BIO NEWS
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Anniversary Edition: 25 Years of Eppendorf BioNews

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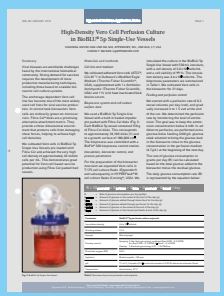
> Centrifuges: Are You Taking Good Care of Your Lab Workhorse?
> Our Technology, Your Success: Customized Cooperation

Application Notes
High-Density Vero Cell Perfusion Culture in BioBLU® 5p Single-Use Vessels · Ultimate PCR Optimization with the Eppendorf Mastercycler® X50 2D-Gradient · etc.



Dear Readers,

What do polymerase chain reaction, the Hollywood movie Jurassic Park®, and Eppendorf BioNews have in common? Well – to put it casually – they each have 25 years under their belt. Hard to believe! Those of you who may be of that age, and who take a little time to reflect on this past quarter-century, may remember quite a bit. Personal and professional successes (and failures), societal and political upheaval as well as quantum leaps in media and technology have accompanied us over the years, and they have challenged and shaped us. Not to mention the breakneck speed of scientific progress! We are proud of our silver anniversary and of your continuous support, and we are highly motivated to continue to provide you with regular insights and updates from the world of Eppendorf.

This issue is ready for you, brimming with reports and Application Notes on a variety of topics, including high-density Vero cell perfusion culture (Application Notes 1–2), PCR optimization using the innovative 2D-Gradient technology of the Eppendorf Mastercycler® X50 (Application Notes 3–4), automated KAPA® HyperPlus DNA library preparation for Illumina® sequencing (Applications 5–6) as well as precise and accurate whole blood dispensing (Application Notes 7–8).

Learn more about stem cell bioprocesses (page 8) or about how you can secure a long life for your centrifuge (page 9). In addition, we are presenting you with a number of new products that will optimize and simplify your daily laboratory routine!

Last, but not least: Our anniversary prize competition offers a total of five "personalized" pipettes with laser engraving. We'll keep our fingers crossed for you!

Enjoy the read!

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. We look forward to your feedback!

Imprint

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Eppendorf BioNews Application Notes

High-Density Vero Cell Perfusion Culture in BioBLU® 5p Single-Use Vessels

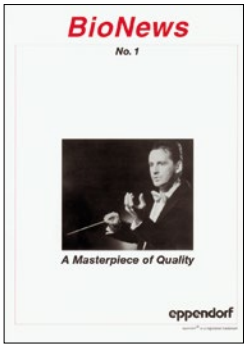
BERRIT HOFF, EPPENDORF AG

25 Years of BioNews, 25 Years of News from the Eppendorf World

The year 1993 was a memorable year. Kary Mullis received a Nobel Prize for the invention of the polymerase chain reaction (PCR) he had made a few years earlier. Steven Spielberg's Jurassic Park® fueled fantasies about the revival of extinct animals such as dinosaurs using PCR and cloning ("Oh my God!"). And Eppendorf – admittedly a little less spectacular – published the first issue of its new customer magazine BioNews.

PCR: scientific milestone and catalyst for product ideas

PCR is a biological technique for amplifying DNA that has revolutionized molecular biology since its invention in 1985. Eppendorf was quick to spot its potential and developed a whole series of matching products for PCR labs. The first generation of Mastercycler® thermocyclers already allowed for short reproducible PCR cycles. Innovative liquid handling instruments and devices for PCR sample preparation and storage facilitated the PCR routine, while laboratory consumables in Eppendorf Biopur® quality met the highest purity requirements to prevent contamination.



BioNews was introduced to present these system solutions and new products in an objective and applicative context. At the time, it was merely more than an exciting challenge in DIN A5 format with unknown future of which no one thought it would once become a customer magazine that is, after 25 years, still regarded by readers as a highly popular source of information.

From "cave art" to digital workflow

Lab methods and techniques have seen a significant development over the past years and so has the production process of BioNews. Compared with the situation today, the way BioNews was produced in the first years after its introduction resembles rather "cave art". Text manuscripts were exchanged using floppy disks with a storage capacity of 1.44 MB. Image data? Far from it! Valuable original diapositives were scanned and digitally edited in a laborious process.

From the end of the nineties, e-mails were used increasingly and, finally, exclusively for internal and external communication. Digitally created image data replaced scanning of diapositives and the now well-established PDF file format allowed exchanging layouts per e-mail and viewing and implementing corrections on the screen. What a simplification!

New technologies, new workflows, new BioNews

CRISPR-Cas9 is currently the "hottest" technology for genome editing and represents the greatest revolution in biology since the invention of PCR. For this method, too, users can rely on Eppendorf for suitable equipment, accessories, and application support in the usual premium quality. Therefore: As long as life sciences develop or enhance exciting methods, Eppendorf will advance, too, in order to meet customer needs. Hence, we will have a lot to report on.

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BHAVIN KOTAK, EPPENDORF AG

DNA – From Discovery to Today's Applications

As scientific research advances, there is an increased requirement to reliably gain access to regions of DNA with more complex sequences. It is as important to address this high complexity as it is to address the variation between different PCR primers. Eppendorf has developed the Mastercycler® X50 family with innovative 2D-Gradient technology that combines the optimization of annealing and denaturation temperature – all in a single run. This is the next milestone in PCR optimization.

DNA – fascinating from the start

For nearly 150 years, DNA has fascinated humankind from its very first observation as a component of the cell nucleus to today's advanced understanding of genetics and how it plays a role in every characteristic. The idea of something inherited from generation to generation has been in existence for a long time. Charles Darwin put forward the theory of evolution occurring by the process of natural selection in his book "The Origin of Species" which was published in 1859. Gregor Mendel, the "father of genetics", shed light on the way in which characteristics are passed from generation to generation by interpreting pea breeding experiments. His ideas of inheriting factors were so far ahead of time that it took more than 30 years for his research to be accepted by the scientific community.

It was in the early 20th century when the three plant researchers Hugo De Vries, Carl Erich Correns, and Erich von Tschermark independently verified Mendel's theory. At the same time, Sir Archibald Edward Garrod was the first, who proved the generality of these plant breeding-based findings by associating them with a human disease. It became clear that the applications derived from the understanding of inheritance were going to reach far and wide.

By the 1940's the knowledge of genetics was refined: Mendel's inheritance factors were named "Genes".

In the meantime, it had become clear that genes are the blueprint of proteins. However, nobody had confirmed what this wonderful molecule looked like. Rosalind Franklin produced two photographs of DNA fibers using X-ray diffraction in 1952. Less than a year later, Watson and Crick discovered the double helix structure of DNA and were awarded the prestigious Noble Prize in 1962.

Kary Mullis invented the technique "PCR" (Polymerase Chain Reaction) in 1983 to allow the amplification of specific DNA sequences. PCR became a central technique in years to come. For the PCR breakthrough, Mullis was awarded the Noble Prize in 1993.

PCR: an indispensable tool of molecular biology

PCR facilitates scientists by amplifying specific regions of DNA for subsequent processing, such as sequencing or cloning. Besides the quality of template molecules, the reaction is highly dependent on the parameters of the reaction. There is no universal recipe: Each PCR needs to be optimized for the target DNA to be amplified and the specific primers used in the reaction. This is to ensure the primers not only bind to the DNA – resulting in amplification, but bind in the right position – ensuring "specific amplification". Gradient technology addresses the need to optimize the reaction when using different primers or multiple primers. It allows the empirical determination of the optimum annealing temperature, often in a single run. The gradient technology developed by Eppendorf for PCR optimization has been used by a number of cycler manufacturers. It was developed to address the difficulties with manual optimization of the annealing temperature, usually carried out by multiple PCR runs with varying conditions (see also Fig. 1).

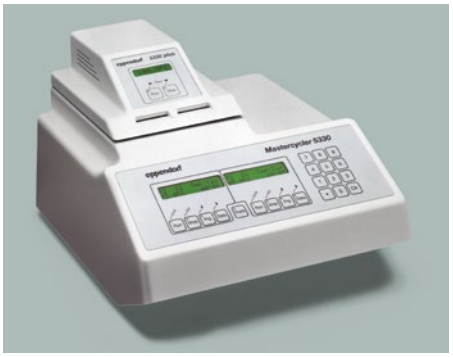


Fig. 1: Mastercycler 5330, one of the first Eppendorf thermal cyclers with gradient technology (1997)

Eppendorf is seeing this trend emerge amongst scientists once again, but this time in relation to the complexities within the template DNA.

How things have changed

In the 1980's, the first use of DNA in a criminal case was noted – at this point the status quo of knowledge and technology only allowed to differentiate very basic and specific repeat sequences. The translated characteristics were unimportant for this purpose, differentiation was the focus.

Fig. 2: Mastercycler X50 with 2D-Gradient technology: the next stage of PCR optimization

In contrast, medical research, plant science, screening of diseases, food science, and other recent scientific focus areas have advanced greatly. There is now a need to understand the impact of the differences that originate within the sequence of DNA. This is a paradigm shift in the requirements of the scientists. It is now vitally important to ensure access to the target region of DNA, irrespective of complexities within the sequence, to enable specific amplification resulting in high yield for subsequent applications, such as Next Generation Sequencing.

Fig. 3: Eppendorf's new 2D-Gradient allows optimization of the annealing and the denaturation temperature in a single run – taking less time than ever before.

As our knowledge of DNA has advanced, so have the complexities of the target regions we are interested in. The consequences of not addressing complex structures can result in little to no amplification. While GC-rich is the simplest of complexities, many more complexities have been identified. These may require more energy, meaning an increased denaturation temperature, to ensure access to the target region. However, consideration needs to be given to the enzymes, because an increased denaturation temperature addressing the DNA also results in an increased rate of polymerase denaturation. After all, it's in the same reaction.

Today, scientists optimize the denaturation temperature in a familiar manual way: by carrying out multiple experiments under varying conditions and comparing the results to find the best parameters. This can now be addressed in a single PCR run. Scientists can today optimize the denaturation and annealing temperature in a single reaction, using the new 2D-Gradient functionality on the Eppendorf Mastercycler X50 family (Fig. 2 and 3). For the first time, scientists don't need to choose between yield or specificity, they can have both – in a single run.

Tip: Please also read the Application Note 3–4 "Ultimate PCR Optimization with the Eppendorf Mastercycler® X50 2D-Gradient" in this issue.

More info at
www.eppendorf.com/mastercycler

Scan the QR code for more information!

News

New: Innova® S44i

Innova S44i biological shaker from Eppendorf is designed for laboratories that will settle for nothing but the best. This innovative new shaker is equipped with maximal platform capacity at a small footprint. Sample capacity can be further increased without sacrificing lab space by stacking up to three shakers.

The proprietary technology of Eppendorf X-Drive allows reproducible shaking of both uneven and heavy loads without compromising performance or longevity of the shaker.

The slide-out platform of the Innova S44i enables easy access to all samples on the platform. Comprehensive multi-step programming is available for automated control of the shaker to save time. Built-in user management allows controlled access, operation, and traceability required in regulated laboratories. This new shaker can be integrated into a central monitoring and data management software using the VisioNize® system from Eppendorf (see page 7). The versatile Innova S44i comes in six different formats and is suitable for culturing microbial and phototrophic organisms.

More information at
www.eppendorf.com/InnovaS44i

Scan the QR code for more information!

TANJA MUSIOL, EPPENDORF AG

Digital Transformation in the Laboratory

Further to digital documentation of experimental details and analysis data, recording and archiving of instrument parameters are becoming increasingly important. It is not only essential to document whether an instrument is subject to regular maintenance. Seamless documentation of instrument performance is nowadays a typical requirement of quality control in the laboratory.

What are the benefits of connected devices in a laboratory?

Remote monitoring of instruments always makes sense since sample material may be sensitive to altered parameters, particularly during storage and incubation. In an IoT* environment, for example, the instrument itself will be able to communicate a deviation. This approach frees the researcher from cumbersome regular monitoring on site, thus saving both time and money. Furthermore, status information enables more efficient planning of workflows and instrument utilization.

*Internet of Things

How do manufacturers such as Eppendorf approach this topic?

The vision of the connected laboratory, with centralized data collection and secure access to the data, is presenting manufacturers of laboratory instruments with entirely new challenges. As a first step towards a digital laboratory, Eppendorf introduced the VisioNize® system for device networking to the market in 2017. This system, which comprises software and hardware components, is currently subject to testing within the everyday lab routine in collaboration with reference customers. The goal is to adapt the functions as well as the scope as closely as possible to the actual requirements of daily laboratory routine.

Modern remote monitoring of instrument parameters using VisioNize

Digital laboratory organization using VisioNize

The VisioNize system offers a modern digital option for remote monitoring of connected devices. In addition, a centralized information and documentation database with user management is available for clear, digital laboratory organization. Further to the organization of current status information and maintenance intervals, the system enables organization of certificate data, locations, documents, and contacts.

VisioNize

In short: The new VisioNize system by Eppendorf will network your laboratory equipment while safely and securely organizing your instruments and instrument data.

More detailed information on the VisioNize system is available at www.eppendorf.com/visionize.

Tip

Time to Have a Good Read

Hot off the press: the new Off the Bench

It is common knowledge that all good things come in threes – on this note, the third issue of the LifeScienceStyle magazine "Off the Bench" has recently arrived on the doorsteps of our customers and colleagues. Another issue with a modern design and carefully-composed contents which addresses interesting and exciting real life stories involving science. For example, in our title story, scientist Madeline Lancaster recounts why she is burning for her research on "mini brains", and how she is planning to unlock the secrets of our brains. Personal, surprising, inspiring.

Such is the tenor of our columns within the LifeScienceStyle magazine, conceptualized with journalistic and scientific depth. Topics such as "bee mortality" and "super plants" belong to the realms of "inspiring science" and "exploring life". Additionally, every issue takes you on an excursion to a new cosmopolitan city – including tips on things to do and places to eat. Furthermore, Eppendorf provides concise information on innovations and new products and services, as well as on current company news. As always, expert tips for daily work in the laboratory are part of the package.

The good news: Three "Off the Bench" issues will not be all; in 2018 we will again publish two new issues. If you never want to miss another issue, please send a short message with your contact information to magazine@eppendorf.com.

ULRIKE BECKEN, EPPENDORF AG BIOPROCESS CENTER, JUELICH, GERMANY

Controlled Stem Cell Cultivation

Stem cell-based technologies lay the basis for pioneering approaches in regenerative medicine, drug screening, and toxicology testing. The development of stem cell bioprocesses for e.g. drug discovery has to meet partly different requirements than stem cell culture for basic research, because industrial applications need much larger cell quantities.

Scale-up challenges

For industrial applications, conventional two-dimensional cell culture systems reach their limits. An important part of research and development in the stem cell field today is the design of scale-up strategies. This comprises the identification of scalable culture systems and suitable growth surfaces, as well as the development of robust cell differentiation protocols and concepts for downstream processing.

Many laboratories trust Eppendorf as an expert partner for upstream stem cell process development. Researchers successfully use scalable Eppendorf BioBLU® c Single-Use Vessels to cultivate anchorage-dependent stem cells on microcarriers or as cell aggregates.

Furthermore, with the Fibra-Cel® disks (see box on the right), Eppendorf offers a promising alternative to conventional growth surfaces for mammalian cells.

The Eppendorf bioprocess control systems allow close monitoring and control of stem cell cultures in BioBLU single-use, as well as conventional glass vessels.

Meeting stem cell experts

Eppendorf fosters close relationships with researchers from the stem cell field all over the globe to fully understand their needs. To support scientific exchange, Eppendorf brought together experts from industry and academia at the 1st Stem Cell Community Day in April 2017 to discuss recent achievements, challenges, and opportunities in stem cell bioprocessing for research and industrial manufacturing. We will continue the exchange of experiences at the next Stem Cell Day, taking place in Dusseldorf, Germany, on April 24, 2018.

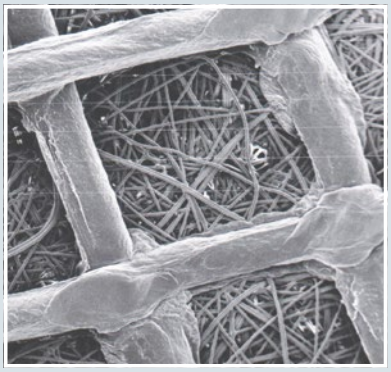
We are looking forward to meeting you there! For more information as well as for a review of the 1st Stem Cell Community Day, please visit www.stemcellday.de.

Close-Up

Growth Matrix for Cell Culture Bioprocesses

Fibra-Cel® disks are a 3D growth matrix for the cultivation of suspension and adherent cells in bioreactors. They are made of polyester and polypropylene and are electrostatically pre-treated to facilitate cell adherence and cell trapping in the fiber system.

Fibra-Cel offers many advantages. The matrix protects cells from damaging shear forces caused by the turbulence of impeller rotation and sparge gas. The disks have a high surface to volume ratio, which increases the biomass that can be maintained in the bioreactor. Because the cells are entrapped in the matrix, culture medium and secreted products can be easily separated from the cells without need for filtration. Like this, Fibra-Cel is ideally suited for perfusion cultures.



The Fibra-Cel disks combined with the Eppendorf basket impeller make your autoclavable or SIP bioreactor ready for a packed-bed process. The BioBLU® 5p Single-Use Vessel is pre-loaded with Fibra-Cel disks: for single-use simplicity.

For use in GMP production, the disks' materials are USP Class VI-certified.

Find more information at www.eppendorf.com/Fibra-Cel.

High-Density Vero Cell Perfusion Culture in BioBLU® 5p Single-Use Vessels

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Summary

Viral diseases are worldwide challenges faced by the international biomedical community. Strong demand for vaccines requires the development of more productive manufacturing techniques, including those based on scalable bioreactor cell culture systems.

The anchorage-dependent Vero cell line has become one of the most widely used cell lines for viral vaccine production. In stirred-tank bioreactors Vero cells are ordinarily grown on microcarriers. Fibra-Cel® disks are a promising alternative attachment matrix. They provide a three-dimensional environment that protects cells from damaging shear forces, helping to achieve high cell densities.

We cultivated Vero cells in BioBLU 5p Single-Use Vessels pre-loaded with Fibra-Cel and achieved the very high cell density of approximately 43 million cells per mL. This demonstrates great potential for Vero cell-based vaccine production using Fibra-Cel packed-bed vessels.

Fig. 1: BioBLU 5p Single-Use Vessel

Materials and methods

Cell line and medium

We cultivated adherent Vero cells (ATCC®, CCL-81™) in Dulbecco's Modified Eagle Medium (Thermo Fisher Scientific®, USA), supplemented with 1 x Antibiotic-Antimycotic (Thermo Fisher Scientific, USA) and 1 % (v/v) heat-inactivated fetal bovine serum.

Bioprocess system and cell culture surface area

We used a BioBLU 5p Single-Use Vessel with a built-in basket impeller pre-packed with Fibra-Cel disks (Fig. 1). Each BioBLU 5p vessel contained 150 g of Fibra-Cel disks. This corresponds to approximately 34,100 disks [1] and to a growth surface of 180,000 cm^2. The bioprocess was controlled with a BioFlo® 320 bioprocess control station.

Inoculation, bioreactor control, and process parameters

For the preparation of the bioreactor inoculum we expanded Vero cells in T-175 cell culture flasks (Eppendorf) and subsequently in HYPERFlask® M cell culture flasks (Corning®, USA). We inoculated the culture in the BioBLU 5p Single-Use Vessel with 530 mL inoculum, with a cell density of 3.0×10^6 cells/mL and a cell viability of 99 %. The inoculation density was 4.6×10^5 cells/mL. The bioprocess parameters are summarized in Table 1. We cultivated Vero cells in the bioreactor for 21 days.

Feeding and perfusion control

We started with a perfusion rate of 0.2 vessel volumes per day (vvd), and gradually increased it to 1.5 vvd at the end of the run. We determined the perfusion rate by monitoring the level of ammonium. The goal was to keep the ammonium concentration below 4 mM. In addition to perfusion, we performed extra glucose bolus feeding (200 g/L glucose stock solution) to bring the glucose level in the bioreactor close to the glucose concentration in the perfusion medium (4.7 g/L) at the beginning of the next day.

The rate of glucose consumption in grams per day (R) can be calculated based on the total glucose added to the bioreactor minus the residual glucose.

The daily glucose consumption rate (R) is represented by the equation below:

$$R = (G_{vessel\text{-}start} + G_{perfusion} + G_{bolus} - G_{vessel\text{-}end} - G_{harvest}) / day$$

R	=	Rate of glucose consumption per day (g/day)
$G_{vessel\text{-}start}$	=	Amount of glucose in the vessel at the start of the day (g)
G_{bolus}	=	Amount of glucose added through bolus feed for the day (g)
$G_{perfusion}$	=	Amount of glucose added through perfusion for the day (g)
$G_{vessel\text{-}end}$	=	Amount of glucose in the vessel at the end of the day (g)
$G_{harvest}$	=	Amount of glucose in the harvest perfusate at end of the day (g)

Parameter	BioBLU® 5p perfusion culture setpoints
Inoculation density	4.6×10^5 cells/mL
Working volume	3.5 L
Sparger	Macrosparger
Gassing control	Sparging: 3-Gas Auto gas mixing, combined flow 0.002 – 0.5 SLPM; Air flow 0.002 – 0.2 SLPM; O_2 flow 0 – 0.5 SLPM Overlay: 3-Gas Auto gas mixing, 0.01 – 1 SLPM flow
Dissolved oxygen (DO)	50 %
Agitation	Basket impeller; 100 rpm
pH	7.1 ± 0.1; Cascade of CO_2 (acid) and cascade of 0.45 M sodium bicarbonate (base)
Temperature	Heat blanket; 37 °C

Table 1: Overview of process parameters and setpoints

High-Density Vero Cell Perfusion Culture in BioBLU® 5p Single-Use Vessels

Crystal violet nucleus counting assay

After the completion of the cell culture process, we cut the vessel open below the head plate and collected three samples of Fibra-Cel disks from two different locations in the basket. We extracted the cell nuclei from the disks, stained them using a Crystal violet dye nucleus count kit (Chemglass® Life Science, USA, CLS-1332-01), and counted the cell nuclei using a Vi-CELL® XR Cell Viability Analyzer (Beckman Coulter®, USA).

The default cell type setting was used for cell counting [1].

Results

We started the perfusion on day 3, because the glucose level became low and the concentration of lactate and ammonium increased. The metabolic profiles of Vero cells in the bioreactor are shown in Fig. 2. For the glucose line, the spikes indicated that a bolus feeding with glucose was performed daily from day 4.

Because the cells adhered to the Fibra-Cel disks, we could not directly measure the cell density during the culture. As an indirect measure of cell proliferation, we tracked the daily glucose consumption rate of the culture. For the first four days, the daily glucose consumption rates were around 2.7 g/day, indicating that cells were in lag phase. From day 5, the glucose consumption rate started to increase to 4.4 g/day, and even doubled to 9.7 g/day on day 6, indicating that cell proliferation had entered the log phase. The glucose consumption rate doubled again to 19.4 g/day on day 17. On the last day of the run, the glucose consumption rate reached 22.4 g/day. In total, the cells consumed 273 g glucose during the 21 days.

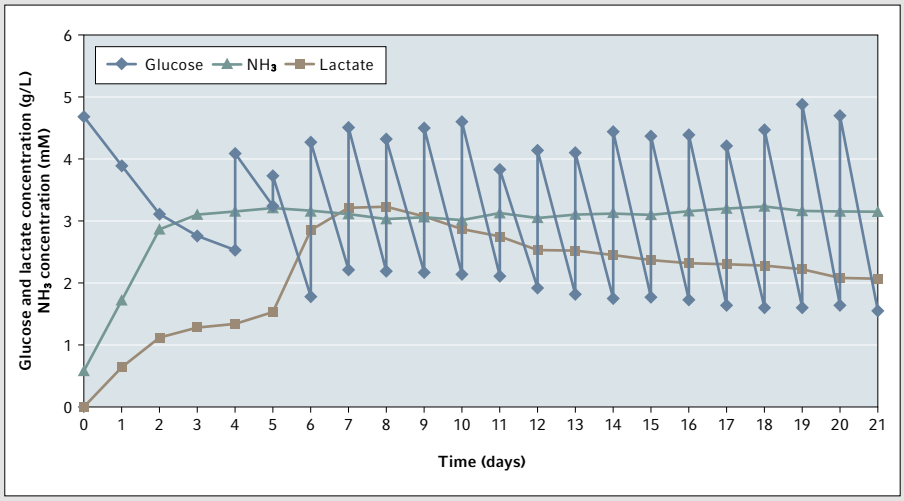

Fig. 2: Metabolic profile of Vero cells perfusion culture. Glucose, ammonium, and lactate concentrations were measured with a Cedex® Bio Analyzer (Roche Diagnostics® GmbH, Germany).

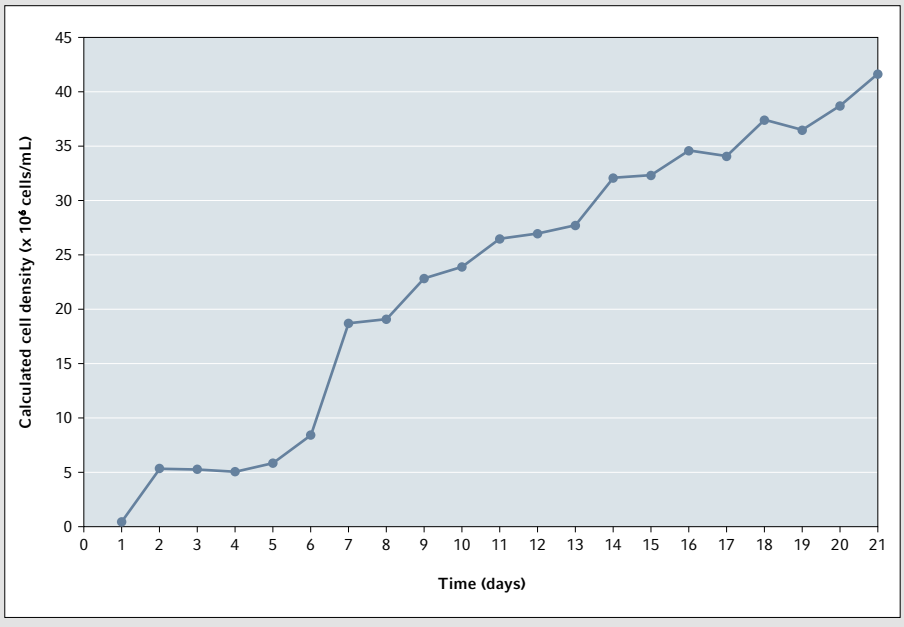

Fig. 3: Calculated Vero cell proliferation curve

After extraction of the Vero cell nuclei from the Fibra-Cel disks, we counted* the nuclei in the samples. The final cell density at the end of the culture was 4.31×10^7 cells/mL.

Based on the cell density at the end of the culture and the glucose consumption rate on the last day, we obtained the glucose-consumption-to-cell-density-conversion ratio at the end of the culture. Assuming that this conversion ratio remained relatively unchanged over the duration of the culture, we converted the daily glucose consumption rate into daily cell density (Fig. 3).

Conclusion

In an Eppendorf BioBLU 5p Single-Use Vessel pre-packed with Fibra-Cel disks we achieved a high Vero cell density of approximately 43 million cells per mL, demonstrating high potential for Vero cell-based vaccine production.

**The nuclei counting method was suggested by Mr. Zhenggang Xie of Eppendorf China.*

Literature

[1] Han K und Sha M. High-Density Vero Cell Perfusion Culture in BioBLU® 5p Single-Use Vessels. Eppendorf Application Note 359. 2017

The complete version of this Application Note can be downloaded in PDF format at www.eppendorf.com/appnote359 or using the QR-code.

Scan the QR code for more information!

Ultimate PCR Optimization with the Eppendorf Mastercycler® X50 2D-Gradient

ARORA PHANG AND TIM SCHOMMARTZ, EPPENDORF AG, HAMBURG, GERMANY

Abstract

The new Eppendorf Mastercycler X50 (Fig. 1) is the only thermal cycler in the market equipped with the innovative 2D-Gradient function. PCR optimization, typically of the annealing temperature using a gradient function, is an established technique. Optimization of the denaturation temperature is less commonly done and typically limited to applications dealing with complex or GC-rich DNA templates.

This is mainly due to the high amount of effort required to obtain useful optimal results from the combination of denaturation and annealing conditions. The 2D-Gradient function allows optimization of both denaturation and annealing temperatures in just one PCR run. This provides users with a great amount of information in the least amount of time and effort, thus greatly shortening the scientific research process.

Introduction

The gradient function is a powerful innovation that targets solving the difficulty in determining the primer-dependent annealing temperature of a PCR system. Thermal cyclers with gradient function can simultaneously provide multiple different temperatures at a certain step, reducing time and effort needed in optimizing the annealing temperature of a primer. As most DNA will be completely denatured at 95 °C, denaturation temperatures generally deviate only slightly from the temperature specified by the manufacturer, and most enzymes have a maximum temperature tolerance around that temperature. However, while not as variable as primers, each DNA template has its own characteristic and hence a certain degree of variation is unavoidable. Thus, while PCR might be successful without optimizing the denaturation step, the quality and yield of the PCR might not be optimal.

To date, it is possible to optimize the denaturation and annealing steps of a PCR system by doing two separate runs. To find the best combination of optimal denaturation and annealing temperatures, one would have to first run a gradient for the annealing temperature. Subsequently, for each of the annealing temperatures tested, a gradient is then repeated for the denaturation step. This would result in multiple PCR runs, which is both time- and resource-consuming.

The new innovative technique called 2D-Gradient allows for ultimate PCR optimization (Fig. 2) with utmost ease and speed.

Materials and methods

PCR reactions were performed on human ß-actin gene using PCRBio Taq DNA polymerase (PCR Biosystems Ltd., UK) and Human Genomic DNA (Roche®) in skirted Eppendorf twin.tec® PCR plates sealed with adhesive PCR film in the Mastercycler X50s. Dispensing was carried out by an Eppendorf ep*Motion*® 5073.

Results and discussion

The 2D-Gradient function enables optimization of both the denaturation and annealing temperatures in one PCR run through a matrix-style temperature set-up. The first gradient at the denaturation step is set vertically while the second gradient at the annealing step is set horizontally. This means that for each denaturation temperature (T_D), 12 samples would be amplified at that temperature: e.g. wells A1–A12 would be subjected to 99 °C T_D while B1–B12 would be subjected to 98.5 °C T_D.

After the denaturation step, samples in the same column would be subjected to the same annealing temperature (T_A), thus giving rise to 12 different T_A across the block: e.g. A1–H1 would be subjected to 51.9 °C T_A and A2–H2 would be subjected to 52.3 °C T_A. At the end of the completed PCR, the best combination of denaturation + annealing temperatures can then be determined.

Fig. 1: New Mastercycler X50

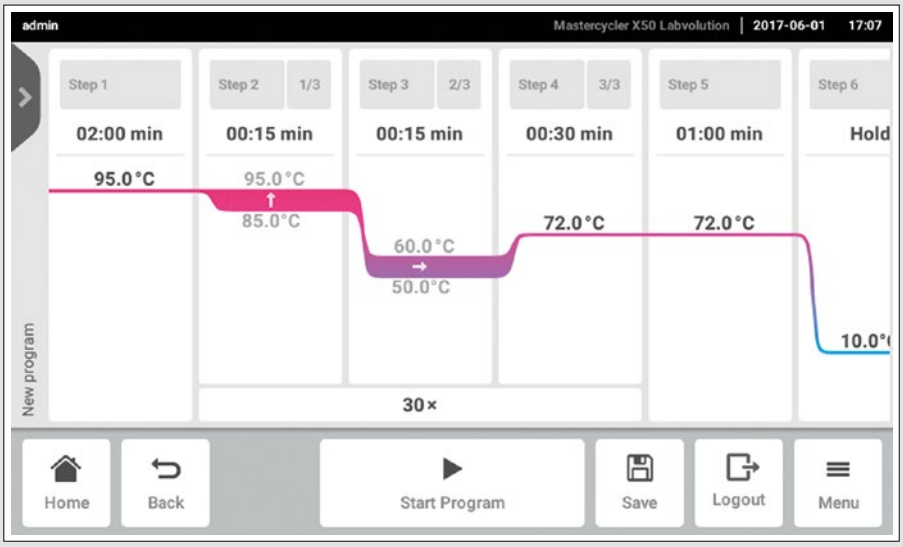

Fig. 2: Mastercycler X50 touchscreen interface showing PCR program with 2D-Gradient function

Ultimate PCR Optimization with the Eppendorf Mastercycler® X50 2D-Gradient

An optimal PCR result is defined by maximum yield of the specific amplicon of interest. While this can be primarily achieved through optimizing the annealing temperature, there is no guarantee that the result obtained is the true "optimal" result. It is always possible that the yield could be increased or the amount of non-specific product be reduced.

Fig. 3 shows the result of the matrix-style optimization technique of the 2D-Gradient in amplifying the human ß-actin gene. Specific amplification will yield 484 bp fragments while sub-optimal condition will give rise to non-specific amplification visible as a 350 bp artefact in the gel. Ordinarily, gradient optimization is only performed for the annealing step at a fixed denaturation temperature at ca. 95 °C.

Taking the example from Fig. 3, when 95.6 °C is used, the gradient result for the annealing step shows that 65.9 °C gives the best yield with a small amount of non-specific product, and at 70.5 °C only specific product will be obtained.

Depending on the objective of the PCR, both temperatures can be considered "optimal" conditions that are usually sufficient for most applications. However, in certain cases, such as low target copy number, a small difference in yield can be crucial to the application. In the example above, it can be clearly seen that 95.6 °C is not an optimal T_D for this PCR system. By lowering the T_D to 93.4 °C, the specific bands almost doubled in intensity. In addition, the results in this study showed that increasing T_D leads to decreasing non-specific amplification.

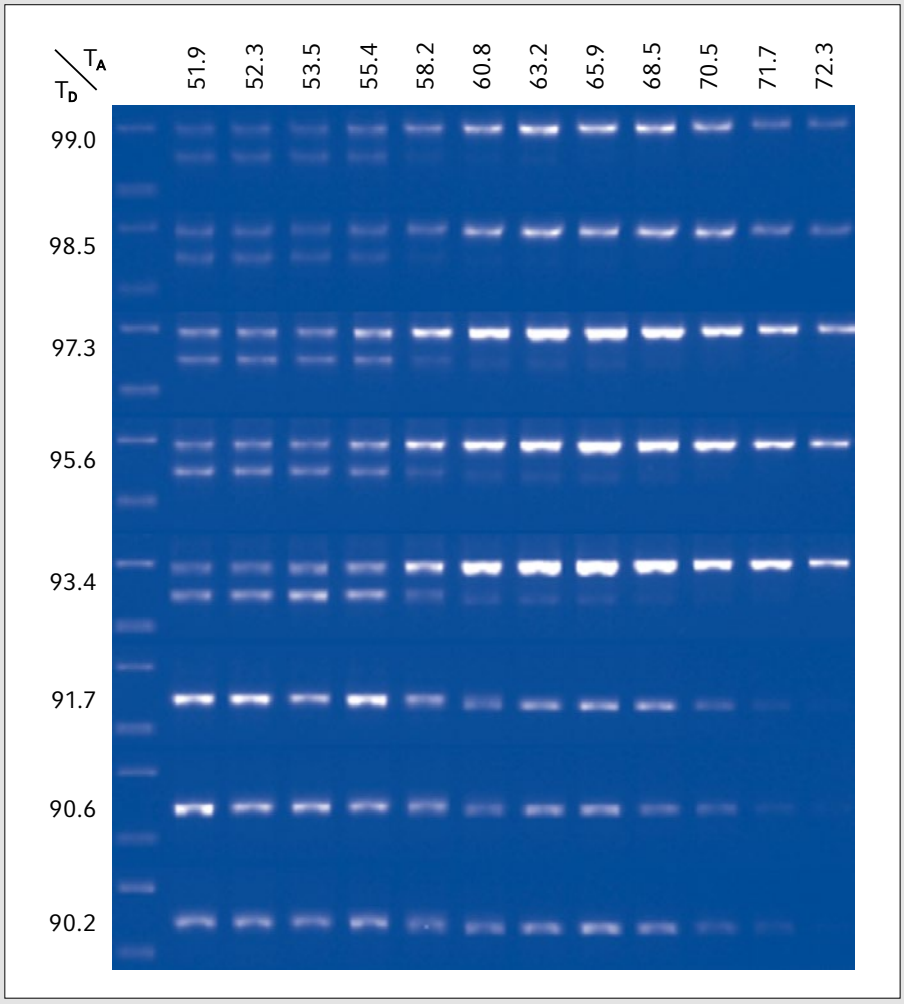

Fig. 3: PCR optimization of ß-actin gene using 2D-Gradient technique

For PCR systems where non-specific amplification is a problem, especially those with multiple bands, running a gradient at the denaturation step would be especially beneficial. Hence the 2D-Gradient allows users to easily obtain a rich amount of information about the characteristics of their PCR system, which in turn is beneficial for various application objectives such as increasing yield or resolving non-specific amplification problems.

Conclusion

The Mastercycler X50 2D-Gradient allows users to simultaneously optimize both denaturation and annealing temperatures to determine the conditions for combined optimal yield and specificity for best PCR results. This function allows users to save much time and effort in their optimization work, has important implications for applications relating to low target copy number and GC-rich targets and is useful for troubleshooting non-specific amplification issues.

The complete version of this Application Note can be downloaded in PDF format at www.eppendorf.com/appnote387 or using the QR-code.

Scan the QR code for more information!

Automated KAPA® HyperPlus DNA Library Preparation for Illumina® Sequencing on the Eppendorf ep*Motion*® 5075t

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ROCHE SEQUENCING & LIFE SCIENCE | KAPA BIOSYSTEMS, WILMINGTON, MA, USA
CHENG LIU, EPPENDORF AG, HAMBURG, GERMANY

Introduction

The KAPA HyperPlus Kit enables rapid construction of DNA libraries for Illumina sequencing. It is compatible with a wide range of sample types and input amounts (1 ng–1 µg), making it one of the most versatile kits on the market. The novel one-tube chemistry streamlines the DNA fragmentation and library construction processes.

This highly optimized protocol, including engineered enzymes, optimally formulated buffers, and minimal cleanup steps, results in efficient conversion of input DNA to adapter-ligated library, enabling deep and uniform sequence coverage.

The method developed on the Eppendorf ep*Motion* 5075t offers an automated solution for preparation of up to 48 samples, with the capability of scaling up to 96 samples per run. Modular programming gives the user flexibility to run a size selection step if desired, before or after PCR amplification. The system's user-friendly interface also guides the user through the run setup, including placement of the labware and required reagent volumes. An optical sensor verifies that all labware is correctly placed before the run starts. Moreover, ep*Motion*'s walk-away potential for generating PCR-free libraries is maximized by an on-deck Eppendorf ThermoMixer® that provides homogeneous reaction mixtures, a thermal module that enables on-deck incubation steps, and a gripper that transports plates to various deck positions. Workflows with PCR require the use of an off-deck thermocycler.

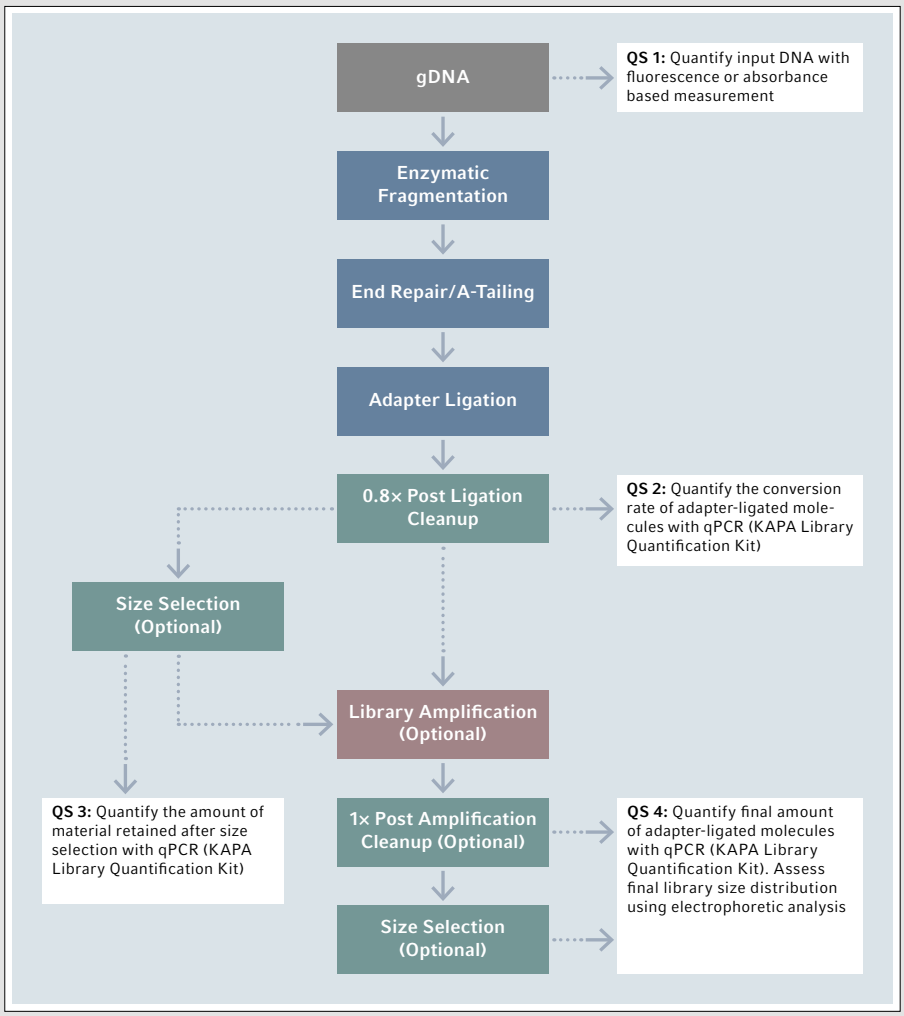


Fig. 1: KAPA HyperPlus workflow shown with optional QC checkpoints. The approximate total instrument time to run the 16 samples was 4 hours.

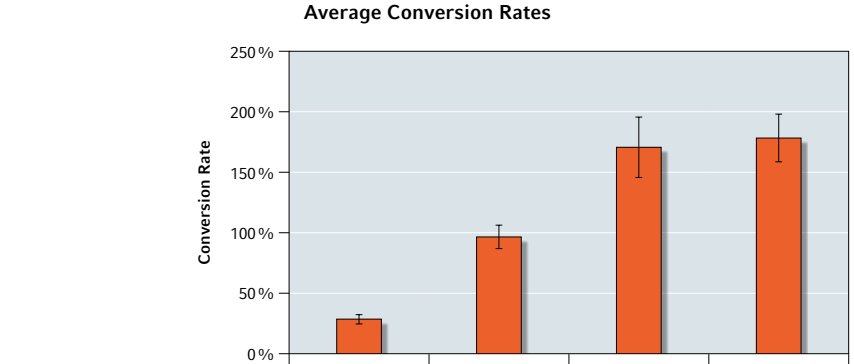
Materials and methods

Sixteen libraries were prepared from varying input amounts of *Escherichia coli* (*E.coli*) genomic DNA. All incubations were performed on-deck, except for the library amplification. Molecular biology grade mineral oil was used to prevent evaporation during the end repair and A-tailing incubation. Four replicates each of 1 ng, 10 ng, 50 ng, and 200 ng inputs were fragmented at 37 °C for 35 minutes. End repair and A-tailing was performed at 65 °C for 30 minutes.

The adapter concentrations were matched to the input amounts according to the kit specifications. The ligation reaction was performed at 20 °C for 15 minutes, followed by a 0.8× post-ligation cleanup and 0.6× – 0.8× size selection. The number of library amplification cycles was adjusted for each input amount to achieve final library yields between 100 ng and 1 µg. The method was completed with a final 1× post-amplification cleanup. Quality Control (QC) samples at several stages in the workflow were collected during the validation of the automated method. QC samples were recovered after the post-ligation cleanup, size selection, and the post-amplification cleanup as shown in Fig. 1.

Samples were quantified using the KAPA Library Quantification Kit (LQK). This quantification is a cost-effective alternative to sequencing that can indicate viability of the prepared libraries prior to downstream sequencing. Post-ligation and post-size selection QC samples were diluted 1:10,000 and post-amplification QC samples diluted 1:100,000. All qPCR samples and standards were run in triplicate on the Eppendorf Mastercycler® thermal cycling device.

Automated KAPA® HyperPlus DNA Library Preparation for Illumina® Sequencing on the Eppendorf ep*Motion*® 5075t



	1 ng	10 ng	50 ng	200 ng
Average of Conversion Rate	28.86 %	96.81 %	170.91 %	178.54 %
StdDev of Conversion Rate	4.11 %	9.97 %	25.24 %	19.99 %
Average of Post Ligation Yield	0.29 ng	9.68 ng	86.46 ng	357.09 ng
StdDev of Post Ligation Yield	0.04 ng	1.00 ng	12.62 ng	39.98 ng

Fig. 2: Post-ligation yields and conversion rates. All input amounts resulted in higher conversion rates than typically observed in manual experiments. Conversion rates above 100 % can be explained by highly efficient adapter ligation with little to no sample loss during the post-ligation cleanup. The addition of the ~140 bp adapters to the DNA fragments increases the overall molecular weight of the samples. If most of the DNA fragments were adapter-ligated and carried through the post-ligation cleanup, then the net yield can be higher than the initial input amount.

Final library size distributions were determined by running 1:5 dilutions of the post-amplification libraries on the Agilent® 2100 Bioanalyzer® with the High Sensitivity DNA Assay.

Results and discussion

The post-ligation qPCR results were used to calculate the percentage of starting material that was successfully adapter ligated, or the conversion rate as shown in Fig. 2.

Higher conversion rates are generally achieved with higher input amounts into library preparation, which results in libraries with greater complexity. With lower input amounts, a larger proportion of material may be lost to DNA adsorption to plastic surfaces, which can contribute to decreased conversion rates. For inputs above 100 ng, conversion rates typically range from 50–100 %.

For inputs between 10 ng and 100 ng, conversion rates range from 10–50 %. For inputs between 1 ng and 10 ng, conversion rates range from 5–20 %. Across all replicates, each input exceeded the expected conversion rates.

The high conversion rates indicate that the Eppendorf LoBind® quality consumables (PCR plates, tubes) may improve sample recovery yields throughout the library construction process.

The post-size selection qPCR results were used to calculate the percentage of material retained from the post-ligation cleanup. Typically, 5–20 % of material is retained from size selection. Across all inputs, the size selection retention fell within the expected range. The post-amplification qPCR results were used to calculate the amplification efficiency. Library amplification efficiency is typically ≥ 80 %, but can vary depending on the quality of sample and number of PCR cycles. With the chosen PCR cycling parameters, both the 1 ng and 10 ng input samples had an average amplification efficiency greater than 80 %. The 50 ng and 100 ng input samples had an average amplification efficiency of approximately 74 %, still being within the normal range.

The Agilent 2100 Bioanalyzer was used to determine the final library size distributions (data not shown). The 0.6×–0.8× size selection was expected to retain fragments between 250 bp and 450 bp. Across all inputs, the average final library sizes were between 305 bp and 330 bp, with narrow and reproducible size distributions. The absence of adapter-dimer (~140 bp) and large fragments (> 450 bp) shows the size selection process to be highly effective.

Conclusion

NGS library construction is a critical step in sample preparation for sequencing on Illumina platforms. The increased demand for high-quality NGS libraries in high-throughput laboratories has necessitated the development of robust, automated methods for library preparation. KAPA Biosystems®, an industry leader in NGS library preparation, has partnered with Eppendorf to automate the KAPA HyperPlus Kit on the Eppendorf ep*Motion* 5075t.

The experimental data shows that the automated method performs within the KAPA HyperPlus Kit specifications at a wide range of input amounts from 1 ng to 200 ng. The highly flexible and modular automated solution for NGS library preparation will allow laboratories to easily scale up experiments without sacrificing quality in the process.

The complete version of this Application Note can be downloaded in PDF format at www.eppendorf.com/appnote383 or using the QR-code.

Scan the QR code for more information!

Precise and Accurate Whole Blood Dispensing with Multipette® E3x (Repeater® E3x) and Combitips advanced®

VINCENT DUFEY AND MURIEL ART, EPPENDORF APPLICATION TECHNOLOGIES S.A., NAMUR, BELGIUM
HANAË HENKE, EPPENDORF AG, HAMBURG, GERMANY

Abstract

Dispensing of whole blood samples is challenging and needs careful consideration of the appropriate pipetting tool. We showed that using a positive displacement system, such as the Eppendorf Multipette E3x (U.S./CAN: Repeater E3x), is more suitable for this liquid than an electronic air-cushion pipette. Furthermore, the Multipette E3x and Combitips advanced system was compared to two competitor instruments showing that the only instrument staying in the supplier's specifications when dispensing whole blood was the Multipette E3x.

Introduction

Two different dispensing principles of pipettes exist: air-cushion and positive displacement [1]. In an air-cushion pipette, a dead air volume is present between the aspirated sample and the piston inside the pipette. In a positive displacement instrument, as the piston is integrated in the consumable, there is no compressible air-cushion between the piston and the test liquid.

The air-cushion can be significantly influenced by environmental parameters as well as by physical properties of the pipetted liquid [2].

In contrast, a positive displacement pipette is not affected by characteristics of the sample or laboratory conditions. Therefore, this option is ideal for dispensing all complex liquids having physical properties different from water, such as whole blood. This fluid is composed of plasma, cells, and a significant amount of proteins. All those components make blood denser and more viscous than water. As several applications require exact and reproducible blood pipetting, the appropriate dispensing system must be selected when blood is involved.

Results and discussion

Selection of the appropriate dispensing system

Human blood density depends on the proportion of its components. The density of the blood used in this study was experimentally determined. The value obtained was 1.058 kg/L, which is higher than the density of water (1 kg/L). Blood viscosity is also higher with 4.5 Pa/s on average, compared to the viscosity of water considered as 1 Pa/s.

To determine the most appropriate dispensing system to deliver an accurate and precise volume of human whole blood, an electronic air-cushion pipette (Eppendorf Xplorer® plus 1–10 mL) was compared to a positive displacement instrument (Multipette E3x). Pipetting and dispensing modes were evaluated for both systems.

Pipetting mode

Air-cushion pipettes, when used in pipetting mode, deliver smaller volumes of high density liquids as compared to aqueous solutions [2].

This theory is confirmed with whole blood. As shown in Fig. 1, the volume delivered by the Xplorer plus pipette is significantly less than expected. On the opposite, by using a positive displacement system, an accurate pipetting of blood is obtained with a systematic and random error value below the error limit (0.52 % and 0.26 %, respectively). As demonstrated by 10 values, the instrument precision is not affected by the liquid density. With random error values below 0.2 %, both instruments generated a random error lower than the acceptable error limit.

Dispensing mode

Data obtained for dispensing mode showed that the systematic error obtained with the air-cushion pipette (1.62 %) is significantly higher than the error obtained with the dispenser (0.39 %). In this case, the volume delivered by the air-cushion pipette is higher than expected (for full data, please see the complete version of this Application Note at www.eppendorf.com/appnote386).

Besides accurate blood dispensing, the positive displacement instrument also permits reproducible liquid delivery whereas the air-cushion system shows higher variation (Fig. 2).

By comparing both liquid handling systems, the results indicate that a positive displacement instrument is the right choice when accurate and precise dispensing of whole blood is required.

Selection of the most reliable dispenser

To determine the most reliable equipment for whole blood dispensing, the

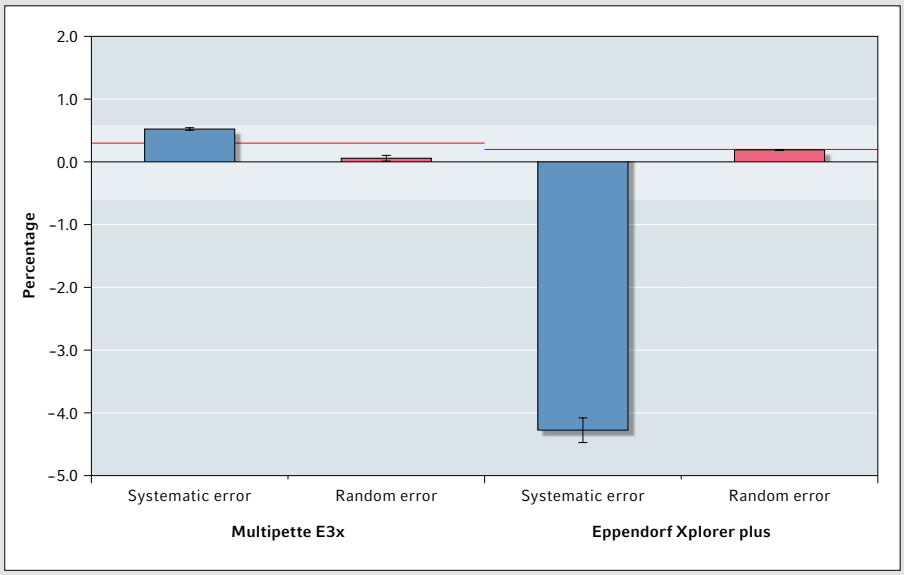


Fig. 1: Results of blood pipetting using Multipette E3x and the electronic air-cushion pipette Eppendorf Xplorer plus. Systematic error (blue) and random error (red) for 10 x 1 mL were compared to technical specifications (n=3).

Precise and Accurate Whole Blood Dispensing with Multipette® E3x (Repeater® E3x) and Combitips advanced®

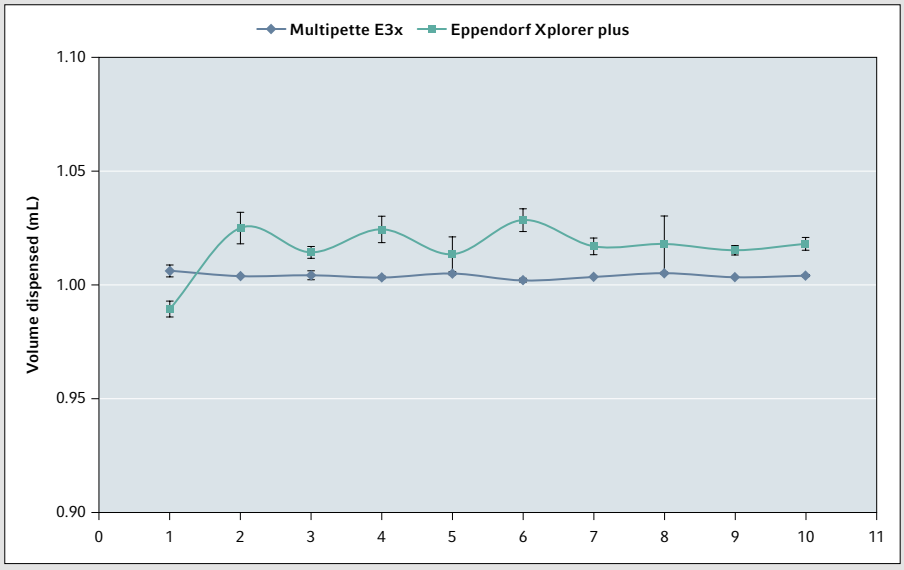


Fig. 2: Individual values of blood dispensing (10 x 1 mL) using Multipette E3x and an Eppendorf Xplorer plus air-cushion pipette (n=3).

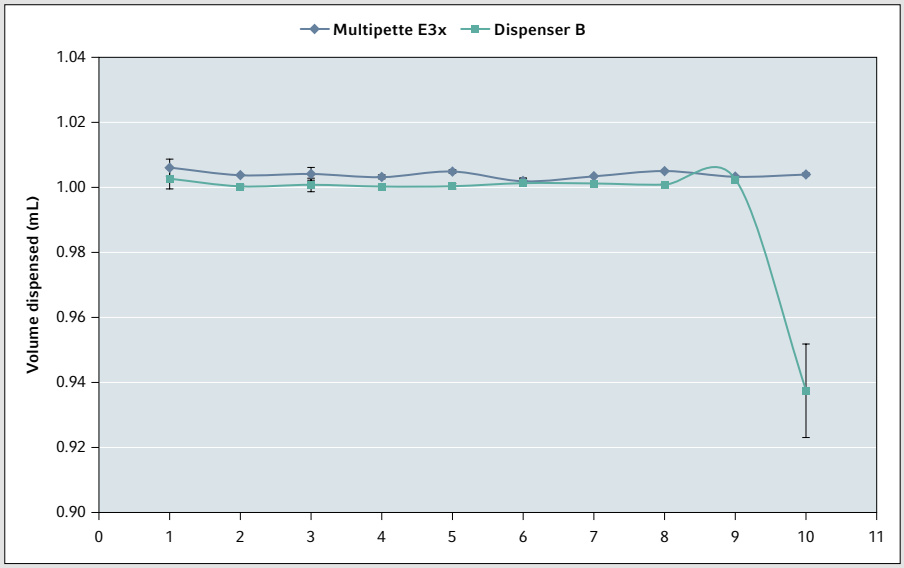


Fig. 3: Individual values of blood dispensing (10 x 1 mL) using Multipette E3x and dispenser B (n=3)

Eppendorf Multipette E3x was compared to two competitors: dispenser B and dispenser R. Each instrument was used with its own consumables and was tested in pipetting and dispensing mode.

Tested with water, dispenser R generated a systematic error above the upper limit defined by the supplier while the Eppendorf Multipette E3x and dispenser B delivered accuracies within the declared specifications, thus appearing to be the most reliable systems. Only these instruments were evaluated for whole blood handling.

When using dispenser B with its 10 mL tip for blood dispensing, air bubbles formed, reducing the liquid volume. The blood volume became insufficient to guarantee 10 successive reproducible dispensing steps, and as demonstrated in Fig. 3, the last volume dispensed was significantly lower than expected. Dispenser B's random error was significantly above the acceptable error limit defined by this supplier.

For an exact and reproducible blood dispensing, the Eppendorf Multipette E3x qualified as the best option. Only this instrument, combined with Combitips advanced, ensured repeated, reproducible blood dispensing in every tested case.

Conclusion

Positive displacement instruments, based on a direct contact between liquid and piston, are not affected by liquid properties and are the appropriate choice to handle whole blood. This was shown by comparing the Multipette E3x, a positive displacement dispenser, to a classic air-cushion pipette (Xplorer plus electronic pipette).

With an air-cushion pipette, used in pipetting mode as well as in dispensing mode, the systematic error increases significantly. On the opposite, Multipette E3x performances stay stable in any circumstances ensuring accurate and precise blood dispensing.

To determine the most reliable dispenser, the Multipette E3x was compared to two competitors: dispenser B and dispenser R. It was demonstrated that only the Eppendorf Multipette E3x, combined with Combitips advanced, reaches accuracy and precision specifications in every case.

The complete version of this Application Note can be downloaded in PDF format at www.eppendorf.com/appnote386 or using the QR-code.

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Literature

[1] Ewald K. Fundamentals of dispensing. Eppendorf Userguide No. 19, 2015. www.eppendorf.com

[2] Ewald K. Influence of physical parameters on the dispensed volume of air-cushion pipette. Eppendorf Userguide No. 21, 2015. www.eppendorf.com

Both documents can be be found at the Eppendorf homepage under Service & Support within our comprehensive Knowledge base.

NICOLE SEELIGMÜLLER, EPPENDORF AG

Centrifuges: Are You Taking Good Care of Your Lab Workhorse?

Cross your heart: Are you taking good care of your centrifuge? Like a car in day-to-day life, a centrifuge is one of the tools in the lab that we can't do without. It is a laboratory workhorse, and we take it for granted that it does its job whenever we need it – and it usually does. But even centrifuges can benefit from proper care to maintain reliable and safe performance. And exactly as with a car, a few points should be considered to prolong the lifetime of your centrifuge.

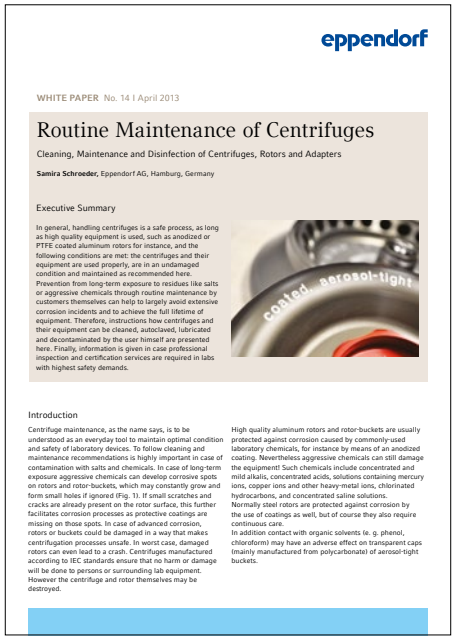
During a centrifugation run, high forces are generated. Since rotors, adapters, and samples are exposed to multiples of *g*, only centrifuges and accessories made of high-quality materials, such as those from Eppendorf, guarantee a secure usage and a long lifetime. Nonetheless, even the strongest material can get damaged, for example, after long-term exposure to residues of aggressive chemicals such as concentrated and mild alkalis, concentrated acids, solutions containing mercury ions, copper ions or other heavy-metal ions, chlorinated hydrocarbons, and concentrated saline solutions.

These chemicals can create weak spots in the material in the medium term which can lead to a failure of the equipment. Eppendorf centrifuges are manufactured and tested e.g. according to IEC standards and therefore ensure that no harm or damage will be done to persons or surrounding lab equipment in case of a material failure (e.g. a rotor crash). However, the centrifuge and the rotor may be destroyed, creating an unwanted financial burden for the lab.

How to learn more on centrifuge routine maintenance

Our White Paper No.14 "Routine Maintenance of Centrifuges" offers detailed instructions for the user, covering the correct cleaning of centrifuges, rotors, and adapters on a day to day basis, as well as decontamination techniques like autoclaving.

It includes recommendations regarding correct cleaning in case of a spillage with infectious/harmful substances as well as a list of recommended disinfection chemicals (internally tested with all relevant materials).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WHITE PAPER No. 14 I April 2013

Routine Maintenance of Centrifuges

Cleaning, Maintenance and Disinfection of Centrifuges, Rotors and Adapters

Samira Schroeder, Eppendorf AG, Hamburg, Germany

Executive Summary

In general, handling centrifuges is a safe process, as long as high quality equipment is used, such as anodized or PTFE coated aluminum rotors for instance, and the following conditions are met: the centrifuges and their equipment are used properly, are in an undamaged condition and maintained as recommended here. Prevention from long-term exposure to residues like salts or aggressive chemicals through routine maintenance by customers themselves can help to largely avoid extensive corrosion incidents and to achieve the full lifetime of equipment. Therefore, instructions how centrifuges and their equipment can be cleaned, autoclaved, lubricated and decontaminated by the user himself are presented here. Finally, information is given in case professional inspection and certification services are required in labs with highest safety demands.

Introduction

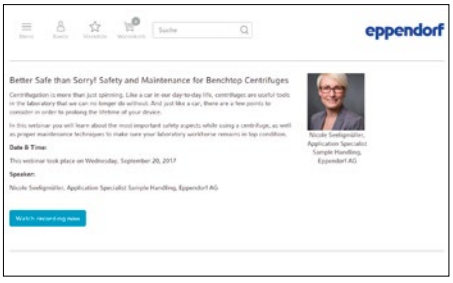
Centrifuge maintenance, as the name says, is to be understood as an everyday tool to maintain optimal condition and safety of laboratory devices. To follow cleaning and maintenance recommendations is highly important in case of contamination with salts and chemicals. In case of long-term exposure aggressive chemicals can develop corrosive spots on rotors and rotor-buckets, which may constantly grow and form small holes if ignored (Fig. 1). If small scratches and cracks are already present on the rotor surface, this further facilitates corrosion processes as protective coatings are missing on those spots. In case of advanced corrosion, rotors or buckets could be damaged in a way that makes centrifugation processes unsafe. In worst case, damaged rotors can even lead to a crash. Centrifuges manufactured according to IEC standards ensure that no harm or damage will be done to persons or surrounding lab equipment. However the centrifuge and rotor themselves may be destroyed.

High quality aluminum rotors and rotor-buckets are usually protected against corrosion caused by commonly-used laboratory chemicals, for instance by means of an anodized coating. Nevertheless aggressive chemicals can still damage the equipment! Such chemicals include concentrated and mild alkalis, concentrated acids, solutions containing mercury ions, copper ions and other heavy-metal ions, chlorinated hydrocarbons, and concentrated saline solutions. Normally steel rotors are protected against corrosion by the use of coatings as well, but of course they also require continuous care.
In addition contact with organic solvents (e. g. phenol, chloroform) may have an adverse effect on transparent caps (mainly manufactured from polycarbonate) of aerosol-tight buckets.

White Paper No. 014. Download at www.eppendorf.com/whitepaper14.

You can download the White Paper at www.eppendorf.com/whitepaper14.

Alternatively, you are invited to watch our Recorded Webinar "Better Safe than Sorry! Safety & Maintenance for Benchtop Centrifuges" at www.eppendorf.com/webinars.



Visit www.eppendorf.com/webinars to find the webinar „Better Safe than Sorry! Safety & Maintenance for Benchtop Centrifuges"

Whatever tool you choose, you'll be perfectly equipped with the knowledge needed to take good care of your centrifuge. If you keep your laboratory workhorse in a good condition, you will benefit from an extended lifetime. Promised!

TIP: On top, Eppendorf offers professional inspections and certification services for customers with highest lab safety demands. Find more information at www.eppendorf.com/epServices.

BERRIT HOFF, EPPENDORF AG

Our Technology, Your Success: Customized Cooperation

Eppendorf premium products are renowned for ease of use, precision, longevity, and quality. The expertise behind all this is highly valued not only by users in the lab, but also by companies like Serosep from Ireland. An OEM* version of the ep*Motion*® platform was successfully integrated into Serosep's EntericBio® Realtime system workflow resulting in a faster and more convenient procedure.

Serosep is a successful manufacturer of innovative kits for clinical labs. Its EntericBio system is a high-throughput, easy-to-use molecular solution for the detection of enteric pathogens. To avoid the time-consuming manual sample transfer into PCR plate wells, Serosep was looking for an automated handling solution.

"We first met with Serosep at a lab automation trade fair", says Mehran Khajooei, Program Manager in the Eppendorf OEM team. The ep*Motion* displayed at the Eppendorf booth had caught the attention of the Serosep representatives. "We started talking about the ep*Motion*, provided a hands-on demonstration, and actually quite soon we concluded that an OEM version of the ep*Motion* 5070 could be the ideal solution to simplify and accelerate the EntericBio procedure", Khajooei continues.

"When deciding to work with Eppendorf in developing an OEM version of the ep*Motion* platform, it quickly became clear that we had made an excellent decision. Eppendorf provided us a laboratory product of excellent quality which we at Serosep could successfully qualify for the detection of enteric pathogens and execute the according registrations at health authorities", says Allin Winter, Managing Director Serosep UK Limited, United Kingdom.

Aoife Beggan (Technical Support Manager) und David Clancy (Unit Manager, Molecular Diagnostics)

"The OEM team has been a pleasure to work alongside, continually providing outstanding professionalism, quality, and technical service."

According to Mehran Khajooei, it can take up to several months from the initial customer contact to a finished OEM product, especially since the most diverse departments within Eppendorf have to be coordinated: from R&D to product management, production, and logistics. "Nevertheless, we managed to deliver a prototype for a Serosep inhouse-exhibition within only one month", he proudly adds.

"This is what we were looking for", says Allin Winter. "We are delighted with our decision to work with Eppendorf. Not only have our goals been achieved, but the partnership has helped enhance the EntericBio brand on a global scale."

Diverse and individual solutions for your success

Besides OEM products, Eppendorf also offers Eppendorf Brand customized solutions designed in line with the customer's requirements, for example, tubes in special colors, microplates with specific surface treatments, and lots more (see www.eppendorf.com/oem for more information).

To ensure consistent product quality and the greatest possible safety, Eppendorf meets globally recognized ISO standards.

Customized product solutions according to the customer's specification

If you want to know more, please mail to customizedsolutions@eppendorf.com.

*OEM = Original Equipment Manufacturer

BARBRO PATTERSON, EPPENDORF AG

Keep Calm and Pipette On!

"How Fit are You?/How Fit is Your Lab?" This was the title of the 2017 online quiz celebrating our double epServices anniversary year: For 10 years, we have been offering our global Eppendorf Performance Plans, carefully designed maintenance and certification services for "lab fitness". In addition, the Eppendorf Training Center has been your professional training partner for lab knowledge fitness for 20 years!

Participants from around the globe tested their knowledge in our quiz about Eppendorf lab services and training and took their chance to win one out of many great prizes. The lucky grand prize winners received epServices prize packages including an Eppendorf Research® plus pipette with a personal "pipette tattoo" plus a free pipette calibration or free participation in a liquid handling training course.

Although the quiz was not about creativity, we received some wonderful ideas of what people would like to have written on their pipette. This made us curious about their inspiration.

Stefanie F., UK: "I am a woman returner to science, with a family and career break of seven years. So, when I was thinking about what to put on the pipette somebody said 'Wonder Woman®', and I liked it!"

Erica C., Italy: "A wish that LUCK is always present in my life; and it is nice to read it and keep in mind any time I handle it."

Rosanne W., Belgium: "Like many PhD students I also have my fair share of days where everything seems to go wrong. Luckily my co-students are always there for me to understand me and they always succeed to cheer me up. In case I have these moments, I can now also have a look at my personalized pipette encouraging me with the text 'Keep calm and pipette on!'"

Timo E., Norway: "Working in basic research for me doesn't only mean to analyze specific processes where the outcome might be more or less predictable, but also every once in a while stumbling over something completely unexpected. The 'Everyday Amazing' engraving will hopefully not only help myself, but also everybody else in the lab using the pipette, to embrace the complexity in biology research and this feeling of amazement when something unexpected comes their way." Speaking for itself is "*Hoc quoque transibit*" ("This too shall pass") requested by Jae-yeon C., South Korea.

Tip! With our epServices program we support all our customers so that they enjoy amazing, wonderful, and lucky days in the lab.

epServices
for premium performance

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Tip

Proper Storage of Pipettes

The proper storage of your pipette in a vertical position helps maintain its performance and extend its lifetime. The new Eppendorf Pipette Holder System offers a variety of storage solutions: highly flexible carousels, stands, and wall mounts for all current manual and electronic Eppendorf liquid handling instruments and most of their predecessors.

The **Pipette Carousel 2** holds up to six manual single- or multi-channel Eppendorf pipettes, including the current and most predecessor models, such as Eppendorf Research®, Eppendorf Research plus, Eppendorf Reference®, Eppendorf Reference 2, and Biomaster®.

The **Charger Carousel 2** (with 50 % more capacity than before) holds six electronic Eppendorf Xplorer® or Eppendorf Xplorer plus pipettes. When used with optional charger shells, up to six Multipette® dispensers E3/E3x and/or stream/Xstream can be stored. The Charger Carousel 2 also perfectly accommodates manual pipettes using optional pipette holders.

www.eppendorf.com/pipetteholder

Scan the QR code for more information!

BRIGITTE KLOSE, EPPENDORF AG

Varispenser® 2/2x: Uncompromising Quality

Bottletop dispensers are needed in any lab where aggressive solutions like lyes, acids, bases, or solvents are dispensed from large supply bottles. They are used day in, day out, need to be universally applicable and of uncompromising quality.

We are continuously working on adjusting our products to actual customer needs. One recent example is the new generation of bottletop dispensers. Varispenser 2 and Varispenser 2x offer a combination of proven quality and technology, optimized features, and a fresh, ergonomic design.

Optimized telescopic aspiration tubes match various bottle heights even at a low level of liquid, additional safety balls in the screw-on discharge valves reliably seal the dispensing channel when the dispensing tube is not attached, and the 360° rotatable valve block on the flask enables the dispensing of liquids from various positions. Varispenser 2 and Varispenser 2x are fully autoclavable without disassembly.

Thanks to the positive displacement principle used, loss of sample or media residue is almost completely avoided. A sealing lip made of PFA attached to the piston wipes off the inner cylinder wall during dispensing to prevent deposits from quickly crystallizing media, thus avoiding costly and time-consuming cleaning procedures.

Varispenser 2/2x: available in 6 sizes for dispensing volumes from 0.2 – 100 mL

The components of the Varispenser 2/2x that are exposed to aggressive liquids are made of materials with high chemical resistance to most acids, bases, and organic solvents, e.g. PTFE, PFA, FEP, or borosilicate glass. This, in combination with the dispensing technology described above, enables the use of the Varispenser 2/2x with nearly all common liquids in the lab.

Optional accessories enhance the scope of applications. A drying tube, filled with the suitable absorbent, is easily attached to the rear side of the Varispenser 2/2x. It allows the dispenser to be used with moisture-sensitive or CO_2-sensitive media. A sterile filter retains sterility of the medium in the bottle, and a flexible blow-out tube with recirculation valve simplifies serial dispensing in small microtest tubes in racks.

Find more details on Varispenser 2/2x and its accessories at www.eppendorf.com/top-performance.

Scan the QR code for more information!

Tip

Simply Well Organized

Benchtop racks are essential laboratory accessories for efficient processing, transport, and short- to mid-term storage of sample preparation tubes, cryogenic tubes, and cuvettes. The new Eppendorf Tube Racks and Eppendorf Cuvette Rack system enables you to tackle these tasks in a user-friendly, space-saving, and safe manner.

The comprehensive system supports a clearly arranged and error-free handling of reaction vessels in manifold laboratory workflows. The racks offer the optimal solution and perfect fit for all tubes and cuvettes commonly used in laboratories. Compact dimensions require minimal space.

Eppendorf Tube Racks and Eppendorf Cuvette Rack

- Six different formats
- Space-saving design, stackable
- Non-slip, safe stand
- Tube rows arranged in different heights: reaction vessels are easily accessible and clearly identifiable
- Numbered tube positions support fast and easy orientation and error-free handling

www.eppendorf.com/tube-racks

Scan the QR code for more information!

BERRIT HOFF, EPPENDORF AG

Greatly Appreciated: Flexibility, Reproducibility, Ease of Use

In 1950, Eppendorf brought its first photometer to the market. Since then our focus has been on developing equipment with maximum benefits while using the simplest handling methods. Eppendorf photometry products are designed to handle a wide range of applications. For this report, we have asked two of our customers in which way Eppendorf spectrophotometers help them in their daily experiments.

Examining virulence factors from *Pseudomonas aeruginosa* is part of Dr. Silvia Ferrara's research in the Department of Life Sciences at the University of Milan, Italy.

"In our laboratory, we use the Eppendorf BioSpectrometer® fluorescence and Eppendorf µCuvette® G1.0 mainly for detecting RNA and DNA. Sometimes we have high and low concentrations, so we need to be very flexible in detecting our samples", says Dr. Ferrara. "With our device, we could detect our samples via absorbance and fluorescence, so we are fully flexible for very low and very high concentration. In addition, we like the reproducibility in detecting small RNA samples with the Eppendorf µCuvette. For our northern blots, we were, for the first time, able to get reliable results for our reference standards", Dr. Ferrara continues.

Her conclusion: "The BioSpectrometer fluorescence […] was the first device that could enable absorbance as well as fluorescence measurement, which is important since we have many samples in various concentration ranges. So, we are well prepared for all upcoming measurements of nucleic acids samples that could be done in a reproducible way." [1]

Another convinced user is Michael Dreifke of the University of Hamburg's Institute of Inorganic and Applied Chemistry in Hamburg, Germany. He studies the function of enzymes immobilized to silica matrices. For functional enzyme tests he employs the Eppendorf BioSpectrometer kinetic.

For Dreifke, a major advantage of the Eppendorf BioSpectrometer kinetic is the temperature-regulated cuvette shaft, enabling him to carry out kinetic studies at different temperatures. He also likes the instrument's ease of use. "It features a very good user interface. Many standard methods are pre-programmed by the manufacturer, which simplifies the workflow", he comments. "Measurement results are saved as an Excel® file which contains all measurement parameter selections as well as screenshots of the measurement process. Importantly, previous measurements can be recalled using the memory function and retroactively saved on a USB. This sophisticated software provides a clear advantage compared to competitors' instruments." [2]

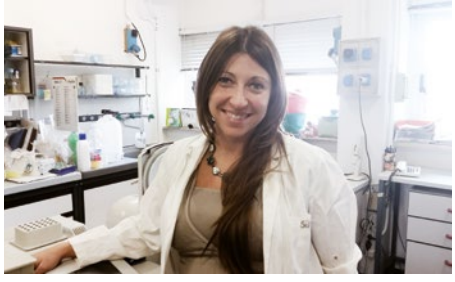
[1] Client Interview with Dr. S. Ferrara (©2017).
www.eppendorf.com/molecular-biology

[2] Client Interview with M. Dreifke (©2016).
www.eppendorf.com/molecular-biology

Visit www.eppendorf.com/detection for more information on Eppendorf spectrophotometers and accessories.

Scan the QR code for more information!

Eppendorf BioSpectrometer fluorescence: UV/Vis + fluorescence measurements in a single device



Dr. Silvia Ferrara, University of Milan, Italy

Michael Dreifke, University of Hamburg, Germany

CAROLYN TAUBERT AND BERRIT HOFF, EPPENDORF AG

Eppendorf Research Prize Winners Visit Eppendorf

Gilad Evrony

Tom Baden

For many years, Eppendorf, has been supporting promising young scientists with two renowned research prizes: the *Eppendorf Award for Young European Investigators* and the international *Eppendorf & Science Prize for Neurobiology*. A part of both prizes is a visit to Eppendorf in Germany.

The Israeli-American scientist Gilad Evrony, M.D., Ph.D., of the Mount Sinai Hospital in New York, USA, visited Eppendorf in summer 2017. Dr. Evrony had won the *Eppendorf & Science Prize for Neurobiology* 2016 for his work on developing technologies to sequence and analyze the genomes of single cells in the human brain (more information at www.eppendorf.com/prize). We asked Gilad on his impressions about our production facilities. "Seeing the manufacturing robots and the precision technology, and just the sheer scale of the process behind making even the most basic lab tools was exciting. I love knowing how things are made, and this was a unique opportunity to see how the tools that I use daily in the lab are created. I will never see a pipette in the same way again!"

A few weeks later, Dr. Tom Baden, Senior Lecturer in Neuroscience at the School of Life Sciences of the University of Sussex in Brighton (United Kingdom) visited Eppendorf. He had won the *Eppendorf Award for Young European Investigators* 2017 for his ground-breaking work on signal processing in the retina (more information at www.eppendorf.com/award). "It was fascinating to see that a global brand such as Eppendorf, with more than 70 years of corporate history, continues to produce the bulk of its produce at or near the original factory site in Northern Germany", Tom said. "It was also exciting to witness how much finesse and testing goes into producing in particular all this plastic ware that we in the lab often take for granted."

As a souvenir of their visit to Eppendorf, both Gilad Evrony and Tom Baden were presented with a personalized pipette with their names laser-printed on it.

Win 1 of 5 Personalized Pipettes

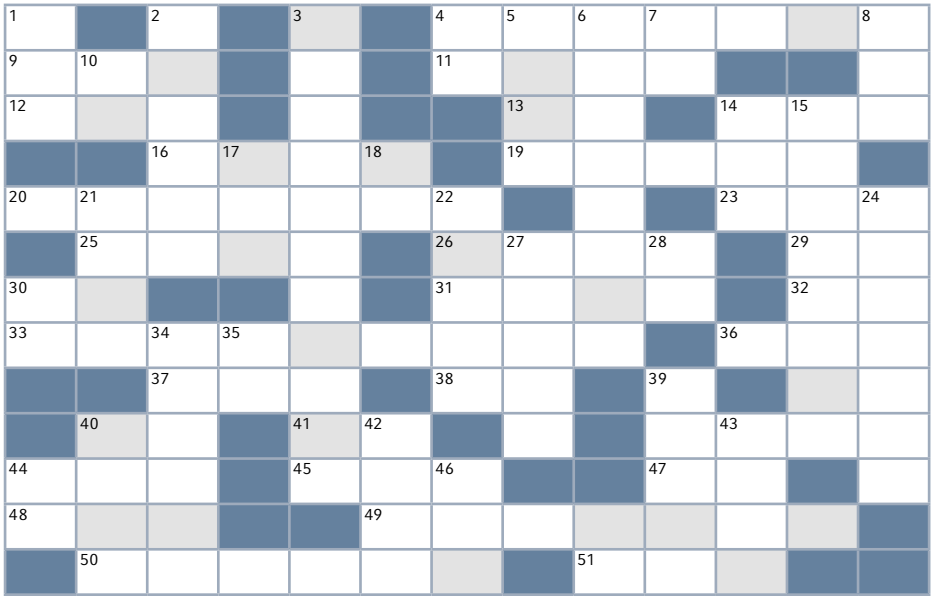
The solution of the prize competition of BioNews No. 46 was "Eppendorf Handling Solutions". Dr. Aleksandra Hac (University of Gdańsk, Poland) won the first prize.

Good luck in our anniversary competition!

How to find out the solution: Simply arrange all letters in the light gray boxes of the crossword in the correct order. Send us the solution until **June 30, 2018**.

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. 50.



1st to 5th Prize:

1 personalized Eppendorf Research® plus pipette (adjustable, single-channel) of your choice

6th to 10th Prize:

1 Amazon® Voucher worth 50.00 EUR

11th to 15th Prize:

400 bonus epPoints® each

(epPoints registration required)

ACROSS

4 Innovative cover of the thermoblock on Eppendorf thermal cyclers
9 Inventing it changed the life of 50 across
11 Consumables needed for pipetting and other advice
12 21st letter of the Greek alphabet
13 *ante meridiem (abbrev.)*
16 Rotate rapidly, whirl
19 Engine in a boat or car
20 Precipitate, sediment
23 French lake
25 To make ready (abbrev.)
26 Please … until you are seated (e.g. in a bar or restaurant)
29 English auxiliary verb
30 Internet protocol (abbrev.)
31 Smallest unit of an element
32 ISO country code for Israel
33 The new Eppendorf Mastercycler gradient features two of these (sing.)
36 French salt
37 Three-letter country code for Australia
38 ISO country code for The Netherlands
40 ISO country code for Sierra Leone
41 Short form of Edwin or Edward
44 Measure of resolution in print (abbrev.)
45 Past tense of run
47 Chemical symbol for sodium
48 Where scientific research takes place (abbrev.)
49 Contains books or 8 down
50 Discovered 9 across
51 Worldwide trend for makers, creators, tinkerers (abbrev.)

DOWN

1 Program for mobile devices
2 Genome-editing technique
3 Handles aggressive solutions
4 12 inches long (abbrev.)
5 Irish male given name
6 Comes as 96, 5070, 5073, and 5075 version
7 Extra small (abbrev.)
8 Includes A, T, C, and G
10 ISO country code for Switzerland
14 Latin for sun
15 Now also available with two of 33 across
17 Edgar Allan's surname
18 Silvery, hard, ductile metallic element (chemical symbol)
21 Nickname for a microtest tube
22 Famous American writer † 1910 (surname)
24 Artwork created by gluing together different elements
27 Ring-shaped reef enclosing a lagoon
28 Trademark (abbrev.)
30 ISO country code for Indonesia
34 City in California
35 "United in Diversity", political and economic union (abbrev.)
39 Peninsula in Northeast Egypt
40 Unsolicited e-mail
42 Spanish surrealist artist
43 This mother speaks words of wisdom
46 N-Iodosuccinimide (abbrev.)

Solution hint for prize competition of BioNews No. 48

	A					I		N							I				S			Y		

Send us the solution until **June 30, 2018**, via e-mail to **bionews@eppendorf.de**, or participate online at **www.eppendorf.com/bn-service**.