BIO NEWS

No. 56 – 2022

eppendorf

Become a Digital Experiment Manager

> Centrifuge 5910 Ri: documentation at your fingertips
> How do you choose your next pipette?
> Save plastic, maintain safety

Application Notes

Xeno-free generation, expansion, and differentiation of human iPSCs ·
Constant RQ fermentation of *P. pastoris* in the DASbox® Mini Bioreactor System · etc.

We are so pleased

that you are taking the time for the latest issue of our BioNews. We hope you will enjoy our potpourri of topics!

We were wondering, how do you feel about digitalization in the laboratory? Are you already on board, or are you still in the planning phase? For Eppendorf, the laboratory of the future will definitely be digital, with various degrees of automation and intelligent solutions. After laying the foundation for the networked laboratory with the VisioNize® Lab Suite last year, our new service "Experiment Management" will now promote you to "Digital Experiment Manager". More on this topic in our leading article on pages 4–5.

Digitalization also benefits experiment documentation. Smart laboratory instruments such as the Centrifuge 5910 Ri save all important parameters of your centrifuge run for the purpose of digital documentation in the eLabJournal®, an electronic lab notebook (p. 6).

Our new (of course, digital) format Eppendorf Lab Channel with virtual webinars and product demonstrations will help you expand your knowledge on all things around the laboratory and start the conversation with our experts (p. 12).

In addition, we would like to share useful ideas with you for your daily laboratory routine – for example, tips regarding the selection of your next pipette (p. 7), ideas for the reduction of plastic waste (p. 11) as well as our Stay Informed infographics (Application Note 1–2).

Last, but not least: perhaps you will reward yourself with an (analog) cup of tea and relax a little with our prize competition? As always, great prizes are to be won (p. 15).

Do you have any ideas or requests? You can reach us via e-mail to bionews@eppendorf.de. We look forward to your feedback!

Your Eppendorf BioNews Team

By the way: Out of responsibility for the environment, Eppendorf is sponsoring the International Freezer Challenge for the fifth time in succession. Curious? Take a look: www.freezerchallenge.org

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www.eppendorf.com/bionews

Editorial team
Berrit Hoff (Editor-in-Chief),
Dr. Jan-Hendrik Bebermeier,
Dr. Tanja Musiol, Natascha Weiß

Design
Holger Paulsen Grafik-Design,
Hamburg, Germany

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Eppendorf BioNews Application Notes


Stay Informed Infographics

ANN-CLAIRE FOETSCH, EPPENDORF SE

Become a Digital Experiment Manager

Digitalization has entered our lives, and over the past two years, we have experienced a tremendous push forward. Video conferences are the "new normal", and communication via Microsoft® Teams or Slack® are part of everyday life. In the laboratory, too, we are increasingly becoming accustomed to electronic lab notebooks; we manage samples, chemicals, and consumables digitally, and we use networked instruments. The laboratory 4.0 is advancing – and with it the promise of more efficiency, decreased use of resources, and improved sustainability. But is it really that simple?

Physical calendars for the reservation of an instrument or workspace, centralized locations for data on a server, or even the maintenance of instrument inventories using Microsoft Excel® are still the methods of choice in many laboratories. However, multiple different approaches are available which will help optimize workflows and improve the utilization of existing resources through digital applications. Tasks such as instrument monitoring, the keeping of maintenance schedules, or documentation of instruments and experiments can be accomplished much more efficiently using software applications with visualization.

Complete traceability of the protocols carried out and seamless documentation of laboratory processes are paramount when it comes to ensuring an efficient and productive laboratory environment.

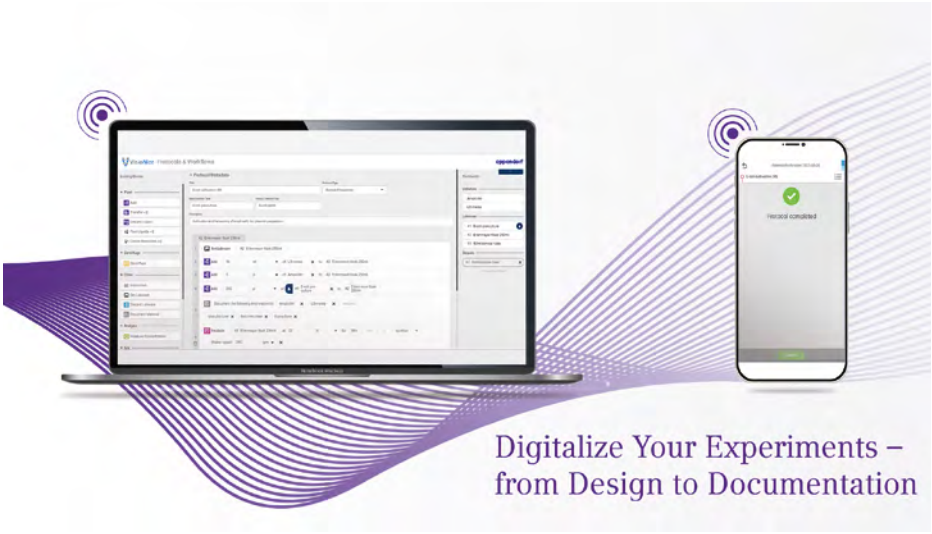
Who in the laboratory is not regularly faced with time-consuming documentation of laboratory processes? Handwritten, loose-leaf protocols that are kept in a lab notebook impede reproducibility.

Even today, more and more scientists are saving their experimental data in an electronic laboratory notebook (ELN) or a laboratory information management system (LIMS) instead of using traditional paper lab notebooks. Switching to digital formats has brought many advantages as well as saved time.

In the case of a failed experiment, tracing the experiment and identifying potential sources of error have become much easier. Data from within as well as from outside the laboratory may be used and shared by multiple parties. The digital tools assist you in the tracing of your samples, and (in combination with an inventory management system) will support you in the management of your consumables, chemicals, and solvents.

"Unstoppable": digitalization in the laboratory

Since more and more laboratory instruments are now suitable for networking, the use of efficient laboratory and inventory management systems will become ever more commonplace – particularly these days as laboratories all over the



world are confronted with novel demands and challenges. These include new standards of social distancing and work-from-home regulations (which, in turn, translate to a more flexible workforce), limited access to laboratory workspaces, and virtual compliance audits.

We at Eppendorf have recognized the increased need for digital software services and the digital networked laboratory. For us, the laboratory of the future is digital – with different levels of automation and intelligent solutions which will allow you to concentrate on what really matters: your research.

With the introduction of the VisioNize® Lab Suite in the spring of 2021, we laid the foundation for the networked laboratory in order to enhance productivity across all routine tasks. As a solution for effective lab management, this platform offers services in the areas of remote monitoring, alarm, instrument and task management.

The VisioNize Lab Suite is continually updated, and it therefore represents a lasting and sustainable investment in the laboratory of the future.

New service "Experiment Management"

With the introduction of the new service "Experiment Management", we go one step beyond the familiar tools, which include ELNs. We offer you the opportunity to digitalize your experiments – beginning with protocol design, all the way to automated documentation of your experiments. "Experiment Management" was launched in early 2022, ensuring improved efficiency in your laboratory by enhancing the productivity and reproducibility of experiments – from design to optimization.

VisioNize Lab Suite Experiment Management offers you:

> Easy creation and management of protocols and workflows with drag-and-drop protocol steps for high reproducibility and adaptability of protocols

> Assurance of proper protocol execution through guided and specified sequences of steps and networking of instruments used within the experiment in order to remain in contact with the experiment

> Complete traceability of staff conducting the experiment, samples, reagents, and instruments used, as well as errors and protocol deviations

Experience a new dimension of digital experiment management now!

More information at
http://eppendorf.global/IZP

Tip

Tips & Tricks for Pipetting

In the new eBook "The Science of Pipetting to Perfection", we have compiled all our experience and insights from the last 60 years. This guidebook will help you carry out good science when dealing with all kinds of liquids in the lab.

Discover what you should consider before selecting a new pipette (see also our article on page 7 in this issue) and how correct handling and careful maintenance will ensure your pipette always operates at optimum performance.

Learn how to maintain and increase the reproducibility and reliability of results and how to design your work processes safely and efficiently. You will also receive suggestions for creating ergonomic, health-promoting working conditions.

Download eBook now at
http://eppendorf.global/m0H

NICOLE SEELIGMÜLLER, EPPENDORF SE

Centrifuge 5910 Ri: Documentation at Your Fingertips

Even today, many scientists still use spreadsheets or traditional notebooks to document their experiments, despite the fact that modern electronic tools are available. Laboratory instruments are now capable of tracking run and user information as well as connecting to digital lab notebooks like eLabJournal® from Eppendorf. eLabJournal allows you to document data generated, for example, by your smart Centrifuge 5910 Ri, in a safe and efficient manner.

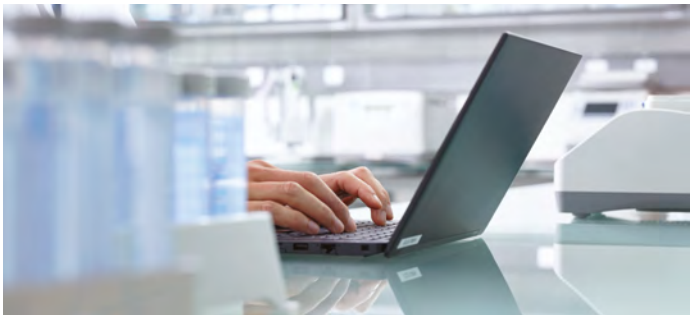
Documentation: a love-hate relationship

Documentation is one of the most important and, at the same time, most unpopular tasks in the laboratory. Traceable documentation of research data is essential in any laboratory, both in academia and in regulated environments such as the pharmaceutical industry. Whether data, text, or figures need to be retained for scientific publications, or for the purpose of meeting GxP/GLP standards – the most challenging aspect is typically not the data creation, but rather the manual documentation effort behind it.

Paper-based documentation – still found in many laboratories today – has the disadvantage of different aspects of the data being documented in different laboratory books (e.g. a centrifuge logbook in addition to a personal laboratory journal). These locally stored data are not accessible to every employee and from every location, and they could be lost or damaged.

Documentation: efficient and safe

Our smart Centrifuge 5910 Ri records all the details of the run that would otherwise have to be noted by hand: user, time, temperature, speed, whether the run was stopped manually, which program was used, etc. The centrifuge stores up to 1,000 of these "run records". The option of filtering by date, user, or program makes it very easy to export only the records you need. Data are exported as either PDF or CSV file, which can subsequently be documented in the eLabJournal.



The eLabJournal software is a fully integrated solution for data, sample, and protocol management. It helps you streamline your workflows, for example, by documenting and searching research data, tracking sample collections, and more.

eLabJournal is web-based, so you can view your data anytime, from anywhere, on any device. Do you work in a regulated environment? With eLabJournal, your lab can work in compliance with Good Laboratory Practice (GLP). Tests can be electronically signed and countersigned in accordance with FDA 21 CFR part 11, protecting them from further modification.

More information at

www.eLabJournal.com

Scan the QR code and learn how the Centrifuge 5910 Ri documents the centrifugation run

SIMON PLATE, EPPENDORF SE

How Do You Choose Your Next Pipette?

Selecting the right pipette or dispenser can be the key to the success of your work. It can boost your efficiency, help you handle different liquid types with ease, and ensure reproducible results. With a vast array of choices (Eppendorf alone offers more than 100 pipette variants), the selection of a liquid handling system should be considered with care, taking into account our five guiding questions.

Spoilt for choice: Eppendorf alone offers more than 100 pipette variants

1. Which volume range?

To maximize reproducibility, you should always select a pipette with a nominal (max.) volume that is as close as possible to the volume you usually need to transfer.

2. Which vessel format?

If you routinely work with individual tubes, single-channel pipettes are ideal. When working with microplates, multi-channel pipettes or hand-dispensers will be your tool of choice. For 384-well-plates you should consider selecting a 16- or 24-channel pipette to easily fill a complete row in one pipetting step. If, on the other hand, you regularly need to switch formats (e.g. from tubes to plates), it's definitely worth looking at an adjustable tip spacing pipette.

3. Which liquid type?

The most commonly used pipettes in labs around the world are air-cushion pipettes, which are ideal for transferring aqueous solutions.

Challenging liquids with a different viscosity, volatility, surface tension, or density than water, as well as hot, cold, or hazardous liquids, are better handled with a positive displacement system such as the Multipette® (US/CAN: Repeater®) with Combitips® advanced. When using an electronic pipette, a digital lab assistant with pre-defined settings for different liquid types (such as the VisioNize® pipette manager) can offer additional assistance when handling challenging liquids.

4. Throughput and 5. Complexity

The definitive criteria to be considered when selecting your liquid handler, however, include the throughput as well as the complexity of your tasks. The higher your throughput and complexity, the more it makes sense to look at electronic pipettes or even automated pipetting robots.

Download a selection chart for Eppendorf pipettes and matching tips at

http://eppendorf.global/IVe

Or scan the QR code

Close-up

Chocolate Muffin with Sugar Icing?

Can you recognize what our close-up is about? Doesn't it look like a chocolate muffin that turned out a little dry, delicately decorated with sugar icing?

Our video "Handling Magnetic Beads during NGS Library Preparation" can help you solve our picture puzzle. The preparation of a high-quality NGS library is a cost-, labor-, and time-intensive process. DNA cleanup using magnetic beads, in particular, requires a great deal of experience. For example, if the beads are over-dried before the final DNA elution step, they will become brittle (like a dry chocolate muffin), and removing the DNA will be difficult.

The video clearly describes the various steps, provides valuable tips for handling magnetic beads and shows why a pipetting robot like the ep*Motion*® is a real labor-saver for this process.

Scan the QR code to watch the video

Find the most important points of the video in an infographic of our "Stay Informed" series.

Scan the QR code to download the infographic

Find more Stay Informed infographics at http://eppendorf.global/m1r

HANAË KÖNIG, EPPENDORF SE

Mixers Can Do So Much More than "Just" Mix

If there is one standard piece of equipment in every laboratory, it will be the thermomixer. Every day, samples are mixed, heated, or cooled. In addition, mixers enable further practical applications – for example, have you ever considered retiring the old waterbath and instead using a mixer to thaw your cell lines? Actually, why not? The advantages are overwhelming. Also: did you know that a PCR-like application, performed at a consistent temperature, is possible using a mixer?

Secure thawing of sensitive cell lines

The step of thawing cell lines is most often carried out in a waterbath. This practice, however, presents a number of disadvantages, including, for example, the labor-intensive cleaning process, the risk of contamination, and the inability to standardize the thawing process. Following the thawing process, vials are wet – they must be dried and sterilized on the outside before it is safe to transfer them to the biological safety cabinet. Frequent opening and closing of the lid of the waterbath, along with parallel use for warming media bottles and medium additives, may lead to temperature fluctuations and thus jeopardize the reliability of the thawing process.

A safer, easier, and more reproducible alternative is presented by the Eppendorf ThermoMixer® C with the Eppendorf SmartBlock cryo thaw and an integrated thawing program for cell lines [1].

Cell lines frozen in 1.8–2 mL cryovials can now be thawed reproducibly, without the use of water, in an environment that is easy to clean and disinfect. Once adapted to the respective sample number, the thawing program will always proceed in the same manner. 1–24 samples with a volume of 1 mL can be thawed in parallel – either directly inside or beside the biological safety cabinet.

Familiar instrument – new possibilities

Research continually brings about new methodologies – for example, loop-mediated isothermal amplification (LAMP). While this method does not replace PCR, it serves as a meaningful addition, allowing a quick check of individual gene sections. LAMP employs 4–6 primers and 6–8 binding sites on the target sequence and a specific polymerase which performs the annealing as well as the elongation steps at a consistent temperature.

Self-hybridizing DNA-loops result, which generate a dumbbell structure.

The speed of the amplification increases, and the result is available after approximately 30 minutes. If a colorimetric LAMP kit is used, the pH of the reaction will change with the incorporation of nucleotides.

If the DNA section of interest is present, the sample will change color from pink to yellow. Examples of applications include the quick detection of bacterial infestations in mosquitoes; COVID; or viruses infecting vines.

More information at
www.eppendorf.com/thermomixer

Eppendorf ThermoMixer C with SmartBlock cryo thaw

Eppendorf ThermoMixer C with SmartBlocks for almost all common tube and plate formats

[1] Standardized and Water-free Cell Thawing using the Eppendorf ThermoMixer® C with the Eppendorf SmartBlock cryo thaw. *Eppendorf Application Note* 437.

Scan the QR code and download for free

Stay Informed Infographics

Infographics help you stay informed

Staying on top of the techniques used in your laboratory is crucial for the research you do. The basic techniques are often the ones that cause most of the problems because they are so widely used.

> Does everyone in your lab know how to do reverse pipetting?

> Does everyone know what is influencing homogeneous cell growth in your cell culture?

> Is everyone preparing the PCR mastermix the same way?

Our "Stay Informed" Infographics Series helps you stay on top of techniques and make sure procedures are consistent throughout your lab.

You will find a first impression of our Stay Informed contents on this and the next page. Visit www.eppendorf.com/stay-informed for comprehensive information. This website also provides links to supportive materials for industrial or specialized applications, e.g. food & beverage, pharma, and forensics.

Precise and Repeatable Pipetting of Problem Liquids

Pipetting seems easy, but some tips and tricks can help you to get consistent and reproducible results – even with difficult liquids.

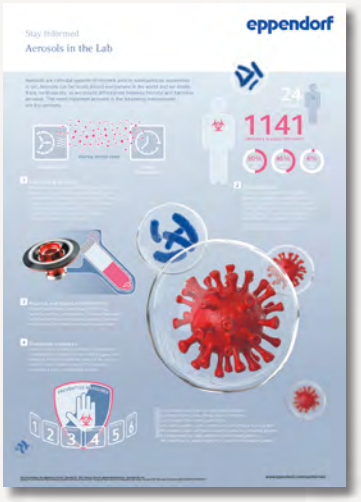
Download infographic at
http://eppendorf.global/m2t

Or scan the QR code to watch the tutorial

Aerosols in the Lab

Aerosols are colloidal systems of droplets and/or solid particles suspended in air. Aerosols can be found almost everywhere in the world and we inhale them continuously, so we should differentiate between harmful and harmless aerosols. The most important aerosols in the laboratory environment are bio-aerosols.

Download infographic at
http://eppendorf.global/m2v



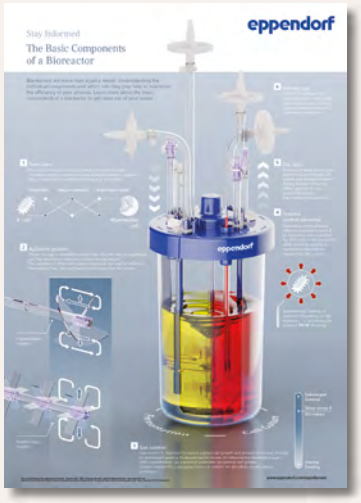
Or scan the QR code to watch the tutorial

Stay Informed Infographics

The Basic Components of a Bioreactor

Bioreactors are more than just a vessel. Understanding the individual components and which role they play helps to maximize the efficiency of your process. Learn more about the basic components of a bioreactor to get more out of your vessel.

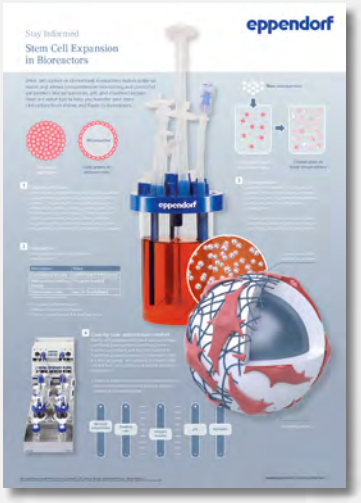
Download infographic at
http://eppendorf.global/m2z



Stem Cell Expansion in Bioreactors

Stem cell culture in stirred-tank bioreactors makes scale-up easier and allows comprehensive monitoring and control of parameters like temperature, pH, and dissolved oxygen. Our infographic provides tips that help you transfer your stem cell culture from dishes and flasks to bioreactors.

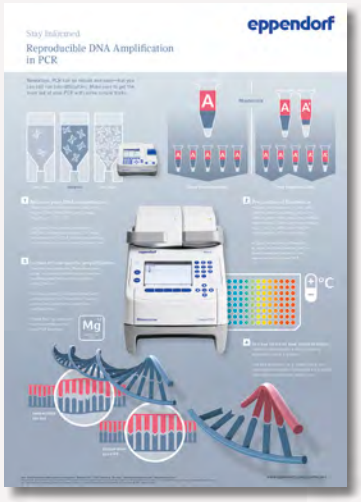
Download infographic at
http://eppendorf.global/m2C



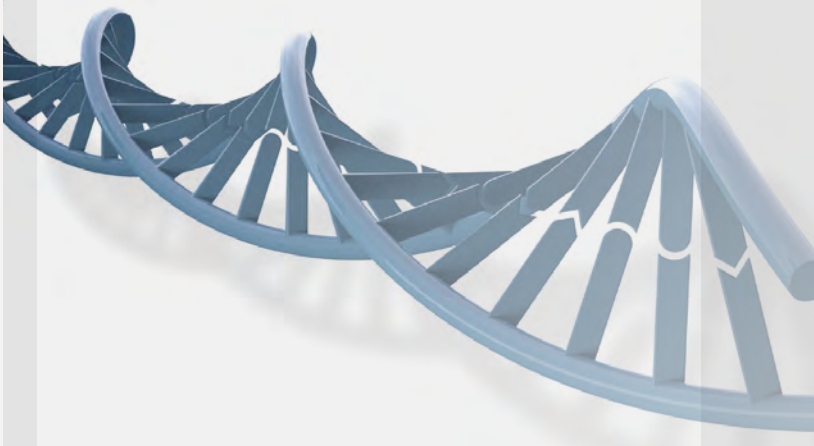
Reproducible DNA Amplification in PCR

Nowadays, PCR can be robust and easy – but you can still run into difficulties. Make sure to get the most out of your PCR with some simple tricks.

Download infographic at
http://eppendorf.global/m2x



Or scan the QR code to watch the tutorial



Optimizing Plasmid Yields in Shake Culture

BLANDINE VANBELLINGHEN, SILVIA TEJERINA, AURELIE TACHENY, EPPENDORF APPLICATION TECHNOLOGIES, S.A., NAMUR, BELGIUM
INES HARTMANN, EPPENDORF SE, HAMBURG, GERMANY

Abstract

Recombinant plasmid DNA is produced in bacterial cultures, mostly in *E. coli*. Plasmid yield and quality depend on multiple factors, including the insert, the selection of host strain, the vector design, and the methodology chosen for cultivation and downstream purification. Here, we focus on how to optimize *E. coli* shake flask cultures examining the influence of culture media, vessel design, fill volume, and shaking speed on bacteria and plasmid yields. We illustrate how larger production range can be obtained by using the appropriate combination between a large incubated shaker, optimized culture conditions, and specifically-designed culture flasks.

Innova S44i with Ultra Yield Flasks

Introduction

Plasmids serve as vehicles in genetic engineering either to clone and amplify DNA fragments, such as genes, or express recombinant proteins. Plasmid DNA (pDNA) can be easily genetically manipulated, produced in *E. coli* in large amounts and a variety of ready-to-use solutions allows easy subsequent downstream purification. Depending on the application, pDNA production ranks from research laboratory scale (up to a few mg) to industrial scales (mg to g scales). Here, we will examine the impact of cultivation with focus on high yield pDNA production in 2.5 L Ultra Yield® flasks.

Materials and methods

E. coli DH5α and JM109 were transformed with pUC19 plasmid and pGEM®-3Z. Glycerol stocks were stored at –80 °C. Lennox-Broth (LB) medium and modified TB was prepared and freshly supplemented with ampicillin and an anti-foam. All experiments were inoculated with the same start amount of 1 % (v/v) from a liquid seed culture and run in triplicates. Experiments were performed in the Eppendorf Innova® S44i with 25 mm orbit at 37 °C using Erlenmeyer flasks (baffled and unbaffled) and Ultra Yield flasks (Thomson). Bacteria densities were measured by OD_{600} nm, biomasses were determined from harvested culture samples and pDNA was isolated and measured by OD_{260} nm.

Results and discussion

Impact of flask design

Shake flasks are the most common laboratory-scale cultivation vessels for production of plasmids. The choice of the adequate flask design is depending on the organism's oxygen requirements and the individual application needs. Different shake flask designs are available nowadays to increase the oxygen transfer to the culture (Fig. 1).

Depicted flask designs were examined to select the best suited one for high yield production in larger volume flasks. The Ultra Yield design performed slightly better than the standard baffled flask (data shown in original Application Note 449*) and was used for subsequent tests.

Impact of media composition

The media supplies the culture with nutrients, such as proteins, minerals, vitamins, and carbohydrates. Figures 2 and 3 show clearly the influence of the media composition on biomass and pDNA yields. The overall bacterial biomass in the enriched TB media resulted in a 2-4-fold higher biomass, depending on fill volume and shaking speed applied, compared to classic LB media (Fig. 2). The resulting pDNA yields gave similar results, with 4-5-fold higher pDNA yields when cultivated in the TB formula (Fig. 3). Classic LB medium formulations are excellent for routine molecular biology applications, but yields saturate at an $OD_{600} \leq 7$, when utilizable carbon sources are exhausted. To achieve high yields with ODs ≥ 20 a buffered nutrient rich medium containing glycerol as additional carbohydrate source is better suited.

Impact of agitation speed

Typical shaking speeds are around 200–250 rpm. The positive impact of using a higher shaking speed is clearly demonstrated here in the TB media culture. The best results in biomass and pDNA yields were obtained in the cultures with 20 % fill incubated at 400 rpm. Compared to the standard agitation at 250 rpm, an increase to 400 rpm resulted in a nearly 2-fold higher biomass after 8 hours (Fig. 2) and a ~30 % increase in pDNA yields (Fig. 3).

*Application Note 449 available for download at www.eppendorf.com/appnote449

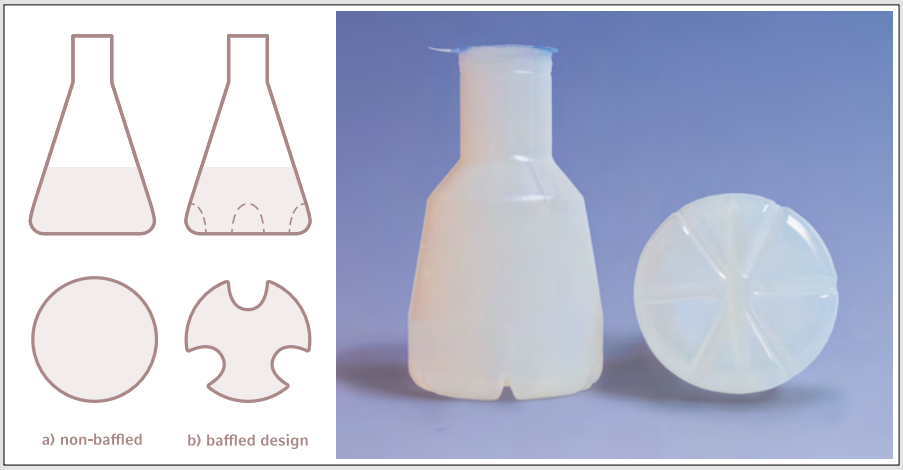


Fig. 1 (from left to right): Erlenmeyer flask design classic (unbaffled) and baffled; Ultra Yield flasks

Optimizing Plasmid Yields in Shake Culture

Impact of working volume

A larger culture volume can also improve plasmid yields. The higher fill volume (at same shaking speed of 250 rpm) resulted in higher yields. Doubling the culture volume from 20 to 40 % resulted in a ~1.4 higher biomass (Fig. 2) and an increase of pDNA yields >15 % after 8 hours (Fig. 3).

This is a bit unexpected, as usually a higher fill results in oxygen limitation. One explanation may be the specific baffled design and flow behavior in the specialized Ultra Yield designs.

Impact of the shaker design

To incubate this many flasks in parallel, large capacity stackable incubated shakers are the product of choice for high yield plasmid production. Depending on fill volume, a volume between 19.5 L (20 % fill) and 45 L (40 % fill) in 2.5 Liter Ultra Yield flasks can be reached e.g. in a triple stacked large capacity shaker as the Eppendorf Innova S44i. The shaker should operate reliably with high weight loads also at high shaking speeds.

Shakers with a multi-shaft drive system stabilize the platform on more than one point, providing maximum stability also at high speed/load scenarios. To compensate the centrifugal force created by the liquid mass, the shaker drive should in addition be equipped with a good counterbalance to prevent imbalance situations and prevent the shaker from wearing out over time.

Conclusion

Optimizing the bacteria cultivation conditions can contribute to enhancing pDNA yields. Using a nutrient rich medium instead of LB, baffled or specialized flask design like Ultra Yield flasks, and an increase in shaking speed can positively impact bacterial growth and thus subsequent production yields. Hence, selection criteria for shakers should be, beside capacity, a robust drive and counterbalance system to operate reliably with high weight and shaking speeds.

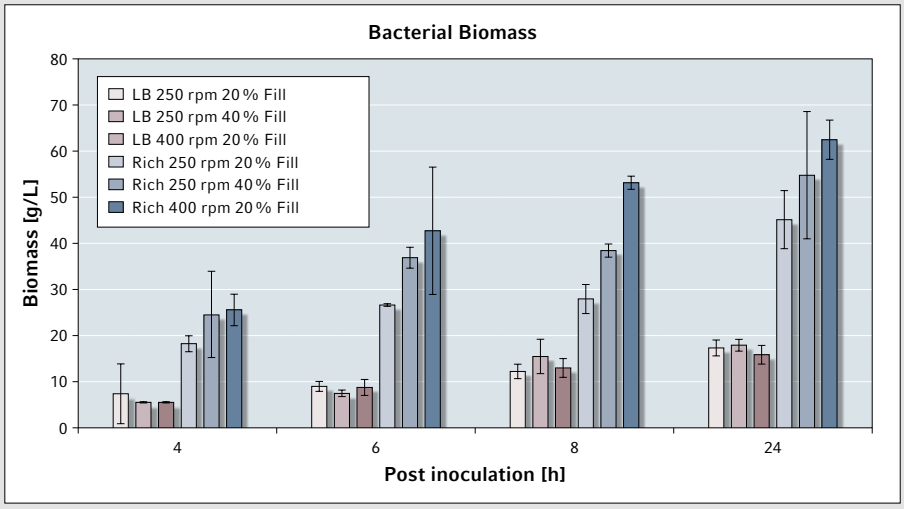


Fig. 2: Bacterial biomass (DH5α with pUC19 plasmid) in 2.5 L Ultra Yield flasks with different media, working volumes, and agitation speeds incubated at 37 °C

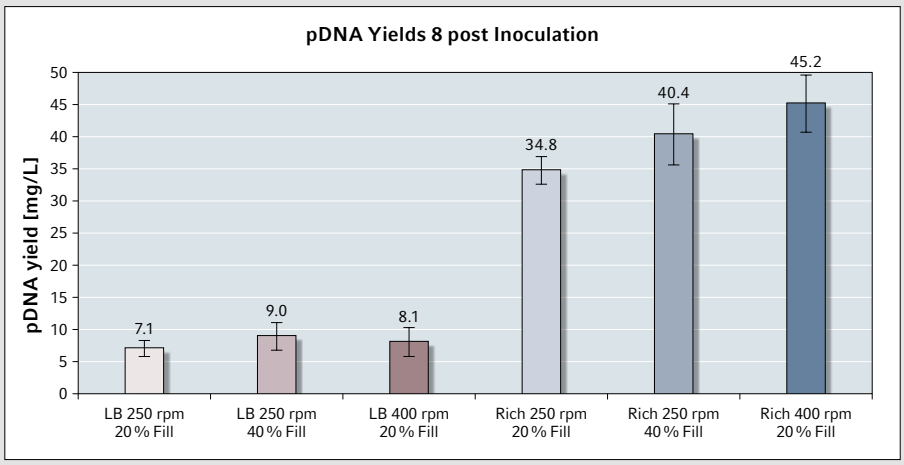


Fig. 3: pDNA yields (DH5α with pUC19 plasmid) in 2.5 L Ultra Yield flasks with different media, working volumes, and agitation speeds incubated at 37 °C

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A Low Oxygen Atmosphere Supports the Xeno-Free Generation, Expansion, and Differentiation of Human Induced Pluripotent Stem Cells

RICK COHEN, RUTGERS UNIVERSITY, PISCATAWAY, NJ, USA

Abstract

We previously demonstrated that using low oxygen tension increased the efficiency of reprogramming human somatic cells to pluripotency [1].

In this study, we extend our findings and further increased the development of this culture paradigm. We are able to observe that post-electroporated fibroblasts cultivated at 4 % O_2 grow on synthetic and biological surfaces. Further, we observed normal spreading of iPSCs and a high level of purity in early iPSC passages. The cells were successfully differentiated into motor neuron and cardiomyocyte lineages emphasizing the efficacy of low oxygen levels during cell cultivation.

Introduction

Reprogramming human somatic cells into the pluripotent state is a subject of thousands of publications since their discovery in 2007. Originally these studies were pioneered with a set of four genes found to be expressed in native human embryonic stem cells; Oct4, Sox2, KLF4, and c-Myc (Lin28 and Nanog), delivered using genetically modifying methods such as retrovirus, and cultured on non-defined matrices.

In the 13 years since these seminal studies many improvements were made such as (1) replacing genetically modifying methods with non-genome altering safer alternatives; (2) replacement of c-Myc oncogene with non-transforming family member, L-Myc; (3) inclusion of small molecules to boost efficiency of reprogramming; (4) optimizing culture conditions which includes the use of low O_2 tension (4–5 %); and (5) use of clinically relevant defined media and matrices.

In this novel study, we combined many of the improvements to demonstrate the successful reprogramming of human foreskin fibroblasts with low O_2 conditions in the CellXpert® C170i CO_2 incubator. We observed that the tested growth substrates offered stable cell adhesion and spreading following electroporation of fibroblasts, which in turn led to a robust production of karyotypically normal iPSC colonies capable of robust expansion in defined media and ability to differentiate into neural and cardiac lineages.

Materials and methods

The reagents and procedures used in this Application Note* are similar to that described previously. When indicated, culture vessels were coated with 5 µg/mL of Vitronectin and used in comparison with a synthetic substrate. Once iPSC colonies appeared in Reprogramming Media, they were transitioned into Animal Free Low Protein hESC media for expansion. iPSC expansion and early steps in neuronal differentiation was carried out with 6-well plates, whereas cells analyzed for immunostaining or terminally differentiated to motor neurons or cardiomyocytes were spin seeded onto 24-well culture plates. Neurons were developed as described previously.

Results and discussion

Expansion and characterization of iPSCs under hypoxic conditions

Rudimentary iPSC colonies gave rise to more mature colonies at day 21–30 post modification and were passaged using gentle non-enzymatic methods. In order to purify the culture of Vitronectin derived cells, manual picking of colonies was used, whereas iPSCs grown on synthetic substrates outgrew any minor number of fibroblasts that were dislodged during the non-enzymatic passaging.

After 7 passages, the episomal plasmid reprogrammed line from the synthetic coating was analyzed for karyotype and found to be normal. Similar cultures were spin seeded onto 24-well dishes and analyzed for routine pluripotency or differentiation markers (Fig. 1). At passage 8, the cultures were found to lack any appreciable expression of SSEA1 and robustly expressed both SSEA4 and Oct4. Likewise, these cells co-expressed Lin28, Nanog, and Tra-1-60.

Differentiation of iPSCs to neuronal and cardiomyocyte lineages

The iPSCs were first differentiated into neuroepithelial cells and then secondly into neural stem cells. After 4 passages in neural stem cell media, the cells appeared to differentiate into a uniform mat, with repeating patterns of rosettes, characteristic of neural stem cell stage. Many of these cells were OTX2+, with a greater number expressing both Pax6 and Nestin.

Since this was the second step out of four to derived motor neurons, we continued to differentiate the stem cells into presumptive motor neuron precursors. The small molecules and growth factors were altered to continue the differentiation protocol (Fig. 2, upper panel), and some of the cells continued to express OTX2 and Nestin, while many began to express Olig2, a marker of motor neuron lineage.

After one week, the cells were finally exposed to a set of cytokines and small molecules to induce the final differentiation into presumptive motor neurons (Fig. 2, lower panel).

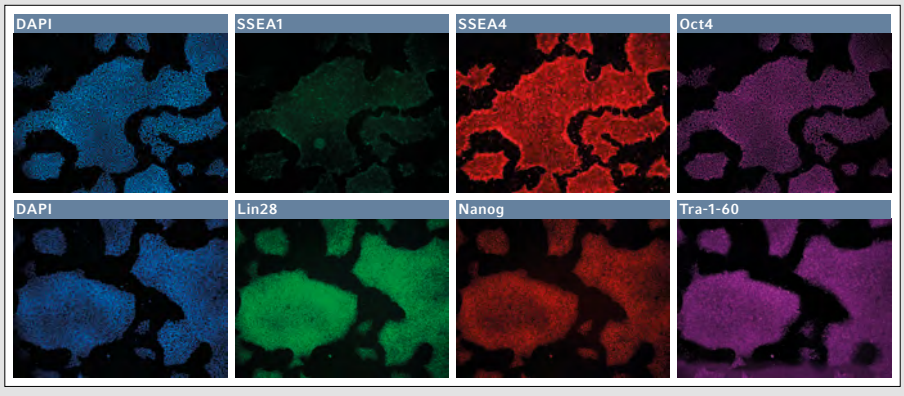

Fig. 1: Immunofluorescence staining of markers for pluripotency and differentiation in low passage iPSCs

A Low Oxygen Atmosphere Supports the Xeno-Free Generation, Expansion, and Differentiation of Human Induced Pluripotent Stem Cells

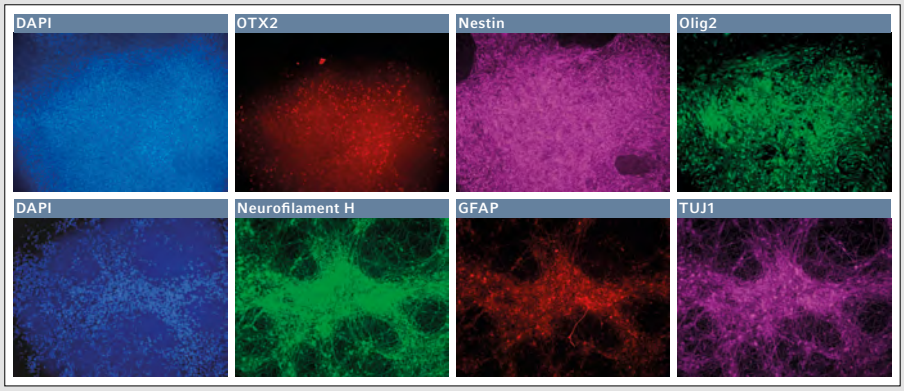

Fig. 2: Differentiation of iPSCs into presumptive motor neuron precursor cells in low O_2 environment

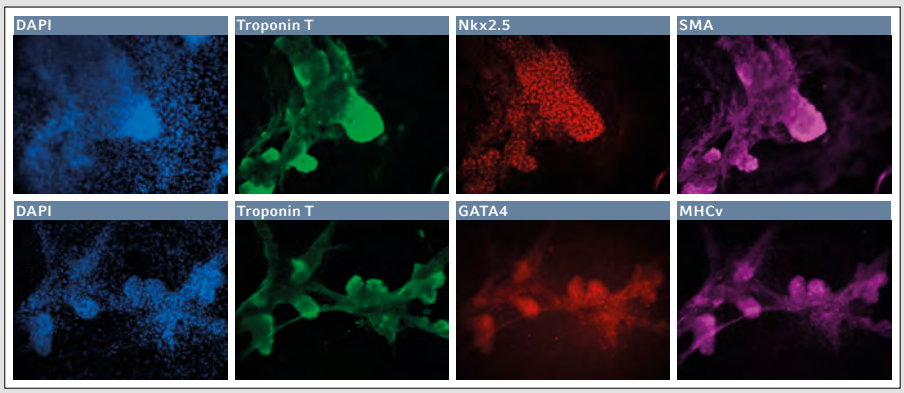

Fig. 3: Differentiation of iPSCs into cardiomyocytes in low O_2 environment

After 7 days in the final media cocktail, the cells organized a fine network of process which were robustly Neurofilament H+, with some remaining TUJ1+ appearance and few GFAP+ glial cells. Together these results showed that hypoxic incubation can be used to derive cells of neuroectodermal lineage. In addition, we were able to first differentiate the cells into definitive endoderm and then finally develop them into cardiomyocytes (Fig. 3).

After 10–11 days in culture the surviving cells organized into small patches. These "nodes" were highly three dimensional and expressed Troponin T, Nkx2.5, and SMA. Further, we observed robust expression of Troponin T along with GATA4 and MHCv. Together this indicates the successful development of the cardiomyocyte lineage in a hypoxic environment.

Conclusion

We successfully demonstrated the reprogramming of human foreskin fibroblasts in low oxygen tension in the CellXpert C170i CO_2 incubator. The iPSC line was characterized as karyotypically normal and found to express expected markers of pluripotency. Further, the low O_2 environment supported the reliable differentiation of iPSCs into various stages of ectoderm, neuronal stem cells, presumptive motor neuron precursors, and finally motor neurons. As well, the hypoxic atmosphere inside the incubator supported the differentiation of the same iPSC line into cardiomyocytes.

*Download the full Application Note 443 at http://eppendorf.global/IR1 or scan the QR code

Literature

[1] Low Oxygen Levels Enhance the Efficiency of Reprogramming Human Somatic Cells to Pluripotency. *Eppendorf Application Note* 338. Download at http://eppendorf.global/IUW

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Constant RQ Fermentation of *Pichia pastoris* in the DASbox® Mini Bioreactor System

NINA SCHRAND, MALTE SCHNEIDER, EPPENDORF SE BIOPROCESS CENTER, JUELICH, GERMANY
MA SHA, BIOPROCESS APPLICATIONS LAB, EPPENDORF, INC., ENFIELD, CT, USA
CONTACT: BIOPROCESS-EXPERTS@EPPENDORF.COM

Introduction

The yeast *Pichia pastoris* is largely used for heterologous protein production in biotechnology. For optimizing the production yield, one of the most important factors is the feeding strategy. One of the leading strategies for protein production-oriented feeding is based on the respiratory quotient (RQ). Constant RQ-based feeding ensures that the respiratory metabolism of glucose/glycerol is optimized for protein production purposes and the formation of by-products is limited [1].

The intention of this study is to demonstrate the feasibility of constant RQ-based feeding for protein production-oriented yeast fermentation using the DASbox Mini Bioreactor System. Further, we compared the results to a standard DO-spike triggered feeding done in the same setup.

The respiratory quotient (RQ)

RQ is the quotient of carbon dioxide produced and oxygen consumed by a culture, expressed with the carbon dioxide transfer rate (CTR) and the oxygen uptake rate (OUR). Under conditions of a constant dissolved oxygen concentration, the OUR equals the oxygen transfer rate (OTR) [2]. The RQ for the metabolization of glucose is 1. This can be explained by the fact that per mol glucose, 6 mol O_2 are needed and 6 mol CO_2 are produced. Which means that the O_2 uptake equals the CO_2 transmission and thus their quotient is 1. Once glucose is fully metabolized the culture starts to consume fermentation by-products, mainly ethanol. As ethanol is more reduced than glucose, the use of ethanol as substrate results in a RQ value below 1.

Thus, the RQ value can serve as an inline parameter indicating which substrate is consumed by the culture, and a constant RQ process can be used to optimize fermentation based on a specific carbon source.

Materials and methods

In this study we used the *Pichia pastoris* strain DSMZ 70382. We used the DASbox Mini Bioreactor System (Eppendorf) for microbial fermentation applications. We connected a DASGIP® GA4 exhaust gas analyzer module (Eppendorf) and used DASware® control software (Eppendorf).

Medium preparation, setup of the bioprocess system, inoculum preparation, and optical density measurements are described in the Eppendorf Application Note 439 [3]. The process parameters are listed in Table 1.

Feed process triggered by DO-spike

The end of the batch phase of a fed-batch culture is often indicated by a DO-spike. At this point, the carbon source within the initial culture medium is consumed, the metabolic activity of the culture and, therefore, its oxygen demand drop rapidly, resulting in a sharp increase of DO in the medium. We took advantage of this DO-spike to trigger an automatic start of the feed pump using an automatic programming script. The details are described in the Eppendorf Application Note 439 [3].

Automatic RQ control by programmed feeding

As stated before, the RQ value can serve as an indicator for which substrate is used by the culture. We implemented a software script which starts automatically after inoculation [3]. After a delay time of 12 h, the RQ value of 1 is used to start the feeding pump, and further feeding is automatically controlled based on the RQ value.

Parameter	Configuration
Vessel	DASbox Mini Bioreactor, autoclavable, microbiology
Inoculation density	OD_{600} = 1
Dissolved oxygen (DO)	30 %, maintained by DO cascade
Agitation	Overhead drive, maximum 1,600 rpm, controlled by DO cascade
Gassing	Automatic gas flow and mix, controlled by DO cascade
Temperature	30 °C, heating and cooling done by Peltier elements in the bioreactor's positions
pH	5.0, one sided control with 10 % (v/v) sterile ammonium hydroxide solution
Sparger	L-Sparger
Feeding	Automatically triggered via reactor script

Table 1: Process parameters used during fermentation

Results

We ran *P. pastoris* fed-batch fermentation two times. In one setup, the feed was started automatically by a script which was triggered by a DO-spike. In the other setup, we implemented a constant RQ-controlled feeding strategy.

The DO-spike based feeding was started once the value of dissolved oxygen raised from 30 % to above 38 % which was the case after 39 h inoculation time [3]. The RQ-controlled feeding was started after a delay time of 12 h. The RQ is controlled towards a value of 1 by turning the feed pump on and off.

After an inoculation time of 36 h, the RQ starts dropping sharply (Fig. 1). When it hits 1, the pump starts feeding glucose. During the duration of the feed, the RQ oscillates between 0.9 and 1.1.

This is caused by a delay in measurement of the exhaust gas components, as the bioreactor's exhaust gas needs to pass the additional tubing between the reactor and the GA4 before being measured. This can be further minimized by optimization of the controller's PID settings.

Constant RQ Fermentation of *Pichia pastoris* in the DASbox® Mini Bioreactor System

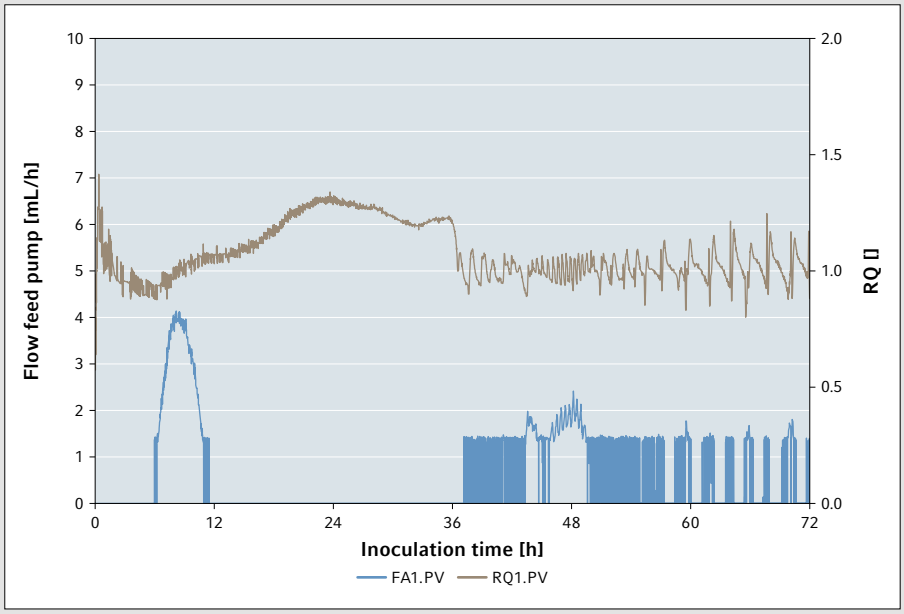

Fig. 1: Fermentation graphs of the flow of the feed pump (blue) and the respiratory quotient (brown) during *P. pastoris* fermentation with RQ-controlled feeding

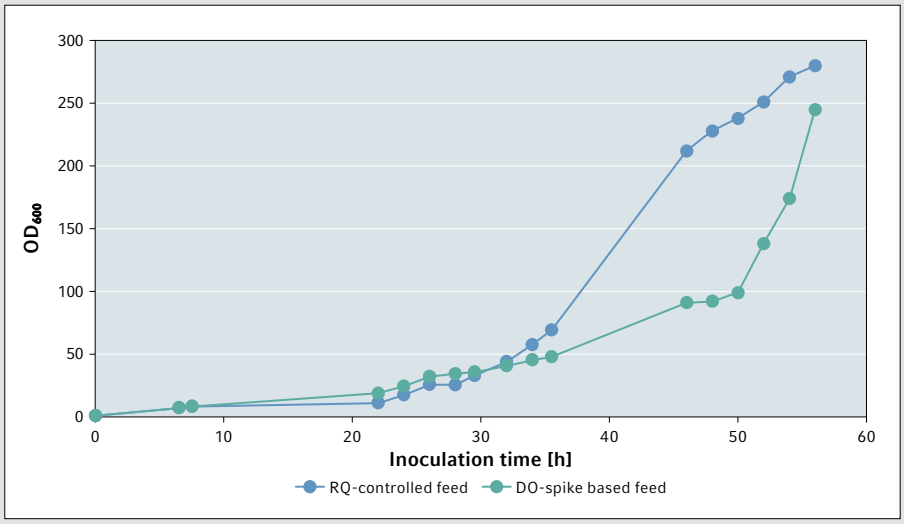

Fig. 2: Growth curves of *P. pastoris* with RQ-controlled feed and DO-spike based feed

During the batch phase, before feed start, the culture's growth in both settings was comparable (Fig. 2).

After the batch growth phase of the culture, the RQ-controlled feeding resulted in a much earlier exponential growth than the DO-spike triggered feeding. After 56 h, the DO-spike based fed-batch culture reached an OD_{600} of 245, while the RQ-controlled culture reached an OD_{600} of 280. The RQ-controlled culture reached an OD_{600} above 200 at an earlier time point during fermentation than the DO-controlled culture.

Conclusion

We demonstrated the implementation of a constant RQ-controlled feeding strategy using DASware® Control 5 and a DASbox fermentation system. If the culture's growth profile and biomass accumulation is an indication of a successful fermentation, it can be concluded that the feeding based on constant RQ has multiple benefits. We observed an OD_{600} of 200 much earlier with the RQ-based feeding strategy compared to the DO-spike triggered feeding strategy.

This can be explained by insufficient feeding in the DO-spike triggered set-up. The feed steps would need to be adapted, which is not the case for the RQ-based feeding. In the latter case, the feeding optimization is automatically self-achieved as the culture creates its own demand for feeding based on the drop of RQ value.

This will save additional experiments and therefore material costs, labor costs, system occupancy, and time.

Literature

[1] Xiong Z. Q., Guo M. J., Guo Y. X., Chu J., Zhuang Y. P., Wang N. S., & Zhang S. L. (2010). *Enzyme and Microbial Technology*, 46(7), 598–602. https://doi.org/10.1016/j.enzmictec.2010.03.003

[2] Clarke K. G. (2013). 8 - (K. G. B. T. - B. E. Clarke Ed.; pp. 147–170). *Woodhead Publishing*. https://doi.org/10.1533/9781782421689.147

[3] Schrand N., Schneider M., Sha M. (2021) *Eppendorf Application Note* 439. Download at http://eppendorf.global/lYo

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ULRIKE RASCHE, EPPENDORF SE BIOPROCESS CENTER, JUELICH, GERMANY

From Data to Control: Process Analytics in Bioprocessing

Reproducibility is essential in the production of biologics. That is why understanding interdependencies between process parameters and establishing control strategies are so important in upstream bioprocess development. Thorough process analytics is a prerequisite. Joerg Schwinde, Key Segment Manager Vaccines and Monoclonal Antibodies at Eppendorf SE Bioprocess Center, shares his perspective on which strategies will help labs best optimize upstream bioprocess analytics both now and in the future.

What are important parameters in biologics upstream bioprocessing?

Joerg Schwinde: Besides process parameters like pH, temperature, and dissolved oxygen, there's the behavior of a strain or cell line – for example, the growth kinetics, the ratio between total and viable cell density, and the productivity of the cells. Metabolite concentrations are also important. Furthermore, the product needs to be characterized: is it indeed the target product? Are there undesirable byproducts that can significantly affect product quality? Monitoring all of this is quite a complex task.

What strategies is industry applying to control relevant bioprocess parameters?

JS: The monitoring of parameters can be offline, as with external analyzers in combination with sampling devices. This sampling can be automated, but the challenges here include the additional manual workload and the absence of automated feedback loops. Automated feedback loops are possible through online analyzers which provide almost real-time data and spare sampling steps. Setting them up requires a bioprocess control software which receives the sensor signals and controls the acting units inside the process control system – for example, an aeration unit or a pump. To ensure communication between the control software and the analyzer hardware, analog and digital options are available.

One communication standard in this context is what's known as open platform communication (OPC). To reduce the workload, speed up the process, and more, the online solution with the option for automated feedback control is very attractive.

In your opinion, which developments will become more important in upstream bioprocessing in the coming years?

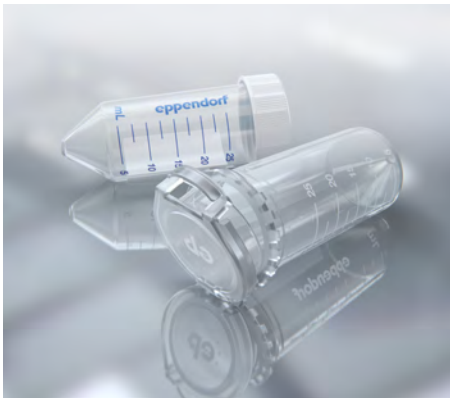
JS: Data acquisition, analytics, and process automation will get faster, even more precise, and more powerful. This will be supported by predictive analyses (i.e., design-of-experiment approaches) and by artificial intelligence. Those options provide tremendous opportunities to simulate processes ahead of time and predict where challenges lie and how to bypass them successfully – all of which contributes to time and cost savings as well as to safety.

More information at
www.eppendorf.com/bioprocess

BRIGITTE KLOSE, EPPENDORF SE

Good Prospects for Conical Tubes 25 mL

Do you work with DNA, protein, or light-sensitive samples? Then you can look forward to further variants of Eppendorf Conical Tubes 25 mL, available with SnapTec® or screw cap. As of approx. May 2022, these tubes will also be available with LoBind® surfaces and in amber.



DNA LoBind or Protein LoBind

Tubes with DNA LoBind or Protein LoBind surface are ideal for use in applications where concentrations tend to be small and sample recovery is vital for assay results. A special, two-component polymer mix creates a hydrophilic surface that guarantees optimal recovery rates and leads to more stable concentrations as well as to more reproducible and reliable experimental results.

Tip! More about LoBind in our Video "Eppendorf LoBind® – How it works"

Amber tubes

Handling light-sensitive samples in non-transparent long tube formats is a frequent source of contamination.

Our amber colored Conical Tubes 25 mL combine the effective protection from energy-rich light in the lower wavelength range with a high degree of transparency. You can fully control the insertion depth of your pipette tip.

All about Conical Tubes 25 mL at http://eppendorf.global/IRc

Or scan the QR code

Tip

For Any Case: Eppendorf Tubes®

Effective light protection in amber tubes

Numerous types of light-sensitive reagents and samples are often used in the laboratory routine. Once exposed to light, their life-time or activity may rapidly decrease and therefore adversely affect subsequent assays and experiments. For this type of samples, Eppendorf offers amber tubes in the following variants:

> Eppendorf Safe-Lock Tubes 0.5 mL, 1.5 mL, and 2.0 mL
> Eppendorf Tubes 5.0 mL
> Eppendorf Conical Tubes 15 mL, 25 mL*, and 50 mL

LoBind® surfaces

No matter in which volume range you work with protein or DNA samples – with our LoBind tubes you get the best out of your applications.

Choice of Protein LoBind and DNA LoBind tubes:

> Eppendorf Safe-Lock Tubes 0.5 mL, 1.5 mL, and 2.0 mL
> Eppendorf Tubes 5.0 mL
> Eppendorf Conical Tubes 15 mL, 25 mL*, and 50 mL

*see also article on the left

JAN-HENDRIK BEBERMEIER, EPPENDORF SE

Save Plastic, Stay Safe

Despite the quest for sustainability, the safety of both the scientist and the samples remains paramount in the laboratory. Although there are good initial ideas, lab waste can still not be recycled efficiently at this time because, for example, regulatory requirements stipulate that it be disposed of as biohazardous waste. How can one at least reduce this type of waste?

In many laboratories, there is no way around disposable tips and tubes, which creates large amounts of biologically or chemically contaminated plastic waste. Innovative recycling concepts are still a long time coming; however, through clever product selection, you can start reducing the amount of plastic used in the laboratory today.

Specific examples:

> Reload pipette tips: for the past two decades, our epT.I.P.S.® refill system consisting of box and reloads has already been available as a plastic-saving refill system. More about our all-round optimized epT.I.P.S. Box 2.0 at www.eppendorf.com/epTIPS-News

> In many cases, a vessel with a smaller volume is sufficient! Changing from 15 mL to 5 mL or from 50 mL to 25 mL reduces plastic waste by half and doubles the storage capacity in the ULT freezer. More information at www.eppendorf.com/5mL and www.eppendorf.com/25mL

A final suggestion

Any object that comes into contact with the bench surface in the laboratory is to be considered contaminated. This risk of contamination also jeopardizes the recycling of packaging material. For this reason, products such as tip boxes should be separated from the packaging material in such a way that they remain "clean".

After all, cardboard, plastic lids or bags are valuable raw materials. Collect them in the appropriate collection bins at your organization.

The new epT.I.P.S. Box 2.0

More information at www.eppendorf.com/sustainability

Or scan the QR code

News

Eppendorf – 100 % Green Energy

Our production facilities have been gradually switched over to power originating from renewable sources. We can proudly announce that since 2021, all products carrying the renowned Eppendorf logo have been assembled using 100 % renewable electrical energy.

However, product assembly is only one aspect of energy-conscious action; the operation of laboratory equipment also requires electricity. Have you checked the source of your laboratory's electricity?

How to reduce your electricity bill

Electricity is required to operate laboratory equipment. Major consumers include ULT freezers, which ideally combine efficient cooling with energy savings.

With the new CryoCube® F440h ULT freezer from Eppendorf, you can achieve reliable –80 °C and minimize your power consumption, as it consumes only 6.8 KWh/day. Tip: Simple maintenance measures such as de-icing the freezer or cleaning the heat exchanger grid reduce power consumption and extend the lifetime of your instrument. Reduce the energy consumption of your Eppendorf centrifuge by using features such as programmable pre-cooling and automatic ECO compressor shut-off.

Further information on the sustainability of laboratory products as well as tips and decision-making aids can be found at http://eppendorf.global/m2a

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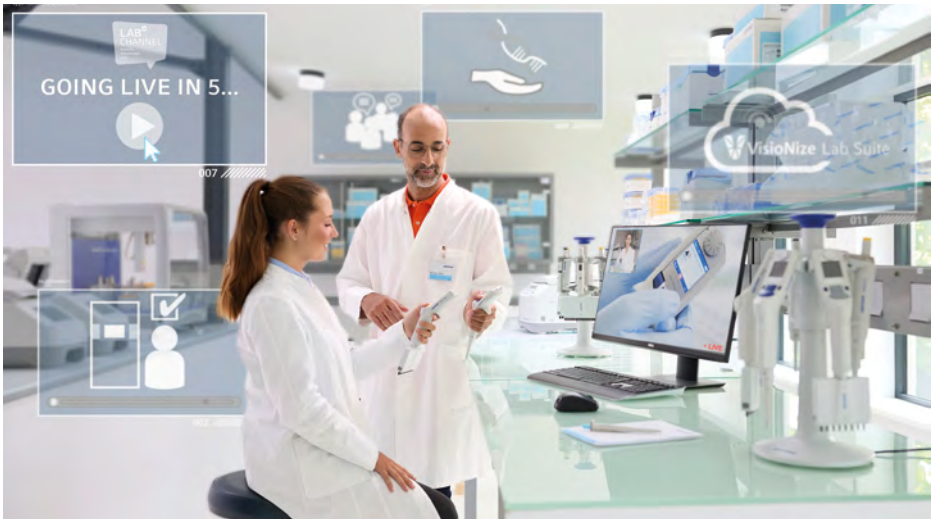
EILEEN DUVE, EPPENDORF SE

Lab Channel: Experts. Knowledge. Live.

Would you like to expand your knowledge on all topics concerning the laboratory? Are you interested in live events and interaction with experts from the lab? Now is your chance. We present the Eppendorf Lab Channel – a new virtual format with webinars and product demos. Live and on demand.



Since at least 2020, homeschooling, online training, and other virtual events have been an integral part of our daily routine. At Eppendorf, too, a lot has happened in our virtual space. In order to continue our lively exchange with you, and to share our expert knowledge, we have established a new virtual format: the Eppendorf Lab Channel.

The Eppendorf Lab Channel is a virtual platform that allows registered participants to watch live and on-demand webinars free of charge. For the first time, on this channel, we also offer you product and application demonstrations. Take a look over the shoulders of the Eppendorf experts and allow yourself to be magically transported into the world of the laboratory.

In our live events, we invite you to ask all your burning questions as well as gather tips and tricks that will help you move forward in your daily lab life.

The kickoff-event, our webinar series "Digitalization in the Laboratory", took place in late 2021. All current events are available under the menu option "Agenda".

LAB CHANNEL
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Knowledge.
Live.

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Tip

From A to Z: Eppendorf on YouTube™

Did you know that Eppendorf provides a great choice of playlists on YouTube? Discover the Eppendorf YouTube universe and find informative videos and educational tutorials on the following topics:

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LUKAS WONRATH, EPPENDORF SE

Catalog 2022: More Compact, More Digital

The Eppendorf catalog is available in over 70 countries; it is published in twelve versions and eight languages. Even if more than 2,100 articles from the Eppendorf portfolio are represented, this year, the catalog, with its 350 pages, is slimmer and more digital than ever before. The catalog team explains how this came about.

"It's the mail slot", describes catalog team-member Ann-Katrin Kardinahl. "It really is the limiting factor for our catalog", she adds with a laugh. And it is also one of the reasons why this year the Eppendorf catalog did not increase in content but instead slimmed down. This was made possible by an optimized concept.

New structure for a clearer overview

Together, the team came up with a novel structure: "The new topic pages in every chapter offer our customers an even better introduction into the product world of Eppendorf", says Ann-Katrin. Immediately thereafter, the reader reaches the helpful "Selection Guides" which enable direct comparisons between products, as well as the focus products of each respective product category.

More compact and more digital

"Even though the catalog 2022 is much more compact, we still show the entire product program", adds Svenja Sterneberg. "And this year, for the first time, our extended centrifuge portfolio also includes high-speed centrifuges and ultracentrifuges."

More in-depth and application-oriented documents are offered digitally via the Eppendorf website, with straightforward access through QR codes or links. In this way, you can easily receive helpful information in a "cross-media" approach.

Your link to the catalog

You can download the Eppendorf Catalog 2022 at www.eppendorf.com/catalog, or you can order the printed version.

News

New "Off the Bench" Edition

Planet Earth is threatened more than ever by greenhouse gas emissions, states the latest report by the Intergovernmental Panel on Climate Change. Not news exactly; however, it does stress that action finally, and urgently, needs to be taken. The dossier of the latest issue of the Eppendorf Magazine "Off the Bench", too, takes a conscious look at the need for leading a life in harmony with nature. It is not only the environment that will benefit – so will humans. After all, living surrounded by, and in harmony with, nature is proven to contribute to health and happiness.

As well as featuring additional current LifeScience topics, this issue of "Off the Bench" once again focuses on selected products and solutions by Eppendorf. Learn more about the Eppendorf pipettes which, for the past 60 years, have continually evolved, as well as about our latest innovations and services.

You can read "Off the Bench" online or download a PDF. You may as well subscribe to the printed magazine and it will arrive at your bench, free of charge and twice a year.

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CORDULA RICHTER & CAROLYN TAUBERT, EPPENDORF SE

Virtual Prize Ceremonies for Tanmay Bharat & Amber Alhadeff

Dr. Axel Jahns (VP Corporate Citizenship and Governmental Affairs), host of the virtual prize ceremony for Tanmay Bharat

Amber L. Alhadeff

As already in 2020, the award ceremony for the *Eppendorf Award for Young European Investigators* could not take place as usual at the EMBL Advanced Training Centre in Heidelberg due to the Corona pandemic. Instead, the ceremony for the award winners of 2020, Prof. Dr. Randall Platt (ETH Zurich, Switzerland) and 2021, Dr. Tanmay Bharat (University of Oxford, UK) was held virtually and transmitted via live streaming.

A recording of the event offers the opportunity to experience the interesting presentations of the two award winners even afterwards. The chairman of the jury, Prof. Dr. Reinhard Jahn, (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany) and jury member Prof. Laura Machesky (Beatson Institute for Cancer Research, Glasgow, UK) gave the laudations. Other speakers were Prof. Edith Heard (Director General EMBL), Dr. Susan Jones (Chief Editor Nature Microbiology), Dr. Georg Winter (CeMM Vienna, Austria), and Dr. Wilhelm Plüster (Chief Technology Officer, Eppendorf SE).

Recording of ceremony:
http://eppendorf.global/m2l

More information at
www.eppendorf.com/award

The American scientist Amber L. Alhadeff, Ph.D. (Monell Chemical Senses Center and University of Pennsylvania, Philadelphia, USA), was as well awarded the 2021 *Eppendorf & Science Prize for Neurobiology* of 25,000 USD in a virtual event.

Amber Alhadeff won the prize for her work on the gut-brain control of hunger circuits. Her research has revealed how hunger-sensitive neurons in the brain receive signals from the gastrointestinal tract, and how they influence food intake and other survival behaviors. Alhadeff demonstrated how neural circuits can filter interoceptive information to prioritize hunger – for example by weakening other signal inputs to enable food seeking. She went on to show that nutrients in the gut rapidly inhibit activity in hypothalamic hunger neurons. Further, different types of food (e.g. fat and sugar) engage different gut-brain pathways to communicate with these neurons.

Recording of ceremony:
http://eppendorf.global/m2K

More information at
www.eppendorf.com/prize

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Win a Move It® Pipette

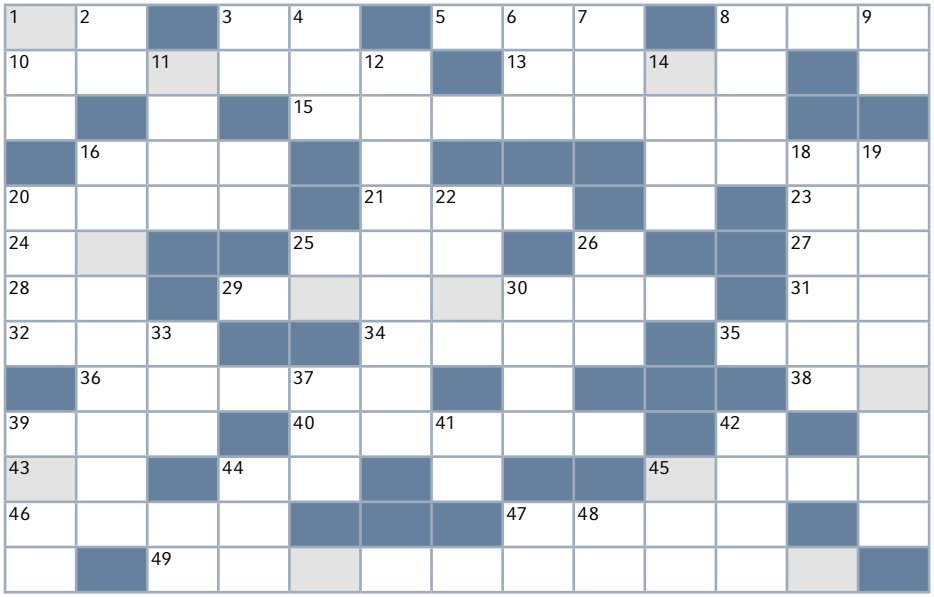
The solution of the prize competition of BioNews No. 54 was "SCIVARIO TWIN". The main prize, an Eppendorf Research® plus multi-channel pipette, went to Alice K., Belgium.

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 **June 30, 2022**.

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. 58.



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

1 User experience (abbrev.)
3 Afterthought to a letter
5 Race, sprint
8 Creates a current of air or a breeze
10 Attribute of certain tubes
13 French Christmas
15 Turned sweet 60 in 2021
16 At the top of the business hierarchy (abbrev.)
20 Ernie's buddy
21 Association of professional golfers (abbrev.)
23 Transition metal used e.g. in corrosion-resistant alloys (chemical symbol)
24 Light silvery-grey metal (chemical symbol)
25 The second p in ppm
27 Its atomic number is 117 (chemical symbol)
28 Kingdom in Western Europe (ISO country code)
29 Handles, controls, directs
31 Ability to perceive, use, understand, and handle emotions (abbrev.)
32 Stands for the good practice quality guidelines and regulations (abbrev.)
34 Air pollution caused by emissions
35 For, not against
36 Infectious, replicates itself within bacteria
38 A person not known yet (abbrev.)
39 Semiconductor light source (abbrev.)
40 Mistake, inaccuracy
43 Gold in French
44 Purifies water, bleaches, stinks (chemical symbol)
45 Music genre
46 Come to a halt
47 Kermit is one
49 Creator of a common conical flask

DOWN

1 Class of freezers for storing biological samples (abbrev.)
2 Symbolizes online hugs and kisses
3 Mathematical constant
4 Genetic marker (abbrev.)
6 French indefinite female article
7 Used to form the negative
8 Bloodsucking good jumper
9 James Bond's counterpart with academic degree
11 Harry and Hermione love it with butter
12 Masters repetitive pipetting
14 One of the world's most active volcanoes
16 Expert for your cell culture
18 Apprentice, trainee
19 Supports the connections in your lab
20 It is big and there is a theory about it
22 He developed a technique for staining bacteria
25 A country of southeast Central America (ISO country code)
26 Tetrapods have four (sing.)
30 Jump statement in programming language
33 Awarded for advanced research (abbrev.)
37 Matrix in electrophoresis
39 Bewildered, confused
41 Human blood group system named after a monkey (abbrev.)
42 Violent, explosive anger
44 Saves lives when done at the right beat (abbrev.)
45 Pleasure, delight, bliss
47 10^{-15} m (abbrev.)
48 Chemical symbol for element 75

Solution hint for prize competition of BioNews No. 56:

		A					R		A	

Send us the solution until **June 30, 2022**. 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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