BIO NEWS
No. 46 – 2017

eppendorf

Centrifuge 5920 R: More Comfort with Universal Rotors

> Bundled Expert Knowledge All Around Cell Culture
> Consumables: New Purity Grade for Forensic Work
> Celebrating 10 Years of epServices

Application Notes

How Pipette Tips Influence Results (Part 3) · Increased Yield of Transcript Species and Reads from NGS Libraries · Scale-Up of *Escherichia coli* Fermentation · etc.

Dear Readers,

We wish you a good start into the year 2017! Perhaps even with a new Eppendorf Centrifuge 5920 R? The special feature (besides its high capacity) is its ability to centrifuge tubes AND plates in the same rotor bucket – a considerable gain in user convenience (p. 4–5).

What started 30 years ago with the introduction of the first Eppendorf micromanipulators has since developed into a comprehensive product line that includes shakers, incubators, and fermentors as well as Cell Culture and Cell Imaging consumables. From manipulation to cultivation, in microbiology and cell culture – Eppendorf Cell Handling products accompany more and more scientists, from research all the way to production.

Intelligent, reliable product solutions and systems that simplify your daily work are but one side of the coin. As your expert partner and advisor, we take pride not only in our competent customer service, but also in our ability to convey current, updated information. We invite you to visit our new Cell Handling portal **www.eppendorf.com/cellexperts**. Here you will find expert knowledge on the topics of contamination, identity, and reproducibility – researched in depth by our Eppendorf cell experts (see p. 12–13).

Eppendorf consumables stand for highest quality and performance. For example, Eppendorf LoBind® products ensure more reproducible and reliable experiments (Application Note p. 1–2) as well as higher yields of transcript species and reads from NGS libraries (Application Note p. 3–4). The new purity grade "Forensic DNA Grade", in accordance with ISO 18385, is to help minimize the risk of contamination during forensic DNA analysis (p. 7).

epServices is celebrating its 10th anniversary of representing Premium Performance, a comprehensive spectrum of services and intensive customer support, e.g. application support and technical support, maintenance and certification as well as seminars and webinars. Tip: The seminar program 2017 of the Eppendorf Training Center with practice-oriented courses (including Cell Handling and Liquid Handling) is available at **www.eppendorf.com/epservices**.

We hope you will enjoy this issue of BioNews, and best of luck with our new prize competition (p. 15).

Your BioNews team

Imprint

Editorial team
Berrit Hoff (Editor-in-Chief), Axel Jahns, Jochen Müller-Ibeler, Tanja Musiol, Natascha Weiß

Publisher
Eppendorf AG, Barkhausenweg 1,
22339 Hamburg, Germany
Telephone: (+49) 40-53801-0
Fax: (+49) 40-53801-556
E-mail: bionews@eppendorf.de
Web: www.eppendorf.com

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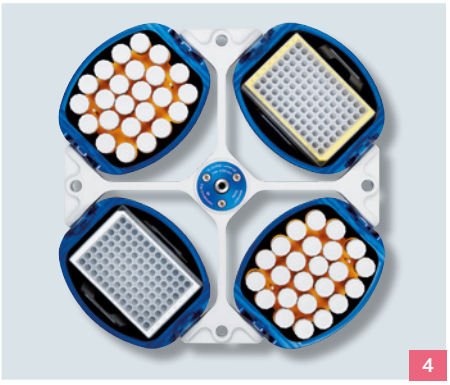
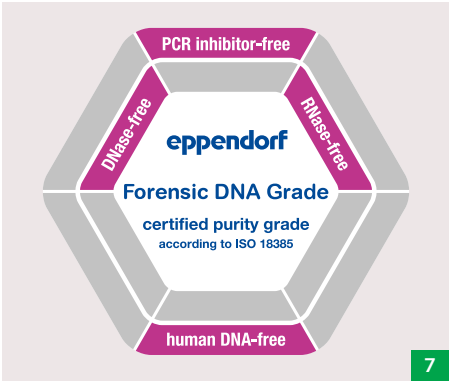
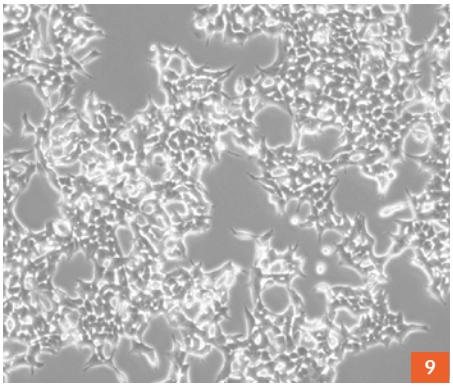
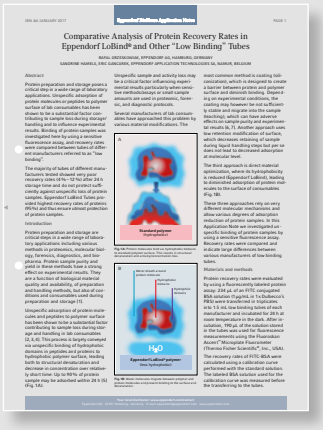






Eppendorf BioNews Application Notes



PETER SCHREINER, EPPENDORF AG

Centrifuge 5920 R: More Comfort with Universal Rotors

Do you spin tubes as well as plates in your laboratory? Are you tired of switching between different rotors or rotor buckets? Then it's time to look forward to the new refrigerated Centrifuge 5920 R. It offers exceptionally high capacity, and thanks to practical universal swing-bucket rotors, you will experience a considerable gain in comfort and convenience. These rotors were designed to accommodate both tubes and plates: The need for tedious exchange of rotors or rotor buckets has been eliminated!

Extraordinary capacity

The Centrifuge 5920 R was specifically designed for applications demanding very high sample throughput. You can spin up to 52 x 50 mL conical tubes or 196 x 5/7 mL round bottom tubes simultaneously. Spin more samples in fewer runs and save time!

New versatile swing-bucket rotor

The new swing-bucket rotor S-4xUniversal-Large is especially versatile. A broad range of different adapters support all major vessel formats, from 1.5 mL tubes to bottles with a volume of 750 mL. The special feature of this new rotor is its capability to accommodate plates (MTP plates, PCR plates, and Deepwell Plates) in the same rotor bucket. This is where the "Plate carrier" is employed: a type of adapter with which the plates are lowered into the bucket and subsequently lifted out of the bucket following centrifugation. Up to two Deepwell Plates or five MTP plates may be stacked into one rotor bucket. The advantages of the new universal buckets are obvious. As the operator, you will no longer have to switch between different rotor buckets (or even different rotors!). Only adapters are exchanged. These are smaller and lighter, and they take up less room in the drawer when not in use.

By the way, all adapter types are color coded. For example, the adapters for 15 mL conical tubes are orange, whereas the adapters for 9 mL round bottom tubes are white. This way, adapters which otherwise look very similar may be identified with ease.

The design of the new universal buckets is especially round and aerodynamic, resulting in lower aerodynamic drag. This, in turn, reduces power consumption, while at the same time facilitating a high maximum speed of 4,400 x *g*. An exceptional value for a swing-bucket rotor of this size! In cases where potentially hazardous samples are to be centrifuged, aerosol-tight Eppendorf QuickLock® caps are an optional choice. Their aerosol-tightness has been tested and verified by Public Health England, Porton Down, UK.

The handle allows the closed rotor bucket to be carried to the biosafety cabinet where it can be opened in a safe environment.

Attractive centrifuge packages

Eppendorf offers attractive pre-configured packages with adapters for either 15/50 mL conical tubes or 13/16 mm round bottom tubes for the Centrifuge 5920 R. Adapters for plates are available separately. Both packages have the advantage of a 10 % discount compared to the price of individual components.

Get +Together campaign

The Centrifuge 5920 R, complete with new universal rotor, is the focus of our new information campaign Get +Together. Experience the Centrifuge 5920 R in action and in combination with Eppendorf Conical Tubes as well as Eppendorf Deepwell Plates and Eppendorf Microplates!

More information at:
www.eppendorf.com/together

At a glance!

Centrifuge 5920 R

> Capacity up to 4 x 1,000 mL / 52 x 50 mL / 108 x 15 mL / 8 x DWP

> Powerful state-of-the-art refrigeration system with advanced product temperature management to keep your samples safe

Rotor S-4xUniversal-Large

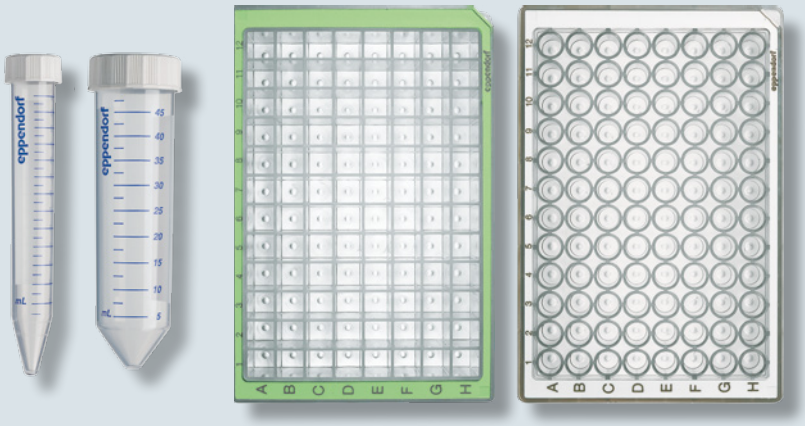
> Universal bucket design accommodates both plates and tubes

> Time saving: No need to exchange buckets

> Cost saving: No need for separate plate buckets

> Space saving: No need to store different buckets

> Aerosol-tight Eppendorf QuickLock® caps optionally available



Tip

Eppendorf Deepwell Plates

Plates are most often used for simultaneous processing of multiple samples within one experiment. In order to obtain reliable and reproducible results, it is vital that the individual sample positions within a plate provide uniform experimental conditions. Of course this also applies in cases where samples are distributed across multiple plates.

Reliability and reproducibility for your data

Highest standards across the entire manufacturing process of the Eppendorf Plates® ensure consistently high quality – lot after lot. Furthermore, Eppendorf Plates offer a range of important features for safe, error-free work:

> g-Safe®: Extraordinary centrifugation stability up to 6,000 × *g* for quick protocols and better sample quality

> OptiTrack®: Up to 30 % quicker well identification and fewer pipetting errors thanks to high-contrast alpha-numeric labeling

> RecoverMax®: Optimized well geometry for maximum sample recovery and superior mixing properties

See for yourself and test the outstanding performance of Eppendorf Plates in your experiments: www.eppendorf.com/consumables

NILS GERKE, EPPENDORF AG

Do Your Conical Tubes Offer Secure Protection from Sample Loss?

Conical Tubes 15 mL and 50 mL are used in many laboratory protocols, making them two of the most widely used tube formats in life science laboratories. Given their broad range of applications, the security of the seal is paramount. The specially developed screw cap of the Eppendorf Conical Tubes (Fig. 1) establishes new standards with respect to handling and sample safety.

Fig. 1: Eppendorf Conical Tubes 15 mL and 50 mL

Cap tightness

The typical areas of application for Conical Tubes include centrifugation, mixing, incubation, transport, and storage of the most diverse samples and solutions. A reliable seal is therefore a basic condition for safe and accurate work. For these reasons, strong emphasis was placed on this aspect during the development of the screw cap for the Eppendorf Conical Tubes. The exceptional tightness of the seal could be demonstrated through comparative experiments such as storage of ethanol, a volatile liquid (Fig. 2).

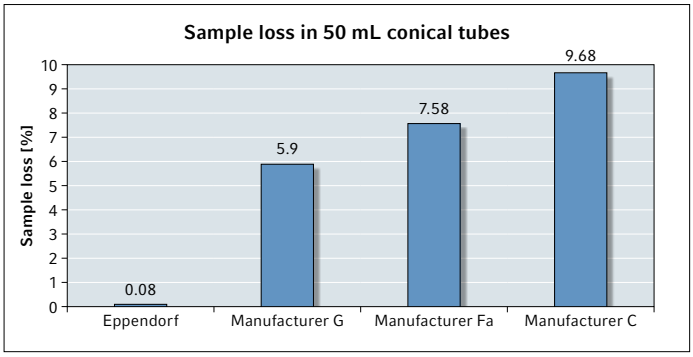


Fig. 2: Sample loss after 24 h of horizontal storage in different Conical Tubes 50 mL at –86 °C. N = 50 Eppendorf Conical Tubes 50 mL and 22 tubes of manufacturers G, Fa, C. Additional information: www.eppendorf.com/appnote343

Tube handling and prevention of contamination

The grooved, multi-surface side contour ensures a non-slip grip while simultaneously allowing secure single-handed opening and closing of the Eppendorf Conical Tubes. Experienced users in particular will handle conical tubes in this fashion for swift and efficient work.

Additional user advantages result from the flat, smooth, and light-colored surface of the screw caps. Whereas other lids, due to dark coloring schemes and uneven or sometimes concave surfaces, do not offer user-friendly labeling opportunities, the smooth, light gray lids of the Eppendorf Conical Tubes allow legible, high-contrast labeling (Fig. 3).

Fig. 3: Numerous user advantages are gained from the light-colored and smooth surface as well as the grooved, multi-surface side contour.

Thanks to their flattened sides, the lids may be placed safely upright on the laboratory bench during sample processing. This minimizes the risk of contamination of the inside surface of the lid as well as the danger of confusing lids during simultaneous handling of multiple samples.

For additional information and the opportunity to order free samples, please visit www.eppendorf.com/conicals.

NILS GERKE, EPPENDORF AG

New Purity Grade for Forensic Work

The prevention of contamination is one of the major challenges in forensic DNA analysis. In order to minimize the risk of contamination, forensic laboratories have established internal standards and accreditations. Even more safety is expected from the recently introduced ISO 18385. It specifies the demands on manufacturers of products which are used in forensic DNA laboratories.

The Phantom of Heilbronn and its consequences

One important reason for the creation of ISO 18385 was the case of the "Phantom of Heilbronn". For more than a decade, police based their investigations of ca. 40 different criminal cases on the involvement of one person with a particular DNA profile. In the end it turned out that the cotton swabs used in the securing of evidence had been contaminated with this very DNA during production.

One of the consequences arising from this case is the ISO 18385. It came into effect at the beginning of 2016 and it defines the demands on manufacturers of products which are used for the collection, storage, and analysis of biological material during the course of forensic investigations.

New! Eppendorf Forensic DNA Grade in compliance with ISO 18385

Eppendorf consumables stand for highest quality and performance. The new purity grade Eppendorf Forensic DNA Grade, compliant with ISO 18385, rigorously carries on this approach.

The product line encompasses consumables for sample processing and PCR set-up for the purpose of forensic DNA analysis. The sophisticated demands of ISO 18385 are met by the high Eppendorf quality standards upheld during the manufacturing process as well as for the final product, which include:

> A high degree of automation to minimize direct contact between staff and products

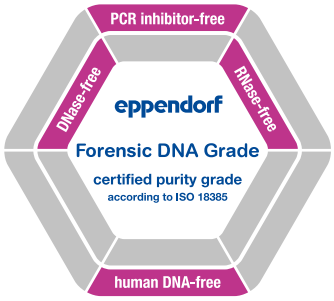
> Restricted access to production areas

> Intensive training measures for staff as well as strict adherence to dress codes

> Continuous product and quality controls

> Testing of production environment for human DNA

> Individual lot-specific certificates are available. Testing is done by an independent external testing laboratory which is accredited in accordance with ISO 17025.



The Eppendorf Forensic DNA Grade is expected to be available as of March 2017 – for more information please visit www.eppendorf.com/forensics

Close-up

Your Tool for Quick, Safe, and Relaxed Dispensing

Eppendorf Multipettes enable precise, quick, and contamination-free dispensing of a multitude of liquids. Whether your work is manual or electronic – the PhysioCare Concept® applies to all current Multipette®* models (*U.S./CAN: Repeater®). It stands for ergonomic product design and product optimization, with a special focus on the individual needs of the user.

Factors contributing to ergonomic product design include the shape and arrangement of control elements, the weight, as well as the force required to operate the instrument. Complementing features such as clear displays and sophisticated technical details allow quick, safe, and relaxed handling.

The Combitip docking site of the Multipette is an example of such detail (see photo): The integrated sensor of the Multipette automatically recognizes the size of the attached Combitip advanced, and the volume is displayed automatically.

Your advantage: Time-consuming volume calculations using tables, which may lead to incorrect dispensing volumes, are obsolete. You can start dispensing immediately!

More information about the Multipette and Combitips advanced® at www.eppendorf.com/multipette-system

NILS GERKE, EPPENDORF AG

Secure Storage of Valuable Samples

"Which properties must a storage box possess in order to be able to ensure safeguarding of valuable samples?" This question remained at the forefront during conception and development of the Eppendorf Storage Boxes. The result is a comprehensive family of products with nine variants for all common tube formats, offering advantages for both application and handling.

NEW! Eppendorf Storage Boxes: innovative, flexible, reliable

In order to ensure a uniform freezing process, the side walls of the boxes for 15 mL and 50 mL tubes feature ventilation slots to facilitate temperature compensation. An even temperature distribution prevents damage or even breakage of the tubes, which is frequently observed when long tube formats are frozen in closed boxes.

The new Eppendorf Storage Boxes offer innovative details, e.g. ventilation slots for temperature compensation.

Flexible and robust

Space is limited in the freezers of most laboratories. Optimum use of available space is therefore paramount! A total of nine variants of the Eppendorf Storage Boxes offer optimized fit for all common tube formats: Boxes for different micro test tubes and cryogenic tubes as well as conical tubes 15 mL and 50 mL are available and may be combined in a flexible and space-saving manner for individual storage requirements.

Dimensional stability is an important topic. Frequently, samples cannot be removed immediately because the storage box has become warped during freezing. The combination of high-quality polypropylene (PP) with a thoughtful design ensures high dimensional stability of the Eppendorf Storage Boxes, thus allowing smooth opening and closing. Users can rely on their Eppendorf Storage Box when freezing samples to –86 °C as well as during autoclaving and during cleaning in the laboratory wash-up facility.

Reliable and user-friendly

Secure sample allocation is indispensable for reliable storage. For this reason, all sample locations in the Eppendorf Storage Boxes have an alpha-numeric laser labeled code. This high-contrast, wipe-resistant, and permanent labeling allows the user to perform quick and reliable sample inspection.

Additional information is available at www.eppendorf.com/storageboxes.

Tip

10 Years epServices

Ten years have passed since we introduced epServices. The name represents: premium performance, comprehensive service, and intensive customer support! It is what we live and breathe on a daily basis.

Training sessions and standards for our international subsidiaries as well as for distributors of Eppendorf products ensure that our services are offered globally at a consistently high level. This is how we guarantee the quality of our support in more than 120 countries worldwide. Continuous improvement of our services, which is made possible in part thanks to your feedback, is important to us. Even after the sales process has been completed, we remain contact partners for our clients.

epServices
for premium performance

Our clients' voices

RJ Bacon, Lab Maintenance Technician, Manus Biosystems (Cambridge, MA, USA)*: *"As a fast-paced research lab, the uptime of our infrastructure is of the highest priority. This is why we work with Eppendorf and their great service team. They are quick and easy to work with, which gives us confidence in their equipment."*

Christophe Lehobey, Biomedical Maintenance Manager, Institut Curie (Paris, France): *"Eppendorf epServices' personnel are specialists with technical expertise, but also partners who listen and enable us, year after year, to meet the requirements of accreditation."*

For full information about epServices please visit www.eppendorf.com/epServices.

*Status: April 2016

Comparative Analysis of Protein Recovery Rates in Eppendorf LoBind® and Other "Low Binding" Tubes

RAFAL GRZESKOWIAK, EPPENDORF AG, HAMBURG, GERMANY
SANDRINE HAMELS, ERIC GANCAREK, EPPENDORF APPLICATION TECHNOLOGIES SA, NAMUR, BELGIUM

Abstract

Protein preparation and storage poses a critical step in a wide range of laboratory applications. Unspecific adsorption of protein molecules or peptides to polymer surface of lab consumables has been shown to be a substantial factor contributing to sample loss during storage/handling and to influence experimental results. Binding of protein samples was investigated here by using a sensitive fluorescence assay, and recovery rates were compared between tubes of different manufacturers referred to as "low binding".

The majority of tubes of different manufacturers tested showed very poor recovery rates (4 % – 12 %) after 24 h storage time and do not protect sufficiently against unspecific loss of protein samples. Eppendorf LoBind Tubes provided highest recovery rates of proteins (95 %) and thus ensure utmost protection of protein samples.

Introduction

Protein preparation and storage are critical steps in a wide range of laboratory applications including various methods in proteomics, molecular biology, forensics, and bio-pharma. Protein sample purity and yield in these methods have a strong effect on experimental results. They are a function of biological material quality and availability, of preparation and handling methods, but also of conditions and consumables used during preparation and storage [1].

Unspecific adsorption of protein molecules and peptides to polymer surface has been shown to be a substantial factor contributing to sample loss during storage and handling in lab consumables [2, 3, 4]. This process is largely conveyed via unspecific binding of hydrophobic domains in peptides and proteins to hydrophobic polymer surface, leading both to structural denaturation and decrease in concentration over relatively short time: Up to 90 % of protein sample may be adsorbed within 24 h [5] (Fig. 1A).

Unspecific sample and activity loss may be a critical factor influencing experimental results particularly when sensitive methods/assays or small sample amounts are used in proteomic and forensic protocols.

Several manufacturers of lab consumables have approached this problem by various material modifications. The most common method is coating (siliconization), which is designed to create a barrier between protein and polymer surface and diminish binding. Depending on experimental conditions, the coating may however be not sufficiently stable and migrate into the sample (leaching), which can have adverse effects on sample purity and experimental results [6, 7]. Another approach uses low retention modification of surface, which decreases retaining of sample during liquid handling steps but per se does not lead to decreased adsorption at molecular level.

The third approach is direct material optimization, where its hydrophobicity is reduced (Eppendorf LoBind), leading to diminished adsorption of protein molecules to the surface of consumables (Fig. 1B).

These three approaches rely on very different molecular mechanisms and allow various degrees of adsorption reduction of protein samples. In this Application Note we investigated unspecific binding of protein samples by using a sensitive fluorescence assay. Recovery rates were compared and indicate large differences between various manufacturers of low binding tubes.

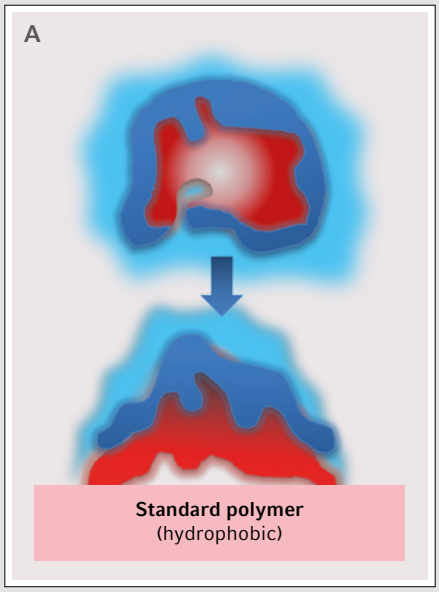


Fig. 1A: Protein molecules bind via hydrophobic domains to standard polymer surface. This results in structural denaturation and activity/concentration loss.

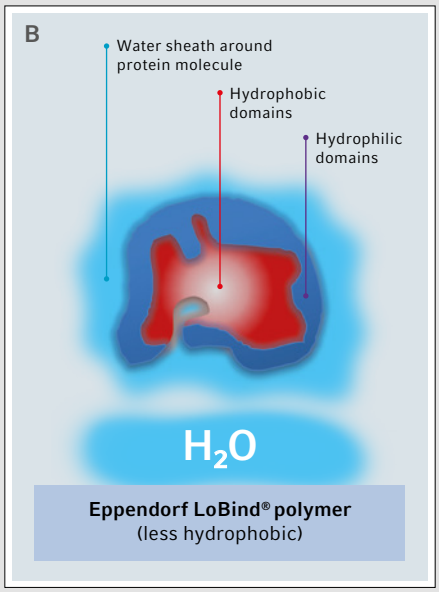


Fig. 1B: Water molecules migrate between polymer and protein molecules and prevent binding to the surface and denaturation.

Materials and methods

Protein recovery rates were evaluated by using a fluorescently labeled protein assay: 234 µL of an FITC conjugated BSA solution (1 µg/mL in 1 x Dulbecco's PBS) were transferred in triplicates into 1.5 mL low binding tubes of each manufacturer and incubated for 24 h at room temperature in the dark. After incubation, 190 µL of the solution stored in the tubes was used for fluorescence measurements using the Fluoroskan Ascent™ Microplate Fluorometer (Thermo Fisher Scientific®, Inc., USA).

The recovery rates of FITC-BSA were calculated using a calibration curve performed with the standard solution. The labeled BSA solution used for the calibration curve was measured before transfer to the tubes.

Comparative Analysis of Protein Recovery Rates in Eppendorf LoBind® and Other "Low Binding" Tubes

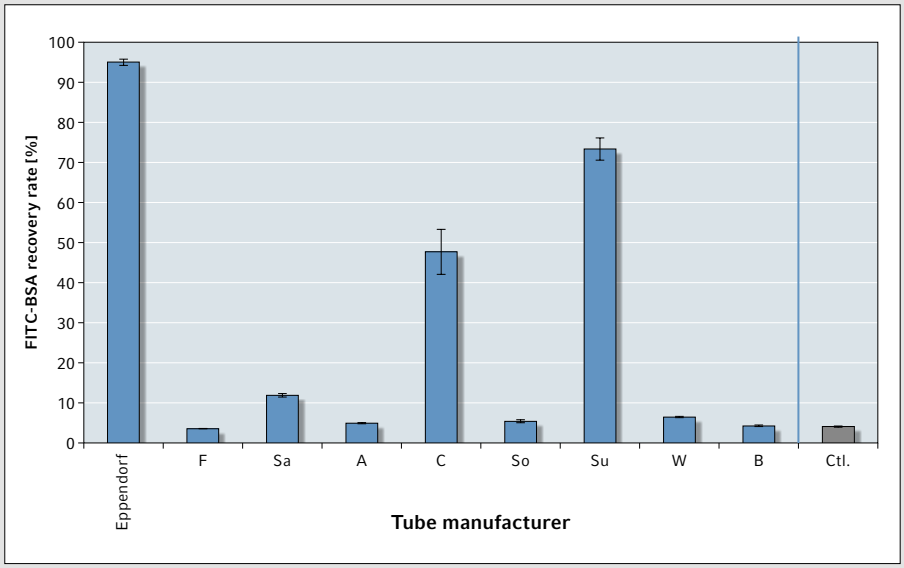


Fig. 2: FITC-BSA recovery rate after 24-hour incubation in 1.5 mL low binding tubes from different manufacturers. Negative control (Ctl.): standard polypropylene tubes. Two independent experiments in triplicates are shown (n=6).

Two independent experiments in triplicates were performed (n=6). Standard polypropylene tubes were used as negative control.

Results and discussion

The recovery rates of the FITC conjugated BSA samples after incubation in the different tubes are presented in Fig. 2. The majority of the investigated tubes referred to as "low binding" (F, Sa, A, So, W, B) showed very poor protein recovery rates ranging between 4 % and 12 %, and were comparable to the standard polypropylene material (Ctl. recovery rate 5 %). This indicates that under applied experimental conditions the majority of the tubes specified as "low binding" offer no or no significant advantage over standard material. Low binding tubes from two manufacturers (C and Su) provided sub-optimal recovery rates of 48 % and 73 %, respectively.

Highest protein recovery (95 %) was obtained with the Eppendorf Protein LoBind tubes after 24-hour incubation. The Eppendorf LoBind tubes showed in two independent experiments very similar results providing consistent and reproducible data.

These substantial differences of recovery rates observed might be due to very different technologies applied by manufacturers to achieve low binding properties of their consumables. Our results indicate that direct material optimization with reduced hydrophobicity offers, under experimental conditions we tested, the most effective approach to minimize unspecific protein adsorption and therefore sample loss. Additionally, since this technology uses no surface modification or silicon coating, it offers higher experimental safety and data reliability.

Conclusion

In this study, we have demonstrated that the sample recovery rates of various tubes referred to as "low binding" vary greatly. Out of nine manufacturers tested, six showed very poor recovery rates (4 % – 12 %) and do not sufficiently protect against unspecific loss of protein samples.

Eppendorf LoBind tubes show the highest recovery rates of protein samples (95 %) and therefore ensure safe protection of protein samples. This leads to more stable protein concentrations and in turn more reproducible and reliable experimental results.

Literature

[1] Goebel-Stengel M, Stengel A, Taché Y, Reeve JR. The importance of using the optimal plastic and glassware in studies involving peptides. *Analytical Biochemistry* 2011; 414(1):38-46.

[2] Kristensen K, Henriksen JR, Andresen TL. Adsorption of cationic peptides to solid surfaces of glass and plastic. *PLoS One* 2015; 10(5).

[3] Hoofnagle AN, Whiteaker JR, Carr SA, Kuhn E, Liu T, Massoni SA, Thomas SN, Townsend RR *et al.* Recommendations for the Generation, Quantification, Storage, and Handling of Peptides Used for Mass Spectrometry-Based Assays. *Clin Chem* 2016; 62(1):48-69.

[4] Kraut A, Marcellin M, Adrait A, Kuhn L, Louwagie M, Kieffer-Jaquinod S, Lebert D, Masselon CD, Dupuis A, Bruley C, Jaquinod M, Garin J, Gallagher-Gambarelli M. Peptide storage: are you getting the best return on your investment? Defining optimal storage conditions for proteomics samples. *J Proteome Res* 2009; 7:3778-85.

[5] Weiß N, Wente W, Müller P. Eppendorf LoBind®: Evaluation of protein recovery in Eppendorf Protein LoBind Tubes and Plates. *Eppendorf Application Note* 180; www.eppendorf.com/appnote180

[6] Smith C. Selecting the Right Tubes or Plates for Your Research. *Biocompare* 2011; Nov. 9.

[7] Bowen RAR, Remaley AT. Interferences from blood collection tube components on clinical chemistry assays. *Biochem. Med.* 2014; 24:31-44.

Eppendorf twin.tec® PCR Plates 96 LoBind Increase Yield of Transcript Species and Reads from NGS Libraries

HANAË A. HENKE, EPPENDORF AG, HAMBURG, GERMANY
BJÖRN ROTTER, GENXPRO GMBH, FRANKFURT AM MAIN, GERMANY

Abstract

Currently the most sophisticated sequencing method is Next Generation Sequencing (NGS). Many factors may interfere with the procedure and impair the quality of the final library. Amongst these factors, the selection of consumables is often overlooked. We compared Eppendorf twin.tec® PCR Plates LoBind 96 with low binding characteristics and standard polypropylene PCR Plates.

To demonstrate the advantage of Eppendorf LoBind® consumables, two NGS libraries (10 and 50 ng total RNA input) were prepared in both plate types, sequenced, and the resulting data were compared. It was shown that samples processed in LoBind plates produced a higher average number of reads, as well as broader variation of transcript species.

Introduction

Nowadays sequencing is one of the most important technologies in almost every research field. Next Generation Sequencing (NGS) is the most sophisticated method [1].

Sample quality and quantity are crucial for successful sequencing. Both should be as high as possible. One method to enable sequencing of low quality and quantity samples is MACE (Massive Analysis of cDNA Ends) [2]. Studies have found that standard laboratory polypropylene consumables may lead to DNA denaturation, multimerization, as well as increased binding of short DNA fragments to the plastic surface [4, 5]. Using plastics that do not affect DNA, such as polyallomers, for the production of laboratory consumables is one approach to answer those challenges [4]. To demonstrate the effectiveness of the low binding feature for NGS workflows achieved by using new materials for PCR plate production, we used Eppendorf twin.tec PCR Plates LoBind 96 (LoBind) with low binding characteristics and Eppendorf twin.tec PCR Plates 96 (standard) for the preparation of NGS libraries. Then we compared their performance by the number of reads from sequencing and the variation of transcript species.

Materials and methods

Comparison of sequencing efficiency of an NGS library of primary cells in LoBind and standard PCR plates

10 and 50 ng total RNA from primary HUVEC cells were used to prepare an NGS library according to the MACE protocol [3] by GenXpro GmbH (Frankfurt a. M., Germany). cDNA synthesis and all following steps were carried out in Eppendorf twin.tec PCR Plates LoBind 96, semi-skirted (LoBind) and Eppendorf twin.tec PCR Plates 96, semi-skirted (standard). Both plates were treated in parallel with three replicates each. Ten PCR cycles were applied for both groups. Sequencing of all products was done on the same Illumina® NextSeq® 500 lane (Illumina, Inc., San Diego, CA, USA).

The adapter sequences had a length of 75 bp. All sequencing data was cleared from artifacts and low quality reads with the TrueQuant method (GenXpro). All MACE sequencing data was annotated on the human genome hg19 using the GenXpro annotation pipeline with Novoalign (Novocraft Technologies Sdn Bhd, Petaling Jaya, Malaysia) [6].

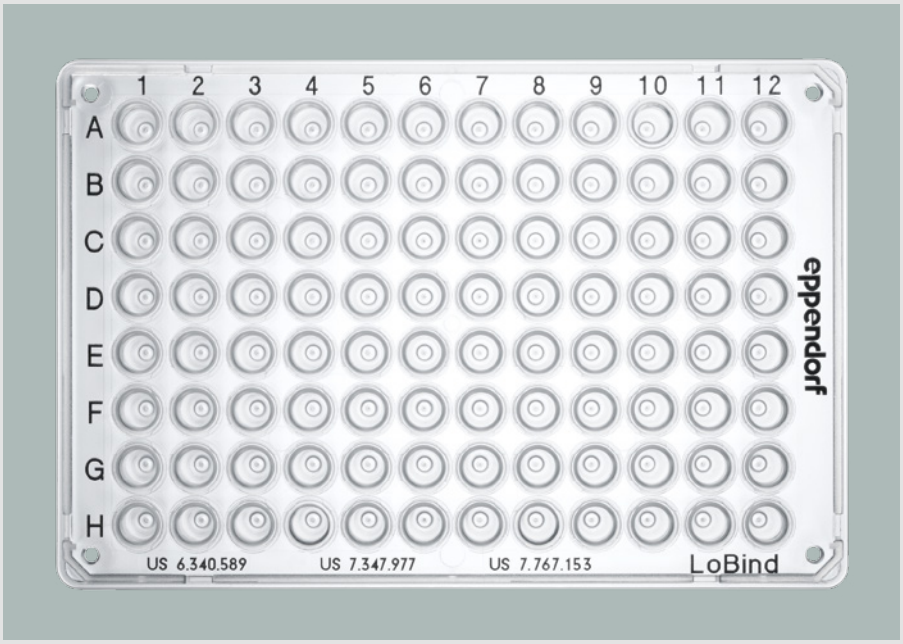
All annotation coordinates were aligned with Refseq-tracks to receive the genetic information. Normalization of the data was accomplished by calculating the number of each transcript over the number of all sequenced molecules followed by multiplication of 106 (TPM = tags per million).

Results and discussion

Comparison of sequencing efficiency of an NGS library of primary cells in LoBind and standard PCR plates

To compare the efficiency of sequencing, NGS libraries were prepared in LoBind and standard PCR plates with 10 and 50 ng total RNA, respectively. Both libraries were sequenced on the same lane and resulted in an average of 549,165 reads from the library prepared in the LoBind plate and 442,699 reads from the standard plate with 10 ng total RNA input.

The data shows a lead of 20 % in reads using the LoBind plates versus the standard ones, which is repeated with 50 ng (~23 % more reads from the LoBind plate). The average number of reads in LoBind plates was 2,836,281, while in standard plates 2,184,589 reads were detected (Fig. 1).



Eppendorf twin.tec® PCR Plates 96 LoBind Increase Yield of Transcript Species and Reads from NGS Libraries

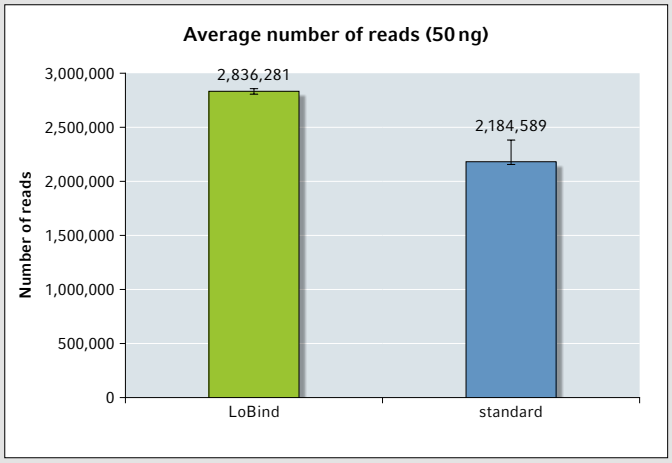


Fig. 1: The average number of reads of a 50 ng NGS library prepared from total RNA of primary HUVEC cells is up to 23 % higher in LoBind than in standard plates.

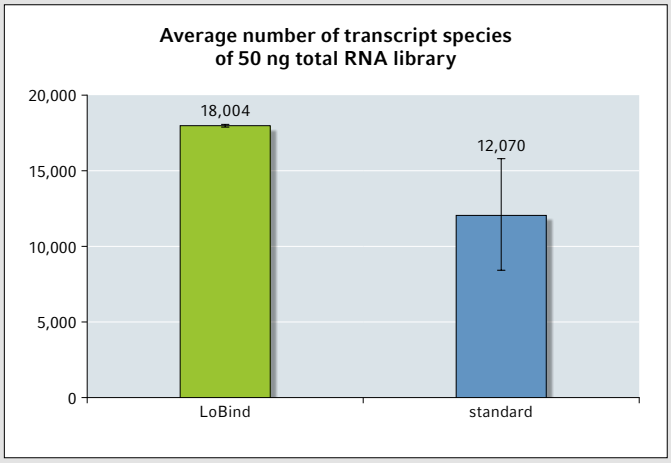


Fig. 2: Average number of transcript species of NGS libraries prepared from 50 ng total RNA of primary HUVEC cells is up to 30 % higher in LoBind than in standard plates.

This indicated that using LoBind plates for the preparation of NGS libraries leads to more raw data output, leaving room for evaluation and a higher chance of catching all desired sequences in the library.

Not only the average read depth is a quality measure of a sequencing run, but also the number of different transcript species detected. It was found that using LoBind plates for the preparation of NGS libraries led to an increase in the average number of transcript species.

13,800 different transcript species in LoBind plates and 12,800 in standard plates from 10 ng total RNA for the library set-up shows that LoBind plates helped to preserve the transcript variability. This enhancement was more robust as the starting material increased (50 ng). 30 % more transcript species from the LoBind plates were reported (Fig. 2).

Conclusion

More reads and transcript species were identified by sequencing the same library in the LoBind plates as compared to the standard plates. These results show that preparation of NGS libraries in Eppendorf twin.tec PCR Plates 96 LoBind increases sequencing data quality and quantity. Even a low quality NGS library prepared in a LoBind plate can have comparable results to a high quality library prepared in a standard plate.

Literature

[1] www.illumina.com/technology/next-generation-sequencing.html

[2] Hofer TP, Zawad AM, Frankenberger M, Skokann K, Satzl AA, Gesierich W, Schuberth M,Levin J, Danek A, Rotter B, Heine GH, Ziegler-Heitbrook L. Characterization of subsets of the CD16-positive monocytes: impact of granulomatous inflammation and M-CSF-receptor mutation. *Blood* 2015 Oct 6. Pii: blood-2015-06-651331

[3] genxpro.net/sequencing/transcriptome/mace-massive-analysis-of-cdna-ends/

[4] Gaillard C, Strauss F. Avoiding adsorption of DNA to polypropylene tubes and denaturation of short DNA fragments. *Science*, Technical Tips online 1998, Vol. 3: 63-65

[5] Belotserkovskii BP. Polypropylene Tube Surfaces May Induce Denaturation and Multimerization of DNA. *Science*, Technical comments 1996, Vol. 271: 222-223

[6] www.novocraft.com

The complete version of this Application Note (No. 375) can be downloaded in PDF format at www.eppendorf.com/appnote375.

The Tip of the Iceberg: How Pipette Tips Influence Results. Part 3: Not Every Tip Tolerates Every Treatment

MURIEL ART, VINCENT DUFEY, ION GLIGOR, EPPENDORF APPLICATION TECHNOLOGIES S.A., NAMUR, BELGIUM
ULRIKE GAST, LAURA KOCH, RONJA KUBASCH, EPPENDORF AG, HAMBURG, GERMANY

Abstract

The pipetting accuracy of pipette tips by certain manufacturers may suffer after autoclaving. This was not the case for Eppendorf tips. We describe the measures which Eppendorf takes in order to avoid the negative influences of autoclaving. Furthermore, we describe how the interference of biological assays by leachables from pipette tips is prevented. A MEA (Mouse Embryo Assay) test proves that sensitive biological systems are not influenced by Eppendorf pipette tips.

Introduction

We have shown in part 1 and 2 of this series (see BioNews No. 44 and 45) that pipette tips influence the performance of the system "pipette and tip" and thus the pipetting result. This was predominantly caused by the tip's shape and its production quality. Autoclaving is a widely applied method for decontamination of pipette tips but it imposes thermal stress. For this reason, calibration was used to determine whether autoclaving of pipette tips would influence the pipetting result.

Recent scientific literature reported evidence of disturbance of a broad range of biological assays caused by leachables, even for pipette tips [1, 2]. Examples include enzymatic and photometric assays as well as alterations in growth rates in cell culture [3]. Eppendorf does not make use of such additives and provides certificates to this effect. However, in order to obtain experimental proof, we have tested Eppendorf pipette tips for inhibition effects on the development of early stage embryos by performing a Mouse Embryo Assay.

Materials and methods

Autoclaving was performed by applying standard methods [4]. The influence of autoclaving on the performance of pipette tips by different manufacturers was determined by calibration [4] with the Eppendorf Xplorer® pipette. The MEA test was performed by an accredited and FDA-registered laboratory [4].

Results and discussion

Autoclaving is a widely applied method for the decontamination of labware such as pipette tips. Thus, one would expect autoclaving to have no impact on the tip's performance. On the contrary, Fig. 1 shows that autoclaving did exert a negative influence on the performance of the tips by manufacturers B, D, and I. Indeed, the system of pipette and tip passed calibration with tips by these manufacturers prior to autoclaving but exceeded the permissible error limits for the systematic error at 1 µL after autoclaving.

For the production of good tips, the impact of autoclaving is already taken into account during the construction phase. The type of polypropylene (PP) and the shape and surface structure decide how much a tip will shrink and in which direction this will occur. Recycled materials typically contain higher concentrations of leachables,

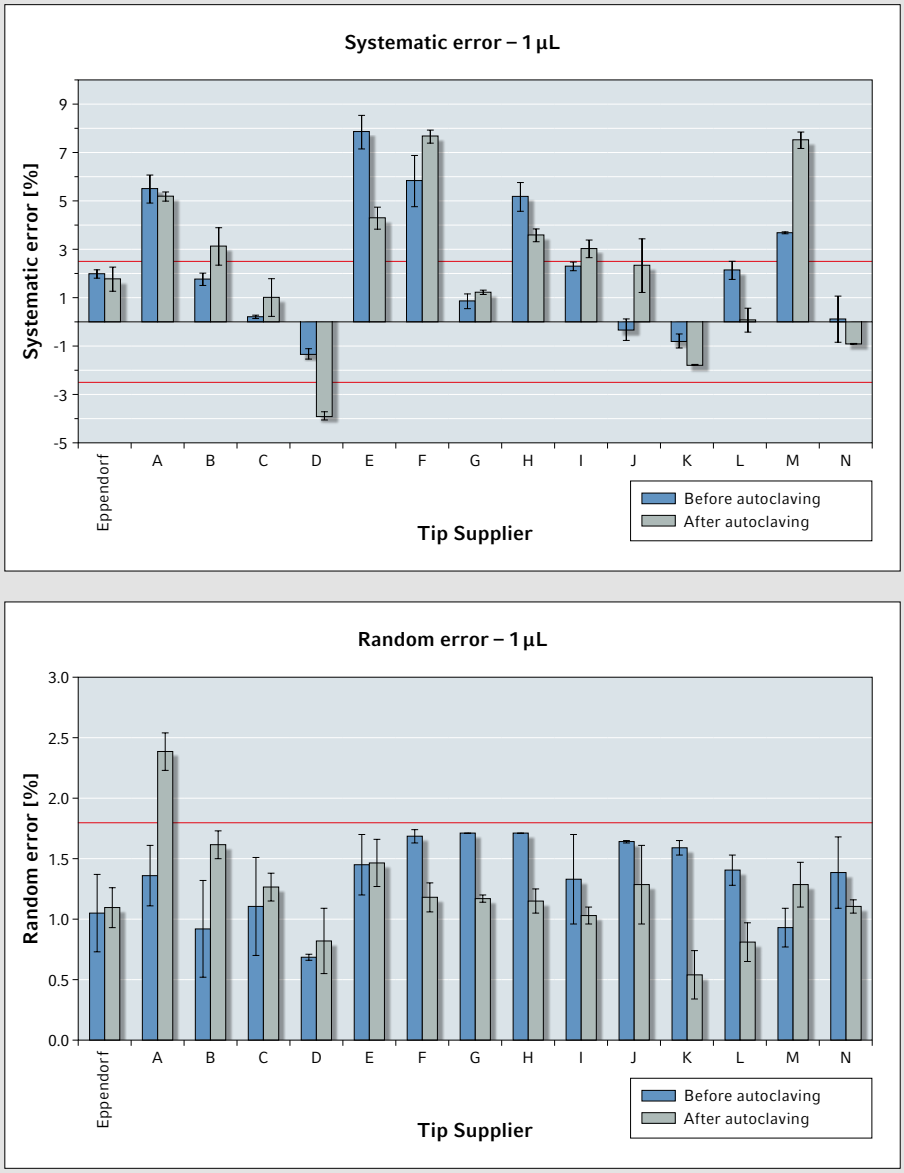


Fig. 1: Calibration results at 1 µL with non-autoclaved and autoclaved 10 µL pipette tips by different manufacturers. Each red line marks the error limits for the Eppendorf Xplorer pipette.

The Tip of the Iceberg: How Pipette Tips Influence Results. Part 3: Not Every Tip Tolerates Every Treatment

Results for MEA test of Eppendorf tips			
	Extraction time		
Pipette tip	10 pipettings	4 h ± 15 min	24 h ± 2 h
epT.I.P.S.® 2–200 µL Biopur® Batch-Nr. D158054Q	90 %	90 %	87 %
epT.I.P.S.® 50–1000 µL Biopur® Batch-Nr. D157726P	90 %	87 %	100 %

Table 1: Results of the MEA test with epT.I.P.S. in Biopur quality. The test is considered passed if the test item has no effect on the growth and development of at least 80 % of tested embryos.

and they may also alter the tip characteristics, for example shrinking behavior during autoclaving. For these reasons, Eppendorf does not use recycled material, nor does Eppendorf reuse material from discarded products.

Furthermore, Eppendorf is very careful with the addition of additives to the polypropylene. Plastics in general need additives in order to ensure certain desired characteristics, e.g. prevention of fast decomposition. These additives cannot be avoided. On the other hand, additives such as slip agents, biocides, and plasticizers are only included to make the production process faster and cheaper. Such additives can be avoided – if the producer has the required expertise and ability to allow a more expensive production process.

Eppendorf pipette tips are made of pure, virgin polypropylene. The material is free from any plasticizers, UV stabilizers, latex, slip and antistatic agents, silicones, bisphenol A, and biocides. Mouse embryos are very sensitive to molecules such as additives from plastics and are therefore good indicators of toxicity such as that posed by leachables. We found no influence on the growth of two-cell embryos towards the blastocyst stage by the Eppendorf pipette tips (Table 1).

Conclusion

The quality of production directly influences tip performance at the user's lab bench. Production does not start with melting the plastics but rather with the tip's design phase. It is absolutely necessary to consider other uses of the product besides pipetting water, such as autoclaving. The international standard ISO 8655 [5] requires re-calibration of the system pipette plus tips if tips of other manufacturers are used. Based on our results, we recommend that laboratories using autoclaved alternative tips calibrate the system after autoclaving. In contrast, with Eppendorf tips, no negative influence on the calibration result was observed after autoclaving. Therefore additional calibration is not required.

Expertise and the determination not to minimize production costs at the expense of quality are essential in the production of pipette tips which will not leach additives. Eppendorf provides certification for the absence of additives which interfere with biological assays – and rightfully so: It has been shown that the epT.I.P.S. examined here did not influence a sensitive biological system.

In the fourth part of this series the influence of tip change on pipetting results will be explained (BioNews No. 47).

Literature

[1] Olivieri A, Degenhardt OS, McDonald GR, Narang D, Paulsen IM, Kozuska JL, Holt A: On the disruption of biochemical and biological assays by chemicals leaching from disposable laboratory plasticware. *Can J Physiol Pharmacol* 2012; 90(6):697-703

[2] Watson J, Greenough EB, Leet JE, Ford MJ, Drexler DM, Becastro JV, Herbst JJ, Chatterjee M, Banks M: Extraction, identification, and functional characterization of a bioactive substance from automated compound-handling plastic tips. *J Biomol Screen* 2009; 14(5):566-72

[3] Grzeskowiak R, Gerke N: Eppendorf White Paper 26: Minimizing the influence of plastic consumables on the laboratory worflows. www.eppendorf.com

[4] Art M, Dufey V, Gast U, Gligor I, Koch L, Kubasch R: Application Note 354: The Tip of the Iceberg: How Pipette Tips Influence Results. www.eppendorf.com/appnote354

[5] DIN EN ISO 8655:2002. Piston-operated volumetric apparatus. Beuth-Verlag, Berlin, Germany

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

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

Introduction

The ultimate goal in bioprocess development is large-scale commercial production. Production-scale process optimization is usually cost prohibitive, so optimization is often performed in smaller scale fermentors and the process is then transferred to pilot scale. We used *Escherichia coli* (*E. coli*) fermentation to demonstrate the scale-up capabilities of Eppendorf fermentation systems from small scale (1 L) to bench scale (10 L) and pilot scale (100 L). To determine suitable parameters for the operation of each fermentor, we investigated engineering parameters critical for scaling-up, such as vessel and impeller geometry, oxygen transfer rate (OTR), power number, and impeller power consumption per volume (P/V). We scaled-up *E. coli* fermentation using the constant P/V strategy. The fermentation runs at each of the three scales produced very similar biomass yields over time, indicating excellent scalability within the Eppendorf fermentation system product family.

Materials and methods

Equipment

The Eppendorf fermentation systems used in this project are shown in Fig. 1.

Investigation of engineering parameters

OTR is the rate at which oxygen is transferred from air to the liquid in a vessel. Since oxygen is often the limiting factor during aerobic fermentation, it is important to select equipment of different sizes with similar OTR capabilities so that the small scale success can be replicated at the large scale. We conducted the OTR measurements using a previously published OTR protocol based on sulfite depletion [1], with the exception of air flow, which was set to 1.5 VVM to match the specification of the BioFlo 610.

The (impeller) power number, Np (also known as Newton number, Ne) is a dimensionless number associated with different types of impellers. Np is commonly used to calculate impeller power consumption during bioprocess scale-up [2]. We experimentally determined the Np by measuring impeller torque using a rotational torque sensor, and calculated the power number using the following equation [2]:

$$\text{Power Number (Np)} = \frac{2\pi(M - M_d)}{\rho N^2 d^5}$$

M: Torque (with full working volume of DI water), (N·m)
M_d: Torque (empty vessel), (N·m)
ρ: DI water density = 1,000 kg/m^3
N: Agitation speed (rps)
d: Impeller outer diameter (m)

It is a common practice in scale-up studies to determine power numbers without gassing. However, gassing greatly reduces impeller torque, and since typical fermentation experiments are conducted under high gassing conditions, we obtained Np under a high gas flow of 1.5 VVM in addition to under "no gassing" conditions.

The purpose of determining Np is to calculate the impeller power consumption per liquid volume (P/V, W/m^3) using the following equation [3]:

$$P/V = \frac{Np \cdot \rho \cdot N^3 d^5}{V}$$

ρ: DI water density = 1,000 kg/m^3
N: Agitation speed (rps)
d: Impeller outer diameter (m)
V: Full working volume (m^3)

E. coli fermentation

We used 90 % of the vessels' maximum working volumes. *E. coli* (ATCC® 25922GFP) was cultured in a chemically defined medium of pH 7.0. We inoculated the growth medium with an inoculum volume of 10 % of the initial fermentation medium volume. The OD_{600} values of the inocula were typically about 12.

To maintain a constant working volume throughout the fermentation process, a continuous fermentation method was used, and volumes of *E. coli* culture identical to the volume of feeding medium added were removed upon feeding. We monitored cell growth offline using samples taken every hour.

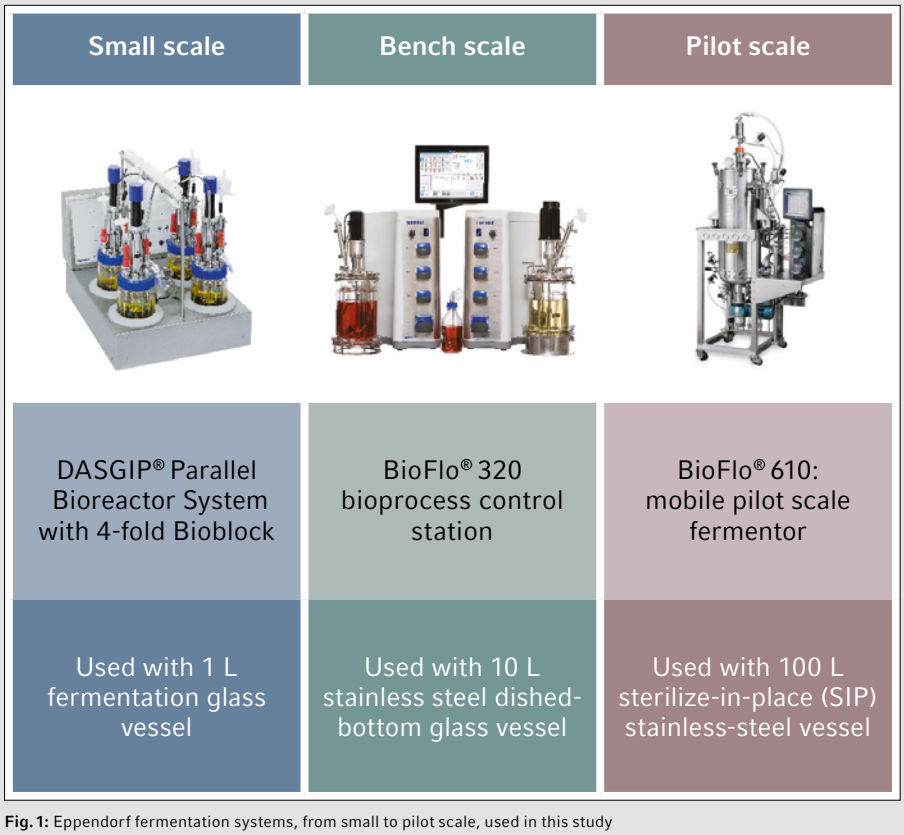


Fig. 1: Eppendorf fermentation systems, from small to pilot scale, used in this study

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

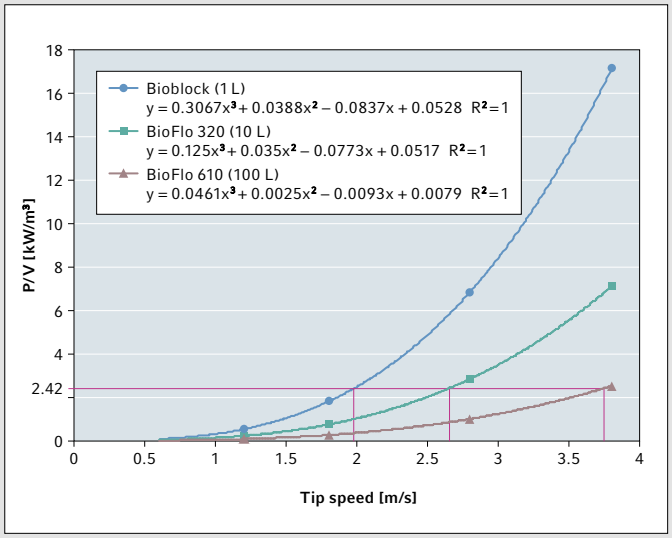


Fig. 2: Determining the constant P/V values for scale-up under 1.5 VVM of air flow

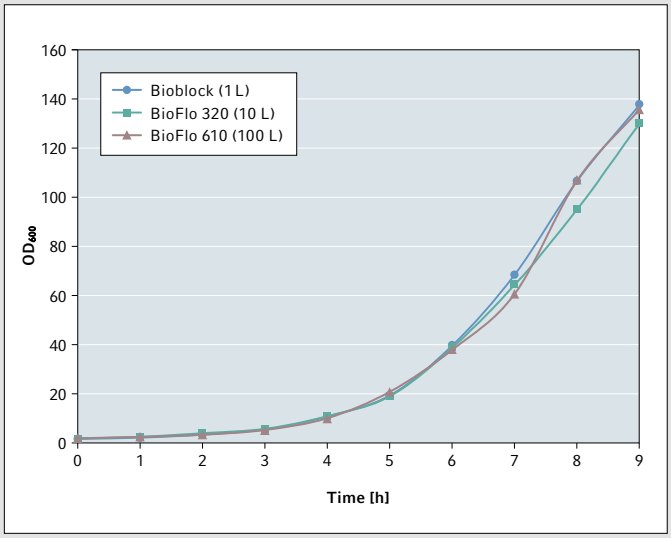


Fig. 3: Fermentation biomass growth curves among all three systems. Fermentations were carried out using a constant P/V value of 2.42 kW/m³, which was determined from Fig. 2.

Results and discussion

All three Eppendorf fermentation systems from small scale to pilot scale were designed following the same vessel and impeller geometrical principles, which laid a good foundation for successful fermentation scale-up. All three systems achieved high levels of OTR, ~350 mmol/L/h or higher. This allowed scale-up fermentation runs to be carried out at high capacities, delivering matching biomass growth curves at very high bacterial densities.

Scalable geometry and matching high OTR provided the foundation and the framework for high density fermentation scale-up experiments, but they did not constitute the scalability strategy in itself. Various strategies have been used for fermentation scale-up including constant tip speed, but the most reliable method to date has been constant power (P/V). It requires the determination of impeller power numbers.

Np is a constant under turbulent conditions [4]. Np numbers varied slightly at different tip speeds, but they were very similar to each other and the average could be considered a constant in guiding fermentation scale-up. We used the Np values obtained under 1.5 VVM air flow. We curve fit the data points and added trend lines (Fig. 2).

The maximum P/V achievable by all three scales was ~2.42 kW/m³, which we selected to be the constant P/V value governing the fermentation scale-up.

Back-calculating the agitations from this P/V value determined that 822, 600, and 433 rpm were the agitation values to be used for Bioblock 1 L, BioFlo 320 10 L, and BioFlo 610 100 L, respectively.

We took samples hourly to monitor the cell growth (OD_{600} value). As shown in Fig. 3, the growth curves from all three fermentation runs produced very similar profiles, indicating that excellent scalability had been achieved using the constant P/V scale-up strategy.

Conclusions

Maintaining constant P/V between different vessel sizes in fermentation scale-up from Bioblock 1 L to BioFlo 320 10 L to BioFlo 610 100 L produced nearly identical *E. coli* growth curves, providing solid proof for the scalability of Eppendorf fermentation systems. In addition, the Np values of the impellers can be used for further scale-up or scale-down studies between Eppendorf and the stirred-tank fermentation vessels of other manufacturers.

Literature

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

[2] Löffelholz C, Husemann U, Greller G, Meusel W, Kauling J, Ay P, Kraume M, Eibl R, Eibl D Bioengineering Parameters for Single-Use Bioreactors: Overview and Evaluation of Suitable Methods. *Chemie Ingenieur Technik*, 2013, 85(1-2):40-56.

[3] Zlokarnik M Scale-up in Chemical Engineering. Second, Completely Revised and Extended Edition. p. 114, 2006.

[4] Raju KS Fluid Mechanics, Heat Transfer, and Mass Transfer: *Chemical Engineering Practice*. 2011.

The complete version of the Application Note 306 can be downloaded at www.eppendorf.com/appnote306.

TANJA MUSIOL, EPPENDORF AG

Proper Handling of Cells in the Lab (II)

What do I have to consider when a new cell line arrives in the lab? How do I establish a cell bank? These are just two of the questions that may arise in the context of establishing a new cell line in the laboratory. Especially if the new cell line was not obtained from an official cell bank, a number of things need to be taken into consideration before these cells are ready to be integrated into routine operations.

Based on data by ICLAC (International Cell Line Authentication Committee), certain estimates state that globally 15–20 % of all cell lines are misidentified or no longer correspond to the original cell line. In the light of these numbers, efforts exist which aim to make cell line authentication mandatory. For example, in early 2016, the NIH (National Institute of Health, USA) released a new guideline stating that only those projects will be funded which employ authenticated cells whose identity has been verified in advance. Furthermore, journals are increasingly leaning towards requesting proof of this nature prior to accepting an article for publication.

How can it be that such a high proportion of cell lines is contaminated with other cells? On the one hand, contamination of cells with cells of different origin is hardly noticeable during routine work. On the other hand, many researchers are not even aware of this type of danger.

This is an unfortunate combination. The risk of contaminating a cell line with foreign cells is just as high as the risk of bacterial contamination. For this reason, sterile technique is always top priority.

In addition, other measures help minimize the contamination risk: New cell lines should be kept in quarantine until their identity has been verified. Prior to transferring a new cell line to routine operations, a master cell bank and a working cell bank should be established. The master cell bank will also serve as a reference for regular verification of those cells used in daily routine, which can help determine whether cells have changed over time. Complete documentation of the individual batches is just as important as the selection of the most suitable method for cell line authentication.

If the basic techniques for proper cell handling in the laboratory are followed, the risk of cross-contamination or a misidentified cell line can be considerably reduced.

Additional information

Additional information around the topic "Working in Cell Culture", as well as links to reference data banks, are available at www.eppendorf.com/cellexperts.

Now online! Bundled expert knowledge all around cell culture: www.eppendorf.com/cellexperts (see also p. 12–13)

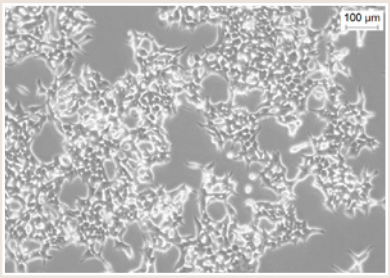
Tip

Performance Test: Eppendorf Cell Culture Consumables

The tissue culture (TC) treated surface of Eppendorf Cell Culture Consumables was tested in regard to cultivation results of the following cell types:

> Primary astrocytes
> Primary chondroytes
> Primary keratinocytes
> Primary melanocytes
> Human mesenchymal stem cells
> LNCaP
> MCF-7
> NIH-3T3
> Vero
> HUVEC
> HeLa
> HepG2
> COS-7
> CHO-K1
> HEK 293

Cells were seeded in appropriate culture medium. Plates were incubated at 37 °C with a humidified 5 % CO_2 atmosphere. Cell growth and cell morphology were monitored on day 1, 2, and 3 post-seeding.*



HEK 293 cells three days post-seeding in an Eppendorf 6-Well Cell Culture Plate

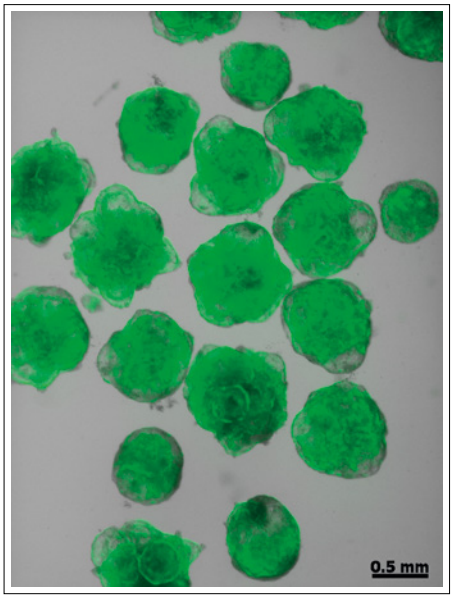
Result: All cell types cultivated in an Eppendorf Cell Culture Plate with TC treated surface showed optimal attachment and homogeneous growth.

*Cultivation conditions and results for each cell type can be found in our detailed Application Notes on the Eppendorf Cell Culture Plates internet page in section „Downloads/Notes & Papers".

ULRIKE BECKEN, EPPENDORF AG, BIOPROCESS CENTER EUROPE, JUELICH

EU Funds Stem Cell Technologies for Heart Repair

Cell-based regenerative medicine holds great promise for the treatment of degenerative diseases, like neurodegenerative diseases, muscle dystrophy, macular degeneration, heart diseases, and others. The concept is intriguing: Damaged tissue in the body is replaced with lab-grown tissues or cells. As starting material for their production adult cells taken from the patient himself are used. To do so, adult somatic cells are first reprogrammed back into a pluripotent state into so called human induced pluripotent stem cells (hiPSCs), and subsequently these cells are differentiated into the desired cell type.



Cystic cardiac speroids of up to 1 mm size rhythmically contracting while floating in suspension culture. The speroids were generated from human pluripotent stem cells differentiated in stirred-tank bioreactors. Green fluorescence indicates cell differentiation into cardiomyocytes (Kempf *et al.* 2014, 2015).

Eppendorf is project partner

Today regenerative medicine is not routine, but researchers all over the world are working on making this kind of therapy reality. Some of them now combine their strengths in the pioneering project "Tools and *TECHNO*logies for *B*reaktrough in h*EA*rt *T*herapies" (TECHNOBEAT), coordinated by the Hannover Medical School, Germany. It will be processed by a pan-European and interdisciplinary consortium of eight partners from industry and science, one of them being Eppendorf.

The scientists, medical experts, and engineers will cooperatively develop effective tools and innovative methods aiming at the production of cardiac microtissue for regenerative medicine.

The project is funded with almost 6 million Euros by the European Union within its Research and Innovation program Horizon 2020. Within the TECHNOBEAT project researchers will explore the use of hiPSCs for the treatment of heart diseases.

In the context of this EU project, DASGIP® GmbH, an Eppendorf company, and the Eppendorf AG Bioprocess Center in Juelich, Germany, will develop innovative bioreactor solutions designed especially for the cultivation of hiPSCs in large scales. "We are pleased to have the Eppendorf Bioprocess Center in the TECHNOBEAT project team. In a long-standing cooperation we already have successfully developed bioreactors for the cultivation of 100 mL hiPSCs", states Dr. Robert Zweigerdt, Principal Investigator at Hannover Medical School and TECHNOBEAT coordinator. "Now, the exciting challenge is to adapt the existing product design to the needs of stem cell cultivation in a larger volume of 1 L", annotates Katharina Kinast, responsible Product Manager Bioprocess at Eppendorf.

Development engineers and product managers at Eppendorf will create novel impeller and vessel designs to optimize hiPSCs culture mixing and shear characteristics.

System-integrated filtering technology will be engineered. Holographic microscopy (industry partner Ovizio Imaging Systems NV/SA, Belgium) will be integrated as well.

According to Katharina Kinast this will "altogether enable tight control and real-time monitoring of cell aggregate formation". With its experience in polymer production and bioreactor design, Eppendorf will further contribute to establishing a GMP-conform hiPSC production process using single-use bioreactor technology.

TECHNOBEAT can provide groundbreaking findings and methods that may revolutionize cell-based heart therapies. „In the future, the microtissues may be grown outside the body in bioreactors and then injected into the patient's damaged heart as a cell implant for curative treatment", outlines Robert Zweigerdt. "Hundreds of patients with cardiac diseases waiting for an organ transplant may benefit from this technology."

The TECHNOBEAT project builds an integrated and application-oriented research approach. For the first time, the consortium combines technologies enabling the mass production of stem cells under defined quality criteria, a strategy for growth of implantable microtissue, methods for evaluation of implant success in mammals as well as designated clinical expertise in cardiology, cardiac surgery, and multimodal imaging.

DASbox® Mini Bioreactor System equipped with BioBLU® Single-Use Vessels

By the cross-border networking of specialists and know-how, TECHNOBEAT supports the expansion and competeveness of the biomedical sector in Europe. The project is funded for a period of four years. Apart from the Hannover Medical School and Eppendorf, the following Institutions and companies are partnering the project: the Medical Centers at the universities of Leiden and Utrecht (The Netherlands), the University of Sheffield (United Kingdom), the Paracelsus Medical University (Austria) and the industrial partners Ovizio Imaging Systems NV/SA (Belgium) and Kadimastem Ltd. (Israel).

Stem cells – from Cell Culture Consumables to bioreactors

As a workflow-oriented provider of lab equipment, Eppendorf offers instruments, consumables, and accessories that perfectly fit the processes in the lab. Scientists working with stem cells benefit from comprehensive solutions for cultivation, genetic engineering, characterization, development, and scale-up. Dishes, flasks, and plates with significantly improved design increase safety and consistency. Traditional cell culture consumables and incubators are indispensable at the research and development stage. However, certain applications require up to 1×10^{10} cells and it is difficult to produce such high cell numbers in conventional, two-dimensional culture systems. Stirred-tank bioreactor systems have clear advantages for the large-scale expansion of cells. They require less lab space than the corresponding number of cell culture flasks, and bioreactors of different sizes but with comparable vessel geometries ease process scale-up. Furthermore, bioreactors allow to monitor and control critical environmental parameters, which is crucial for the development of stable, reproducible processes, and a valid documentation.

In proof of concept studies Eppendorf has already demonstrated the suitability of its bioprocess controllers and BioBLU single-use bioreactors for the cultivation of mesenchymal stem cells in working volumes up to 3.75 L and for cultivation of hiPSCs in working volumes up to 100 mL. Within the TECHNOBEAT project Eppendorf will contribute its know-how in polymer production and bioreactor design to help establish a GMP-compliant, large-scale hiPSC production process using single-use bioreactor technology.

News

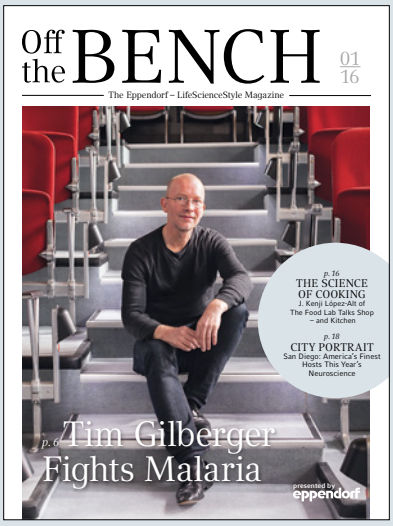
"Off the Bench" – the New Eppendorf Magazine

October 2016 saw the release of the first issue of our LifeScienceStyle Magazine "Off the Bench". In addition to exciting interviews with personalities from science and research, the magazine presents the reader with career tips as well as with lots of information and links about the city hosting the next large life science exhibition. You will also find news from the exciting world of Eppendorf. Entertaining, well-researched, and definitely outside the box.

In order to receive future issues of the magazine free of charge, please write to us at: magazine@eppendorf.com or register online at corporate.eppendorf.com/de/news-media.

Discover "Off the Bench" online at www.eppendorf.com/OTB.

Look forward to the next issue of "Off the Bench" in April.



BERRIT HOFF, EPPENDORF AG

Now Online! Bundled Expert Knowledge All Around Cell Culture

Is my cell culture medium free from contamination? How do I verify the identity of my cell lines? How do I ensure reproducible results? We have learned from numerous conversations that many researchers deal with these and other, often recurrent, questions. A team of Eppendorf cell experts has taken this opportunity to present expert knowledge and the latest findings on the topic of cell handling on a new online platform.

The Eppendorf cell experts

"Every member of this team of application specialists, product specialists, and product managers is backed by in some cases years of experience in the cell culture laboratory and is intimately familiar with the problems at hand", says Project Leader Tanja Musiol. "We quickly agreed that information on this topic area was widely dispersed across the internet and often hard to find. In a case of acute need, in-depth research will quickly turn into a time-consuming exercise for the user", adds Product Manager Samira Schroeder.

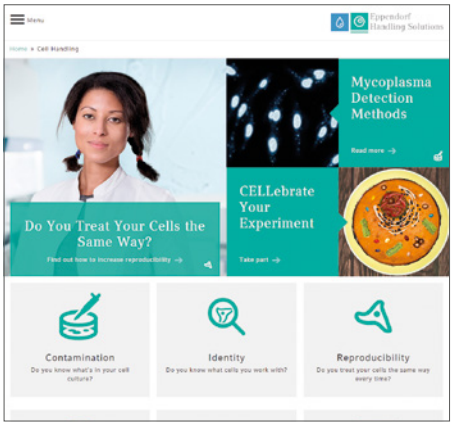
Goal and approach

The common goal was the creation of a central platform which would support the cell culture users in a competent manner during their daily work. "We performed in-depth research and viewed diverse materials. We combined our results and determined those areas which required additional information. We then went ahead and closed those gaps by creating the additional materials ourselves in the form of text, info-graphics, images, or video", explains Tanja Musiol. The result is a data base of knowledge around cell cultivation. It was processed accordingly and compiled on the new website

www.eppendorf.com/cellexperts

Visitors will find sound, comprehensive content on the topics of contamination, identity, and reproducibility. Jennifer Schlichting, Project Manager Communication: "Following an introduction to the topic, we clarify the scientific background and offer recommendations for action for each workflow."

Eppendorf cell experts (left to right): Samira Schroeder, Wolf Wente, Nadine Mellies, Jessica Wagener, Tanja Musiol, Carrie Hipolito, Jennifer Schlichting



Now online: Bundled expert knowledge all around cell culture: www.eppendorf.com/cellexperts

www.eppendorf.com/cellexperts

"This new site is all about cell handling in the laboratory", says Carrie Hipolito, Project Manager Online. "In addition to the information researched by the cell specialists, we make documentation, photos and tutorials, info-graphics, interactive content, and links to external references and data bases available." Tanja Musiol: "The common goal is to present the researcher with comprehensive information, bundled and easily accessible."

Topic 1: Contamination

Everyone working in cell culture fears contamination with bacteria and knows how to detect bacteria under the microscope. In the worst case, contamination is so obvious that the media become turbid and change color. Other types of contamination, as well as their sources and methods of detection, are not as well known. These are explained in detail, supported by images.

A high priority is placed on the prevention of contamination during daily workflow routine: "With the help of a virtual, interactive laboratory, we point out hot spots of possible contamination and offer tips on how to avoid such contamination", says Jessica Wagener, Application Specialist. "This is supported by resources such as video tutorials, Application Notes, and White Papers as well as templates for cleaning schedules for instruments used in the cell culture laboratory."

Topic 2: Cell line identity

Are you sure about the cell line you are working with? Misidentified cell lines may enter circulation through labeling errors, or they may originate from cross-contamination. In order to check whether a cell line is authentic or not, multiple factors must be taken into consideration. Several methods exist which can verify the identity of a cell line.

"STR-profiling is definitely the most widely known method", explains Application Specialist Wolf Wente. "The individual methods are explained in detail in our new online portal. This is also where we provide tips on how to establish a new cell line in the laboratory."

www.eppendorf.com/cellexperts presents you with overviews on how to establish a master and a working cell bank as well as links to useful resources such as ICLAC (International Cell Line Authentication Committee), DSMZ (German Collection of Microorganisms and Cell Cultures), and the NCBI (National Center for Biotechnology Information).

Topic 3: Reproducibility

Ensuring the reproducibility of experiments in cell culture is not as easy as one may expect. "For example, if serum is added to the medium for cell cultivation, this constitutes a biological raw material which is subject to natural variation", explains Application Specialist Nadine Mellies. For this reason, the new Cell Handling portal covers this topic with information and tips on how to make culture conditions as reproducible as possible and, as a result, the data obtained from two experimental series as comparable as possible.

A dynamic concept

Similar to the established Liquid Handling portal www.eppendorf.com/pipetting, the new www.eppendorf.com/cellexperts is a dynamic site. New seminar offers and webinars all around the topic of cell culture are continuously added. The column "Did you know?" is host to a growing list of answers to questions frequently asked by researchers.

Tanja Musiol: "Our work continues in order to keep the portal exciting and relevant! We therefore value each opportunity for contact with users in seminars and at exhibitions as well as on social networks. We want to learn about the questions of each person working in a cell culture laboratory."

The virtual, interactive cell culture laboratory on www.eppendorf.com/cellexperts
Click on the blue dots to learn more about each contamination hot spot.

CAROLYN TAUBERT AND BERRIT HOFF, EPPENDORF AG

Welcome to Eppendorf: Shigeki Watanabe & Adrian Liston

Shigeki Watanabe. To learn more about his work visit www.watanabelab-emanias.com.

The Japanese scientist Shigeki Watanabe, Ph.D., Assistant Professor at Johns Hopkins University, Baltimore, USA and winner of the 2015 *Eppendorf & Science Prize for Neurobiology* visited Eppendorf in spring 2016. Shigeki Watanabe had won the prize for his work on synaptic vesicle endocytosis (more info including a video and a podcast at www.eppendorf.com/prize).

We asked Shigeki Watanabe what surprised him most about our headquarters as well as the Eppendorf pipette and consumables production plants.

"I imagined that the production would be automated but I didn't think about the scale. The machines were so huge. A spectacular view! I was surprised by the effort that goes into the details of the products. It was also a great opportunity for me to learn about Eppendorf's history and how the company has evolved over the last 70 years."

Shigeki Watanabe is convinced that winning the prize will help his career and research. He started his own research group at the beginning of 2016 and is now applying for funding and recruiting people to his new lab.

A few weeks later, the 2016 winner of the *Eppendorf Award for Young European Investigators*, Prof. Adrian Liston, Group leader at VIB Translational Immunology Lab, University of Leuven, Belgium, visited Eppendorf. Adrian Liston and his team work on both the discovery (Treg biology, diabetes) and applied (genetics, human disease) aspects of translational immunology (see also www.eppendorf.com/award).

"I was really surprised to see that the PCR machines were so lovingly put together by hand, more an engineering enterprise than a factory floor. At the other end of the scale, the plastics factory was almost complete automation. But even there the almost obsessive attention to quality was obvious – with most of the set-up dedicated to quality control."

Both winners were presented with a personalized pipette with their names laser printed on it. Adrian Liston: "I haven't done any pipetting for seven years now, but the pipette has a place of honor on my desk!"

Prof. Adrian Liston with his personalized pipette. More info on Adrian Liston's work at http://liston.vib.be.

Win a Multichannel Pipette!

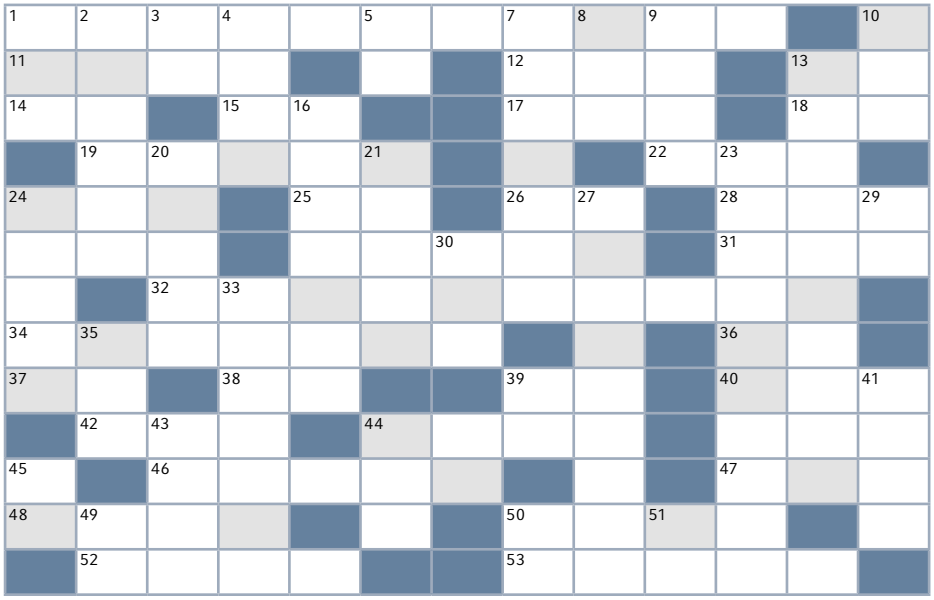
The solution of the prize competition of BioNews No. 44 was „In Touch With Life". Julia Ma (Baruch S. Blumberg Institute, Doylestown, PA, USA) won the first prize.

Good luck in our new 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, 2017**.

You can either send us 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. 48.



1st Prize:

1 Eppendorf Reference® 2 Pipette (multichannel, adjustable-volume) 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 Rude, insolent
11 Enduring, steady, constant
12 Welcome or farewell in Latin
13 ISO country code for Sweden
14 ISO country code for Oman
15 Reverse transcriptase (abbrev.)
17 Opposite of min., also a male given name
18 European Community (abbrev.)
19 Piece of classic music
22 Polymerase used in PCR
25 Chemical symbol for aluminum
26 Chemical symbol for barium
28 Large open vessel
31 American actress (first name)
32 The "G" in NGS
34 Listed in the periodic table (sing.)
36 ISO country code for the Seychelles
37 Second largest city in the USA (abbrev.)
38 Chemical symbol for iridium
39 Polypropylene (abbrev.)
40 Female given name
42 Unit of pressure
44 Winnie who?
46 Brief appearance of a celebrity in a single scene of a movie
47 The self of an individual
48 Small missile aiming at the bullseye
50 Russian male given name
52 Adolescent
53 Internet-related prefix

DOWN

1 Identified flying object (abbrev.)
2 Flower closing its leaves when touched (genus name)
3 Public Relations (abbrev.)
4 Turkish male given name
5 Chemical symbol for tantalum
7 Country in southern Africa
8 Female given name
9 Nearest or adjacent to
10 Abbrev. for December
13 The "S" in NGS
16 Coach, sports instructor
20 Infectious, replicates itself within bacteria
21 Science-fiction classic film (1979)
23 Gas layer around a celestial body
24 Famous prize founder (family name)
27 Cellular recycling mechanism
29 ISO country code for Bulgaria
30 Method, skill, facility
33 Territory ruled by an emir
35 Facility to conduct scientific research
39 Longest river in Italy
41 Smallest unit of an element
43 Unit of area used in certain English-speaking countries
44 Writing instrument
45 Anno Domini (abbrev.)
49 ISO country code for Austria
50 Integrated circuit (abbrev.)
51 River in western Siberia

Solution hint for prize competition of BioNews No. 46:

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Send us the solution until **June 30, 2017**, via e-mail to **bionews@eppendorf.de**, or participate online at **www.eppendorf.com/bn-service**.

10 ways that *Science* Careers can help advance your career

1. Register for a free online account on **ScienceCareers.org**.
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5. Watch one of our many webinars on different career topics such as job searching, networking, and more.
6. Download our career booklets, including Career Basics, Careers Beyond the Bench, and Developing Your Skills.
7. Complete an interactive, personalized career plan at "my IDP."
8. Visit our Career Forum and get advice from career experts and your peers.
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