mean flow velocity of Eppendorf and competitor C tips. The "w" denotes a wide-bore variant (n=5).

Competitor	Mean orifice diameter (mm)	Volume flow (mL/s)	Mean flow velocity at orifice (m/s)
Eppendorf	0.83	1.11	2.1
C	0.68	1.11	3.1
Cw	1.89	1.11	0.4

Pipetting HEK 293 cells using the electronic Xplorer plus pipette at its maximum speed led to a slightly decreased proliferation after two days of cell culture compared to control (Fig. 1). This finding was accompanied by a slight increase in DNA release supporting the results for cell proliferation (Fig. 2).

Interestingly, the difference in cell proliferation between the wide-bore and the standard tips was found to be only small. It was too weak to be correlated by a significant increase in DNA release. In cell culture (and when handling genomic DNA) wide-bore tips are often used to decrease the shear stress. Our results suggest that the topic is much more complex to become focused on the orifice diameter, only.

Of course, a small tip orifice increases the mean flow velocity (Table 1). But if the pipette's piston speed – and thus the flow velocity – is kept small enough the influence of the orifice diameter is also small. Spoken with other words: even the maximum pipetting speed of the Xplorer plus pipette is slow enough to keep the influence of the factor "orifice diameter" on HEK 293 cells small.

Instead, the general slight decrease in cell proliferation was created by a methodical influence: the five successive pipetting steps.

Minimizing Shear Stress when Using Liquid Handling Systems

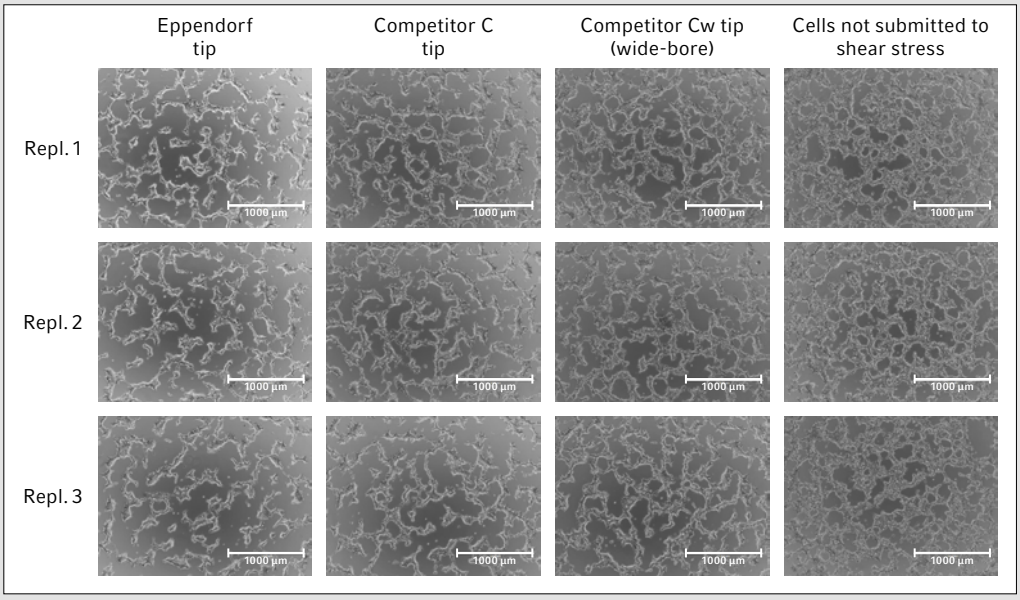

Fig. 1: Cell proliferation of HEK 293 cells according to the pipette tip model (Eppendorf and competitor C) associated to the Xplorer plus set to maximal piston speed.

The cells have been stressed by 5 successive pipettings. The "w" denotes a wide-bore variant.

Magnification: 4x.

The influence of duration of shear stress on the integrity of cells and tissues is discussed controversially in the scientific literature [1, 2, 3] and may vary between the different functional units. For our system with HEK 293 cells, we conclude that its influence was greater than the influence by the orifice diameters. Please note that with other electronic pipettes and their different speed range, the picture can be different. Especially with the faster ones the use of wide-bore tips may be indicated to buffer the higher mean flow velocity. From our findings we can derive the following recommendations for the pipetting of cell cultures in general:

> Always work with a slow piston speed as this positively influences the mean flow velocity at the tip's orifice.

> Decrease the number of pipetting steps as they influence the integrity of your cells.

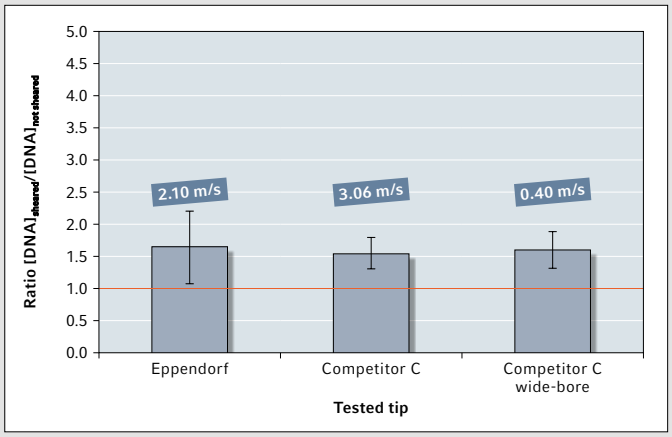

Fig. 2: DNA release of HEK 293 cells in culture according to the consumable model (Eppendorf and competitor C) associated to the Xplorer plus pipette at maximal piston speed. The cells have been stressed by 5 successive pipettings. The DNA release is represented by the ratio of the mean DNA concentration in the supernatant of sheared cells divided by the mean DNA concentration in the supernatant of not sheared cells (n=3). The "w" denotes a wide-bore variant. The mean flow velocity at the orifice is given by the figures above the bars.

Literature

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*Please see Application Note 442 for deeper insights into the interesting topic of shear stress at www.eppendorf.com/appnote442 (in preparation)

PCR Optimization for Single-Molecule Experiments Using the Mastercycler® X50 2D-Gradient

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SCHOOL OF MEDICINE AND SURGERY, UNIVERSITY OF MILAN-BICOCCA, ITALY

Abstract

We illustrated the powerful capabilities of the Mastercycler X50 2D-gradient in producing suitable DNA filaments for single-molecule applications. Our results indicate that its characteristic of simultaneously modulate both the annealing temperature (T_A) and the denaturation temperature (T_D) results in a very potent tool for GC-rich template applications. This innovation in gradient technology allows the setting-up of the best conditions to obtain an efficient amplification of a long DNA sequence with a high GC-content in a fast and convenient way.

Introduction

Single-molecule techniques such as Magnetic Tweezers (MT), Optical Tweezers (OT), and Atomic Force Microscopy (AFM) [1, 2, 3] are the methods of choice to study the nanomechanical properties of nucleic acids *in vitro*. The investigation on DNA properties, especially based on OT and MT, requires DNA molecules with stringent and peculiar characteristics, i.e. sufficiently long (2,000–20,000 base pairs) and nicked-free. However, for the study of DNA denaturation and supercoiling in a single molecule, the imposition of a torsional constraints over a DNA filament is mandatory, and the presence of nicks in the sequence, as a result of the gel extraction procedure, would impede the torsion. Hence, the only way to obtain an intact double helix is via PCR.

However, PCR amplification, especially of long sequences, has its own challenges. Ideal results include sufficiently high yield and low amount of shorter or non-specific amplification products. To reach these optimal conditions, the T_A and time must be modulated. Moreover, the local characteristics of DNA (e.g. GC-content, distribution frequency) were reported to be associated with DNA metabolism and DNA-associated pathologies [4][5].

However, high GC-content (>70 %) regions in the DNA added another dimension to the challenge in amplifying long DNA sequence. GC-rich DNA sequences have a higher melting temperature. This implies the need for simultaneous modulation of T_D on top of the previous need in modulating T_A, to find the best conditions for amplification. A classical thermal cycler could only independently vary the T_A and T_D one at a time. This translates to an overall large expenditure of both time and resources.

Material and methods

To obtain a 6,098 bp amplicon with a 78 % GC-content, the pSC-77 % GC_ΔAsc vector [5] was used as a template for the PCR. The PCR was performed using the Mastercycler X50 with the 2D-gradient function in a final volume of 15 µL using cycling conditions specified in Table 1.

Following amplification, the PCR products were subjected to an agarose gel electrophoresis (with 0.5 g/mL ethidium bromide) and visualized using the Amersham® Imager 600 (GE Healthcare-Life Sciences).

Table 1: PCR cycling condition using 2D-gradient (both at denaturation and annealing step) settings.

Header (Eppendorf settings)	Lid	105 °C
	TSP/ESP	ON
	Lid auto-off	ON
	Temperature mode	Fast
Initial Denaturation		98 °C/1 min
	Denaturation	Gradient at 95–99 °C/20 s
	Annealing	Gradient at 52–62 °C/15 s
	Elongation	72 °C/2 min
Post-Cycle Elongation		72 °C/2 min
Storage	Hold	10 °C

Results and discussion

The opportunity to simultaneously optimize both T_A and T_D is a specific feature of the Mastercycler X50 equipped with an innovative 2D-gradient function. It has been shown that this facilitates optimization of difficult amplifications [6]. For this reason, we decided to leverage this technology to optimize the amplification of a 6 kb DNA sequence with high GC-content (78 %), for use in single-molecule experiments. Three T_D (95, 97.5, 99 °C) and five T_A (54, 56, 58, 60, 62 °C) were scanned.

Fig. 1 clearly shows that lower T_D was not sufficient to allow the amplification of the desired sequence (approximately 6,000 bp), where only non-specific bands, which showed up mainly as smears, can be detected at a lower molecular weight of around 500 bp, and only in some more permissive T_A.

PCR Optimization for Single-Molecule Experiments Using the Mastercycler® X50 2D-Gradient

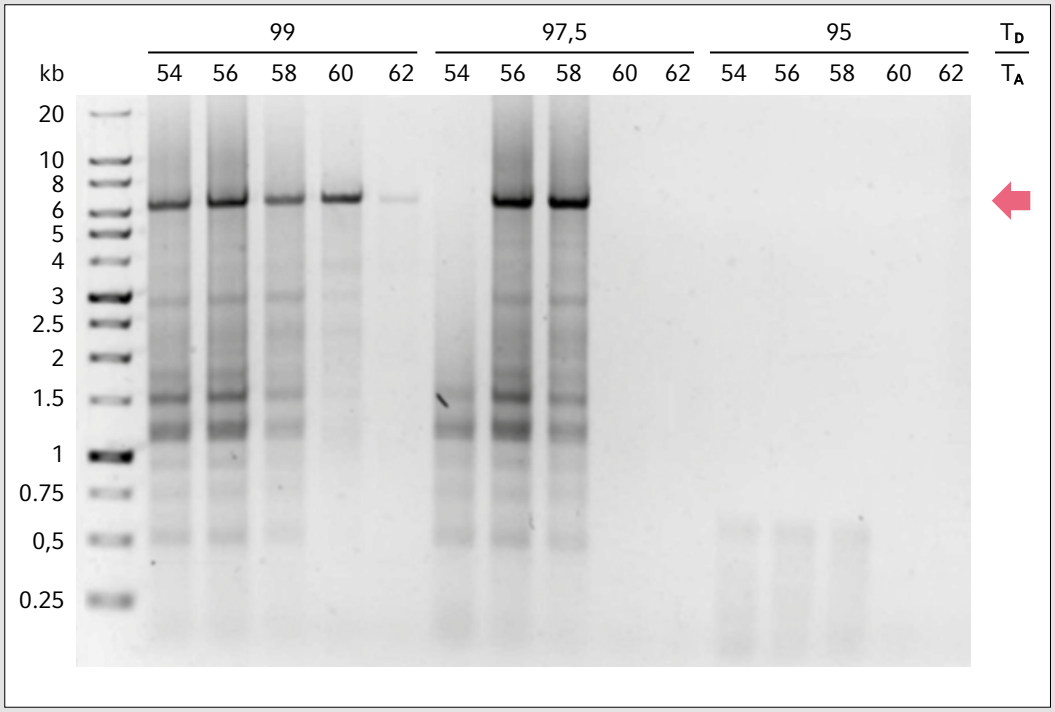

Fig. 1: PCR optimization of 6 kb-long amplicon (arrow) with a 78 % GC-content, using the 2D-gradient technology. One representative run out of three independent experiments.

At 97.5 °C T_D, a thick and specific band is present at 56 °C T_A (theoretical optimal T_A) and at the more stringent T_A of 58 °C. However, in both cases, undesirable non-specific bands ranging from 500 bp to 3 kb are also present in abundance. When T_D was increased to 99 °C, a general improvement in PCR specificity was observed.

This result confirmed that a higher T_D is required to allow the amplification of long amplicons with a high GC-content. Moreover, it is worthwhile to note that at the highest T_D, the most stringent T_A tested (60 °C) showed most specific amplification, with significant reduction of non-specific bands at the lower molecular weights. Thus, we can conclude that this temperature combination (99 °C T_D + 60 °C T_A) fulfilled the demand of highest specificity for obtaining the most suitable construct for single-molecule experiments.

The results show that the Mastercycler X50 2D-gradient is an extremely powerful tool. Only one PCR experiment was sufficient to obtain the desired conditions, which took approximately 2 hours. Compared to using conventional one-dimensional gradient technology where three PCRs would be required, the 2D-gradient technology helped in significantly reducing the operational time, making a complex and time-consuming screening easier and faster. Moreover, concurrent modulation of both T_D and T_A allows run-to-run variations between three different PCR experiments to be avoided. The possibilities and benefits offered by this tool would surely help in simplifying the production of complex DNA constructs necessary for single-molecule experiments.

Conclusion

We optimized the amplification of a 6,098 bp-long DNA sequence with 78 % CG-content by taking advantage of the 2D-gradient technology. Our results clearly indicate that this exclusive feature of the Mastercycler X50, which can modulate both the T_A and T_D simultaneously, is crucial to turning a difficult and time-consuming procedure into a rapid and easy process.

The original Application Note 428 can be downloaded at http://eppendorf.global/IDX

Literature

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How Scale Up Assist Using the BioFlo® 720 Bioreactor Control System Can Help Your Antibody Production Workflow

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Introduction

To scale upstream bioprocesses, an effective scale-up strategy is required to ensure the reproducibility of both cell culture growth and batch yields at large working volumes, ideally with little or no additional optimization. This study details the use of the new Scale Up Assist feature of the BioFlo 720 Bioreactor Control System to scale CHO batch cultures from 3 L to 10 L BioBLU® Single-Use Bioreactors and from 10 L to 50 L Thermo Scientific™ HyPerforma™ single-use bioreactors (SUB).

Material and methods

Inoculum preparation

For all experiments, we used a proprietary suspension CHO cell line producing a human monoclonal antibody (hmAb) from TPG Biologics, Inc., cultivated in Dynamis™ AGT™ Medium (Thermo Fisher Scientific®). We prepared our cell culture inoculum by cultivating the cells in single-use, baffled bottom shake flasks (Corning®) with a 20 % maximum fill volume.

We first thawed the cells from a previously cryopreserved vial and inoculated them into a 125 mL flask at a seeding density of 3×10^5 cells/mL. After our shake flask was seeded, we placed it into a New Brunswick™ S41i CO_2 Incubator Shaker. We set the shaking speed to 125 rpm and the CO_2 to 8 %.

A critical aspect to designing any cell culture bioprocess, especially regarding scale up, is to achieve matching growth curves and production yields from shaker to pilot scale. Reducing shock factors such as temperature fluctuations can greatly reduce negative impact on your culture.

After the initial thaw we allowed our cells to grow for a few passages before scale-up, to acclimate them to their environment. We determined that under our conditions the optimal passage schedule is every other day. After monitoring cell growth and viability (determined to be >95 %), we increased the culture volume from 125 mL to 250 mL, and finally, to 1 L shake flasks. During this expansion, flask inoculation density, percentage fill, and other parameters remained the same. Using this culture method, each bioreactor in this experiment was inoculated with cells that were approximately at the same passage and duration of the culture post-thaw.

For each experiment, the number of flasks per scale-up step varied, based on the amount required for optimal target inoculation and working volume of each bioreactor. For the 50 L SUB run, we needed a larger volume of inoculum. To reach this goal, we inoculated a BioBLU 10c Single-Use Bioreactor with a working volume of 10 L. For all experiments, our target inoculation density was 5×10^5 cells/mL.

Bioreactor control stations and vessels

> *Working volume 3 L:*
BioFlo 320 bioprocess control station equipped with BioBLU 3c Single-Use Bioreactor

> *Working volume 10 L:*
BioFlo 320 bioprocess control station equipped with BioBLU 10c Single-Use Bioreactor

> *Working volume 40 L:*
BioFlo 720 bioprocess control station equipped with 50 L Thermo Scientific HyPerforma single-use bioreactor. The single use bioreactor was inflated using the BioFlo 720 Auto Inflate feature.

Process parameters

The DO setpoint was set to 50 % for all experiments and was controlled at setpoint using 3-gas auto mixing. The pH setpoint was set to 7.0 with a deadband of 0.1 and was controlled via a cascade to CO_2 (acid) and 0.45 M sodium bicarbonate (base). For all experiments, temperature was controlled at 37 °C and remained constant for the duration of the runs.

Scale Up Assist feature

The Scale Up Assist feature of the BioFlo 720 bioprocess control station allows operators to tailor their processes using constant power or tip speed. This allows users to pursue either strategy or compare them to determine which one works best for their application. Scaling-relevant characteristics of the BioBLU Single-Use Bioreactors and the Thermo Scientific HyPerforma 50 L

The new BioFlo 720 bioreactor control system with 250 L single-use bioreactor

How Scale Up Assist Using the BioFlo® 720 Bioreactor Control System Can Help Your Antibody Production Workflow

single-use bioreactor, such as impeller diameter and power numbers, are pre-programmed into the software feature. A user-defined vessel option facilitates to manually enter parameters of other vessels. Users select their vessels from the drop-down menus. The Scale Up Assist then automatically calculates the critical parameters, such as agitation and maximum gassing, to successfully scale their culture from one vessel to another.

We based our scale-up strategy on using a constant P/V ratio of 20. Using this P/V ensured all bioreactors could be operated at reasonable tip speeds for their vessel size and geometry. The Scale Up Assist calculated all critical parameters needed, including gas flow rates and agitation with vessel specific data automatically populated in the software.

Sampling and analytics

We took two samples from each bioreactor daily, one in the morning and one in the evening, to check offline values such as cell density, viability, glucose, ammonia, lactate, and hmAb concentration. We measured glucose, ammonia, glutamate, lactate, and hMAb using a Cedex® Bio Analyzer (Roche Diagnostics®). We measured cell density and viability (via the trypan blue exclusion method) using a Vi-Cell® XR Viability Analyzer (Beckman Coulter).

Results and conclusion

Throughout our experiments, we monitored metabolic profiles twice a day, to observe how our culture was growing and to intervene if we detected any changes in growth or the health of our cells. The metabolic profile for the single use bioreactor run at 40 L is described as an example. The actively growing cultures depleted the initially supplied glucose by day 5. Lactate remained under 2.5 g/L for the duration of the run. Ammonia concentrations rose every day to a toxic level of 10.2 mmol/L by day 6. We reached 164 mg/L of IgG by day 6. By using the parameters calculated by the BioFlo 720 Scale Up Assist feature, we were able to match the growth profiles across all platforms in multiple batch runs (Fig. 1).

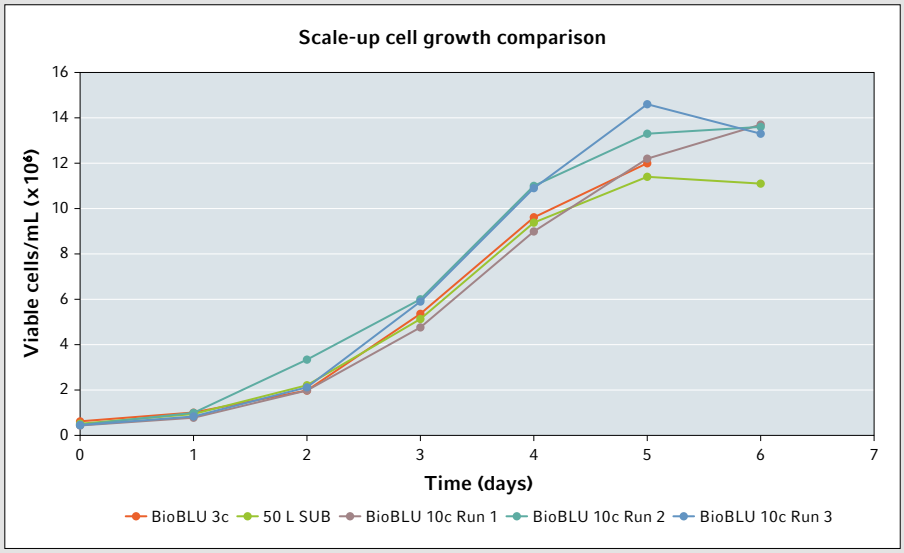


Fig. 1: Cell culture comparison of runs with working volumes of 3 L (BioBLU 3c), 10 L (BioBLU 10c), and 40 L (50 L SUB)

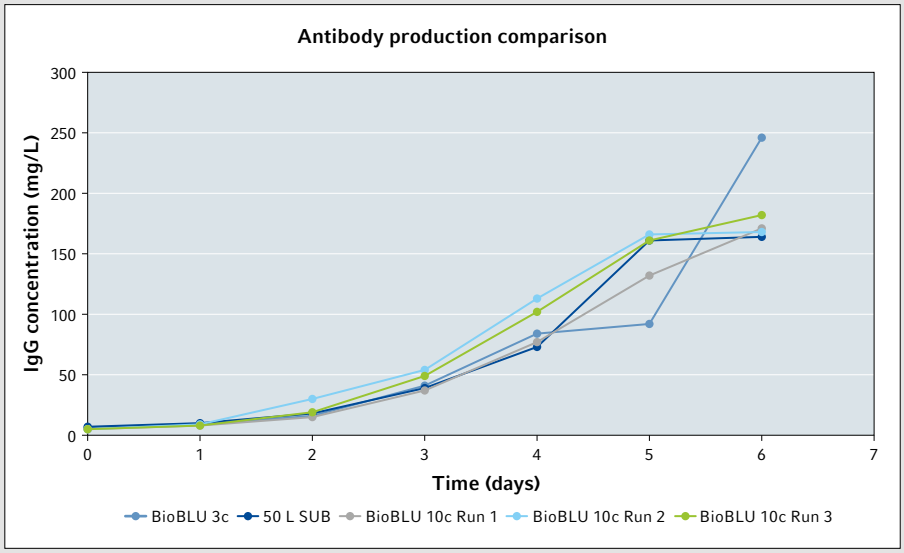


Fig. 2: Antibody production at working volumes of 3 L (BioBLU 3c), 10 L (BioBLU 10c), and 40 L (50 L SUB)

These results demonstrate that the new Scale Up Assist feature can maintain cell yields across vessel sizes and bioreactor platforms. Antibody production is shown for all runs in Fig. 2. All our runs had similar results in IgG production when they reached completion.

Find more experimental details in the full application note at http://eppendorf.global/IDZ

ULRIKE RASCHE, EPPENDORF AG BIOPROCESS CENTER, JUELICH, GERMANY

Expand Your Bioprocess with Ease

New biologics hold great promise in improving disease treatment and prevention. However, developing these solutions in a commercially viable manner requires close control of development costs and time to market. To help you with these challenges, Eppendorf provides solutions to increase efficiency and reproducibility of your bioprocess workflow. The new BioFlo® 720 bioprocess control station for applications at pilot and production scale is one significant step forward in helping ease scaling up.

An integral part of biologics manufacturing is the upstream bioprocess during which cells are multiplied in bioreactors and express the molecule of interest. Factors determining upstream bioprocess performance include:

> *Scale-up:* Streamlined process transfer is desired to reproduce product titers and quality optimized in small working volumes at production scale with little additional optimization.

> *Efficiency:* Ideally, bioprocess workflows save time on routine laborious tasks and prevent errors.

Boosting bioprocess efficiency and reproducibility

Automating repetitive process steps helps save time and increases reproducibility. DO sensor calibration, for example, takes time and different users calibrating the same sensor can introduce variability.

The BioFlo 720 bioprocess control station is compatible with the Thermo Scientific HyPerforma 5:1 single-use bioreactors with working volumes of 50 L and more

Automating it, like with the BioFlo 720 Auto Calibrate feature, saves time, and standardizes the procedure, adding to reproducible DO control. The controller's Auto Inflate feature, which automatically controls the inflation process for flexible single-use bioreactors, is another example of efficiency.

Streamlining biologics scale-up

Common scale-up strategies include keeping one or more parameters constant between bioreactors of different sizes, such as k_La, tip speed or power input per volume. This requires the knowledge of vessel parameters, like power number and impeller diameter, and the calculation of process parameters, like agitation and maximum gas flow.

The Scale Up Assist feature of the BioFlo 720 bioprocess control station is an example of how automating the calculations simplifies scale-up. Scale Up Assist is populated with relevant engineering parameters of pilot and production-scale Thermo Scientific™ HyPerforma™ 5:1 single-use bioreactors as well as of small and bench scale BioBLU® Single-Use Bioreactors and calculates relevant parameters for scale-up based on constant tip speed or power input per volume.

Antibody production workflow

For an example of how smart software features helped in an antibody production workflow, read the Application Note 7–8 in this issue.

The BioBLU c Single-Use Bioreactor portfolio covers working volumes of 100 mL to 50 L. In combination with the BioFlo 720 bioprocess controller Eppendorf offers one of the most scalable single-use portfolios, including manufacturing solutions for regulated environments.

More information at
www.eppendorf.com/bioflo720

SIMON PLATE, EPPENDORF AG

60-Year Anniversary of a Pipetting Masterstroke

2021 marks a key milestone for anyone working in a lab. 60 years ago, Eppendorf changed the ways of liquid handling forever with a new product type that enables today's scientists to talk and pipette simultaneously! In 1958, German physician Heinrich Schnitger at the University of Marburg filed for a patent describing a *"device for the fast and exact pipetting of small liquid volumes"*. Eppendorf recognized the potential and developed it into the first industrially manufactured piston-stroke pipette.

A world first

Launched in 1961, Eppendorf's "Marburg Pipette" featured the same basic elements to those found in today's labs: a spring-loaded piston that stops exactly at a set volume level and a removable plastic tip. This alternative to cumbersome and risky mouth pipetting with glass tube pipettes changed the face of pipetting forever.

The new piston-stroke pipette paved the way for modern clinical analytics and biochemical research by enabling safe, easy, and accurate liquid handling in the microliter range. With its launch and the complementary reaction tubes (launched in 1963), which are known around the world as Eppendorf Tubes® (or "Eppi®"), Eppendorf laid the foundation of its premium liquid handling business.

Continuing innovation

Since then, by applying our proven expertise and innovation, Eppendorf has continued to set industry standards in precise manual and automated dispensing of small volumes. For example, in 1978, we redefined liquid handling again with the introduction of the Multipette® (US/CAN: Repeater®) pipette with Combitips® tips system – the first handheld repetitive dispenser and an entirely new concept of disposable tips with integrated pistons.

We have always worked closely with our customers to understand their precise needs and develop the right pipette or solution for every application. Through this partnership, we recognized the growing requirement for increased throughput with the arrival of 96-well plates, and in 1994 launched the Titerman, our first manual multi-channel pipette for high sample throughput.

Still accelerating research

Over the years we have continued to evolve the pipette through the advent of electronic pipetting and automated liquid handling systems for the utmost reproducibility of micro- and nanoliter volumes. And in today's digital era, Eppendorf is still setting liquid handling benchmarks to meet the needs of future lab work. Our latest introduction in pipetting connectivity – the VisioNize® pipette manager – is designed to redefine speed and workflow management to further accelerate research.

At Eppendorf, we combine state-of-the-art instruments, high-quality consumables, and leading added-value services to ensure that the past, present, and future of pipetting continues in safe hands.

www.eppendorf.com/60-Years

ANN-CLAIRE FOETSCH, EPPENDORF AG

A New Era for Pipetting

VisioNize pipette manager – a system that is constantly being expanded with new features

To support the ever-increasing need for accuracy and speed in the lab of the future, Eppendorf is continuing the evolution of its ground-breaking pipette portfolio by improving connectivity and using an Internet-of-Things approach.

Designed to support digitization of liquid handling workflows to help scientists improve speed, accuracy, and collaboration, we are redefining pipetting routines with the introduction of the **VisioNize® pipette manager** – our first step towards comprehensive digital liquid handling.

The external control panel of the VisioNize pipette manager turns your Eppendorf Xplorer® pipettes into fully connected devices for faster volume transmission and support when working with challenging liquids. Ideal for scientists managing high workloads with bench tasks requiring many intricate pipetting steps: the VisioNize pipette manager accelerates your pipetting and improves your results – for example in projects involving multiple users or challenging liquids in terms of viscosity, volatility, or foaming.

Volumes can be input rapidly via the VisioNize pipette manager with all settings transmitted instantaneously to all connected electronic pipettes, and the embedded software provides helpful guidance to ensure accuracy when working with varying liquid types. And there's so much more to come!

More information at
www.eppendorf.com/
visionize-pipette-manager

Tip

Are Your Results Reliable?

Pipettes are precision instruments with parts subject to normal wear and tear. Therefore, regular maintenance and calibration are fundamental for reliable results. With our newly designed global pipette calibration service portfolio we follow the highest standards possible, for Eppendorf and other brand pipettes.

For our highly regulated customers working in the Pharma or Industry Research and Production environment, we now offer various ISO 17025 compliant pipette calibration services.

To learn more about our new service portfolio, visit the Pipette Service website where you can also download our brochure

https://www.eppendorf.com/
pipette-service

Do you already know our video "Eppendorf Pipette Service: Spa & Wellness for Your Pipettes"? Simply scan the QR code and watch it right away.

ANN-CLAIRE FOETSCH, EPPENDORF AG

Stay Connected to Your Lab

Every user in the laboratory has their very own needs and goals that they attend to on a daily basis. For this reason, Eppendorf has developed the VisioNize® Lab Suite, a laboratory management platform that is completely customizable and can still be utilized by different users.

Depending on their function, laboratory employees pursue different goals, tasks, and problems in their daily routine. With the **VisioNize Lab Suite (VNLS)**, they can now experience comprehensive support in improving their workflows.

For example, using the VNLS platform and thanks to IoT ("Internet of Things"), a lab manager can get a complete overview of all his lab equipment at the push of a button; be it to support the planning of an upcoming maintenance activity or to quickly identify the source of error in an experiment, for example, the use of an uncalibrated instrument.

A quality manager could likewise benefit from VNLS to prepare for an audit, where all instrument documentation is recorded centrally and always linked to the asset for quick retrieval. Scientists, on the other hand, stay informed with VNLS if there is a change in the status of their experiments.

Thanks to remote monitoring and real-time notifications, they can react quickly and accurately if, for example, manual interaction is required or if they receive an error message.

VisioNize Lab Suite does not need to be installed and/or updated locally. It is easily accessed via the Internet – without complex software and hardware management. As you and your lab change or expand over time, VNLS will also evolve to meet your needs with new future services.

More information about VisioNize Lab Suite at www.eppendorf.com/visionize

Tip

Are Pipetting Ergonomics Important?

Whether on board the International Space Station, on a research vessel, in a high-security laboratory, or at other non-automated laboratory workplaces, one thing applies to them all: manual pipetting is one of the most central and, at the same time, most error-prone tasks in the lab.

While pipettes can be calibrated, devices can be adjusted, and measurement protocols can be perfected, the human factor remains comparatively unpredictable.

"The main risk of error in pipetting is the operator," explains Peter Schmidt, Business Manager for Pipettes at Eppendorf. "When you take up the liquid, you should immerse vertically and only to a certain depth, so that the liquid is not pressed in more than intended. Then wait long enough for the liquid inside to reach a steady state – this is especially crucial for large volumes – and only then dispense it, sliding along the wall of the target vessel."

According to Schmidt, pipetting has to be carried out in the same way, with high precision, making fatigue a major problem: "A tired hand cannot work as well as a rested hand. The more ergonomic the pipette is, the longer good results can be achieved."

Read the full article on
http://eppendorf.global/lrm

CORDULA RICHTER, EPPENDORF AG

The Eppendorf Life Improving Program

It has always been the belief of Eppendorf that success should go hand in hand with taking responsibility. In this context, and in accordance with the wishes of its founding fathers Dr. Netheler and Dr. Hinz, the company has created the "Eppendorf Life Improving Program".

In celebration of Eppendorf's 75th anniversary last year the company provides the symbolic sum of 75,000 Euros to the **Eppendorf Life Improving Program**. This sum will be donated to a global aid organization every year to provide substantial support for their work.

When selecting the projects that it supports, Eppendorf is guided by its mission to improve people's living conditions. The first one to be selected for this year and next year is a project by **Plan International**, an independent, global aid organization for children who are working to improve the living conditions of children and adolescents in over 70 countries. One of Plan International's focal points is the education and empowerment of girls.

All Eppendorf employees were given the opportunity to vote for one of three projects proposed by Plan International. Eppendorf employees from all over the world decided to support a project for extending the drinking water supply of 36 communities in Ghana. This will make a direct contribution to improving the living conditions of around 32,000 people.

Tip

Discover Eppendorf as an Employer

Eppendorf builds on a foundation of collective experience, history, and knowledge. We know that our products make great things happen in research and science and help improve human living conditions. It is often the achievement of our interdisciplinary scientists who contribute their experience and thus initiate trend-setting changes.

Do you also want to make your contribution and use your expertise to develop new ideas? At Eppendorf, highly specialized teams in state-of-the-art working environments are waiting for you.

Discover what makes working at Eppendorf so special.

Visit our website career.eppendorf.com to find comprehensive information about

> Departments

> Entry opportunities

> Application process

CAROLYN TAUBERT & CORDULA RICHTER

Eppendorf Prize Winners 2020/2021: Chris Zimmerman & Tanmay Bharat

Christopher Zimmerman
https://wittenlab.org

The American scientist Christopher Zimmerman, Ph.D. (Princeton Neuroscience Institute, New Jersey, USA), won the 2020 *Eppendorf & Science Prize for Neurobiology* of 25,000 USD.

Christopher Zimmerman won the prize for his work on the neural circuits that govern thirst and drinking behavior. Zimmerman discovered that sensory signals originating throughout the body come together within individual neurons in the brain to produce the sense of thirst.

He demonstrated that this new class of body-to-brain signals predicts changes in hydration before they occur and, as a result, adjusts our level of thirst preemptively. Zimmerman's research has revealed fundamental principles of ingestive behavior of foods and liquids and provided neural mechanisms to explain aspects of everyday human experience.

Christopher Zimmerman describes the neurobiology that underlies a phenomenon everyone has experienced multiple times. His work helps us understand, for example, how we can quickly feel thirst, how the sensation changes during meals, and why cold drinks have a thirst-quenching power.

www.eppendorf.com/prize

Tanmay Bharat
https://bharat.path.ox.ac.uk

The 2021 *Eppendorf Award for Young European Investigators*, endowed with € 20,000, went to Dr. Tanmay Bharat, Sir William Dunn School of Pathology, Oxford, United Kingdom.

The jury panel was impressed by his pioneering work on the structure and function of extracellular surface layers that surround and protect prokaryotic cells. In particular, his work elucidated how filamentous phages (symbiotic viruses released from the bacteria) protect pathogenic bacteria from the attack of certain antibiotics.

His work has far-reaching general implication and also opens the door for the development of new antibiotics that are urgently needed.

Due to the ongoing COVID-19 crisis situation, there was again no award ceremony in 2021 at the usual venue, the EMBL Advanced Training Centre in Heidelberg. Instead, Tanmay Bharat and Randall Platt (winner 2020), the jury, and other speakers were connected in a virtual award ceremony via live stream.

www.eppendorf.com/award

Win a Move It® Pipette

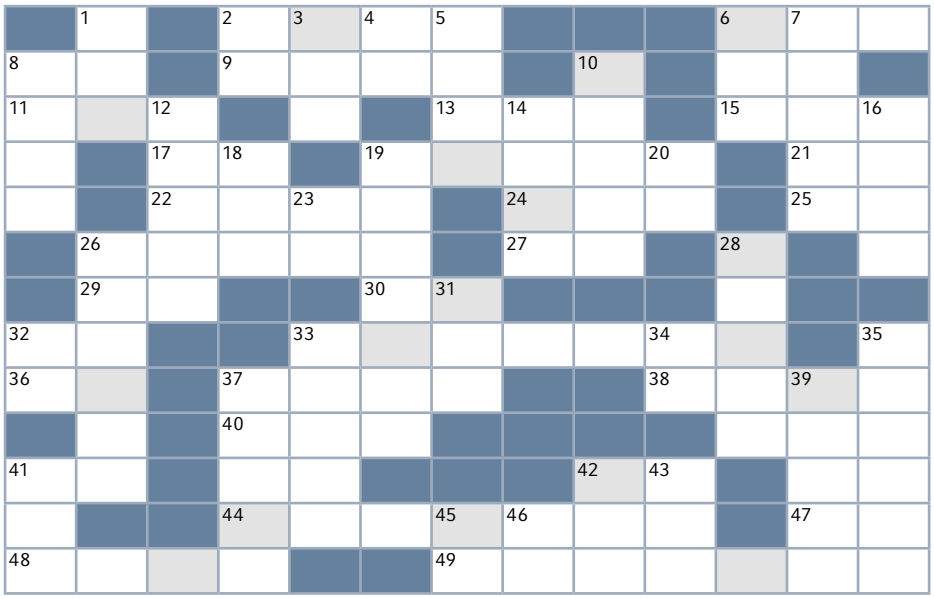
The solution of the prize competition of BioNews No. 53 was "EPSERVICES FOR PREMIUM PERFORMANCE". The three main prizes, one Eppendorf Research® plus multi-channel pipette each, went to Silvia C. (United Kingdom), Julia P. (Germany), and Rafal N. (Poland).

Good luck in our new competition!

Simply arrange all letters in the light gray boxes of the crossword in the correct order. Send us the solution until **October 31, 2021**.

Participate online at **www.eppendorf.com/bn-service** or e-mail the solution to **bionews@eppendorf.de**.

All correct answers will be considered for a prize for use in your lab. Winners will be notified in writing. Cash payment of the prize is not possible. No recourse to legal action. The judges' decision is final. Eppendorf employees and their families may not participate. The winner of the first prize will be published in BioNews No. 57.



1st Prize:

1 Eppendorf Research® plus Move It® pipette of your choice

2nd to 5th Prize:

1 Amazon® Voucher worth 50.00 EUR

6th to 10th Prize:

500 bonus epPoints® each

(epPoints registration required)

ACROSS

2 Part of the name of a new conical tube
6 Flying saucer
8 Also referred to as Pine Tree State (abbrev.)
9 Country in West Africa
11 Type of music in which words are spoken
13 Unprocessed computer data
15 Completes 2 across
17 Completes Points, Motion, Tips
19 Ideally good
21 Located on the Eastern coast of the Baltic Sea (ISO country code)
22 In the UK, a female member of an order of knighthood
24 Snakelike fish
25 Ankara is the capital (ISO country code)
26 Detection system using radio waves
27 Baltic state, south of Latvia (ISO country code)
29 0.1 m^3 (abbrev.)
30 Country in the horn of Africa (ISO country code)
32 Kuala Lumpur is the capital (ISO country code)
33 Turning sweet 60 in 2021
36 1 million tons (abbrev.)
37 Story of a book, film, play
38 A must-have since 2020
40 Race, sprint
41 Atomic number 100 (abbrev.)
42 Déjà … what?
44 Its executive capital is La Paz
47 French male article
48 Preferred position in a car race
49 Used for cold sample storage

DOWN

1 Aromatic beverage
2 Atomic number 62 (abbrev.)
3 Very relaxing when combined with power
4 Silver gray metal (chem. symbol)
5 One half of a very hot spice
6 Class of freezers for storing biological samples
7 Group of ships, planes, cars under one control
8 So small, so effective
10 Sugary, lovable, just …
12 Foot operated lever
14 Cain's brother
16 Birthplace of the Word Wide Web (abbrev.)
18 Flat mat for mice
19 Form or variant of a type or original
20 Ljubljana is the capital (ISO country code)
23 Kingdom in Northwest Africa (ISO country code)
26 Stands for the R in R&B
28 Thoughts, suggestions
31 Make a choice or decision
32 0.001 metres (abbrev.)
33 Dwarf planet
34 Marks intellectual property (abbrev.)
35 On the move on a board or on ice
37 Labeled fragments of nucleic acids
39 The way something is said, done, performed
41 Variant of 38 across
42 C'est la …
43 Abu Dhabi is the capital (abbrev.)
45 In the event that, on the condition that
46 Popular in video games (abbrev.)

Solution hint for prize competition of BioNews No. 55:

				O		I					B		U			

Send us the solution until **October 31, 2021**. Participate online at **www.eppendorf.com/bn-service** or e-mail the solution to **bionews@eppendorf.de**.
Information about the use of your personal data can be found at **www.eppendorf.com/gdpr**

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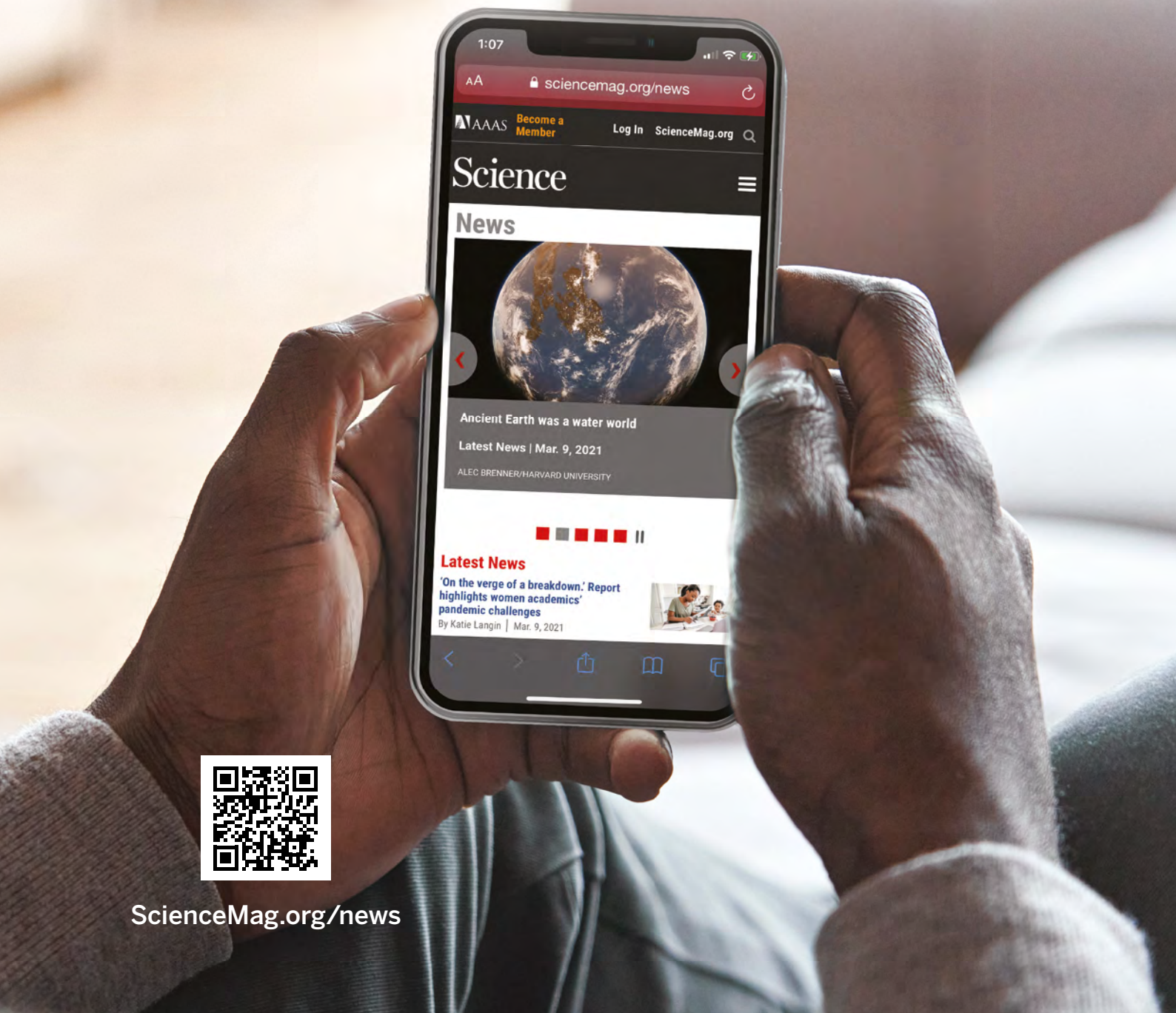

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