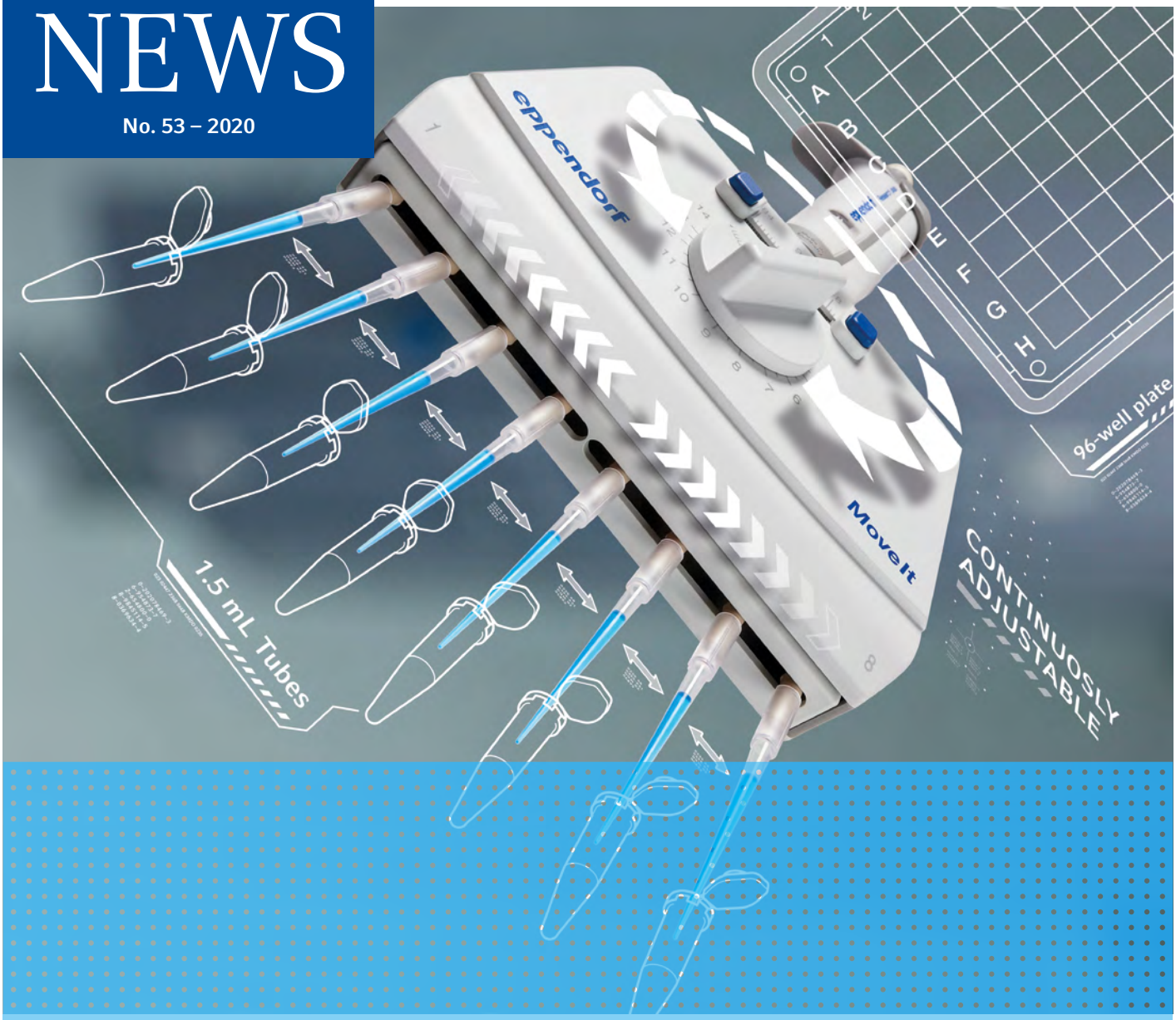
BIO NEWS

No. 53 – 2020

eppendorf



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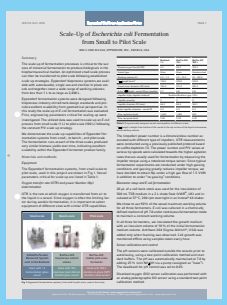
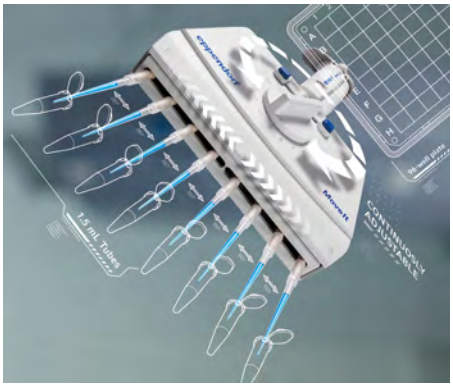

Scale-Up of *Escherichia coli* Fermentation from Small to Pilot Scale



Dear Readers,

The coronavirus SARS-CoV-2 continues to affect our lives, and it is currently not possible to foresee how long this situation will last. All over the world, research teams are working around the clock to develop effective vaccines and medications; during this process, ensuring the highest safety for people as well as samples is of the utmost importance.

We support scientists worldwide in their work with

> Centrifuges with innovative aerosol-tight rotors
> Systems for automated liquid handling to speed up workflows and generate accurate, reproducible results
> Bioprocessing solutions for rapid progress in vaccine research and development
> Manual pipettes and pipette tips for ergonomic, safe, and accurate sample transfer
> CO_2 incubators which provide a safe, easy to sterilize environment for cells

And development continues to move forward. For example: our customers have asked us about an efficient, safe, and ergonomic solution for multiple sample transfer between different formats. The answer is "Move It®" – a new generation of multi-channel pipettes with adjustable tip spacing. More on this topic on pages 4–5. The need for increased sustainability is not sparing the laboratory. After 17 successful years of epT.I.P.S.® pipette tips on the market, we are now taking a measurable step towards saving plastic through a design revision of our single-use racks (pages 6–7). Additional topics within this issue address sample identification and process tracking in the laboratory. More details on pages 10–11.

Making your daily laboratory experience easier and more efficient as well as supporting you in reaching important decisions about the future – this is what has been driving Eppendorf for 75 years, and what will continue to drive Eppendorf – fully in accordance with our mission to contribute to the improvement of human living conditions.

You would like to learn more about 75 Years Eppendorf?
Visit http://eppendorf.global/kuR

Your Eppendorf BioNews Team

Note: In response to the rapidly changing situation of the coronavirus outbreak, the **Young European Investigators Conference** (see last issue) has been rescheduled to June 24, 2021. More details at http://eppendorf.global/kwG

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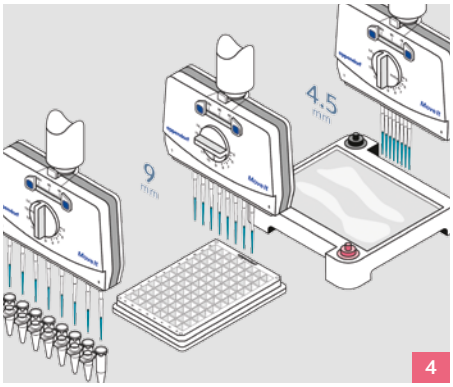

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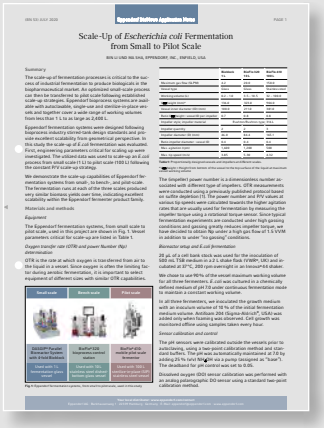
Eppendorf BioNews Application Notes


Scale-Up of *Escherichia coli* Fermentation from Small to Pilot Scale

STEFANIE RÖSEL & SAMIRA SCHROEDER, EPPENDORF AG

Just Keep Moving: Move It®!

Are you busy pipetting samples between different vessel formats? Perhaps even between tubes and 96-well or 384-well plates, using a single-channel pipette? What an inefficient, cumbersome, and tiring process. And what's more: increasing throughput will result in reduced performance. The new adjustable tip spacing pipettes "Move It" allow you to transfer multiple samples simultaneously and complete your task three times faster. Our test clients commend: "Move It really boosts performance in the laboratory."

A challenge taken on and mastered

For the longest time, multi-channel pipettes could only be used for distribution of e.g. mastermixes, buffers, or cell culture media; the single-channel pipette remained the instrument of choice when it came to the transfer of individual samples for PCR, ELISA, FACS, etc. between different formats of reaction tubes, plates, and agarose gels.

Loading a microplate with up to 384 wells meant pipetting up to 384 times! This not only takes a long time, it also requires the utmost concentration, and it is inefficient. If errors occur during setup, the results will no longer correspond to the samples.

For these reasons, our customers have asked us for an efficient and safe solution for multiple sample transfer, which at the same time offers comfortable and ergonomic working conditions.

The Eppendorf engineers have taken on the challenge, and with "Move It", they have created a new generation of pipettes. Their motto: double the performance through maximum efficiency and secure sample transfer.

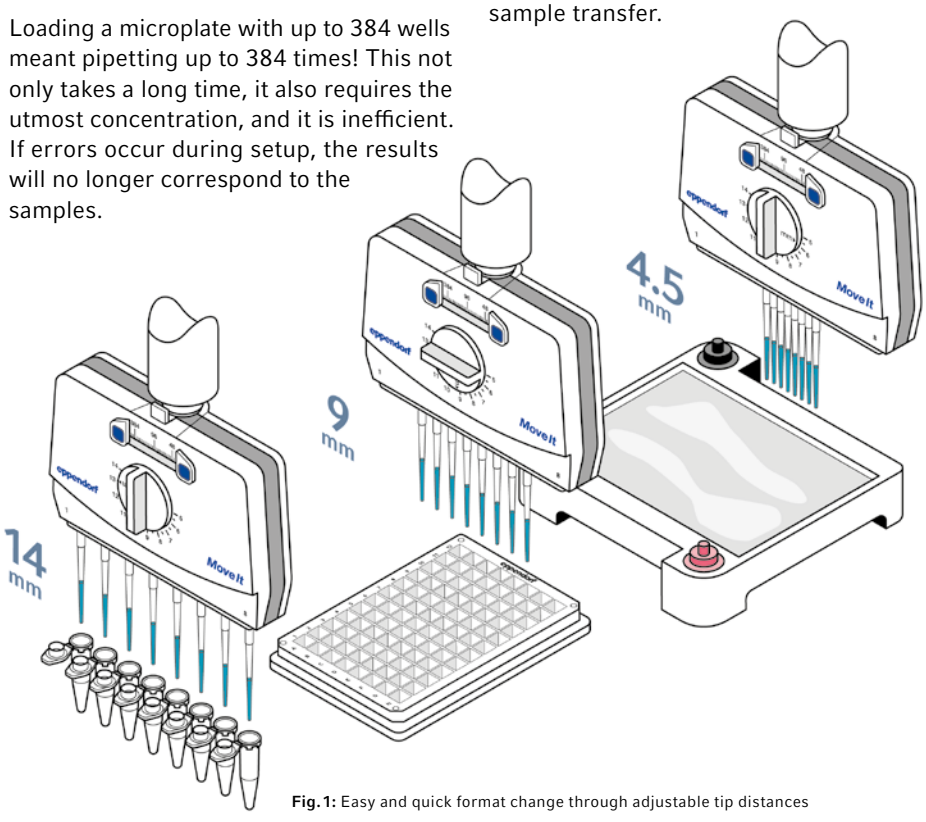


Fig. 1: Easy and quick format change through adjustable tip distances

"Move It means efficiency"

Goodbye, single-channel pipette

Transferring multiple samples between reaction vessels of different formats instead of repeated pipetting using single-channel pipettes? Switching between formats as needed? No problem! A rotary knob allows you to adjust the distance between the tips manually – easily and quickly (Fig. 1). Transfer four to twelve samples simultaneously – from reaction vessels to microplates or agarose gels.

Increase your speed 3-fold

With Move It, you will reduce your processing time by up to 70 %. Pre-defined settings enable easy and quick change of format. Thanks to integrated spacing control, spacings between tips are quickly adjusted to both vessels of origin and target vessels. Our test clients summarize: "Pre-selection of the tip spacing considerably eases the workflow. Switching between them is easy and fast, and it can even be done without looking."

Work with less fatigue – all day long

Fast, efficient workflows also require comfortable handling of the pipette. "During the development of pipettes with adjustable tip spacing, our customers' request for perfect balance when holding the pipette proved to be one of our biggest challenges", reports Peter Schmidt,

Business Manager at Eppendorf. "Our product designers have developed an ingenious design concept. Handling remains natural and relaxed while fatigue is reduced to a minimum. Users need fewer breaks and are able to work all day in a relaxed fashion."

No bending and twisting

The upper part of the pipette permits full rotation of 360° around its own axis. In this way, you will be able to read the display, and all the important parameters, quickly, comfortably and from every position, without the need to twist your neck or back (Fig. 2). This feature thus supports an ergonomic posture at all times.

"Move It means secure sample transfer"

Transferring samples in a precise and reproducible fashion

Move It pipettes are designed without tubing (Fig. 3). The piston cylinder system and the cones are directly connected to one another. The advantages are manifold: the direct connection reduces the size of the air cushion many times over, and with it the influence of the heat generated by the motor or the hand of the user, which may cause imprecise pipetting volumes and compromise reproducibility. Increased temperature stability enhances precision and reproducibility.

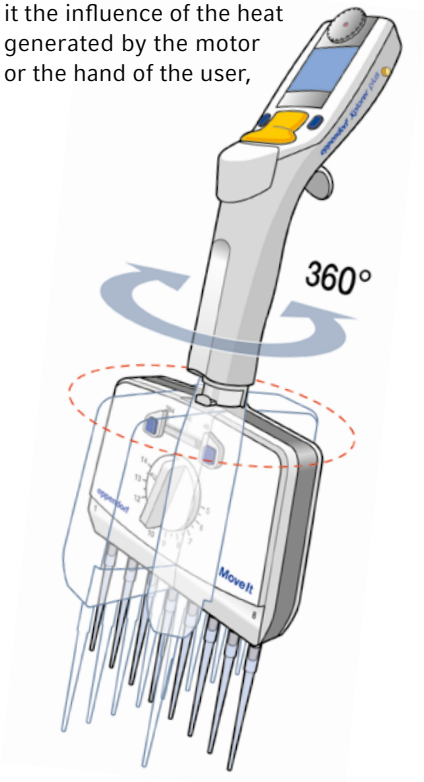


Fig. 2: Comfortable readability of the display from every position as the lower part of the pipette pivots by 360°

Risks posed by cable clutter, porous tubing, and leakage are also prevented. The reduction of movable parts within the pipette further reduces the risk of wear; it is durable and your guarantor of lasting precision. To reduce the contamination risk for your samples, you can also autoclave* your Move It pipette.

*Eppendorf Research plus: autoclavable in full; Eppendorf Xplorer plus: lower part is autoclavable

No safety gap

Motor vibration, which may occur during the adjustment of tip distance, is a known challenge of working with adjustable tip spacing pipettes. In the worst case, these vibrations cause the formation of droplets – a potential source of sample loss and cross-contamination. In such cases, experiments must be repeated, costs will incur, and valuable time is lost.

For these reasons, Move It pipettes operate without a motor. Tip distances are adjusted via manual adjusting knob, entirely without vibration – for a secure sample transfer without droplets.

Learn more

Starting in the summer of 2020, our multichannel pipetting systems Eppendorf Xplorer® plus (electronic) and Eppendorf Research® plus (manual) will be available with "Move It" function.

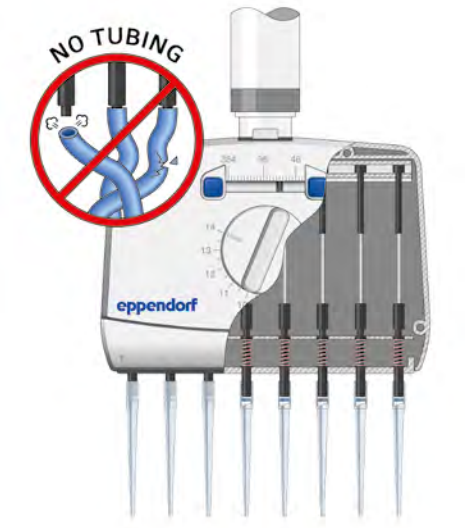


Fig. 3: Secure sample transfer thanks to tubing-free system

To learn more, or to register for a product demo, please visit
http://eppendorf.global/kjS

Tip

Software Update: Do It Yourself!

Regular software updates help keep your electronic pipettes and dispensers current. On one hand, technical bugs can be removed, and on the other hand, new, valuable features may be installed to extend the life spans of your instruments.

You can now perform these software updates yourself, comfortably at your bench. Begin the updating process on the Eppendorf homepage in the area Service & Support and download the update program to your computer. Simply connect your pipette* or dispenser with your computer via USB-port and start the updating process. The latest software version is now being installed on your device. The process only takes a few minutes, and you can consecutively update as many instruments as you wish.

Stay independent and flexible, and never miss another product improvement.

More information:
http://eppendorf.global/kle

*All pipettes with push-ON/Off key are update-capable.

BRIGITTE KLOSE, EPPENDORF AG

Upgraded: Design and Sustainability

Already in 2002, at the time of the development of the epT.I.P.S.® Box for pipette tips, sustainability was an important consideration. Autoclavable for up to 100 times, this reusable box was meant to be refilled with epT.I.P.S. pipette tips (e.g. "reloads"). Only pre-sterilized pipette tips such as ep Dualfilter T.I.P.S.®, were provided with disposable racks. After 17 successful years on the market, we have now revised our design with a particular focus on sustainability in the case of single-use racks, as well as on optimized functionality in the case of our reusable boxes.

Tried and tested features remain unchanged

The new design of our boxes and racks is a consistent and modern advancement of the familiar brand design of the epT.I.P.S. pipette tips. Key features, such as the blue lid color of the boxes and racks, as well as the color of the closing button on the box, have remained unchanged. The characteristic horizontal line design of the front view of the reusable box, too, is recognizable in modified form on the new box and the new rack.

In contrast, the shape of the closing button of the box was modernized, and the colors of the boxes, as well as the lower parts of the racks, are noticeably lighter. The rack itself is no longer double-walled and thus significantly streamlined.

Optimized functionalities

At the same time, the new design carries with it changes which significantly improve handling and function. The prominent visual slots on the back of the box have for the most part disappeared. These openings, a cause of concern for some of our customers due to the possible contamination risk of the tips inside the box, were removed almost entirely. In the two small box sizes for tips up to 1,250 µL, they have vanished completely. While the box containing tips 2.5 mL and up has retained a small opening, this is not connected to the interior space of the box housing the pipette tips.

Oblong indentations on the sides of the lids of racks and boxes ensure optimized stackability. In contrast to the precursor rack, the new lid has a closure. Especially during sterile work, the new single-use racks may be closed securely, each and every time, thus precluding the risk of accidentally losing pipette tips. The integrity of the product is safeguarded by a new purity sticker placed exclusively on the closure.

The circumferential tape, which had a tendency to stick to and damage gloves worn during sterile work, has been removed. Other components of the epT.I.P.S. Racks, Reloads, and Boxes were also optimized. New, clearly designed product labels with unambiguous pictograms of the type of tip contained within the package simplify secure handling.

Our contribution to sustainability in the laboratory

Eppendorf is keenly aware of its social responsibility when it comes to sustainability and the environment. At the same time, we know about the key roles that plastic consumables play in the laboratory. Many tasks within the realm of research and development could not be performed to the current high standards of quality, precision, and reproducibility without vessels, pipette tips, or plates made from plastic. The balance between the demands of modern science and the concern for the environment with respect to plastic waste represents a central challenge for the management of a life science laboratory.

We at Eppendorf have taken on this challenge by following the "Reduce & Reuse"-principle whenever and wherever possible. We have already achieved this goal with our new packaging formats of the single-use racks and reusable boxes: the new racks are designed in such a way that, depending on the size of the rack, 20 % to 35 % less polypropylene* is required. Expressed in numbers: for a hypothetical monthly demand of 100 medium-sized racks (e.g. pipette tips for 1,000 µL), 46.3 kg of polypropylene (or 84.7 liters of crude oil) are saved annually during production.

*Polypropylene (PP) is a thermo-plastic that is produced via the chain-polymerization reaction of propene. It belongs to the group of polyolefines, and it is semi-crystalline and non-polar. Its properties resemble those of polyethylene; however, it is slightly harder and more thermo-resistant.

Propene (propylene) is a colorless, flammable gas. It is obtained through thermal decomposition (steam cracking) of the benzenes arising during the processing of crude petroleum.

Like its predecessor, the new epT.I.P.S. Box is guaranteed to be autoclavable up to 100 times. For this reason, more and more customers employ the reusable box and refill epT.I.P.S. pipette tips from reloads or bags (bulk) in the volumes 10 µL to 5 mL.

Reduce & Reuse – this will continue to be our motto for the development of new Eppendorf laboratory consumables. The potential for the reduction of raw materials used in production on the one hand and the reusage of product delivery formats on the other hand, has not been exhausted.

We continue to invest large amounts of capital, as well as time, in the research of more environmentally friendly alternatives to the commonly used single-use petroleum-based plastics.

These alternatives include plastics made from renewable biomass as well as biodegradable plastics. The biggest challenge will be to assure that a switch will not compromise the products' quality and function and, as a result, the quality of the results generated in the lab.

So – stay curious and expect only the best from Eppendorf – also when it concerns sustainability in the laboratory.

Learn more about optimized handling in our video at **http://eppendorf.global/kla**

Tip: Our pipette tips, too, have news for you! ep Dualfilter T.I.P.S. 0.25 – 2.5 mL are now available for your electronic pipette Eppendorf Xplorer®/Eppendorf Xplorer® plus, as well as manual Eppendorf Reference® 2 and Eppendorf Research® plus pipette with 2.5 mL volume.

Tip

Free Up Your Bench Space!

Is space a limited resource in your lab or are you looking for an ergonomic positioning of your Eppendorf centrifuge? The new Eppendorf mobile tables save precious benchtop space and provide the freedom and flexibility to use your Centrifuge 5804/5804 R, 5810/5810 R, 5910 R, or 5920 R wherever you need it. You can also transport and use the centrifuge in other labs.

Whether you choose the low design model for under bench storage of your centrifuge or the high design model for ergonomic loading and unloading of rotors and samples, both mobile tables have a very sturdy design for safe centrifugation and are equipped with heavy-duty transport wheels and brakes. And both follow the same extremely high Eppendorf standards in safety and reliability.

More information at **http://eppendorf.global/krB**

DAVID SOLBACH, EPPENDORF AG BIOPROCESS CENTER, JUELICH, GERMANY

From the Lab to Closed-Loop Cell Growth Systems

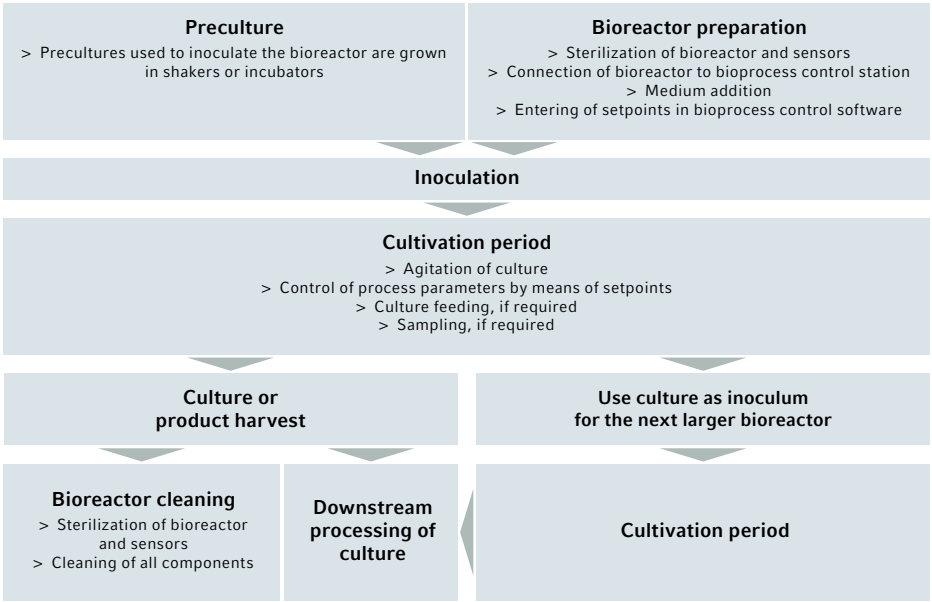
Modern biotechnology must be robust, reproducible, and cost-efficient to remain competitive. Increasing productivity typically requires growing working facilities. For biological cultivation applications, this is not entirely true; a variety of cells can be cultivated in bioreactors/fermenters, thus reducing the footprint in the lab while strongly increasing the quality and yield of a process. However, the first cells used to inoculate a bioreactor have already had a long beginning.

Process development

Cultivation of cells in flasks or on plates is one of the first steps in the long growth journey of a cell. Shakers and incubators are useful tools for early process development and to test different single parameters such as temperature and nutrient supply. Even though online monitoring of all running process parameters is not possible, advanced shakers and incubators are available to tightly control environmental conditions and ensure reproducible growth of healthy cells. The cells produced in this first step enable the upscaling and process control that drive modern biotechnological industries.

Small-scale production

But before the cells can eventually produce large amounts of a desired product in a tank-sized bioreactor, the distinct advantages of small- and bench-scale parallel bioreactors come into play: real-time monitoring and parallel control of several bioreactors to run different processes, where all important parameters can be systematically tested to set up an efficient, reproducible, and reliable process. Culture conditions such as stirring speed, pH, dissolved oxygen, nutrient concentrations, and temperature can be monitored, collected, and evaluated by software solutions.



Scaling up

The next step in the cell journey is scaling up to larger bioreactor sizes to avoid batch-to-batch variations. In the initial steps, we are speaking of small mL to liter volumes, but industrial processes are conducted in large pilot and production scale volumes of 2,000 L and beyond. For all steps, reliable software that tracks changes and reacts automatically will alleviate variations in real time. The long growth journey of a cell that starts in a freezer and ends in large bioreactors will run smoothly if all steps are in line and the different needed systems work together. Software solutions like VisioNize®-onboard are eminent for consistent user-experience across a variety of devices. Such close interplay between the devices simplifies the workflow and mitigates the risk of failure.

To learn more about up-scaling your process and boosting productivity with bioreactors, download our eBook RESOLVING CULTIVATION BOTTLENECKS: THE BIOPROCESSING JOURNEY at http://eppendorf.global/kjT

Scale-Up of *Escherichia coli* Fermentation from Small to Pilot Scale

BIN LI UND MA SHA, EPPENDORF, INC., ENFIELD, USA

Summary

The scale-up of fermentation processes is critical to the success of industrial fermentation to produce biologicals in the biopharmaceutical market. An optimized small-scale process can then be transferred to pilot scale following established scale-up strategies. Eppendorf bioprocess systems are available with autoclavable, single-use and sterilize-in-place vessels and together cover a wide range of working volumes from less than 1 L to as large as 2,400 L.

Eppendorf fermentation systems were designed following bioprocess industry stirred-tank design standards and provide excellent scalability from geometrical perspective. In this study the scale-up of *E. coli* fermentation was evaluated. First, engineering parameters critical for scaling up were investigated. The utilized data was used to scale-up an *E. coli* process from small scale (1 L) to pilot scale (100 L) following the constant P/V scale-up strategy.

We demonstrate the scale-up capabilities of Eppendorf fermentation systems from small-, to bench-, and pilot-scale. The fermentation runs at each of the three scales produced very similar biomass yields over time, indicating excellent scalability within the Eppendorf fermenter product family.

Materials and methods

Equipment

The Eppendorf fermentation systems, from small scale to pilot scale, used in this project are shown in Fig. 1. Vessel parameters critical for scale-up are listed in Table 1.

Oxygen transfer rate (OTR) and power Number (Np) determination

OTR is the rate at which oxygen is transferred from air to the liquid in a vessel. Since oxygen is often the limiting factor during aerobic fermentation, it is important to select equipment of different sizes with similar OTR capabilities.

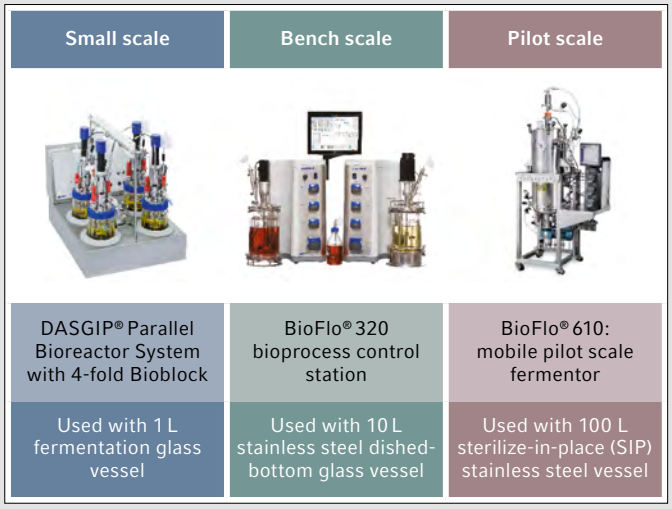


Fig. 1: Eppendorf fermentation systems, from small to pilot scale, used in this study

	Bioblock 1 L	BioFlo 320 10 L	BioFlo 610 100 L
Maximum gas flow (SLPM)	4.2	20.0	150.0
Vessel type	Glass	Glass	Stainless steel
Working volume (L)	0.2 – 1.0	3.5 – 10.5	32 – 100.0
V_{max} height (mm)*	136.0	323.0	904.0
Vessel inner diameter (ID) (mm)	100.0	211.0	381.0
Ratio V_{max} height : vessel ID per impeller	0.7	0.8	0.8
Impeller style; impeller material	Rushton/Rushton-type; 316 L		
Impeller quantity	2	2	3
Impeller diameter (D) (mm)	46.0	84.4	165.1
Ratio impeller diameter : vessel ID	0.4	0.4	0.4
Max. agitation (rpm)	1,600	1,200	500
Max. tip speed (m/s)	3.85	5.30	4.32

Table 1: Proportionally designed vessels and impellers at different scales.

*V_{max} height = Height from bottom of the vessel to the top surface of the liquid at maximum vessel working volume

The (impeller) power number is a dimensionless number associated with different type of impellers. OTR measurements were conducted using a previously published protocol based on sulfite depletion [1]. The power number and P/V values at various tip speeds were calculated towards the higher agitation rates that are usually used for fermentation by measuring the impeller torque using a rotational torque sensor. Since typical fermentation experiments are conducted under high gassing conditions and gassing greatly reduces impeller torque, we have decided to obtain Np under a high gas flow of 1.5 VVM in addition to under "no gassing" conditions.

Bioreactor setup and E. coli fermentation

20 µL of a cell bank stock was used for the inoculation of 500 mL TSB medium in a 2 L shake flask (VWR®, UK) and incubated at 37 °C, 200 rpm overnight in an Innova® 44 shaker.

We chose to use 90 % of the vessel maximum working volume for all three fermenters. *E. coli* was cultured in a chemically defined medium of pH 7.0 under continuous fermentation mode to maintain a constant working volume.

In all three fermenters, we inoculated the growth medium with an inoculum volume of 10 % of the initial fermentation medium volume. Antifoam 204 (Sigma-Aldrich®, USA) was added only when foaming was observed. Cell growth was monitored offline using samples taken every hour.

Sensor calibration and control

The pH sensors were calibrated outside the vessels prior to autoclaving, using a two-point calibration method and standard buffers. The pH was automatically maintained at 7.0 by adding 25 % (v/v) NH_4OH via a pump (assigned as "base"). The deadband for pH control was set to 0.05.

Dissolved oxygen (DO) sensor calibration was performed with an analog polarographic DO sensor using a standard two-point calibration method.

Scale-Up of *Escherichia coli* Fermentation from Small to Pilot Scale

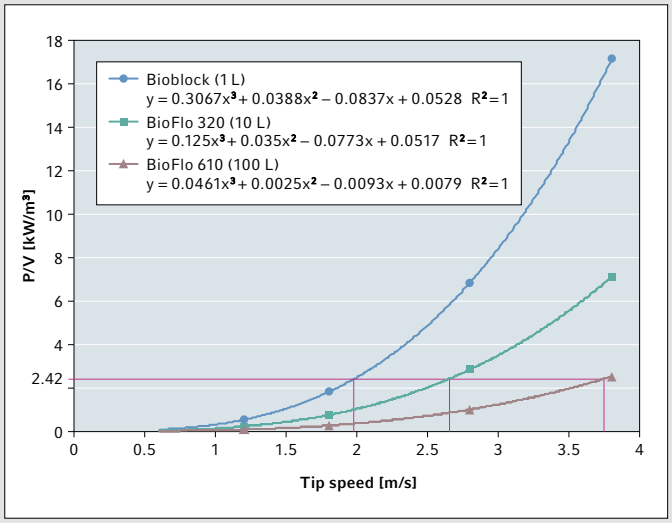


Fig. 2: Determining the constant P/V values for scale-up under 1.5 VVM of air flow

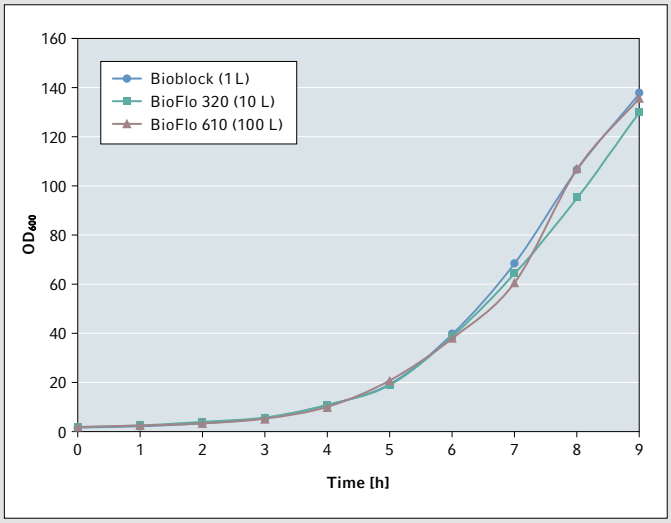


Fig. 3: Fermentation biomass growth curves among all three systems. Fermentations were carried out using a constant P/V value of 2.42 kW/m³, which was determined from Fig. 2.

Results and discussion

All three fermentation systems were designed following the same vessel and impeller geometrical principles, which laid a good foundation for a successful scale-up. Selecting equipment of different sizes with high OTR capabilities is also important, so that the different fermentation scales can match each other in top line performance, and the small-scale success can be replicated in large scale. The three fermentation systems achieved high levels of OTR of ~350 mmol/L/h or higher. This allowed scale-up fermentation runs to be carried out at high capacities, delivering matching biomass growth curves at very high bacterial densities.

Scalable geometry and matching high OTR provide the foundation and the framework for high-density fermentation scale-up experiments, but they did not constitute the scalability strategy in itself. Various strategies have been used for fermentation scale-up including constant tip speed, but the most reliable method to date has been constant power (P/V). It requires the determination of impeller power numbers (Np). The impeller Np for Eppendorf fermentation vessels are ~10 without gassing and ~5 with 1.5 VVM of air sparging.

Maintaining constant P/V between vessels is one of the most accepted strategies for scale-up approaches. From the measured Np numbers, we calculated the impeller power consumption per liquid volume (P/V, W/m³).

The Np values obtained under 1.5 VVM air flow were used. The maximum P/V achievable by all three scales was ~2.42 kW/m³, which we selected to be the constant P/V value governing the fermentation scale-up (Fig. 2). Back calculating the agitations from this P/V value determined that 822, 600, and 433 rpm were the agitation values to be used for Bioblock 1 L, BioFlo 320 10 L, and BioFlo 610 100 L, respectively.

We conducted three fermentation runs and took samples hourly to monitor the cell growth (OD_{600} value).

The comparable growth curves of all three runs are shown in Fig. 3, indicating that excellent scalability has been achieved using the constant P/V scale-up strategy.

Conclusions

The Eppendorf fermenters from Bioblock to BioFlo are of geometrically and proportionally similar stirred-tank design. All three systems can deliver high OTR values, providing excellent capability for high density aerobic fermentation in a scalable manner. Maintaining constant P/V between different vessel sizes in fermentation scale-up from 1 L to 100 L produced nearly identical *E.coli* growth curves, providing solid proof for the scalability of Eppendorf fermentation systems.

In addition, the Np values of the impellers can be used for further scale-up or scale-down studies between Eppendorf and the stirred-tank fermentation vessels of other manufacturers.

Literature

[1] How to Measure and Calculate OTR Using a New Brunswick™ Fermenter. 2013, Applications Training Document, Eppendorf, Inc.

For additional information, download the full-length Application Note 306 at http://eppendorf.global/kq0

Using the Mastercycler® X50 and Its 2D-Gradient to Increase Yield and Specificity of Your PCR

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FLORIAN HILBERS, EPPENDORF AG, HAMBURG, GERMANY

Abstract

The discovery of the polymerase chain reaction (PCR) in 1983 led to the need for semi-automated devices to lessen the workload and improve reproducibility and productivity in the field of PCR. The development started with water bath-based devices, led to Peltier heated devices, added a heated lid to reduce evaporation and to get rid of oil-overlays and subsequently to the invention of the annealing temperature gradient for easier optimization of PCR conditions. The latest addition to this line of inventions is the 2D-Gradient. This Application Note shows the use of the 2D-Gradient to eliminate unspecific signals and to increase product yield. The 2D-Gradient allows for a temperature gradient during the denaturation step AND the annealing step in the same PCR run. This allows for 96 different conditions in one PCR run and thus to quickly screen for the optimal temperature combination, which finally saves time and resources.

Introduction

PCR is routinely used in research, diagnostics, and industry. This technique was discovered in 1983 and was at that time a tedious, time and resource consuming process [1, 2]. Therefore, it is not surprising that the discovery of PCR was followed by the development of the first thermocycler [3] and via Peltier heated and cooled metal blocks [4], a heated lid [5] and a temperature gradient to run up to 12 different annealing temperatures in one go using a 96-well cycler [6] to the current Mastercycler X50.

The annealing temperature is the temperature that is classically optimized. Optimizing the denaturation temperature on the other hand is usually not a focus, since the impact of the annealing temperature is considered much bigger than the one of the denaturation temperature. However, different denaturation temperatures, especially with GC-rich templates, may lead to higher yield and thus should be kept in mind when talking about optimizing PCR. Our aim is to show that optimizing the denaturation temperature does yield benefits for many PCR reactions. The 2D-Gradient of the Mastercycler X50 offers for the first time a tool to optimize both temperatures in one PCR run thus providing 96 different conditions in a single PCR run.

One might argue that analyzing 96 different conditions is tedious, time and resource consuming as well; thus, we decided to minimize the effort but maintaining an efficient number of different temperature combinations by using an established pipetting and tempering scheme (see full Application Note No. 423*).

The full Application Note shows that modulating the denaturation temperature does indeed change the outcome of the PCR, i.e. by increasing the specificity and/or yield of the PCR product. Three different targets that are used in the lab were optimized, namely one plasmid construct, a mouse originating gene, and a zebrafish gene. We could show three different benefits with these three templates.

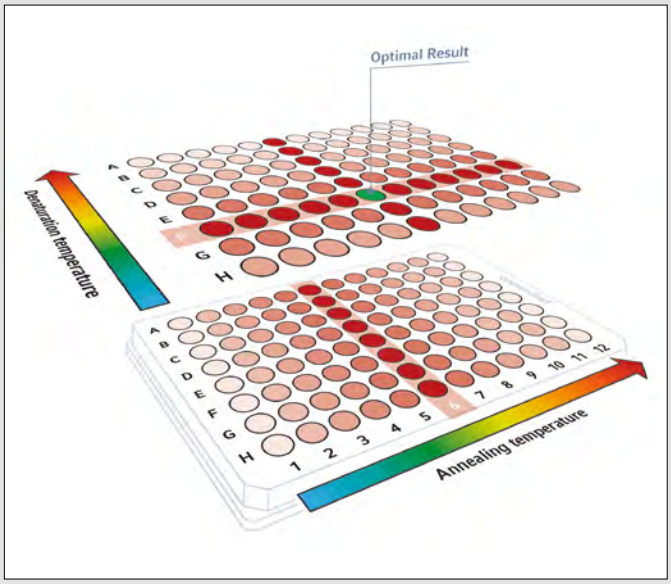

Fig. 1: Principle of the 2D-Gradient. The traditional gradient during the annealing step is depicted in the bottom part of the picture while the second dimension is added by applying a temperature gradient during the denaturation step, thus generating 96 different conditions across the PCR plate.

Firstly: An increase in specificity for the amplification of the gene plasmid construct.

Secondly: An increase in yield for the mouse originating gene.

Thirdly: We could show that changing the denaturation temperature leads to a failure of amplification of one of the two specific target products in a genetically modified zebrafish line.

This short version focuses on one of the three mentioned benefits, namely the increase in yield.

Materials and methods

All experiments were performed on a Mastercycler X50s (Eppendorf) with the lid temperature set to 105 °C, energy-saving mode on, the temperature mode set to "standard" and the block settings set to "Silver 96".

Eppendorf twin.tec® PCR Plates 96 (semi-skirted and skirted) were used in all experiments. Plates were sealed with Eppendorf PCR Film or Eppendorf Heat Sealing Film.

Murines Gen B

The 10 µL reaction setup contained 5 µl REDExtract-N-Amp™ PCR ReadyMix™ (Sigma ordering number R4775), 3.2 µL ultrapure water, 0.4 µM forward Primer Gene B (5'-AAA GTC GCT CTG AGT TGT TAT-3'), 0.4 µM reverse Primer Gene B (5'-GGA GCG GGA GAA ATG GAT ATG-3'), 30 ng total genomic DNA (2-propanol purified).

Using the Mastercycler® X50 and Its 2D-Gradient to Increase Yield and Specificity of Your PCR

The following PCR program was used for amplification:

Initial Denaturation	95 °C	120 s
Cycles 35x	Gradient 98–90 °C	30 s
	Gradient 50–70 °C	30 s
	72 °C	60 s
Post Cycle Elongation	72 °C	60 s
Storage	15 °C	Hold

Temperature combinations used are depicted in the results part. PCR products were analyzed by Agarose Gel-Electrophoresis (1.5 % agarose in 1 x TAE, run for 45 min at 100 V in 1 x TAE).

Results and discussion

Optimizing a PCR can be a very resource consuming process, especially if the desired product is not appearing or if the bothering unspecific bands are not disappearing. One possible reason could be the lack of optimization of the denaturation temperature, which is usually considered to be "just hot". This work shows that modulating the denaturation temperature indeed does influence specificity, yield and in the extreme case also influences the presence or absence of specific bands.

Mouse located gene B

The target amplicon here has a size of 600 bp and is present in all samples except for one. There are no unspecific products visible, hence an optimization on this side is not necessary. However, running a 2D-Gradient shows variation in yield if the denaturation temperature is modulated. The product yield is acceptable in a denaturation temperature range of 96.5 °C to 90.2 °C. The highest yield is achieved in a denaturation temperature range of 91.5 °C to 90 °C and an annealing temperature of 56.2 °C (Fig. 2).

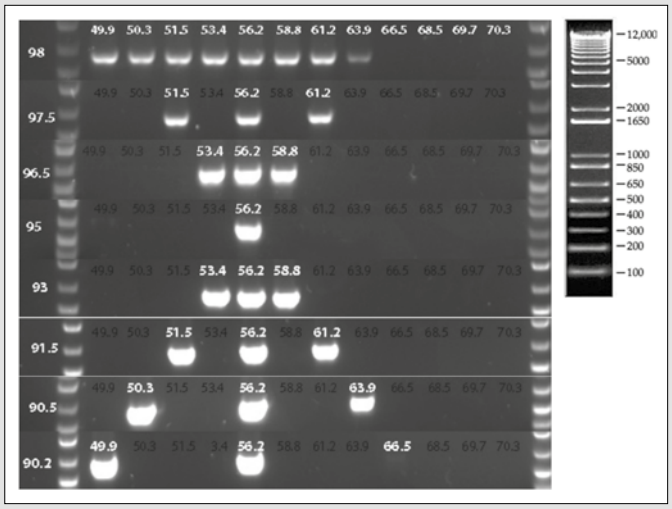

Fig. 2: Agarose-Gel analysis of the mouse located gene B. This experiment shows an increase in yield if the denaturation temperature is decreased. This data shows that the most optimal yield can be achieved at a denaturation temperature of approximately 91 °C.

The initially used denaturation temperature was 94 °C thus delivering product in an acceptable range but not being in the optimal denaturation temperature. This leads to a decreased product yield that can be easily optimized using a 2D-Gradient.

This shows that not only the annealing temperature needs optimization but also the denaturation temperature. Both factors have an impact on product yield. Thus, optimizing both temperature conditions could lead to better yield *without* increasing the number of PCR cycles in one PCR run, and thus drastically saving time and resources. Optimization is easily achieved in one PCR run if the 2D-Gradient is used. If not, at least 8 PCR runs must be performed to achieve the displayed results.

Conclusion

We could show for three cases with different organisms of origin and different target genes that the denaturation temperature has a crucial effect on the PCR results, be it specificity, yield, or false negative results. Optimizing the annealing and the denaturation temperature however proves to be a very time consuming and tedious process using conventional thermocyclers with 1D-Gradients. This setup would require at least 8 different PCR runs to obtain the wanted results.

Using the Mastercycler X50 with its 2D-Gradient cuts down this optimization time to one single PCR since all gradients can be run in one go on one thermoblock.

Acknowledgement

We would like to thank the Genotyping Service at MPI-CBG for providing the mouse and zebrafish gDNA and the Protein Purification and Characterization facility at MPI-CBG for isolating the in-house *Taq* DNA polymerase and providing the 10 x PCR buffer mix.

*Download at http://eppendorf.global/kpZ

Literature

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Total Sample Recovery in Eppendorf Protein LoBind Conical Tubes

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Abstract

Protein preparation and storage are critical steps in a wide range of laboratory applications including various methods in the fields of proteomics, molecular biology, forensics, and bio-pharma. Non-specific adsorption of protein/peptide molecules to polymer surfaces of lab consumables has been shown to be a substantial factor contributing to sample loss and degradation. This may adversely influence experimental results, particularly when sensitive methods/assays or small sample amounts are used. In this study, recovery rates of low concentration protein samples were compared between conical tubes from different manufacturers by using a sensitive fluorescence assay. Among the conical tubes tested, Eppendorf Protein LoBind Conical Tubes showed highest protein recovery rates (mean of 100 %) and thus ensured the highest protection from sample loss.

Introduction

Preparation and storage of protein samples are crucial steps in various protocols in the fields of proteomics, molecular biology, forensics, and bio-pharma. Non-specific adsorption of protein and peptide molecules to polymer surfaces of laboratory consumables has been shown to be a substantial factor contributing to structural denaturation, diminishing activity and decreasing sample concentration [1, 2, 3].

These effects are particularly prominent when sensitive methods/assays or small sample amounts are used in proteomic and forensic protocols. In this Application Note, we investigated non-specific binding of low concentration protein samples by using a sensitive fluorescence assay. Sample recovery was compared between standard polypropylene conical tubes from various manufacturers and Eppendorf Protein LoBind Conical Tubes.

Materials and methods

Protein recovery rates were evaluated by using a fluorescent-labeled protein assay in 15 mL polypropylene tubes: Eppendorf Protein LoBind Conical Tubes and standard polypropylene conical tubes from the manufacturers Sa, Co, Gr, Bd, Nu, Br. The assay was set up as follows: 256 µL of a FITC conjugated BSA solution at 1 µg/mL (1 x Dulbecco's PBS) were transferred into each tube and incubated for 24 h at room temperature in the dark. After incubation, 190 µL of the solution stored in each tube were used for fluorescence measurements using the Fluoroskan Ascent™ Microplate Fluorometer (ThermoFisher). The remaining FITC-BSA concentration and the recovery rate percentage were calculated using a calibration curve established with the original solution. Two independent experiments were performed in triplicate (n=6).

Results and discussion

The recovery rate of the FITC conjugated BSA samples following incubation for 24 hours in the different tubes is presented in Fig. 1.

The analysis revealed that most of the protein sample was adsorbed to the wall of standard PP tubes and that the overall recovery rate after 24 h of storage was only approximately 15 %.

All investigated tubes, Sa, Co, Gr, Bd, Nu, and Br, with the exception of Eppendorf Protein LoBind, showed very poor protein recovery rates, ranging between 10 % and 17 %. This indicates that under applied experimental conditions the standard polypropylene material used in these tubes poses a high risk of losing significant amounts of protein sample. In contrast, Eppendorf Protein LoBind Conical Tubes showed very high recovery rates of the tested protein samples: virtually no loss was observed after 24 h of incubation, with a mean recovery rate of 100 %. This guarantees utmost sample protection and assures reliable results for respective downstream applications.

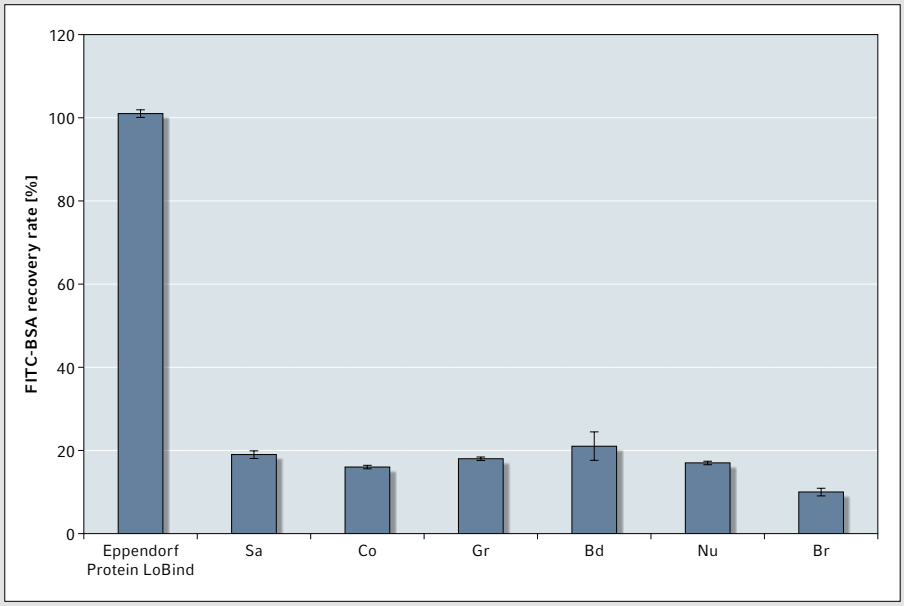

Fig. 1: FITC-BSA recovery rate after 24 h of incubation in 15 mL conical tubes from various manufacturers. Bars represent mean values of two independent experiments, each performed in triplicate (n=6).

Conclusions

In this study, we employed a sensitive fluorescence assay to measure sample recovery rates in various standard conical tubes, as well as Eppendorf Protein LoBind Conical Tubes, following incubation at room temperature in the dark over a period of 24 h. We showed that the recovery rates of samples incubated in standard conical tubes from various manufacturers were low (10 % to 17 %) and that these tubes may therefore not sufficiently protect against the non-specific loss of protein sample.

Total Sample Recovery in Eppendorf Protein LoBind Conical Tubes

In contrast, the Eppendorf Protein LoBind Conical Tubes allowed nearly complete sample recovery (mean recovery rate of 100 %) and thus ensured the highest possible protection of samples, assuring the integrity of the sample and thus safeguarding various downstream applications.

Literature

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> Eppendorf LoBind material ensures optimized sample recovery for improved assay results

> Free of surface coating (e.g., silicone) to minimize the risk of sample interference

> Lot-certified PCR clean purity grade: free of human DNA, DNase, RNase, and PCR inhibitors

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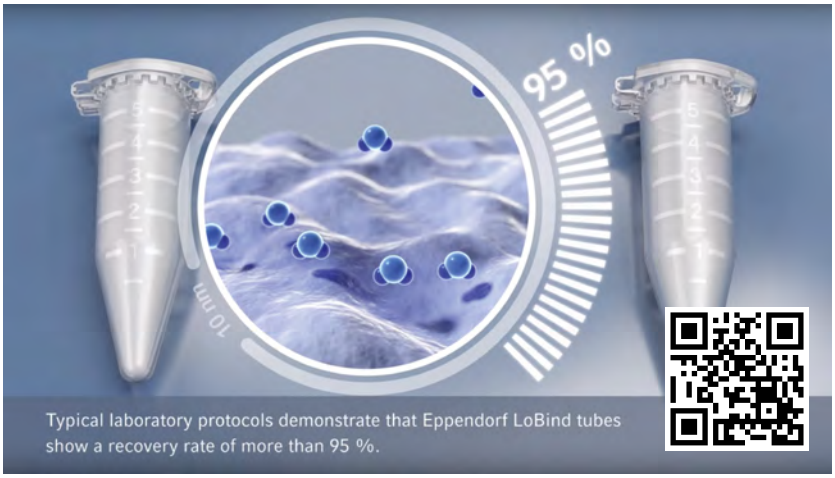
> Precise lid sealing to minimize evaporation

When biological samples are stored or incubated in standard vessels, over 90 % of the sample can be lost within 24 hours as a result of binding to the plastic surface. Eppendorf LoBind Tubes maximize sample recovery by significantly reducing sample binding to the surface.

A special, two-component polymer mix creates a hydrophilic surface that ensures optimized recovery rates of valuable samples. Protein LoBind Tubes have been specially designed for use in protein research or with sensitive proteomic test methods, and frequently provide significantly improved analysis results.



To learn more, watch our video "Eppendorf LoBind® – How it works" http://eppendorf.global/kjR

Optimal Bioreactor Inoculum Preparation in Shake Flasks

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Abstract

While monitoring and control of inoculum characteristics at the seed fermenter stage are well established, the very first steps of culture expansion in shake flasks are usually neglected. Especially for organisms with complex metabolic regulation, the utilization of suboptimal inocula can severely worsen the bioprocess outcome. Using a *Saccharomyces cerevisiae* ethanol production process as an example, we present an integrated approach for optimal inoculum preparation with automated seed inoculation, conditioning, and online monitoring, which allows the generation of reproducible inocula and the flexibility to adjust the harvesting time according to the operator's requirements.

Introduction

Fermentative ethanol production using the yeast *Saccharomyces cerevisiae* is a typical example of a bioprocess with high susceptibility to inoculum quality. Ethanol is one of the largest-volume biotechnological products with a global production above 100 million tons [2][3].

As for any other bulk chemical, ethanol production is cost-sensitive, so that suboptimal inocula considerably lower the profitability of the overall bioprocess due to lower fermentation yields. Several growth characteristics complicate the preparation of optimal *S. cerevisiae* inocula, first its diauxic growth behavior on glucose with an initial respiro-fermentative growth phase of desired ethanol production, followed by a respiratory growth phase of undesired ethanol consumption [4].

This diauxy comes along with growth phase dependent differences in the cell composition, storage carbohydrate utilization and morphology [5]–[8].

The underlying metabolic fluxes and their regulation persist over generations and shape the lag-phase [9]. Inoculum preparation is further complicated by the fact that *S. cerevisiae*'s growth rate and productivity depend to a considerable degree on the inoculation cell density [10]–[12].

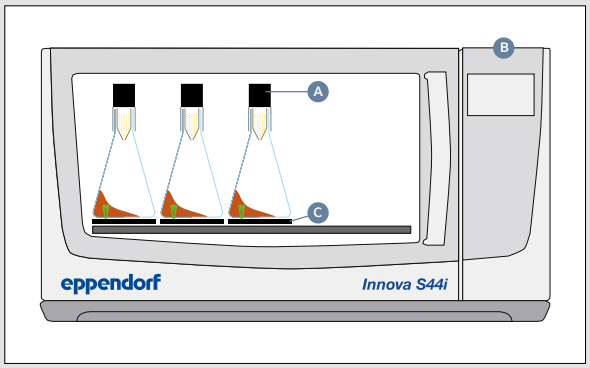

Fig. 1: Integrated approach for optimal inoculum preparation **A:** Automated seed inoculation (LIS, aquila biolabs), **B:** Conditioning (programmable incubated shaker Innova S44i, Eppendorf) and **C:** Online monitoring (CGQ, aquila biolabs).

Materials and methods

The integrated inoculum preparation approach is based on the Eppendorf programmable Innova® S44i and aquila biolabs' liquid injection system LIS and cell growth quantifying system CGQ (Fig. 1).

For automated seeding, three different concentrations of *S. cerevisiae* seeding solutions were transferred into LIS cartridges and mounted with preprogrammed LIS drives on top of the shake flasks. The Eppendorf Innova S44i was preprogrammed to 10 °C and 100 rpm for a preconditioning phase with a subsequent shift to optimal growth conditions of 30 °C and 200 rpm. Cell growth was monitored in real-time by CGQ backscatter measurements. Further information on the handling of CGQ and LIS is available on the aquila biolabs website www.aquila-biolabs.de

Results and discussion

An optimal inoculum for ethanol production with *S. cerevisiae* should be harvested during the respiro-fermentative growth phase. Results of a representative inoculum generation for the bioprocess of ethanol production with *S. cerevisiae* are shown in Fig. 2.

From 0–2 h, the cells inside the cartridge were preconditioned in a cold atmosphere with low rpm in a glucose-free cultivation medium to prevent cell growth and to allow the cells to adapt their metabolism to the other medium contents.

Following this, the Innova S44i was preprogrammed to automatically increase temperature and shaking speed to the desired growth phase conditions. From the time point of seed injection, the cultures started growing with almost no lag-phase, demonstrating the usefulness of a controlled preconditioning phase. In good accordance with the literature [10]–[12], different acceleration phase lengths and maximum cell densities were observed, depending on the seed inoculum biomass concentrations. By using the approach of three different seed inoculation biomass concentrations with different respective growth acceleration phases, it was possible to prolong the optimal harvesting time with growth rates in the maximal growth rate window from approximately 0.6 h for a single cultivation to more than 2 h for the combination of cultivations, thus giving the bioprocess operator more flexibility when planning the inoculation schedule.

Instead of harvesting the cultures at the optimal time, the cells were grown further to demonstrate the diauxic shift, which occurs in *S. cerevisiae* cultures as soon as glucose is exhausted. This diauxic shift towards respiratory growth on the accumulated ethanol is clearly visible, which resembles the typical *S. cerevisiae* growth curve as described in the literature [4], [5], [7].

Optimal Bioreactor Inoculum Preparation in Shake Flasks

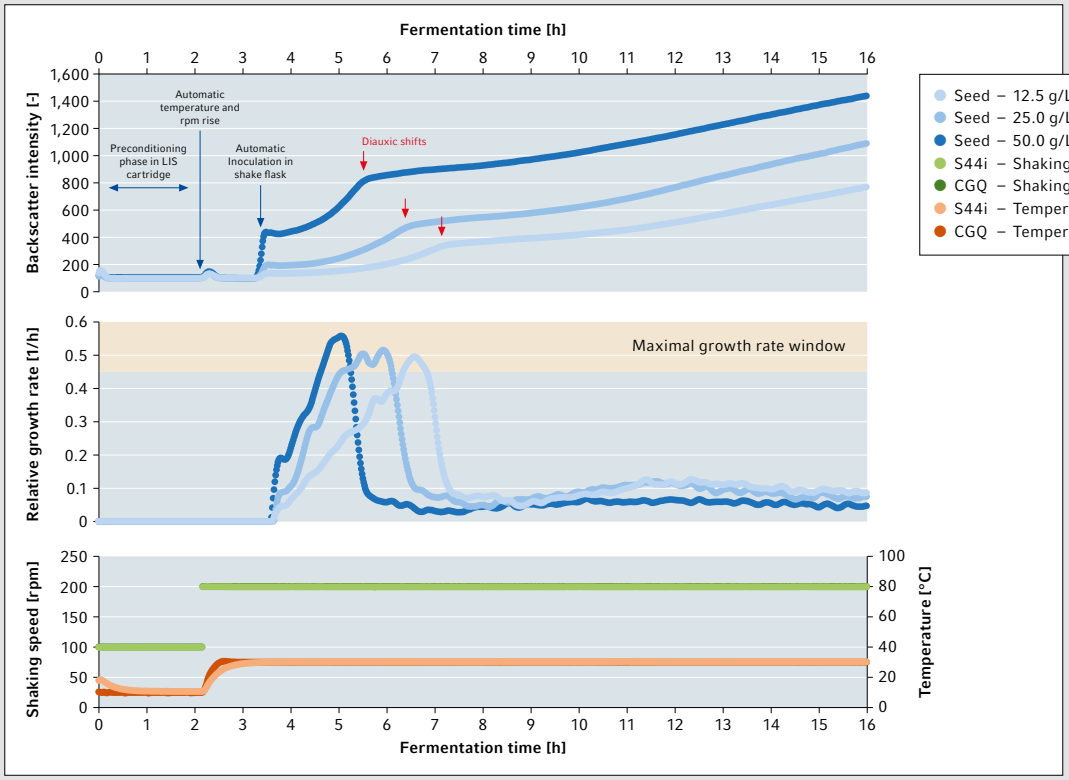

Fig. 2: Process data for the preparation of *S. cerevisiae* inocula using the integrated approach

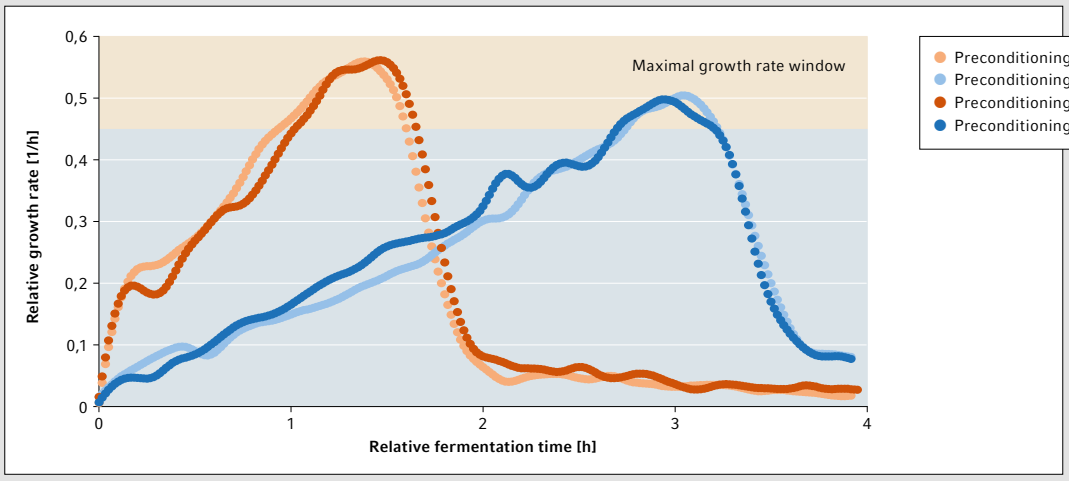

Fig. 3: Growth rates with 2 h and 4 h of preconditioning at 10 °C and 100 rpm. The fermentation times are adjusted relative to the start of seed inoculum injection.

It becomes obvious that the chance of harvesting the inoculum from an uncontrolled overnight culture at a suboptimal time point is extremely high.

Using the integrated approach, the reproducibility and quality of the inoculum can be enhanced, and the harvesting time can be adjusted according to the operator's requirements by setting the lengths of the preconditioning phases.

As depicted in Fig. 3, the growth rate curves show no significant differences, regardless of the length of the preconditioning period.

Conclusion

With this process, the user can plan and prepare inoculation processes reproducibly and harvest optimal inocula at predefined time windows. Programming the Innova S44i preconditioning and growth phases as well as the LIS injection time allows for optimized seed inoculation timing and turns nights and weekends into productive times for the preparation of optimal inocula.

Literature

See full Application Note 431 at http://eppendorf.global/kuw

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Controlled thawing in the Eppendorf ThermoMixer

By scanning the ID on the CryoStorage Vial, you can start to document the next step in your process: within eLABJournal, you combine the process documentation with information about the thawing process (time and temperature).

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HAFID OUAZZANI, EPPENDORF AG

Why Product Design Is Key

The design of a product is not only a matter of looks, it is so much more. It also includes a product's functionality, how it is produced, and the user experience it brings. Looking at most of the successful products today, they have one thing in common: a convincing combination of inner and outer qualities – or in brief – a great design.

Customer-centric, expert partner, innovation … these nice marketing buzzwords lose their luster if a product doesn't live up to customers' expectations. For a great customer experience, it is vital for both our engineers and designers to understand your exact needs and to translate these findings into well thought-out and unique product solutions. With this objective, product designers strive to blend form and function to make products truly desirable. Ideally, the resulting product offers easy handling, intuitive operation, high quality and durability, plus, as the cherry on top, a distinctive appearance.

Product design awards are a validation by external professionals that manufacturers have done a good job in understanding their users and in creating a great customer experience.

Eppendorf is actively participating in product design awards to ensure that our designs are evaluated so that we may continue to offer you great product designs. We have been awarded multiple prestigious product design awards with products such as our new Pipette Holder System, the Eppendorf Research® plus pipette, the Centrifuge 5920 R, and the Mastercycler® X50. Our latest success to be acknowledged is the CellXpert® 170i, a new family of Eppendorf CO_2 incubators. It has won prestigious awards in many regions for its exceptional design and user experience.

Awards like these make Eppendorf proud and validate that we are on the right path to enable you to get the best out of your work and achieve your goals.

Tip

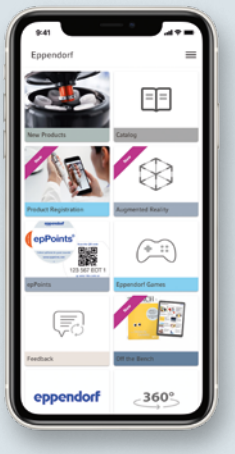
Relaunch of the Eppendorf App

The Eppendorf App appears in new splendor and is based on the latest technology to provide better user-friendliness. Browse through the product catalog to find the right product for your application. Take advantage of many ways to find out about our products or to compare them. Thanks to the improved and new Augmented Reality ("AR") function*, it is now easier than before to place products virtually in your laboratory. Have a look inside our products or exchange accessories in the instruments at your convenience. Experience the diversity of Eppendorf on a scale of 1:1.

Further highlights

> Enjoy our magazines online
> Simply register all your Eppendorf instruments
> Scan your epPoints® quickly and easily
> Calculate the amount of crude oil/PP that you could save by using our newly designed epT.I.P.S.® reusable boxes or ep Dualfilter T.I.P.S.® disposable racks (see article on p. 6–7).

Download the Eppendorf App at http://eppendorf.global/klb



To share your feedback, please write us at app@eppendorf.com or use the feedback button in the app.

*AR function available for Apple® devices from 2016 onwards, currently not available for Android™ devices

CAROLYN TAUBERT AND BERRIT HOFF, EPPENDORF AG

Eppendorf Prize Winners 2019/2020: Lauren Orefice & Randall Platt

eppendorf & Science PRIZE FOR NEURO BIOLOGY

The American scientist Lauren Orefice, Ph.D. (Massachusetts General Hospital and Harvard Medical School, USA), won the 2019 *Eppendorf & Science Prize for Neurobiology* of 25,000 USD.

Lauren Orefice won the prize for her work on the causes and potential therapies for autism spectrum disorders (ASD). She found that peripheral somatosensory neurons – neurons outside the brain that control the sense of touch – are key areas where autism-associated gene mutations have a critical impact.

She showed how abnormal function of peripheral somatosensory neurons causes touch over-reactivity and how this over-reactivity during development contributes to altered brain function and some autism-related behaviors in mice.

Lauren Orefice's work changes how we think about the causes of ASD, providing a surprising revision of widely-held views that link ASD exclusively to brain function. She highlights peripheral somatosensory neurons as a possible novel therapeutic target for improving some ASD-related symptoms.

www.eppendorf.com/prize

EPPENDORF AWARD FOR YOUNG EUROPEAN INVESTIGATORS – 25 Years

The 2020 *Eppendorf Award for Young European Investigators*, endowed with € 20,000, went to Prof. Dr. Randall J. Platt, Assistant Professor of Biological Engineering, ETH Zurich, Switzerland. The Award was given for his pioneering work developing a method to record timelines of gene expression events using a CRISPR-Cas system.

This system works by small pieces of RNA being inserted into the genome of a non-pathogenic bacterium in response to environmental changes. This system enables progress toward future development of a gut microbiome (normal intestinal flora) that can report experiences of the host organism and shows potential for use as a diagnostic tool to predict individualized precision interventions for both humans and other animals.

www.eppendorf.com/award

In response to the rapidly changing situation of the Corona virus outbreak (COVID-19), the Award ceremony at the EMBL Advanced Training Centre in Heidelberg, which was planned for June 25, 2020, within the Young European Investigators Conference, was postponed to June 24, 2021.

www.eppendorf.com/award/25years

Win a New Pipetting System

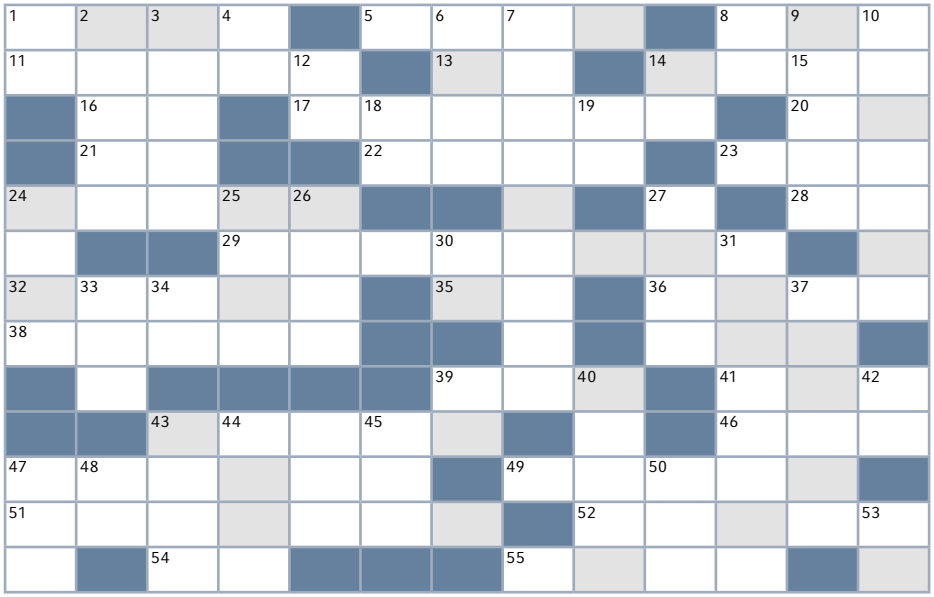
The solution of the prize competition of BioNews 51 was "Inside Cell Culture". The three main prizes, one Eppendorf Research® plus 16 or 24-channel pipette each, went to Wooi K. (Malaysia), Mike S. (Australia), and Steven F. (Belgium). Congratulations!

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, 2020**.

You can either send an e-mail to **bionews@eppendorf.de**, or participate online at **www.eppendorf.com/bn-service**.

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 winners of the first three prizes will be published in BioNews No. 55.



1st to 3rd Prize:

1 Eppendorf Research® plus 16 or 24-channel pipette of your choice

4th to 6th Prize:

1 Amazon® Voucher worth 50.00 EUR

7th to 15th Prize:

400 bonus epPoints® each

(epPoints registration required)

ACROSS

1 Liquid handling consumable
5 Every breath you take, every … you make
8 Opposite of on
11 To be in an upright position
13 Group of islands in the Caribbean Sea (ISO country code)
14 Painter born in Barcelona
16 Vienna is the capital (ISO country code)
17 Separated from Russia by the Bering Strait
20 French gold
21 French male article
22 Not fat
23 Opposite of SSE
24 May the … be with you
28 Metal used as orthopedic implant material (chemical symbol)
29 Ahead of the times, progressive
32 Shop
35 Mathematical constant
36 Famous for his "yabba dabba doo" (first name)
38 Signified authority, validity, or identity
39 Original equipment manufacturer (abbrev.)
41 Resort providing therapeutic baths
43 The way in which something is said, done, or performed
46 Formal honorific address for men
47 Female given name from Ancient Greek
49 Herbivorus mammal inhabiting jungle and forest regions
51 In love with Rosalind
52 French painter
54 Small kingdom in Northern Europe (ISO country code)
55 Circular band

DOWN

1 The musical "Cats" was based on his book (first name initials)
2 Prefix related to a South European Country
3 Latin for father
4 Dakar is the capital (ISO country code)
6 Shape of a famous office
7 Supports the connections in your lab
9 Forward part or surface
10 Notably advanced or developed
12 Atomic mass unit named after John D. (abbrev.)
14 Mother (very short)
18 Enclaved kingdom in South Africa (ISO country code)
19 1.60934 of this make a mile (abbrev.)
24 Quick, swift
25 Handle with …
26 Famous biblical garden
27 Not right
30 American News agency (abbrev.)
31 Thousand Island, French, or Caesar?
33 Starring in Mission: Impossible (first name)
34 Central button on a remote control
37 … State of Mind of Jay-Z and Alicia
40 Where Crockett and Tubbs worked undercover
42 Interactive experience (abbrev.)
43 Too late, this house is …
44 Wood typically used for furniture and shipbuilding
45 Used in lamps and digital displays
47 … favor!
48 Medical parameter, measured in bpm (abbrev.)
50 Lived in Neverland (surname)
53 French personal pronoun (2nd person, sing.)

Solution hint for prize competition of BioNews No. 53:

					V		C					R					I				R				M			

Send us the solution until **October 31, 2020**, via e-mail to **bionews@eppendorf.de**, or participate online at **www.eppendorf.com/bn-service**
Information about the use of your personal data can be found at **www.eppendorf.com/gdpr**