BIO NEWS

No. 50 – 2019

eppendorf

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Redox Potential Monitoring for Improved Anaerobic Fermentation Using the BioFlo® 320 Bioprocess Control Station

Application Notes

Redox potential monitoring for improved anaerobic fermentation · Reduced PCR runtimes and increased yields · Eppendorf Amber Conical Tubes: maximal sample protection and visibility · etc.

Dear Readers,

Many thanks for all the good wishes on the 25th anniversary of BioNews. We feel delighted and motivated by your feedback. The personalized pipettes that could be won in our anniversary prize competitions were very popular!

There is many a "secret recipe" for handling problematic liquids. The key to success, however, lies in identifying the best combination of pipette and tip for your specific application. To this end, in-depth understanding of pipetting techniques and current pipetting systems is a prerequisite. Read our leading article on pages 4–5 to learn how Eppendorf can help you become a pipetting expert – or even a Pipetting Ninja.

Ten years ago, the (Eppendorf) New Brunswick™ Premium U570-G was one of the very first ULT freezers in the market that used green cooling liquids. With our longstanding experience in R&D, production, logistics, and service in the field, we are happy to see the concept globally confirmed. A growing number of users take green ULT freezers for granted. Turn to page 6 for more background information.

PCR is currently the quickest and safest method for DNA amplification. At the same time, this standard method harbors potential pitfalls and is subject to a number of influencing factors. The need to speed up and optimize PCR runs is thus ever-present, and it can be approached in different ways. In our article on pages 12–13, we introduce five ways.

Additionally, in this issue you can find further news and stories, e.g. from bioprocessing, as well as a section with four detailed Application Notes.

Your Eppendorf BioNews Team

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

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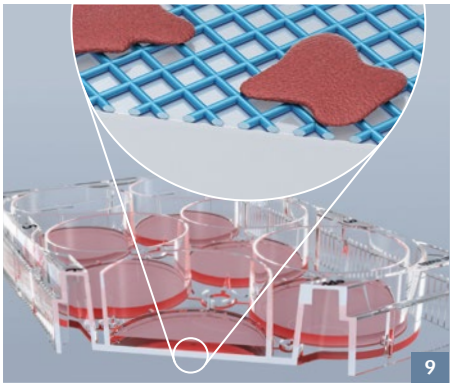
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Eppendorf BioNews Application Notes


Redox Potential Monitoring for Improved Anaerobic Fermentation Using the BioFlo® 120 Bioprocess Control Station

SAMIRA SCHROEDER, EPPENDORF AG

Which Pipette Is the Right One for My Application?

There is many a "secret recipe" for handling problematic liquids that has been handed down. The pipette tip is cut off in order to render viscous liquids pipettable. Ethanol and acetone are pipetted in a race against time, at the fastest possible speed, for liquids could simply drip out of the tip. At times, even detergent residue inside the tip is tolerated. This doesn't sound overly precise? There is another way.

Ever since the first microliter pipette came on the market in 1961, models and techniques have evolved; however, most pipettes are still based on the initial piston stroke principle. In this case, an air cushion resides between the piston and the liquid. Humidity, temperature, and the physical properties of liquids influence the air cushion and, as a result, the pipetted volume. Air cushion pipettes are calibrated for water, which ensures excellent precision for most applications – but there are exceptions.

One air cushion pipette for everything?

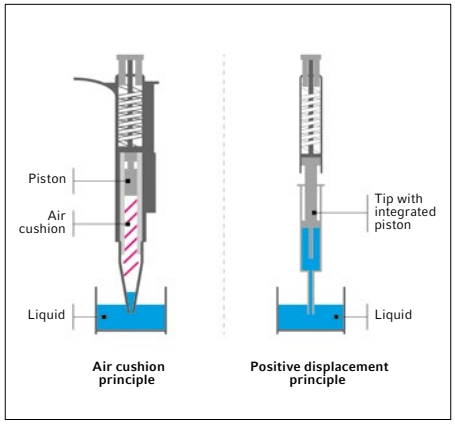
More often than expected, users employ classic air cushion pipettes, independent of the liquid to be pipetted. When working with liquids whose physical properties, such as viscosity, volatility, surface tension, or density, differ from those of water, the air cushion pipette will soon reach its limitations. It drips and it sticks; it flows slowly and air bubbles develop.

What to do? Speed up or slow down? Pre-wet? Use reverse pipetting? Users report other, quite individual techniques, which in itself highlights a certain level of distress around this topic.

Achieving better results with air cushion pipettes

It is indeed possible to process challenging liquids in a more precise manner using air cushion pipettes, simply by observing a few tricks. The challenge with viscous liquids such as glycerol, for example, is the fact that due to their decreased ability to flow, these liquids are difficult to aspirate, and it is not immediately obvious when exactly the correct amount of liquids has been aspirated. Air bubbles are quick to form, and liquid residue remains on the inner tip wall.

As a precaution, it is possible to pipette more slowly or to employ reverse pipetting. More security and precision, however, are afforded by a direct displacement system.



Comparison of air cushion and direct displacement principle

Working in a precise and secure fashion using the direct displacement system

Are you tired of resorting to tips that have been cut off and potentially compromising precision? Direct displacement pipettes such as, for example, the Multipette®*/ Combitips advanced® systems (*U.S./CAN: Repeater®) simplify the pipetting process and thus ensure precise pipetting results, independent of the physical properties of the liquid.

Unlike air cushion pipettes, the piston of the tip is in direct contact with the liquid. Dispensing of the liquid is direct, and it is achieved without an air cushion: The sealing lip of the piston removes all residual liquid from the tip. Since the liquid is located inside a special hermetically sealed tip, the risk of aerosol-contamination is minimized, and both the user and the pipette are protected from hazardous liquids at the same time.

Let it flow! The new ViscoTip specializes in highly viscous liquids

It's easy to become a pipetting expert

Easy handling of creams and honey with the ViscoTip® pipette tip

Most likely, you are aware of the limitations when it comes to pipetting viscous liquids. Thanks to the latest addition to the Combitips advanced family for the Multipette, cumbersome weighing of such liquids is no longer necessary.

The novel ViscoTip specializes in highly viscous liquids such as, for example, creams, honey, and high percentage glycerol. The system consisting of Multipette and ViscoTip is particularly well suited to the areas of food analytics and cosmetics, as well as the petrochemical industry.

Learn more in our webinar "Handling of Viscous Liquids – Basics, Techniques and Tricks" (see box on the right) and at www.eppendorf.com/let-it-flow

No compromise where precision and accuracy are concerned

You hold the quality of your pipetting results in your own hands. Combine the equipment – pipette and tip – that best supports your individual application with your new and enhanced knowledge of pipetting techniques.

Become a pipetting expert …

We help you expand your pipetting knowledge: with advanced information, problems and solutions, articles, posters, videos, and Application Notes.

Available at https://handling-solutions.eppendorf.com/liquid-handling or via QR-Code.

… and a pipetting Ninja!

You have the opportunity to test your brand-new knowledge in our online test and print your own personal certificate that attests your skills.

We hope you will enjoy expanding your knowledge and abilities further.

Scan the QR code for more information!

Tip

Pipetting Viscous Solutions Using the Proper Technique

In the daily laboratory routine, one comes across any number of liquids of different viscosities. From only slightly viscous, to considerably or even highly viscous – anything is possible. But how are liquids classified by viscosity? How does viscosity form? And, above all, how do I pipette viscous liquids correctly?

These are the questions that were addressed in our webinar "Handling of Viscous Liquids – Basics, Techniques and Tricks", the video recording of which we warmly recommend to you.

The video focuses on the different pipetting techniques and types of pipettes that are indispensable in order to attain reproducible and accurate dosing of viscous liquids. It is further demonstrated how the switch from classic air cushion pipettes to direct displacement pipettes will have a positive effect on the pipetting result.

Learn how you can enhance precision and accuracy, as well as the speed of pipetting, by using the appropriate pipette in combination with the correct technique.

Watch the free webinar at www.eppendorf.com/webinar/viscosity

Scan the QR code for more information!

JAN-HENDRIK BEBERMEIER, EPPENDORF AG

CFC, HFC, HC? Just a Green Freezer, Please!

Global warming is a challenge for mankind. Besides direct CO_2 emissions, the hydrofluorocarbons (HFC) used in cooling systems like ULT freezers increase global warming. HFCs cause multiple times the damage of CO_2. As a result, there is a move from hydrofluorocarbons to hydrocarbons (HC), also known as green gases. Already ten years ago, Eppendorf was one of the first movers for green gases at –86 °C.

Sustainability discussions mainly focus on energy consumption. Even energy efficient ultra-low temperature (ULT) freezers consume lots of energy to maintain extremely low temperatures 24/7. Besides energy consumption, the type of cooling liquid has increasingly come into focus.

When the ozone depleting CFC*-based cooling liquids (*chlorofluorocarbons) were phased out some years ago (based on the Montreal Protocol), they were replaced by alternative compounds such as hydrofluorocarbons (HFCs). Despite being better for the environment, HFCs like R508b and R404a still have a high Global Warming Potential (GWP). R404a, for example, has a GWP of 3,922, meaning that 100 g of this substance has the same GWP as 392 kg of CO_2-aequivalent.

Hydrocarbons

In 2014, the European Union announced a ban of all non-hydrocarbon-liquids (EU_517/2014) by 2020. Hydrocarbons are also known as green or natural gases. The two major representatives are propane (R290) and ethane (R170).

According to IEC 60335-2-89, no additional safety instructions for using green gases in ULT freezers are necessary.

The EU ban includes all cooling devices except instruments for temperatures below –50 °C. Based on this exception, ULT freezers of –86 °C may continue to run with HFCs. Still, it makes sense to replace HFC cooling liquids in ULT freezers as well to counteract global warming.

With this goal in mind, Eppendorf will replace all ULT freezers using classic coolants within the next few years. Recent steps with respect to switching to future-proof, green cooling liquids have been accomplished in models F740h, F740hi, and F740hiw (water-cooled) of our flagship series of CryoCube® F740 freezers. But this is only a part of a longer Eppendorf story.

Looking back to 2008

The (Eppendorf) New Brunswick™ Premium U570-G was one of the very first ULT freezers in the market that used green cooling liquids. After ten years of experience in R&D, production, logistics, and service in the field, we are happy to see the concept globally confirmed. Nowadays, the majority of ULT freezers in Europe are sold as green units. Asia and America are following suit. A growing number of users take green ULT freezers for granted, and more and more ULT freezer suppliers switch to green ULT freezers. Welcome to the green club!

For additional information, please visit
www.eppendorf.com/freezers

Scan the QR code for more information!

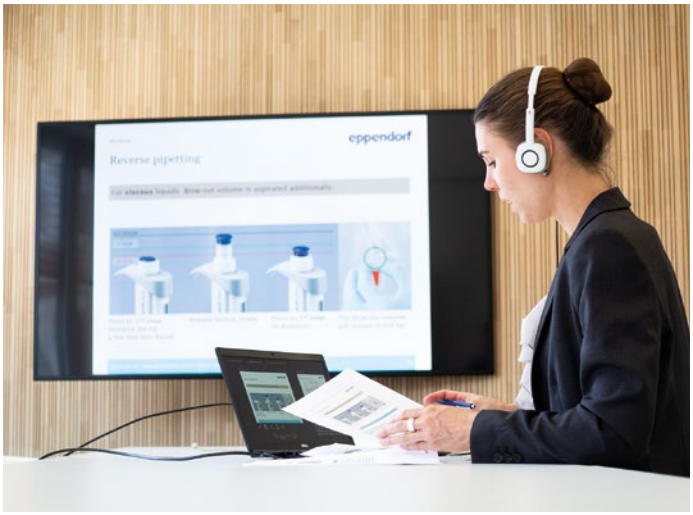
HANAE KÖNIG AND BERRIT HOFF, EPPENDORF AG

Backstage at an Eppendorf Webinar

10, 9, 8, 7, 6, 5, 4 … the countdown is on. I have butterflies in my stomach. In just a moment, I am going to go live, and several hundred people from all around the world will listen to my voice and follow the presentation. 3, 2, 1, and go! The moderator introduces me; I start the presentation. After the first three slides, my voice has lost its ever so slight quiver, the tension has lifted, and the fun begins.

This is how Hanaë König, experienced webinar presenter at Eppendorf, describes the start of a webinar. A webinar is exciting, but it does come with a lot of work during preparation as well as during follow-up. Planning begins as early as a year prior to the live event. Webinar topics are based on questions which frequently reach the Eppendorf Support Hotline, on typical problems arising in the laboratory, or on current research topics. As soon as the topic has been decided, date and time are finalized together with the webinar provider. "It is important to us to ensure that as many interested people as possible will be able to participate worldwide. This is not easy, considering the different time zones", says Hanaë König.

"Once the framework is complete, I start thinking about content, figures, and anecdotes. It is my goal to present the topic in an engaging fashion while at the same time conveying lots of content. And all that in a helpful way that makes the content easy to understand. The participants should feel that they have learned something useful that will help them during their daily work routine", adds Hanae König.



The webinars are advertised early to ensure that they reach as many interested parties as possible. Two to three weeks prior to the live event, the hot phase begins. Hanaë: "The number of participants goes up, and nervousness ensues when I look at the number of registrations."

On the day of the webinar, Hanaë König goes through the presentation a few more times and polishes the text that she will speak with each slide. "I also consider the questions that participants may have following the presentation." Even if she has presented many webinars, stage fright is her companion. "It is like a radio show, only with live questions from the audience at the end. My answer has to be prompt, so that I do not embarrass myself." Once the webinar is over, she is relieved and happy. Now, however, the elaborate follow-up begins. Hanaë König receives all those questions that could not be addressed live due to time constraints per e-mail. Participants who had registered for the webinar have the option of listening to the recording and submitting questions at a later time. "Up to four weeks following a webinar, individual questions will still arrive in my inbox", says Hanaë König. "Following this period, we then analyze the event in order to learn for future webinars and to continue to improve."

Her conclusion: "I am sure that all other Eppendorf webinar presenters will agree – it is a lot of fun to prepare for and conduct a webinar. The interaction with the participants during the question period is marvelous; it is a challenge, and it offers unique insights into current topics and research that concern those who are asking the questions. This alone makes it worth the effort!"

Scan the QR code for more information!

Visit www.eppendorf.com/webinars to find scheduled and recorded webinars!

ULRIKE BECKEN, EPPENDORF AG BIOPROCESS CENTER, JUELICH, GERMANY

Everything for Bioprocessing – Online!

Without bioprocessing, modern medicine, drug discovery, and life sciences could not exist, let alone the chemical, food, and agricultural feed industries. As a leading provider of laboratory equipment and bioprocess solutions, Eppendorf presents a new website section for bioprocess knowledge.

The new website www.eppendorf.com/bioprocess offers application examples, insights from industry experts, and latest news.

This knowledge platform was created for all bioprocess professionals in the above-mentioned fields. Not only does it give a big picture view, it also adds detailed application examples and advice, and addresses specific scientific problems with solutions to overcome them.

Applications at a glance

Bioprocess applications are many and varied, and the bioprocess section on the Eppendorf page reflects this diversity. With well-prepared information and a clear layout, it provides information about the hot topics of bioprocessing: How to scale up antibody and hormone production? What are the challenges when developing new processes to increase vaccine yields? What advances have been made in the expansion of stem cells for drug discovery applications? These are only a few of the questions dealt with on the site. The platform provides information on bioprocess applications at a glance – from different perspectives and in various formats.

Eppendorf systems tailored for every bioprocess need

User reports

Exchange between experts is crucial to advancement. Most information on the new bioprocess page comes from bioprocess professionals in industry and academia. International experts present case studies in the form of application notes, journal articles, and scientific posters. They discuss some of the most relevant questions in bioprocess development today.

For example, the visitors will find an e-book on process automation and data analysis, results from cell culture scale-up in single-use bioreactors, documents on controlled stem cell cultivation, and much more. Those who prefer to listen can find recordings of the latest bioprocess webinars and even a podcast. In webinars just recently, we talked about biosimilar process optimization by comparing batch, fed-batch, and perfusion culture, and discussed the possibilities of multivariate data analysis in process development. With resources like these, beginners can learn about well-established techniques and new processes. Professionals can learn from each other and from Eppendorf, a respected leader in bioprocess technology.

Tailor-made product recommendations

With bioprocess equipment, the demands of individual processes are so specialized that often there is no run-of-the-mill product solution. The Eppendorf bioprocess section helps by providing application studies that show how technical challenges were solved, and which products were successfully used. That is product search in reverse!

Always informed with our newsletter

We warmly welcome you on our new bioprocess website, but we would also be delighted to meet you in person. A list of the upcoming "Bioprocess Events" in which we will participate enables you to plan meetings at trade shows, conferences, and workshops. You can keep in touch through the new bioprocess section of the Eppendorf website. Remember that the Bioprocessing News section keeps readers informed of all Eppendorf bioprocess updates. Finally, make sure to subscribe to our newsletter!

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www.eppendorf.com/bioprocess

Redox Potential Monitoring for Improved Anaerobic Fermentation Using the BioFlo® 120 Bioprocess Control Station

YING YANG AND MA SHA, EPPENDORF INC., ENFIELD, CT, USA

Summary

In fermentation, redox potential is an important physiochemical factor that measures the tendency of the culture medium to acquire electrons. It can directly influence bioprocess efficiency. One example is the anaerobic fermentation of *Clostridium* to produce industrial solvents. Solvent production by *Clostridium* species follows two consecutive metabolic steps. In an early culture, acidogenesis produces mainly acetic and butyric acid. In the late exponential growth phase, the metabolism switches to solventogenesis, which produces largely organic solvents including butanol [1].

To direct the electron flow towards butanol production, regeneration of the NAD(P)$^+$ pool in the *Clostridium* species is essential [1]. The intracellular ratio of NAD(P)H and NAD(P)$^+$ is highly related to the redox potential. Therefore, control of the redox potential can be used to alter biomass accumulation and solvent production [2].

We cultivated *C. beijerinckii* under anaerobic conditions using a BioFlo 120 bioprocess control station equipped with BioBLU® 3f Single-Use Vessels. When we kept the redox potential close to –500 mV, bacterial growth and butanol production drastically increased compared to a process without redox potential control. The study demonstrates the advantages of redox potential monitoring during *C. beijerinckii* fermentation.

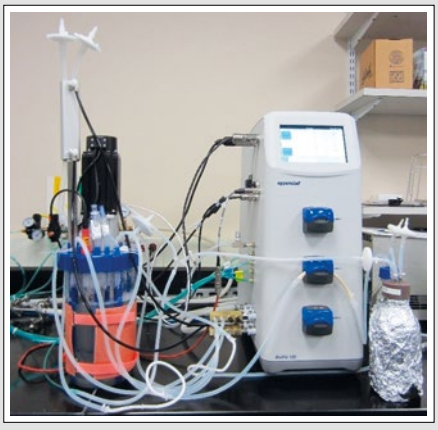


Fig. 1: Bioprocess equipment

Parameter	Device/setpoint
Inoculation density	1:100 (v/v)
Working volume	3 L
Sparger	Macrosparger
Gassing control	100 % constant nitrogen flow at 0.1 vvm (0.3 SLPM), first 4 hours through sparging, after 4 hours through overlay
Agitation	Magnetic drive; 50 rpm
Temperature	37 °C; controlled by heat blanket and cooling baffles

Table 1: Overview of process parameters and setpoints

Material and methods

We performed batch fermentations under anaerobic conditions. The preparation of inoculum and the medium composition are described in [3].

Anaerobic conditions were established by constantly gassing with nitrogen at 0.1 vvm. We conducted two batch fermentation runs. In the first run we did not control the redox potential. In the second run, we controlled the redox potential to a relatively low and stable redox potential of –500 mV. The process parameters and setpoints common to both runs are summarized in Table 1.

We took two 1 mL samples every 24 hours and quantified the concentrations of glucose and butanol [3] and measured the optical density at 600 nm (OD_{600}) to monitor bacterial growth.

Monitoring and adjustment of redox potential

We monitored pH and redox potential online using two ISM® pH/redox sensors (Mettler Toledo®, Switzerland). The same sensor type can be utilized to measure either pH or the redox potential, and the choice can be made in the setup section of the bioprocess control software. We calibrated the pH and redox sensors outside the vessel, using 2-point calibration methods [3].

We adjusted the redox potential of the fermentation broth with 35 g/L sodium sulfide nonahydrate (Sigma-Aldrich®, USA). To avoid oxygen introduction we pre-sparged the solution with nitrogen for 15 min before pumping it into the vessel. The pH of the solution was 12.84.

To maintain the redox potential at approximately –500 mV, 24 h, 32 h, 48 h, and 120 h after inoculation we turned on the pre-calibrated pump to add 2–3 mL sodium sulfide solution to the broth.

Results

We compared bacterial growth and butanol production in two processes. In one process we kept the redox potential relatively stable at –500 mV. In the other process we did not experimentally alter the redox potential.

Redox and pH trends

At the beginning of the fermentation run, the redox potential was around 0 mV. During the first 24 h, and with the exponential growth of *C. beijerinckii*, it dropped drastically to –500 mV. When we did not experimentally alter the redox potential, it varied between –600 and –300 mV from 24 h after inoculation onward (Fig. 2A).

When we added sodium sulfide nonahydrate to the culture, a relatively stable redox potential of –500 to –400 mV was maintained.

During the exponential growth phase in the first 24 h of the culture, the pH decreased from 6.5 to 5.1 in both runs. It is likely that acidogenesis took place, and acetic and butyric acid were produced. In the run without redox control, pH stayed relatively low at 5.0-5.1 with no trend toward rebound throughout the entire culture period, indicating the lack of a dominant solventogenesis phase. By contrast, in the redox-controlled fermentation the pH began to recover after 24 h, approached a value of 6.0 after 70 h, and then gradually dropped

Redox Potential Monitoring for Improved Anaerobic Fermentation Using the BioFlo® 120 Bioprocess Control Station

to 5.5 until the end. It is likely that the curved pH trend was caused by a shift in the microbial metabolism. Butanol production may have required the consumption of acetic acid, leading to a product shift from butyric acid to butanol. The decrease in acid concentration likely caused the pH increase.

Bacterial growth

During the first 24 h, we observed comparable exponential growth in both cultures. In the run without redox control, the growth slowed down significantly after 24 h and finally ended at an OD_{600} of 0.788 at 124 h. In contrast, *C. beijerinckii* continued to grow robustly when we maintained the redox potential at –500 mV. The final OD_{600} at 124 h was 1.565. This is two times higher than in the run without redox control (Fig. 2B).

Conclusion

When we experimentally adjusted the redox potential to a relatively stable value of –500 mV, the final biomass was two times, and the butanol yield three times higher than in the run without redox control. This gives an example for bioprocess optimization through monitoring and adjustment of the redox potential throughout the duration of the culture.

Literature

[1] Wang S, Zhu Y, Zhang Y, and Li Y. Controlling the oxidoreduction potential of the culture of Clostridium acetobutylicum leads to an earlier initiation of solventogenesis, thus increasing solvent productivity. *Appl Microbiol Biotechnol.* 93: 1021-1030. 2012

[2] Sridhar J, and Eiteman M. Influence of redox potential on product distribution in Clostridium thermosuccinogenes. *Appl Biochem Biotechnol.* 82: 91-101. 1999

[3] Yang Y, Sha M. Redox Potential Monitoring for Improved Anaerobic Fermentation Using the BioFlo® 120 Bioprocess Control Station and BioBLU® 3f Single-Use Vessels. *Eppendorf Application Note* 358. 2018; download at www.eppendorf.com/appnote358

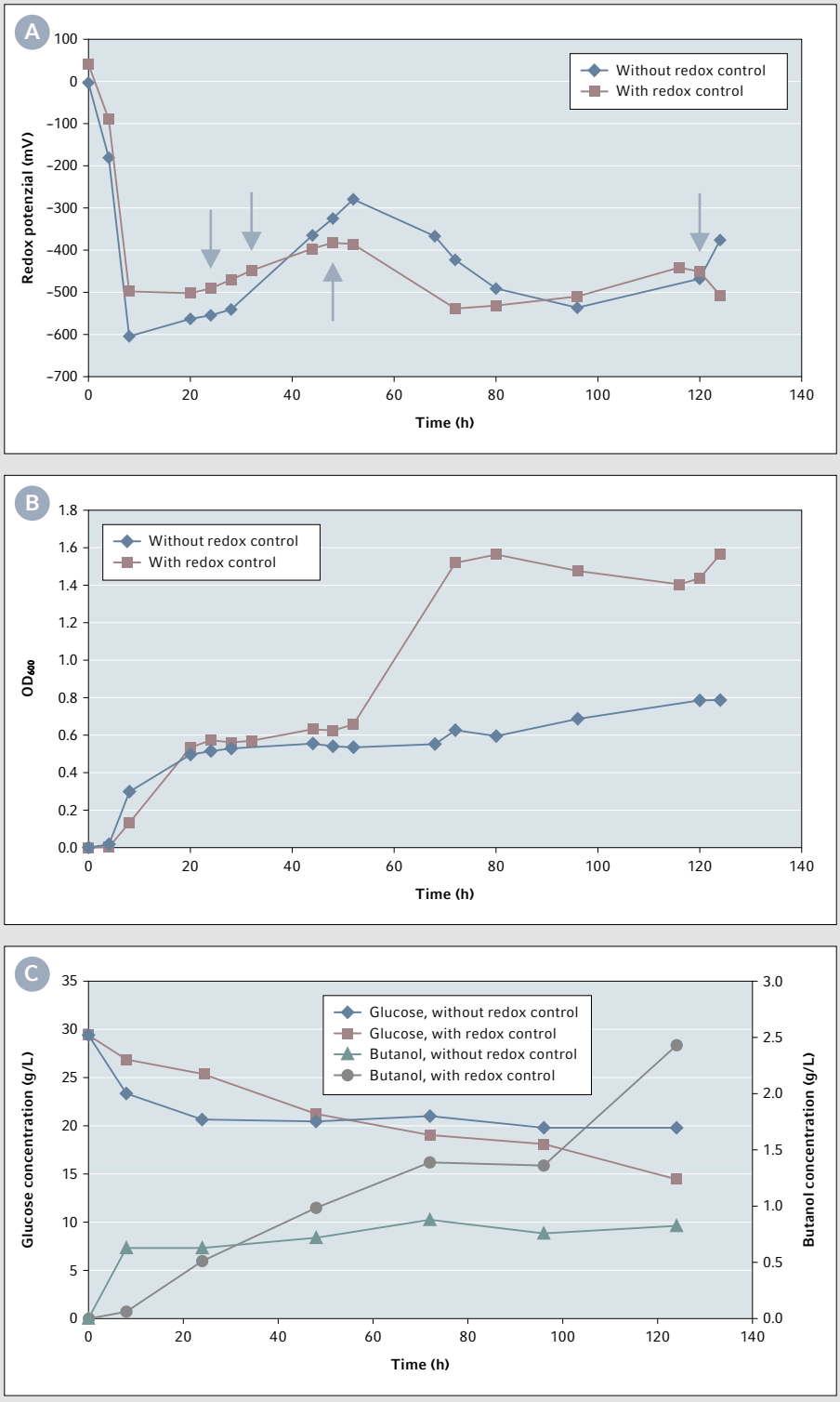


Fig. 2: A) Redox potential trends. In the run with redox control, sodium sulfide nonahydrate was added 24 h, 32 h, 48 h, and 120 h after inoculation (arrows). **B)** *C. beijerinckii* growth curves. **C)** Glucose and butanol concentrations

Reduced PCR Runtimes and Increased Yields Using Eppendorf Fast PCR Consumables

ALISA GRUSCHKA, KERSTIN ISERMANN, ARORA PHANG, EPPENDORF AG, HAMBURG

Abstract

Here we show that the Eppendorf Fast PCR consumables allow an easy transfer from a standard to a fast PCR protocol without compromising reaction efficiency. The Fast PCR Tube Strips are made from polyethylene, offering better heat transfer properties than polypropylene material which is typically used for PCR consumables. The improved thermal conductivity results in higher amplicon yields under fast PCR conditions in comparison to standard and other fast PCR consumables. This helps to streamline workflows and to increase working efficiency in the lab.

Introduction

A decrease in amplification time and thus higher throughput are advantageous for every lab. A lot of efforts have been put into speeding up PCR reactions. One approach is the establishment of two-step thermal cycling protocols. Other developments include thermocyclers with higher ramp rates and special kits containing polymerases with faster extension rates. However, a significant reduction in cycle times often requires specialized equipment and adapted procedures which may not be easily implemented in a standard lab. Therefore, typical standard end-point PCR protocols still take about an hour or more.

One reason fast or rapid PCR protocols are not widely used yet is that the transfer of an established PCR protocol to a fast protocol often compromises PCR yield and specificity. Furthermore, rapid protocols are often linked to very low reaction volumes (<5 µL) and these are rarely applicable for standard applications. The use of special fast PCR master mixes does reduce runtimes but their full potential can only be realized in combination with a fast thermocycler.

The limiting factor here is the heat transfer from the block to the sample. In the past, this limitation was mainly addressed by making the vessel walls thinner. Eppendorf's approach with the Eppendorf Fast PCR consumables is using an alternative material with better heat transfer properties to reduce runtimes.

Materials and methods

All experiments were done on Mastercycler® X50 silver block models (X50s and X50i, Eppendorf) with the following settings:

Lid temperature:	105 °C, energy-saving mode on
Temperature mode:	Fast
Block settings:	Silver 96

Human Genomic DNA (Roche®) was used as template for the fast PCR protocols with SpeedSTAR™ HS DNA Polymerase (TaKaRa) or the 2x GeneAmp® Fast PCR Master Mix (Applied Biosystems®).

PCR reaction master mix for the SpeedSTAR HS DNA Polymerase was prepared using 1x Fast Buffer I, 0.2 mM dNTP-Mix, 0.5 µM of each primer, 30 ng of DNA template, and 0.25 U of DNA polymerase in a total volume of 10 µL. PCR reaction master mix for the GeneAmp Fast PCR Master Mix (2x) was prepared using 1x Fast PCR Master Mix, 0.2 µM of each primer, and 20 ng of DNA template in 10 µL total volume.

The following primers were used for the amplification of a 536 bp sequence from the human ß-globin fragment:

Forward Primer 5'-GCT CAC TCA GTG TGG CAA AG-3'

Reverse Primer 5'-GGT TGG CCA ATC TAC TCC CAG G-3'

Cycling conditions:

	SpeedSTAR™ protocol	GeneAmp® Fast protocol
Initial Denaturation	94 °C/60 s	96 °C/15 s
Cycles 35x	96 °C/2 s 65 °C/6 s	96 °C/1 s 62 °C/16 s
Post Cycle Elongation	72 °C/60 s	72 °C/10 s
Storage	10 °C	10 °C

PCR reactions were carried out in the following consumables:

Eppendorf	Fast PCR Tube Strips
Eppendorf	PCR Tube Strips
ThermoFisher Scientific®	ABI MicroAmp® Fast 8-Tube Strip, 0.1 mL
Bio-Rad®	0.2 ml 8-Tube PCR Strips without Caps, low profile
Analytik Jena®	8 Well Strip (0.2 ml, low profile), transparent with lid

The PCR products were detected via agarose gel electrophoresis using GelRed® (Biotium®) and visualized using the Gel Doc XR+™ (Bio-Rad®).

The ThermoFisher Scientific® GeneRuler™ 50 bp DNA Ladder was used as marker.

Results and discussion

Significantly reduced runtimes can be achieved using fast PCR reagents on thermal cyclers with fast ramp rates but standard PCR consumables are time-limiting. Polypropylene, the standard material for PCR consumables, limits the speed of the heat transfer from the thermoblock to the sample even when the tubes are ultra-thin walled. This usually leads to lower amplicon yields and accuracy under fast PCR conditions.

Reduced PCR Runtimes and Increased Yields Using Eppendorf Fast PCR Consumables

The results presented here show a successful conversion of a standard to a fast PCR protocol using the Eppendorf Fast PCR Tube Strips made of polyethylene (Fig. 1).

PCR protocols using two different fast PCR reagents were successfully established on the Mastercycler X50s and X50i. Whilst the standard PCR protocol generally takes about an hour, the fast PCR protocols were completed in 12 min (SpeedSTAR) to 16 min (GeneAmp). This is a reduction of the runtime by more than 70 %.

Furthermore, a comparison between Eppendorf Fast PCR Tube Strips, standard PCR consumables, and other fast PCR consumables showed the superior PCR performance of the Eppendorf Fast PCR polyethylene consumables with higher amplicon yields (Fig. 2).

Fig. 1: Eppendorf Fast PCR Tube Strips

Fig. 2: SpeedSTAR Protocol (above), GeneAmp Fast PCR Protocol (below)

Conclusion

The better heat transfer properties of polyethylene enable an easy conversion of standard PCR protocols to fast cycling protocols with higher amplicon yields. In combination with fast PCR reagents it is possible to fully exploit the advantage of high ramp rates on fast thermal cyclers such as Mastercycler X50s/X50i and reduce cycling times by more than 70 % without tedious optimization. This allows users to reduce the time to get PCR results and to enhance sample throughput by using thermal blocks and consumables in standard formats, thus eliminating the need for specialized equipment.

For more details see our Application Note No. 400. www.eppendorf.com/appnote400

Economic DNA Determination in the Eppendorf BioSpectrometer® fluorescence Using Qubit™ Assays

MARTIN ARMBRECHT, EPPENDORF AG, HAMBURG

Abstract

Different concentrations of dsDNA samples were determined by the Qubit dsDNA assay using the Eppendorf BioSpectrometer fluorescence. Both the UVette® and the Eppendorf µCuvette® G1.0 were used for the measurements. In order to determine the sample concentration, different regression possibilities of the standard curve were applied. In addition, the results were compared with measurements in the Qubit device, which were performed in parallel with the same samples. We show that the Qubit assay can be used with both the UVette and the Eppendorf µCuvette G1.0. In both cases, the assays can be performed at a significantly lower volume compared to measurements in the Qubit device. Applying BioSpectrometer fluorescence and UVette requires only half of volume of the Qubit reagent, thus doubling the sample capacity of the kit.

Introduction

Besides the classic method of determining the concentrations of dsDNA via absorbance measurements at 260 nm, quantification methods based on fluorescence are well-established. It is easier to perform absorbance measurements as the sample can be directly determined without standard curve. However, photometric measurement will soon reach its limits regarding accuracy and precision especially when samples of very low concentrations are concerned.

A widely used method for determining dsDNA is the Thermo Fisher Scientific Qubit method comprising fluorescence dye and fluorimeter.

Some of the Qubit assays, e.g. the Qubit dsDNA HS Assay Kit, can also be employed on the Eppendorf BioSpectrometer fluorescence. For method optimization, different evaluation steps were carried out, e.g. a variety of standard curve regression options. The corresponding measurements were carried out both in the UVette and the Eppendorf µCuvette G1.0. The results gained from these measurements were then compared with the results obtained from the Qubit fluorimeter [1].

We present the most relevant findings in this report.

Methods

For measurement in the Qubit instrument, the dsDNA standards supplied in the kit are prepared (0 and 500 ng/mL). Operation of the Qubit is carried out in accordance with the experimental instructions included in the kit.

The standards provided in the Qubit kit are also used with the BioSpectrometer fluorescence. Additional concentrations are prepared through dilutions using the Qubit buffer (0, 50, 100, 250, 500 ng/mL), thus allowing the use of different regression options on the BioSpectrometer. A measuring series with four sample concentrations is prepared (Table 1).

Each sample concentration is measured five times. Depending on the measuring system, different sample and standard volumes, respectively, are prepared (Table 2).

Operation of the Qubit is in accordance with the instructions provided in the kit manual. The pre-programmed Qubit HS method is used for measurements in the BioSpectrometer [1, 2]. For measurement in the UVette 100 µL, for measurement in the µCuvette G1.0 5 µL each are used.

Results

Method evaluation for the Qubit dsDNA HS Assay Kit on the BioSpectrometer fluorescence shows that both the UVette and the µCuvette G1.0 can be used. Regression analyses show that, as on the Qubit instrument, a 2-point calibration (linear interpolation) is sufficient for the UVette [1].

Figure 1 shows the corresponding comparison of measurements in the UVette (BioSpectrometer) and the Qubit system. The precision of the respective series of measurements (A) and the deviation from the target value (B) are compared. With respect to precision the results obtained in the UVette are comparable to those measured with the Qubit and are even better regarding the target value achieved (Fig. 1B).

When measuring in the UVette half of the reagent volume can be saved. Only 100 µL instead of 200 µL are needed for the UVette.

The µCuvette G1.0, too, can be used for the assay. Method evaluation shows that the best results are achieved applying quadratic regression [1]. Figure 2A and 2B, respectively, show the results for precision and deviation from target value.

With respect to precision the measurements in the µCuvette G1.0 are comparable to those of the Qubit for nearly all sample concentrations (Fig. 2A) and even better regarding the target value (Fig. 2B).

Initial concentration [µg/mL]	Final concentration [ng/mL] (following 1:20 dilution in Qubit reagent)
10	500
5	250
2	100
1	50

Table 1

	Sample or standard volume [µL]	Buffer volume for dilution [µL]	Measuring volume [µL]
Qubit™	10	190	200
Eppendorf BioSpectrometer® + UVette®	5	95	100
Eppendorf BioSpectrometer® + Eppendorf µCuvette® G1.0	2	38	5 (40*)

Table 2: Measuring volumes in the different measuring systems

*Although a volume of only 5 µL is sufficient for measurement in the µCuvette, a total sample volume of 40 µL is selected to ensure error-free pipetting of samples and standards.

Economic DNA Determination in the Eppendorf BioSpectrometer® fluorescence Using Qubit™ Assays

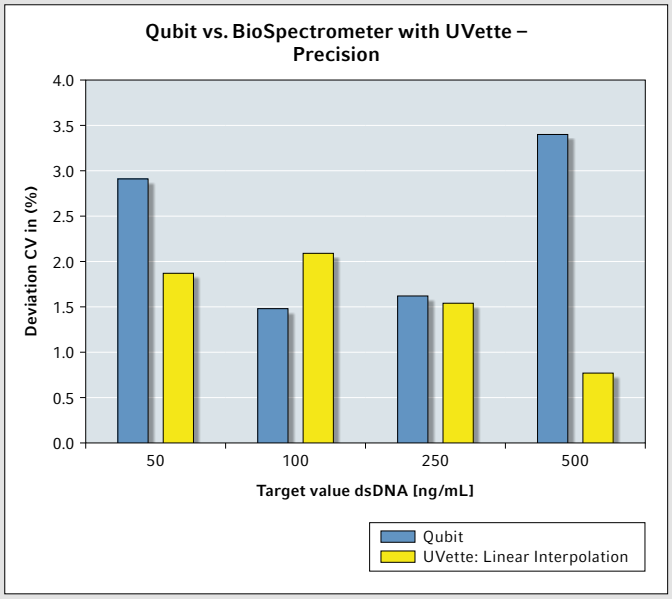


Fig. 1A: Precision of the respective series of measurements at different concentrations

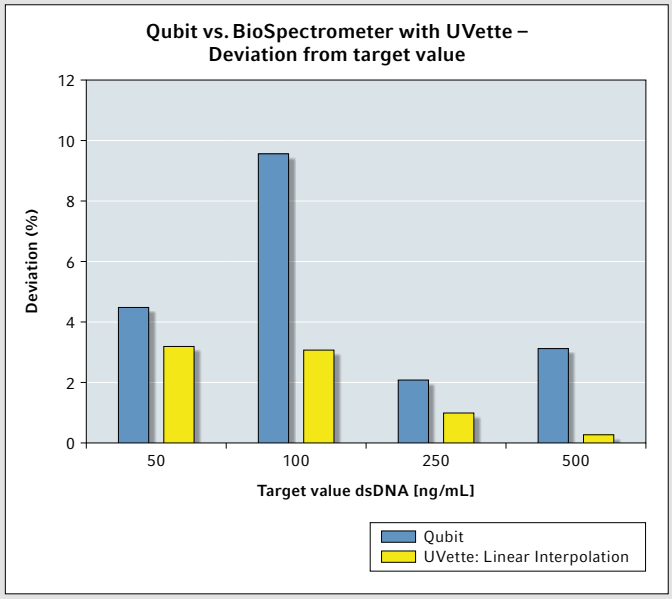


Fig. 1B: Deviations from target value/dsDNA concentration [%]

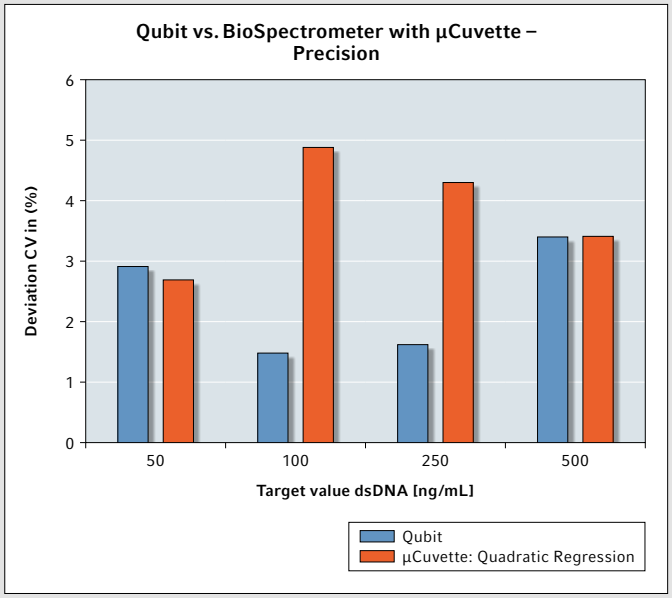


Fig. 2A: Precision of the respective series of measurements at different concentrations

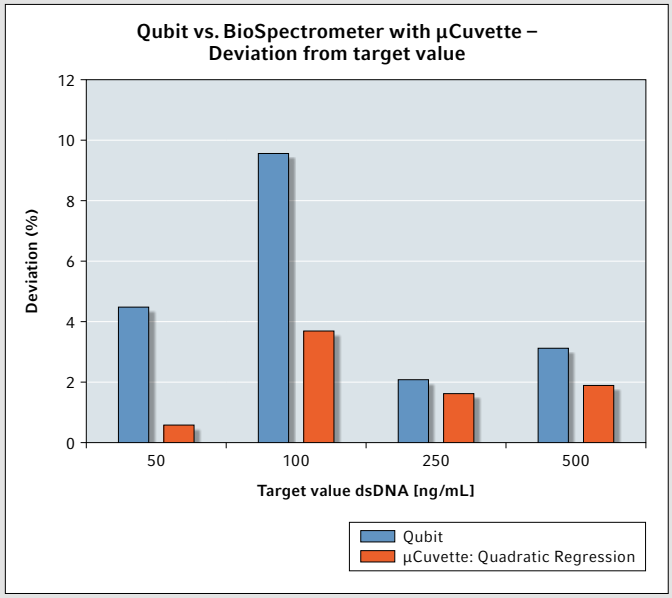


Fig. 2B: Deviations from target value/dsDNA concentration [%]

Even though four standards are needed for the standard curve, substantial reagent savings can be achieved as only 40 µL per measurement are needed.

Conclusion

1. When using the UVette a 2-point calibration is sufficient, as on the Qubit. The results are comparable and/or better compared with the Qubit.

2. Compared with the Qubit only half of the reagent volume is needed when using the UVette. This doubles the kit capacity when used in the BioSpectrometer + UVette system.

3. Using the µCuvette G1.0 provides results that are comparable to Qubit measurements.

4. At least four standards are needed when using the µCuvette G1.0, but substantial reagent savings can be achieved.

For more evaluation details see Eppendorf Application Note 402 [1] or Short Protocol 036 [2].

Literature

[1] Armbrecht M. Economic DNA Determination in the Eppendorf BioSpectrometer® Fluorescence Using Qubit™ Assay kits. *Eppendorf Application Note* No. 402 (2018). Download at www.eppendorf.com/appnote402

[2] Armbrecht M. Using the Qubit™ dsDNA HS Kit on the Eppendorf BioSpectrometer® fluorescence. *Eppendorf Short Protocol* No. 036. Download at www.eppendorf.com/sp36

Eppendorf Amber Conical Tubes: Maximal Sample Protection and Visibility

RAFAL GRZESKOWIAK, EPPENDORF AG, HAMBURG, GERMANY
SANDRINE HAMELS, EPPENDORF APPLICATION TECHNOLOGIES S.A., NAMUR, BELGIUM

Abstract

Handling and storage of light-sensitive reagents or samples is commonly performed in amber-colored plastic tubes. The majority of them have however a major drawback: They are opaque and do not allow direct sample visibility. In this Application Note photo-protection of the samples (fluorescein recovery assay) and sample visibility (transmission spectrum) in several amber conical tubes (15 mL, 50 mL) were investigated. All of the tubes tested showed good photo-protection properties and only the Eppendorf Amber Conical Tubes provided adequate sample visibility (in the range above 550 nm) and thus provide a clear advantage regarding sample handling and prevention of contamination.

Introduction

Handling and storage of light-sensitive reagents or samples is commonly performed in amber-colored plastic tubes. While providing good sample protection, the majority of these tubes are opaque and offer poor visibility of the sample – especially in larger formats such as 15 mL and 50 mL tubes. This disadvantage is generally accepted as unavoidable in order to prevent degradation of reagents, which may have negative impact on the experiment. In this Application Note we investigated both sample light protection (fluorescein recovery assay) and sample visibility (transmission spectrum) in several amber conical tubes.

Materials and methods

Sample photoprotection: fluorescein recovery measurement

Amber conical tubes of various manufacturers (Eppendorf, Gr, V, Ar, Lc) were filled with 30 mL of a 30 nM fluorescein solution (0.1 M NaOH) and incubated for 92 hours at average light conditions in the laboratory or in the dark. Standard (colorless) Eppendorf Conical Tubes 50 mL were used as a negative control (no light protection) and standard (colorless) Eppendorf Conical Tubes 50 mL, wrapped in aluminum foil, were used as the positive control (maximal light protection). After incubation, 190 µL of each sample were used in triplicate for fluorescence measurements (Fluoroskan Ascent™, ThermoFisher Scientific). Two independent experiments were performed.

Sample visibility: transmission spectrum measurement

From each 50 mL amber conical tube (Eppendorf, Gr, V, Ar, Lc) two 5 x 5 mm wall sections were taken and absorbance spectrum (200 nm to 650 nm) measurements were done in the Eppendorf BioSpectrometer. Blank sample: measurement without a section (air). The absorbance data (A) were converted into percentage transmittance data (T) based on the following equation:

$$A = 2 - log10\ \%T.$$

Results and discussion

The amber conical tubes from different manufacturers show significant differences in terms of color intensity and opacity. The color range varies from medium brown to black and sample visibility is nearly none (Fig. 1).

Eppendorf Amber Conical Tubes show significantly less coloring intensity and they are the only tubes that are transparent. This represents a major advantage in terms of sample handling. It enables users to fully control the insertion depths of pipette tips and accurately see the liquid levels as well as avoid pipette contamination.

The recovery rates of fluorescein samples (sample photo-protection) after 92 hours of incubation in Eppendorf Conical Tubes 50 mL (colorless and amber) are presented in Fig. 2.

As compared to the colorless variant, the Eppendorf amber tubes show very high recovery rates of fluorescein samples and thus provide nearly 100 % protection. Recovery rates of fluorescein samples incubated in other amber tubes were similar (data not shown).

The analysis of the controls confirms that fluorescein is sufficiently sensitive to light (negative control: low recovery rates in colorless tubes incubated under daylight conditions), whereas no unspecific degradation of fluorescein could be observed (positive control: normal recovery rates in tubes covered with aluminum foil). To further evaluate possible leaching of other fluorescently active substances from the amber tubes, which might also influence and bias the measurements, an additional control experiment was performed.

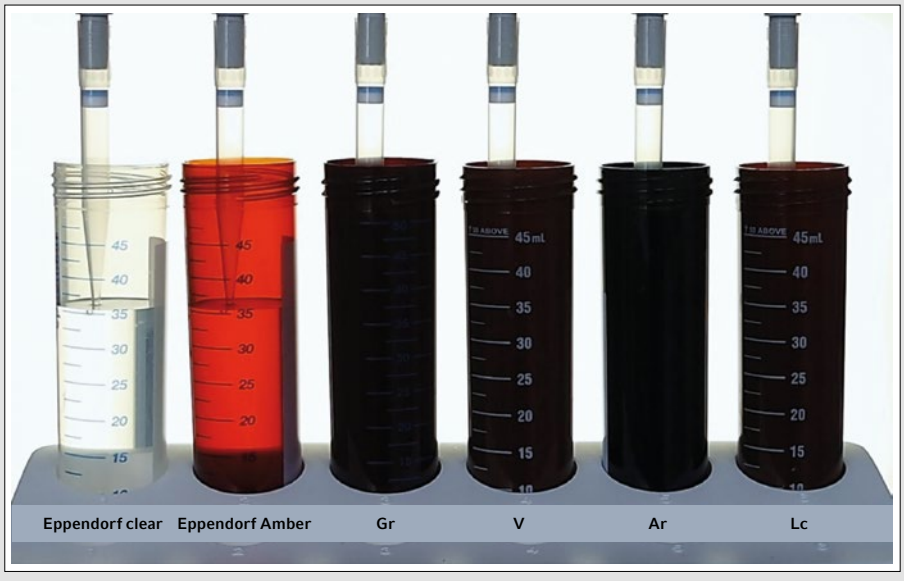


Fig. 1: Visibility of samples and pipetting in various amber conical tubes

Eppendorf Amber Conical Tubes: Maximal Sample Protection and Visibility

After 92 hours of incubation of only the fluorescein dilution solution (0.1 M NaOH), no increase in fluorescence was detected for samples incubated in any of the tubes tested (data not shown).

In Fig. 3 the transmission spectra (percentage of light transmission at different wave lengths) of amber conical tubes are compared. The good photoprotection properties of all tested amber conical tubes are correlated with the light transmission percentage that is close to 0 % when measured in the range between 200 nm and approximately 550 nm.

This constitutes the active range of the majority of dyes and fluorophores used in life sciences as well as in diagnostic and other applications. Above 550 nm the Eppendorf Amber Conical Tubes show a moderate increase in transmission, which allows good sample visibility and which correlates with the light brown coloring of the tubes.

This transmission spectrum is similar to that of amber glass vessels used routinely in life sciences, pharma and chemical laboratories and follows respective limits of spectral transmission set as standard in this field [2].

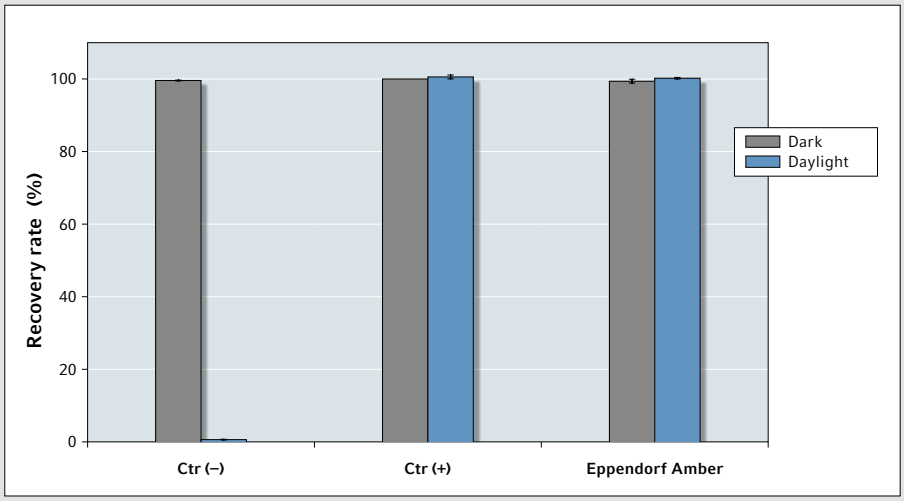


Fig. 2: Fluorescein recovery rate (percent) after 92 hours of incubation in 50 mL amber conical tubes at daylight or in the dark. **Ctr (-):** Eppendorf 50 mL Conical Tubes colorless; **Ctr (+):** Eppendorf 50 mL Conical Tubes colorless wrapped in aluminum foil

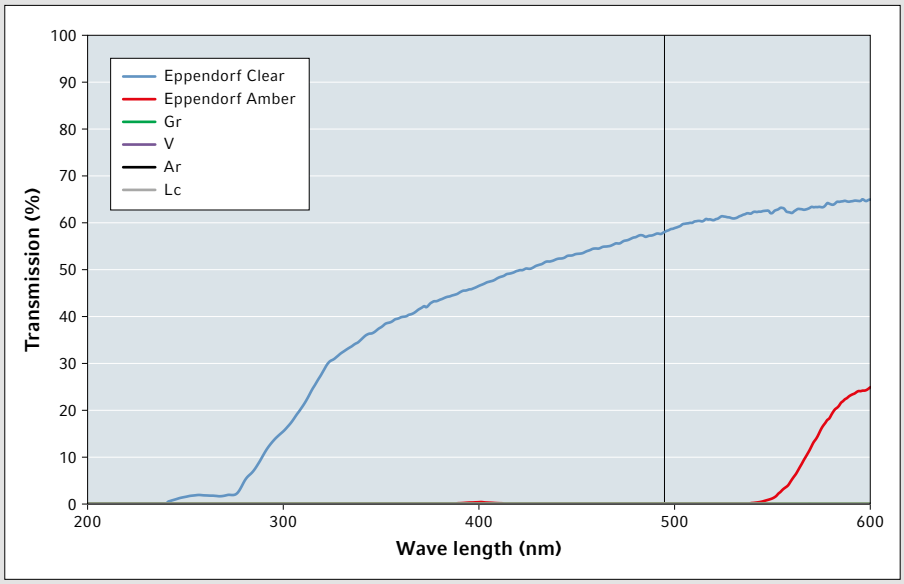


Fig. 3: Transmission spectra (transmission percentage as a function of wave length) of amber conical tubes. The fluorescein excitation (λ ex) maximum is depicted as a solid black line.

Conclusion

All of the 50 mL amber tubes tested in this Application Note provide good protection of light-sensitive samples. This is indicated by high (nearly 100 %) recovery rates of fluorescein samples following 92 h of incubation under regular light conditions. This result is also confirmed by the very low (close to 0 %) light transmission in the energy-rich range of wave lengths between 200 nm and approximately 550 nm.

The Eppendorf Amber Conical Tubes are currently the only tubes that are transparent in the range above 550 nm, thus providing a significant advantage of good sample visibility and handling along with maximal protection of light-sensitive reagents.

Literature

[1] Glass Containers for Pharmaceutical Use (3.2.1) p. 363; *European Pharmacopeia* 7.0; 01/2008

[2] Spectral Transmission for Colored Glass Containers; 4 (660) Containers—Glass / *Physical Tests*; USP Guidelines 36

Download the full version of this Application Note at www.eppendorf.com/appnote403

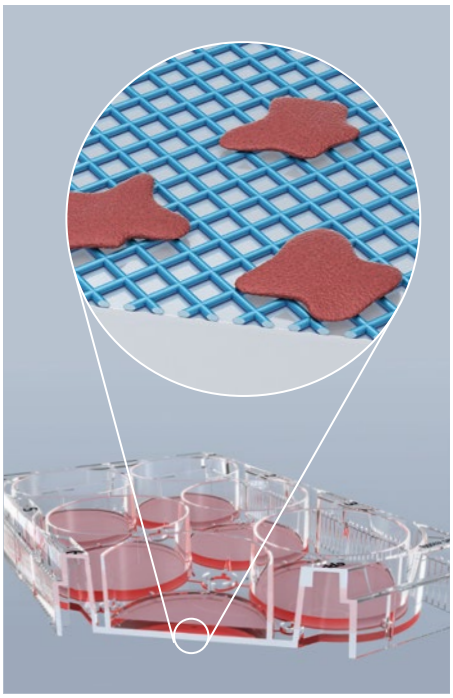
CHRISTIAN HABERLANDT, EPPENDORF AG

CCCadvanced® FN 1 motifs Cell Cultureware

Since the groundbreaking discovery of human induced pluripotent stem cells (hiPSCs) by Shinya Yamanaka in 2006, the use of pluripotent stem cells (PSCs) is booming.

Due to their extensive self-renewal properties and ability to differentiate into many different cell types, PSCs, and especially hiPSCs, offer many promising applications e.g. in regenerative medicine. New ready-to-use cell culture vessels for cultivating stem cells shall increase the required reproducibility of experiments.

The major challenge in iPSC cultivation is to preserve their properties until differentiation is induced. To support this, a growth surface mimicking the extracellular matrix is crucial.



Biological coatings commonly used are inherently complex and non-defined which often results in reduced experimental reproducibility. This is exacerbated by variations between different production lots of the coating media and by an increased contamination risk during self-coating of vessels.

Therefore, ready-to-use, fully synthetic culture systems that are free of animal and human components are of great interest for defined and reproducible culture conditions in research and production. However, until now, such cell culture vessels for iPSCs have not been commercially available.

The new ready-to-use CCCadvanced FN 1 motifs cell cultureware from Eppendorf has a synthetic surface with fibronectin-derived motifs to support long-term hiPSC expansion. The products are also suitable for cultivation of other stem cell types and ECM-dependent cells in xeno-free and restrictive culture conditions.

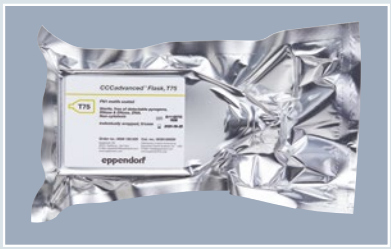
Find more information at
www.eppendorf.com/ccc-advanced

Tip

Your Stem Cell Culture Will Benefit

How reproducible are your iPSC or MSC cultures and their downstream analyses today? If you are using cell culture vessels with feeder cells, biological coatings, or self-coated vessels, there is certainly room for significant improvement as well as for time savings. Synthetic surfaces such as the new CCCadvanced FN1 motifs cell culture vessels inherently offer a high degree of consistency between different manufacturing lots. The fact that they are ready-to-use allows you to save time while simultaneously significantly lowering the risk of contamination in comparison with vessels that are coated manually. This further contributes to enhanced reproducibility.

Central purchasers will also benefit from the new surface: The broad range – compared with many other forms of coating – of successfully tested and compatible cells, media, and dissociation reagents covers manifold applications. Easy storage and the tremendously long shelf-life of three years at room temperature secure a larger investment while at the same time freeing up capacity in refrigerated units.



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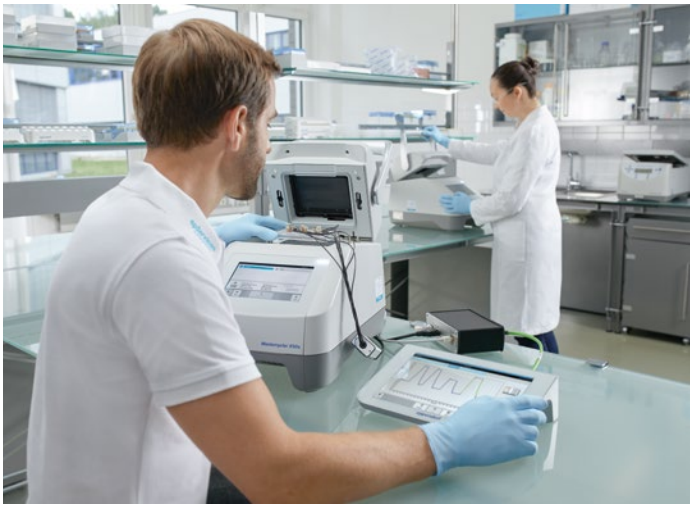

Eppendorf service professional performing a multi-channel temperature verification of a Mastercycler® X50s

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HANAË KÖNIG, EPPENDORF AG

How to Clean and Maintain Your Lab Tools

Day after day, laboratory tools are faced with contamination, aggressive chemicals, and are used by many different people. When maintenance is neglected, this can lead to wear and tear of parts or decreased accuracy and precision. Our series of step-by-step instructions and videos about cleaning and routine maintenance that you can carry out yourself, will help you prolong the lifespan of your devices.

Laboratory tools need regular maintenance. These sensitive devices are built to deliver accurate and precise results. Pipettes, e.g., include many parts made of varied materials such as metal, polymers, and silicone. Contact with sample liquid can cause contamination inside the pipette and, thus, cross contamination between samples. Furthermore, working with strong chemicals may attack certain parts of the pipette and cause corrosion.

Centrifuges, even if they are heavy and robust devices, are exposed to high forces during centrifugation and frequently also to chemical vapors evaporating from the vessels. Some cleaning and maintenance steps such as lubricating the rotor as well as cleaning the rotor cavities and the inner chamber of the centrifuge can be done by yourself.

In the Eppendorf Handling Solutions webspace you can now find online guidance for proper cleaning of pipettes and centrifuges. A series of articles and videos will guide you through the cleaning process. To learn more, please use the following links:

> For pipettes: **www.eppendorf.com/bn50/pipette**

> For centrifuges: **www.eppendorf.com/bn50/centrifuge**

Quality Management Systems increasingly ask for regular maintenance and certification services for pipettes, centrifuges, and rotors to ensure a safe working environment and compliance with manufacturer specifications. Our Performance Plans (s. page 10) support you to keep your instrument in perfect working order. They also support compliance with your laboratory guidelines with the necessary certification documents.

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News

What Our Readers Say

We regularly get feedback from our valued readers. We love to hear that many of you rate the BioNews contents informative and useful and very often delightful and inspiring, too.

Here are some reader comments:

"Really enjoyed the crossword! Also, I loved the article on pipetting! It's been a recent debate in my lab on when and how to use reverse pipetting, and how we were never really taught how to pipette properly! I will definitely be showing this issue to the other people in my lab!" (Charlotte C., UK)

"I applaud Eppendorf in their continuous improvement in products. This makes the lives of us scientists much easier. I find the VisioNize System to be quite interesting and practical. As many times in the lab we have overnight runs, but no way to monitor these runs. With this system we could monitor everything right from a phone." (Rashell W., Jamaica)

"Despite having three talks to prepare for a conference in a few days, my 'need' for a personalised pipette has led me to finish the crossword from the BioNews magazine (that, and the unexplained phenomenon of doing anything but the task at hand)!" (Helen O., UK)

"I don't have to ask my colleague to pinch me, because I know I am not dreaming even if it feels like I am. I get my own personalized pipette! This is just crazy and the cherry on the cake of the (not so) gray everyday lab routine. […] You made my day!" (K. Henriette R., Germany)

Want to leave us your feedback? Just send us an e-mail to **bionews@eppendorf.de**

HANAË KÖNIG & TANJA MUSIOL, EPPENDORF AG

Five Ways to Optimize and Speed up Your PCR Runs

Multiple PCR reactions are conducted daily in almost every laboratory worldwide. PCR is currently the quickest and safest method for DNA amplification. At the same time, this standard method harbors potential pitfalls and is subject to a number of influencing factors. The need to speed up and optimize PCR runs is thus ever-present, and it can be approached in different ways. In this article, we will introduce five ways.

In PCR, being considered almost foolproof, we most often rely on commercially available kits that promise optimal yield along with a quick procedure. However, sample preparation, as well as all instruments and consumables involved in the complete PCR process, will influence the result in ways that are underestimated all too frequently.

Each PCR reaction is unique, and each primer pair and each DNA sample has different requirements with respect to temperature. Therefore, most often, one optimization step also means waiting to complete an entire PCR run. Quicker performance of PCR and higher experimental and analytical throughput are of the essence which, in turn, translates into a need for faster optimization.

1. The right material increases heating and cooling rates

Heat-conducting materials, which are employed as alloys for the PCR block inside the PCR instrument, provide increased heating and cooling rates. While aluminum is the standard material, a block made from silver alloy will achieve twice the heating rate (Table 1).

Thermoblock	Aluminum	Silver
Heating rate	5 °C/s	10 °C/s
Cooling rate	2.3 °C/s	5 °C/s

Table 1: Heating and cooling rates, dependent on the material composition of the thermoblocks

Faster arrival at the desired temperature automatically leads to a quicker PCR run. It is important that the temperatures are reached reliably, with minimal deviation and minimal overshoot and undershoot. In order to ensure that temperatures are reached in a reliable fashion, and that your PCR instrument functions optimally, it is recommended that it be tested in regular intervals, ideally by an accredited service using the Temperature Verification System (TVS).

2. Novel technologies maximize the options for optimization

Ever since the discovery of PCR, the market for PCR instruments has evolved rapidly.

One groundbreaking innovation, for example, was the ability to program temperature gradients in order to achieve a different temperature in each row of the PCR block. This feature has revolutionized and massively accelerated the determination of the optimal annealing temperature.

However, in order to determine the optimal denaturation temperature, a further PCR run had to be carried out. The Mastercycler X50 is the first instrument to enable programming of a 2D-Gradient (Fig. 1).

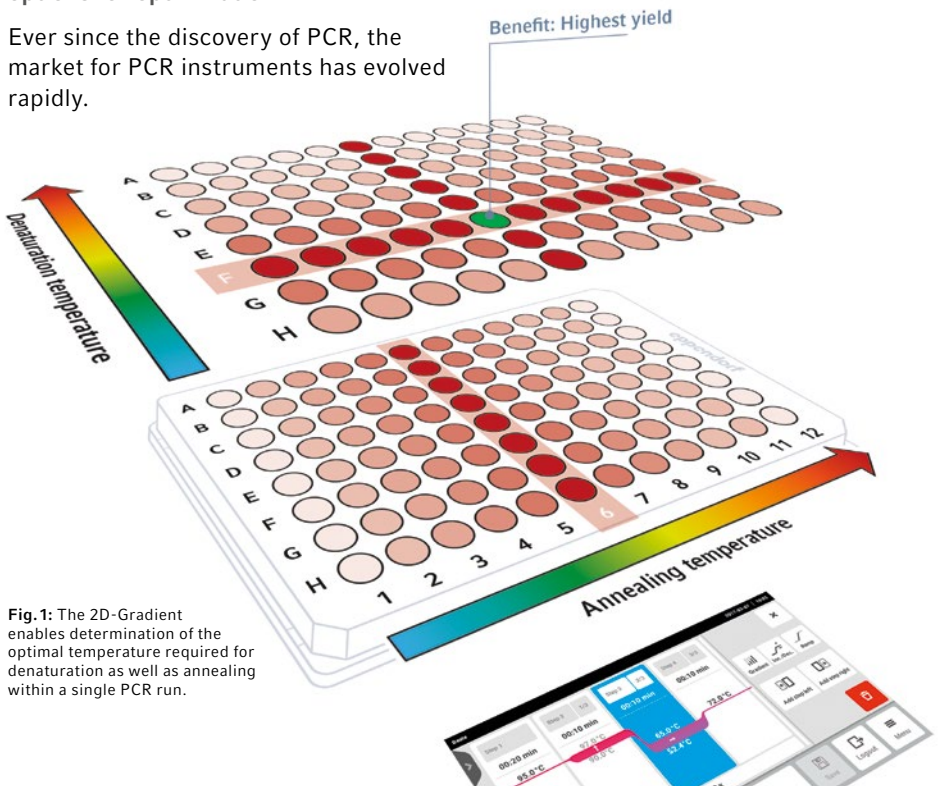
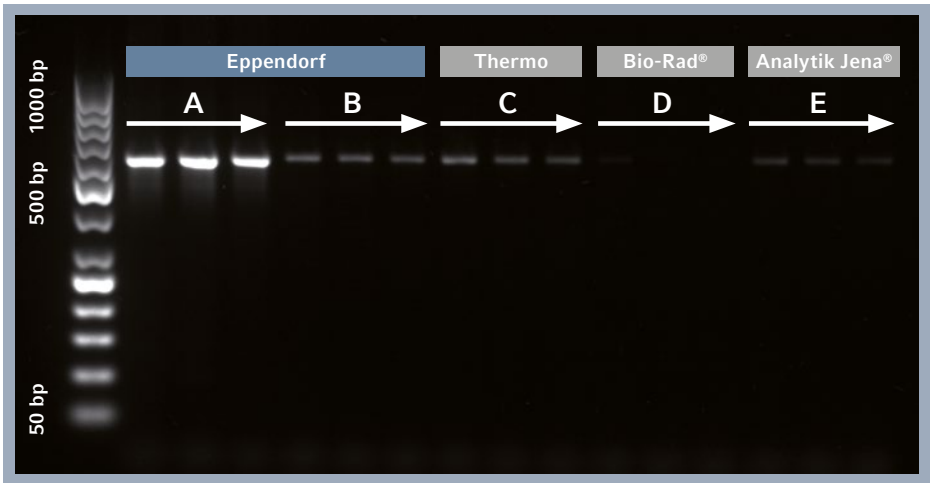


Fig. 1: The 2D-Gradient enables determination of the optimal temperature required for denaturation as well as annealing within a single PCR run.



A: Fast PCR Tube Strips
B: PCR Tube Strips
C: ABI MicroAmp® Fast 8-Tube Strips
D: 0.2 mL 8-Tube Strips
E: 8 Well Strip (0.2 mL, low profile)

Fig. 2: GeneAmp Fast PCR protocol. The use of Eppendorf Fast PCR consumables, in combination with the GeneAmp Fast PCR protocol, leads to a massive increase in DNA yield as compared to other manufacturers. 20 ng of DNA in a 10 µL PCR reaction.

In this way, a different denaturation temperature may be selected in each row, while simultaneously a different annealing temperature is selected in each line. Optimal temperature conditions will thus prevent incomplete denaturation and mispriming. This feature enables the determination of the respective temperatures which will lead to maximum yield as well as optimal PCR reproducibility [1].

3. Optimized consumables speed up PCR runs

Another way to speed up PCR runs includes the use of specially developed fast PCR kits in combination with fast PCR consumables. These consumables feature exceptionally thin walls while at the same time maintaining their stability – a feature which allows quicker heating and cooling of the sample. The temperature inside the sample is reached in a more uniform manner, and with the help of the fast PCR kits, one PCR run is completed within a mere 16 minutes [2]. As a bonus, the use of fast PCR consumables delivers an increased DNA yield (Fig. 2).

4. In a high-throughput PCR laboratory, centralized control of all instruments enhances speed

If more than one PCR instrument is required to run the same program, as is frequently the case in routine PCR laboratories, centralized control of several instruments will ease the work. Up to 50 eco-modules may be connected via one network and controlled through one software application (Eppendorf CycleManager X50, linked with the Mastercycler X50). In this way, for example, 20 instruments may be started on one program, while simultaneously 30 instruments are started on a different program. For laboratories with lower throughput, up to nine eco-modules may be connected to, and controlled by, one master. This saves time during instrument programming.

5. Tips and tricks for PCR optimization

It is further possible to optimize PCR sequences via adjustments to primer design, to the composition of the PCR master mix, or to the process of sample preparation. In addition, adaptation of the denaturing temperature and the annealing temperature, as well as the duration of the elongation step, may be crucial for the specific amount of DNA, its purity and quality. It is worth testing all options before buying a new kit or ordering new primers. Tips and tricks for the optimization of PCR, alongside fascinating background information, are available online at

www.eppendorf.com/bn50/pcr

Through articles and videos, you will learn interesting facts that will make your everyday routine easier while expediting your work.

Conclusion

There are many ways to improve PCR runs – starting with preparation, via consumables and instruments, all the way to new technologies. Within this article, we were able to point towards a few of these options. Even if the discovery of the optimal combination may take a little time, the initial effort will be richly rewarded – in the form of cost and time savings and with the good feeling that comes with knowing that one can rely on each and every PCR.

[1] Phang A, Schommartz T. Ultimate PCR Optimization with Eppendorf Mastercycler® X50 2D-gradient. *Eppendorf Application Note* 387. www.eppendorf.com/appnote387

[2] Isermann K, Phang A. Reduced PCR runtimes and increased yields using Eppendorf Fast PCR Consumables. *Eppendorf Application Note* 400. www.eppendorf.com/appnote400. You can find a short version of this Application Note on pages 3–4 in the middle section of this BioNews issue.

CAROLYN TAUBERT AND BERRIT HOFF, EPPENDORF AG

Welcome to Hamburg: Flavio Donato & Andrea Ablasser

Flavio Donato at the Eppendorf Training Centre

The Italian scientist Flavio Donato, Ph.D. of the Kavli Institute of the Norwegian University of Science and Technology in Trondheim, Norway, visited Eppendorf in summer 2018. Dr. Donato won the 2017 *Eppendorf & Science Prize for Neurobiology* for his work on how neural networks mature during development to represent space in the brain.

After visiting our production facilities Flavio said, "I have a newfound respect for every single pipette tip I have used in my life, after seeing how much technology and care is put into producing every single one!".

In 2019 Flavio Donato will start his own laboratory at the Biozentrum of the University of Basel. Here he will study how neural circuits acquire the ability to learn and form memories during the development of a child.

More information at **www.eppendorf.com/prize**

Memories of Hamburg: Andrea Ablasser with her personalized pipette

Dr. Andrea Ablasser, winner of the 2018 *Eppendorf Award for Young European Investigators* also paid a visit to Eppendorf. Andrea Ablasser, Assistant Professor at the Swiss Federal Institute of Technology, Lausanne, Switzerland, won the Eppendorf Award for her contributions to a key step in the innate immune response, which triggers a frontline defense when cells are attacked by microorganisms. Her work sheds light on the mechanisms by which other cells are informed about the presence of foreign DNA. She could also show that the same pathway can be triggered in ageing cells, contributing to senescence.

Just as Flavio Donato some weeks before, Andrea Ablasser presented her research work to an interested audience of Eppendorf employees. Additionally, she got insights into different production areas, the Eppendorf Training Centre, and our "museum". Andrea was impressed by the visiting program and the Eppendorf spirit, and like Flavio, she was especially happy about a pipette with her name laser-printed on it.

More information at **www.eppendorf.com/award**

Win a Pipette 3-Pack!

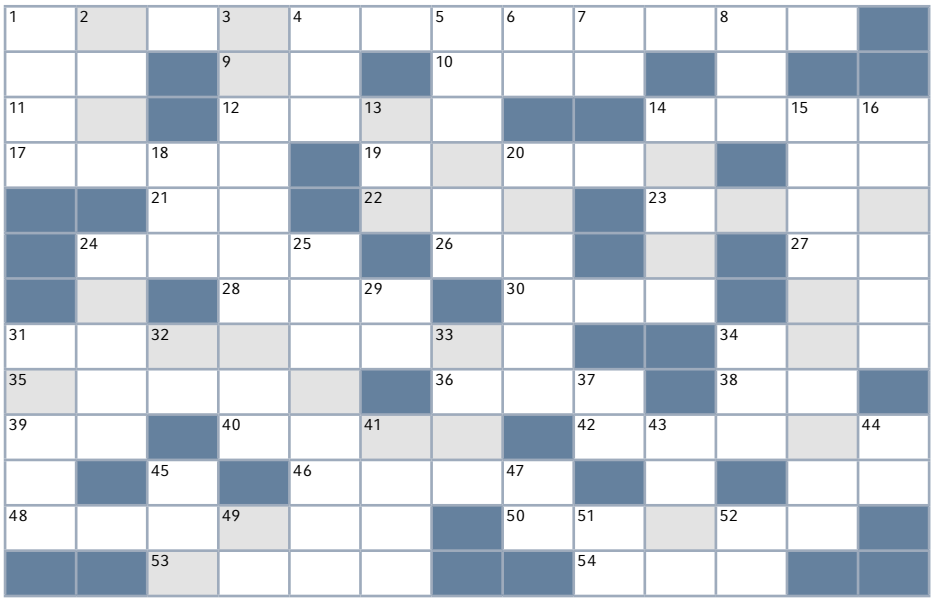
The solution of our anniversary prize competition of BioNews 48 was "Happy BioNews Anniversary". The five main prize winners were happy about a personalized pipette each: Younghyun Wy (Korea), Katharina-Henriette Rasp (Germany), Julie Wilson (United Kingdom), Joanna Michalska (Poland), and Gaston Bonenfant (USA).

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, 2019**.

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. 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. 52.



1st Prize:

3 Eppendorf Research® plus pipettes (3-Pack of your choice)

2nd to 5th Prize:

1 Amazon® Voucher worth 50.00 EUR

6th to 15th Prize:

400 bonus epPoints® each

(epPoints registration required)

ACROSS

1 Character from One Thousand and One Nights
9 Venice river
10 Genus of macaws
11 New type of intelligence (abbrev.)
12 One small … for man
14 Very important persons (coll.)
17 Area of water
19 Music drama
21 US medical TV saga (abbrev.)
22 Fleur de …
23 Virtuous, beneficial
24 Programming language
26 Rhenium symbol
27 Nashville is the capital (abbrev.)
28 Stock market launch (abbrev.)
30 Abbrev. from spectroscopy, complements UV
31 White wine from the Loire valley
34 Female given name
35 Important PC key
36 Influential British musician (family name)
38 Caracas is the capital (Iso code)
39 Afterthought to a letter
40 Female given name
42 Say or declare
46 Speak, communicate
48 Proof of absence (pl.)
50 Honey colored
53 World Cup football organization (abbrev.)
54 Chart, plan, travellers aid

DOWN

1 To make airtight or waterproof
2 Seeds, alleged to be superfood
3 Dedicated to maintain your Eppendorf equipment's performance
4 Sausage + bun, this dog is …
5 Jay-Z is one
6 Nobel gas (abbrev.)
7 Pretoria is its executive capital (Iso code)
8 Measure of printing resolution
13 Greek dawn goddess
14 Important nerve
15 Measures light intensity
16 Australia's biggest city
18 New Zealand parrot
20 Number of players forming a football team
24 Denim trousers
25 Drink before dinner
29 Gold in French
31 Retro photo filter
32 Part of the Christian bible (abbrev.)
33 Existing, not false
34 Female given name
37 Open source or operating system (abbrev,)
41 U.S. space agency
43 Brass instrument
44 Lovable alien
45 Mountainous region in Morocco
47 1,000 amperes (abbrev.)
49 Prefix meaning two
51 1,000 of these form a metre
52 Prefix for Tips, Motion, Services, and more

Solution hint for prize competition of BioNews No. 50:

				N							N				L					S			B	

Send us the solution until **June 30, 2019**, via e-mail to **bionews@eppendorf.de**, or participate online at **www.eppendorf.com/bn-service**