BIO NEWS
No. 52 – 2020

eppendorf

Eppendorf Conical Tubes 25 mL: The Next Level

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> Eppendorf Digital Solutions: Digitize your sample management
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Application Notes

Optimized bacterial culture and plasmid purification with Eppendorf Conical Tubes 25 mL · hMSCs: Reliable and robust expansion on CCCadvanced® FN1 motifs surface · etc.


Optimization of Bacterial Culture and Plasmid Purification with Eppendorf Conical Tubes 25 mL

Dear Readers,

Do you often work with sample volumes larger than 15 mL but significantly smaller than 50 mL? And are you sometimes annoyed about having to resort to a conventional 50 mL tube, which is actually too large for your sample? Then you can look forward to the new Eppendorf Conical Tubes 25 mL offering multiple advantages. This tube type is considerably shorter, allowing for improved handling as well as space savings during storage. The resulting lower use of raw materials during production helps save resources while reducing laboratory waste. Brand new: a tube version with snap cap enabling single-handed opening and closing. Read more on pages 4–5.

Gerald Vallentin from Leipzig, Germany, has worked with Eppendorf products for about 48 years. Before retiring, he sat down and wrote an amazing review about his lab career (pages 11–12). Thank you for sharing, dear Mr. Vallentin!

Sample labeling, sample storage, sample safety, sample identification, sample documentation, sample management: Two articles provide you with valuable information and suggestions how to handle your precious samples (pages 6–7 and page 9).

As always, many other reports and four new Application Notes on diverse topics round off this BioNews issue.

We hope you like it!

Your Eppendorf BioNews Team

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

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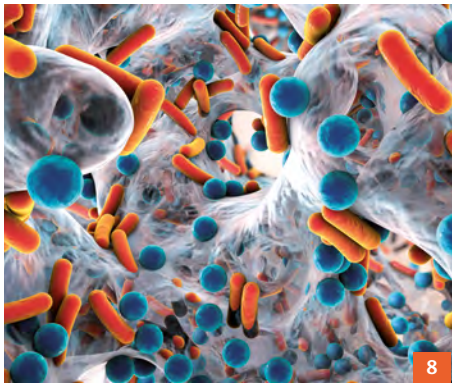


Eppendorf BioNews Application Notes


Optimization of Bacterial Culture and Plasmid Purification with Eppendorf Conical Tubes 25 mL

BRIGITTE KLOSE & BERRIT HOFF, EPPENDORF AG

Eppendorf Conical Tubes 25 mL: The Next Level

Specifically for sample volumes larger than 15 mL, but significantly smaller than 50 mL, Eppendorf has developed a conical tube in the 25 mL format. It closes the gap between the conventional conical tubes with volumes of 15 mL and 50 mL, respectively. This innovative vessel type is available with either screw cap or the patented SnapTec® cap. A complete system of accessories allows the use of the 25 mL tube throughout the different steps along the workflow.

The new 25 mL format (center) closes the gap between the conventional conical vessels with volumes of 15 mL and 50 mL, respectively.

During the culture of bacteria and other microorganisms, as well as for purification of plasmids/biomolecules, in cell culture, or during the preparation of assays, volumes between 15 mL und 25 mL appear to be the predominant sample size.

For lack of alternatives, users will often resort to conventional 50 mL tubes, fully aware that these are actually too large, and that therefore – annoyingly – valuable raw materials will be wasted. Not to mention the storage space that vessels of this size claim in the freezer.

A not entirely unwarranted question

"Why, then, is there no conical tube with a volume between 15 mL and 50 mL?" was a frequently asked question throughout laboratories worldwide – a question that we repeatedly encountered when speaking with our customers. The developers at Eppendorf were only too happy to come up with an answer.

Our solution

Specifically for sample volumes above 15 mL, but significantly smaller than 50 mL, Eppendorf has developed the Conical Tube 25 mL. It is available with the new patented SnapTec* cap or as a screw cap variant. Like all other variants of Eppendorf Tubes®, the 25 mL tubes are manufactured from high-quality raw materials, without the use of slip agents, plasticizers, or biocides.

*Protected by European Patents EP 2 965 816 A1, EP 2 654 958 A1

Saves space and resources

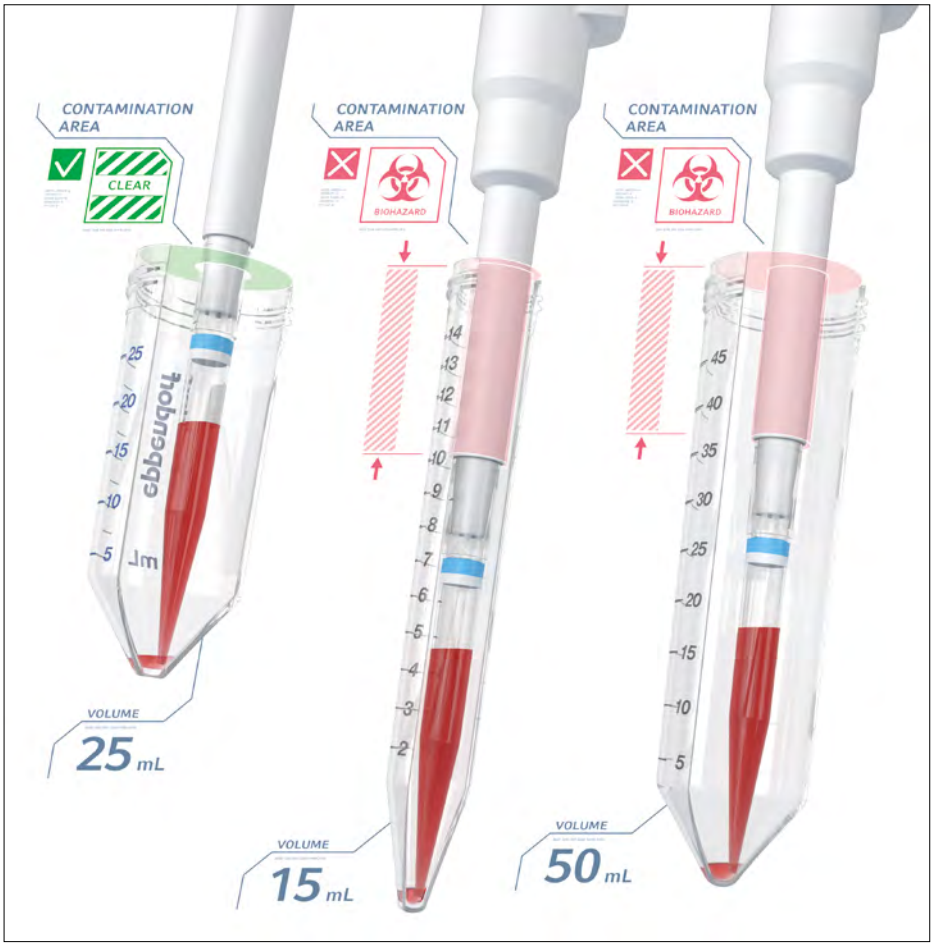
The 25 mL tube format features the same diameter as conventional 50 mL conical tubes, whereas their height has been reduced by 20 %. This not only saves storage space. Thanks to the lower height, the amount of valuable raw materials required for producing the SnapTec cap variant could be reduced by 20 %, and by even 26 % for the screw cap variant, as compared to conventional 50 mL conical tubes. A noticeable avoidance of laboratory waste, and a responsible utilization of resources.

Improved sample processing

The previously mentioned wide opening, combined with the reduced height of the 25 mL tube, facilitates access to the sample while at the same time improving sample recovery. When working with low-volume pipettes and tips in particular, the risk of cross-contamination between pipette and tube through contact with the inner tube wall has been minimized.

Screw cap or snap cap?

The screw cap is grooved, which ensures safe and ergonomic handling. In addition,



Improved sample access, improved sample recovery

its sides are flattened, preventing the lid from rolling off and allowing upright placement on the laboratory bench. The contamination risk is thus minimized. The users of the Eppendorf Conical Tubes 15 mL and 50 mL already trust in this intelligent lid design.

SnapTec cap enables single-handed opening and closing

The patented SnapTec cap of the 25 mL tube is unique among conical tubes. The lid is securely connected to the tube, and it therefore never comes into contact with the laboratory bench. The risk of cross-contamination is reduced, and accidental confusion with other lids has been ruled out.

The SnapTec cap allows single-handed opening and closing for quick liquid sampling or sample addition. Especially during multi-step laboratory protocols, this feature will save time and, as a result, costs. In addition, the 25 mL tube with SnapTec cap is autoclavable.

SnapTec: put to the test

Plasmid purification from bacterial culture is a mainstay in life science laboratories. Media preparations are typically carried out in 15 mL conical tubes with screw cap. The Eppendorf application specialists researched whether improvements could be achieved using the 25 mL tube format.

In a low copy plasmid DNA extraction, they were able to demonstrate that the use of 25 mL tubes with SnapTec cap led to considerably higher productivity of the bacterial culture. It also resulted in a higher DNA yield in comparison to using conventional 15 mL conical tubes with screw cap. More on this topic on pages 1–2 of the Application Notes inside this issue.

Consistent system concept

With all our love for the tiniest technical detail, it is our primary concern that every one of our tubes may be seamlessly integrated in existing laboratory workflows. As such, a complete system of accessories for centrifugation, heating, mixing, automation, sample preparation, and storage allows immediate use of the 25 mL tube.

Conclusion

The new member of the extensive family of Eppendorf Tubes closes the gap between the conventional conical vessels of the 15 mL and 50 mL volumes, respectively. A complete system of accessories allows the use of the 25 mL tube in the different steps along the workflows.

We have visualized the outstanding strengths of the Eppendorf Conical Tube 25 mL

> Single-handed operation (SnapTec cap)

> Reduced contamination risk

> Improved sample access, improved sample recovery

> 30 % space saving during storage

in a short video. Simply scan the QR-code or enter bit.ly/2LhxTKd

Find additional information on our landing page www.eppendorf.com/25mL, where you can also order a free sample.

ANN-CLAIRE FOETSCH & JAN-HENDRIK BEBERMEIER, EPPENDORF AG

Your Sample Goes Digital

Have you ever estimated the value of the samples stored in your freezer? Assuming an average 10 EUR per vial, the total value easily amounts to 500,000 EUR. In other words: your freezer is a true treasury! Careful sample labeling will simplify your life when it comes to storing your precious samples in a safe manner while at the same time allowing for easy identification and access.

Not in the mood to label all 20 tubes? Typing it all up by hand while you have so much more to do? Numbering from 1 to 20 would also be an idea. There must be a simpler method! If these thoughts are familiar to you, then Eppendorf has the answer: Eppendorf Digital Lab Solutions.

Suffering from illegible samples?

Clear sample labeling is recommended to make reading as easy as possible. There is complete agreement on this in all labs, but, in reality, you find vessels in your freezer without any labeling or with illegible labeling. One of the main reasons is that reliable sample labeling is usually a painstaking task.

Smart labeling of your high-value samples is crucial for safe identification and ultimately for reliable results. Printed labels on vessels in plain writing are the minimum required for safe reading. Barcodes go one step further toward fast and clear sample identification.

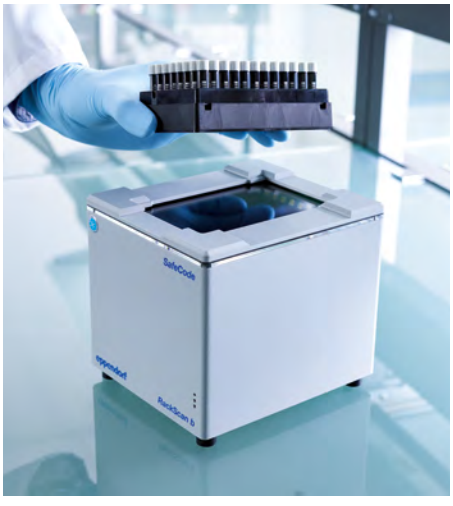
From 2020 onwards, unreadable samples will be a problem of the past. Eppendorf will offer you pre-labeled off-the-shelf consumables for immediate use. Your samples go digital by receiving a reliable long-term label for safe sample identification.

You have more than one sample?

Now, you have taken the first step. Your samples are labeled in a smart and readable way. The next hurdle you need to overcome is storage. When storing your samples, it is vital to keep them safe and accessible. Your colleagues need to be able to find the samples they are looking for. Grab the rack, load it with your pre-labeled vials, and easily scan your samples in parallel. The RackScan instrument for data matrix-coded samples offers a comfortable and easy plug-in system which helps to read more than one sample. The RackScan instruments work perfectly in combination with the Digital Lab Solutions from Eppendorf – the eLABInventory and eLABJournal® software.

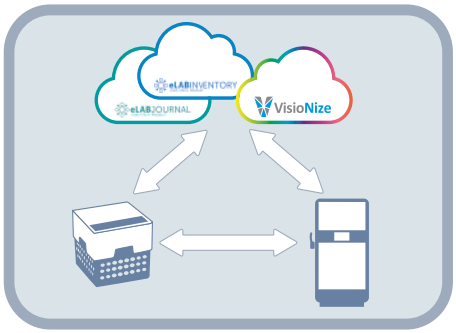
Lost in samples and processes?

Now you can combine accurate barcode labeling of your samples with records of your treasures in the freezer. By using the eLABInventory software, you can easily manage your samples. The software is aware of free spots in your freezer and the barcode reading assures easy sample identification, so you won't lose track of your samples. Your colleagues will thank you. eLABJournal also documents all your working steps in the lab. What else could you want?



Concerned about your sample safety?

With our CryoCube® F740hi freezer, concerns about sample safety are a thing of the past. The freezer is equipped with our VisioNize® touch interface, allowing you to check freezer performance whenever you want – directly on-site. In case of a power outage or a temperature loss, a visible and audible alarm is a common



standard for a freezer. With VisioNize you will experience even greater safety for your stored, digitized samples because you will receive alarm as well as event notifications – no matter where you are. Monitor your freezer remotely from wherever you want and decide the next steps in case of an alarm. Safe sample storage – 24/7, enforced by digital remote surveillance.

Digitize your sample management path

eLABInventory

Switch from paper-based lists or spreadsheet files to intuitive and safe sample management software and take advantage of the structured organization of your samples. eLABInventory is provided by Bio-ITech (a member of the Eppendorf group). Data from barcoded vessels like the CryoStorage Vials with SafeCode™ can easily be integrated into the software. Supplementary documents can be stored as well. By using external label printers, you can also generate your own customized barcodes with eLABInventory. Collect all your information about your valuable samples online – safely and securely.

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Tip

Spin the Bottle

If throughput matters in your lab, capacity is key. Increase capacity and throughput with large volume centrifuge bottles for Centrifuge 5910 R. The new 400 mL and 1,000 mL centrifuge bottles are a reusable solution for large-volume, low-speed applications. This includes harvesting of bacteria, algae, yeast, and mammalian cells. The 1,000 mL bottle was specially designed for swing-bucket rotor S-4xUniversal and upgrades Centrifuge 5910 R into a 4-liter system. The 400 mL bottle fits into swing-bucket rotor S-4x400.

The new bottles provide comfortable and ergonomic handling. They have a tight seal and are easy to open and close. For a low risk of contamination bottles and lids are autoclavable, UV-resistant, and dishwasher-proof. Low opacity of polypropylene allows high sample and pellet visibility.

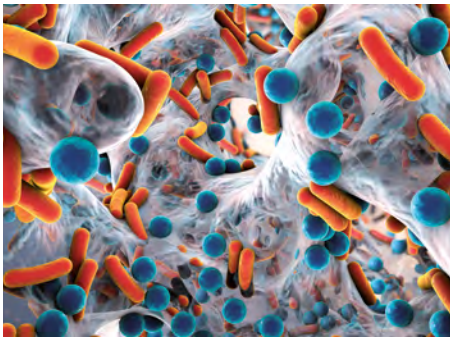
For safe centrifugation of hazardous samples bottles are fully compatible with optional aerosol-tight caps.

Visit Centrifuge 5910 R landing page
www.eppendorf.com/next-benchmark

HANAË KÖNIG, EPPENDORF AG

The Advantages of Standardization in Microbiome Research

Microbiome research yields valuable knowledge that is helpful in the diagnosis and treatment of diseases. This area of research, however, is extraordinarily complex, and it places high demands on the researcher and the laboratory. Standardization of processes and methods, as well as appropriate laboratory equipment, play a crucial role in providing reliable, reproducible conditions for sample processing.



Analysis of the microbiome can reveal information about the health status of a patient.

We share our world with billions of microscopic single-celled organisms – bacteria. They live on us, in us, and all around us – from the depths of the oceans, all the way into space. The composition of the bacterial population, the microbiome, is unique as well as characteristic and essential to each habitat. Analysis of the microbiome, therefore, has the potential to reveal information about the health status of a patient. If the species and the numbers of the bacteria are out of balance, supporting "good" bacteria may solve multiple problems. However, to identify deviations among different microbiomes, and to determine what is healthy and what is not, large datasets are needed.

Success factor standardization

In order to guarantee the statistical validity of data, standardized processes concerning sample handling, as well as consistently high sample quality, are imperative. Further to sample acquisition and storage, the concept of standardization applies to extraction methods, to the processes and the instruments which are used to generate the Next Generation Sequencing (NGS) library, as well as to the consumables and the thermocycler that is used for the PCR.

Several laboratories from universities and industry are now collaborating on large-scale projects dedicated to microbiome research. It is therefore even more critical that all collaboration partners work with the same methods and instruments to be able to compare their data and draw accurate conclusions.

To prevent errors from occurring during sample processing, and to significantly increase reproducibility, tedious dosing tasks are carried out by automated pipetting systems like the ep*Motion*®.

Automated pipetting systems like the ep*Motion* carry out tedious dosing tasks.

Unlike manual sample processing, standardized automated methods for the purification of DNA/RNA and the generation of the NGS library consistently yield comparable, high-quality results. Since the ep*Motion* also takes care of incubation steps, operators are free to pursue other tasks and thus save valuable time.

A further factor of success is a reliable PCR – it requires a high-quality thermocycler such as, for example, the Mastercycler® X50, which provides the necessary temperature stability, as well as rapid heating and cooling rates. In general, the use of high-quality consumables precludes sample loss and contamination throughout the entire sample processing chain.

Conclusion

Especially for large studies and international collaborations, investments in standardization are proving to be enormously helpful. Standardization protects against inadequate results and it allows the generation of comparable, reproducible data that are valid on a global scale.

Additional information at
www.eppendorf.com/automation

Optimization of Bacterial Culture and Plasmid Purification with Eppendorf Conical Tubes 25 mL

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SANDRINE HAMELS, BLANDINE VANBELLINGHEN, EPPENDORF APPLICATION TECHNOLOGIES, S.A., NAMUR, BELGIUM

Abstract

Plasmid purification from bacterial culture is a commonly performed protocol in molecular biology and life science laboratories. For medium scale preparations, 15 mL conical tubes with screw caps are typically used which often pose handling drawbacks and don't enable achieving optimal productivity of bacterial culture and maximal yields of DNA.

Eppendorf Conical Tubes 25 mL with snap cap address these disadvantages. In this Application Note, we show that the bacterial culture productivity and DNA yields using 25 mL tubes were much higher when compared to standard 15 mL conical tubes and at the same time provided improved handling and performance of the workflow.

Introduction

Bacterial culture and subsequent plasmid purification are undoubtfully one of the most commonly performed protocols in molecular biology and life science laboratories. Despite increasing availability and affordability of plasmid purification kits from numerous providers, the standard method of alkaline lysis [1] is still widely used and remains mainstream in the academic sector. It is cost effective, scalable and typically produces high yields of pure plasmid DNA, which can be directly used in various down-stream applications like DNA digestion, cloning, or sequencing.

For medium scale preparations, 15 mL conical tubes with screw caps are typically used which offer tight lid closure and good centrifugation stability, but often pose handling disadvantages (screw cap handling, cross contamination, difficulty in reaching sample at the bottom of the tube) and often don't enable the optimal productivity of a bacterial culture and maximized yields of DNA – particularly of low copy plasmids.

These disadvantages have been specifically addressed by Eppendorf Conical Tubes 25 mL with snap cap (SnapTec®), which markedly improve one-hand handling, while offering the same safety and intermediate volume range for applications between 15 mL and 50 mL.

In this application, the bacterial culture and subsequent purification of low copy plasmid DNA using standard alkaline lysis protocol were compared using Eppendorf Conical Tubes 25 mL and standard 15 mL conical tubes. The bacterial culture productivity and plasmid DNA yields using 25 mL tubes were much higher and at the same time provided improved handling and overall performance of the workflow.

Materials and methods

Bacterial culture

Escherichia coli bacteria (DH5α, Invitrogen™) transformed with low copy plasmid DNA (pBR322TM, Invitrogen) were cultured in LB medium with ampicillin in triplicates for 16 h (37 °C, 250 rpm, Innova® S44i shaker, Eppendorf).

Cell growth was evaluated by optical density at 600 nm (OD_{600}) using Eppendorf BioSpectrometer®, and *E. coli* cell number was estimated using the following conversion formula:

OD_{600} of 1.0 ≈ 5 x 10^8 cells/mL

Low copy plasmid DNA extraction

Plasmid DNA extraction was performed using standard alkaline lysis protocol: 7.5 mL of bacterial cultures were centrifuged at 10,000 *x g* (5 min, RT) and pellets resuspended in 1.5 mL of solution 1 (50 mM glucose; 10 mM EDTA; 25 mM Tris pH 8.0; 100 µg/mL RNase A). After 5 min incubation, 3 mL of solution 2 (0.2 NaOH; 1 % SDS) were added, further incubated (10 min, ice) and 2.25 mL of solution 3 (3M KOAc, pH 5.4) were added.

Samples were mixed and centrifuged at 17,000 *x g* (30 min, 4 °C). The supernatants were transferred to new tubes, precipitated with same volumes of isopropanol, mixed and centrifuged at 17,000 *x g* (30 min, 4 °C). Pellets were rinsed and resuspended in 200 µL of TE buffer.

For 15 mL bacterial culture the alkaline lysis buffers were respectively scaled up by factor 2. Centrifugation was performed using Eppendorf Centrifuge 5810 R with FA-45-6-30 rotor and respective tube adapters. Plasmid yield and quality were estimated by absorbance measurement at 260 nm (Eppendorf BioSpectrometer).

Optimization of Bacterial Culture and Plasmid Purification with Eppendorf Conical Tubes 25 mL

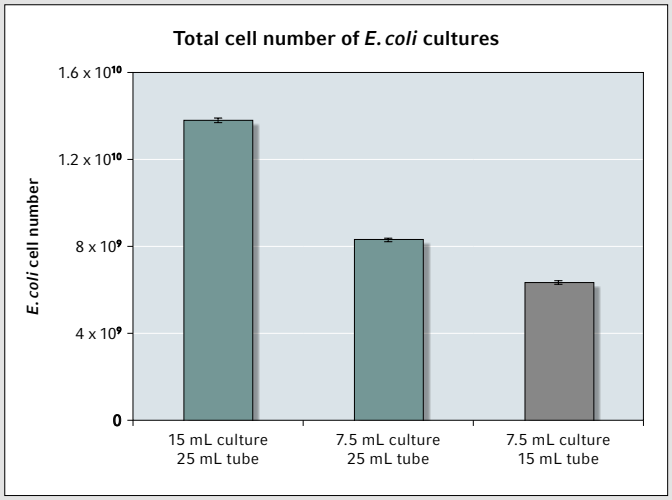

Fig. 1: Total cell number of *E. coli* cultures incubated in Eppendorf Conical Tubes 25 mL with snap cap and standard 15 mL conical tubes

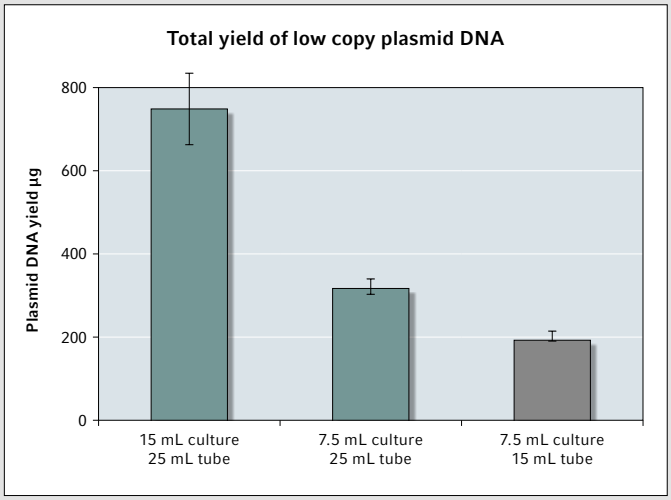

Fig. 2: Total yield of low copy plasmid DNA (PBR 322) purified from bacterial cultures incubated in Eppendorf Conical Tubes 25 mL with snap cap and standard 15 mL conical tubes

Results and discussion

Comparison of bacterial culture growth and productivity is depicted in Fig. 1. Bacterial culture density and total cell number was higher for both 7.5 mL and 15 mL culture volumes incubated in Eppendorf Conical Tubes 25 mL with snap cap. This indicates better growth rates and overall productivity due to more efficient aeration and mixing properties in Eppendorf Conical Tubes 25 mL as compared to standard 15 mL Tubes.

Low copy plasmid DNA extraction

Bacterial cultures were directly used for extraction of low copy plasmid DNA (pBR322, Invitrogen) using standard alkaline lysis extraction protocol. Notably, in this method several high-speed centrifugation steps (up to 17.000 *x g*), as well as mixing and phase collection steps take place. Tight cap closure and safety of Eppendorf Conical Tubes 25 mL was equal to standard 15 mL conical tubes with screw caps.

In addition, snap caps allowed faster tube handling and reduced the cross-contamination risk, which may occur when numerous screw cap tubes are processed in parallel.

As shown in Fig. 2, the plasmid DNA yields obtained using Eppendorf Conical Tubes 25 mL were substantially higher than those obtained with standard 15 mL conical tubes. For 7.5 mL and 15 mL bacterial culture volumes the yield was higher by 70 % and 400 %, respectively, indicating high culture density and improved growth parameters allowing larger production of plasmid DNA. OD ratios indicated highly pure DNA preparations: A260/280 > 1.9 und A260/230 > 2.0.

Conclusion

The results indicate that both the bacterial culture productivity and the resultant plasmid DNA yields obtained in Eppendorf Conical Tubes 25 mL with snap cap (SnapTec), were much higher (70 % to 400 %) as compared to standard 15 mL conical tubes. The significantly better growth rates obtained in 25 mL tubes were due to more efficient aeration and mixing properties of the bacterial culture.

Tight lid closure and safe handling, as well as centrifugation stability of the Eppendorf Conical Tubes 25 mL were equal to those of the 15 mL conical tubes with screw caps, while at the same time providing markedly improved handling for intermediate volume range applications between 15 mL and 50 mL.

In addition, Eppendorf Conical Tubes 25 mL allowed better sample accessibility, and the risk of cross contamination in the purification workflow was reduced, which offers significant handling improvements in various molecular biology and life science protocols.

Literature

[1] Birnboim, HC. A rapid alkaline extraction method for the isolation of plasmid DNA. *Methods Enzymol.* 1983; 100: 243–255.

Easy Automation of Metagenomic Library Preparation with the ep*Motion*® 5073m NGS Solution

SANDRINE HAMELS & ERIC GANCAREK, EPPENDORF APPLICATION TECHNOLOGIES, S.A., NAMUR, BELGIUM
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Abstract

Metagenomic studies can determine the genetic composition of microbiological samples. These studies usually rely on the use of marker genes, such as 16S rDNA, for the phylogenic classification. Which specific marker gene and DNA region will be examined and how deeply sequenced will depend on the aim of the study. Nevertheless, the basic approach of library generation is common to these experimental designs. It is important to have a robust workflow to correctly analyze the samples. In this Application Note we demonstrate such a workflow, which may be customized and scaled to specific customer needs.

Introduction

Scientific questions around the microbiome such as case-control and longitudinal studies require best practices and standardized plus reproducible laboratory workflows to process hundreds, sometimes thousands of samples for the identification and comparison of microbial community structure, composition, and genetics, as well as functional variation [1]. Automation of library preparation not only minimizes the loss of samples, wasted reagents, and sequencing delays, but also reduces inter-operator variability as well as errors in sample tracking. This Application Note describes the automated processing of microbial DNA samples on the ep*Motion* (Eppendorf) into sequencing ready libraries.

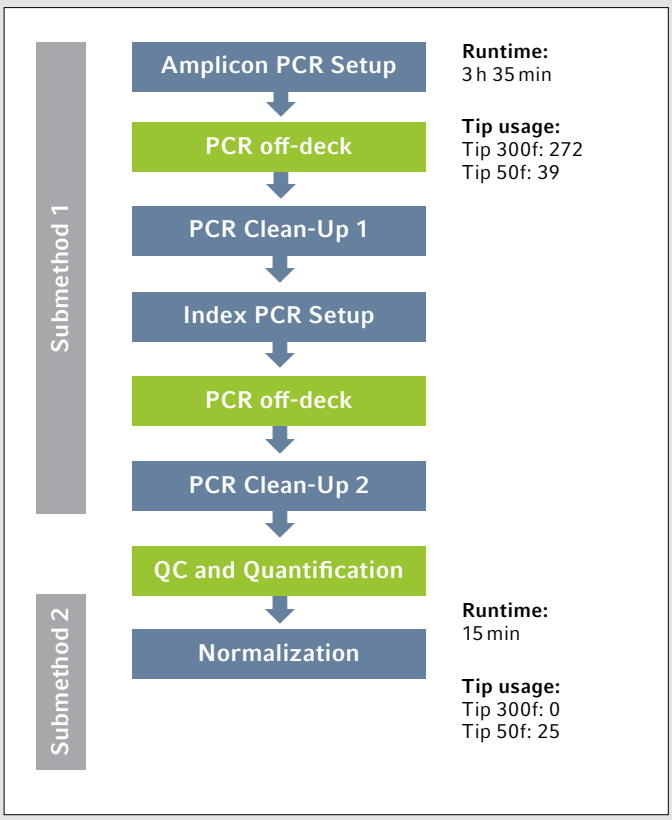

Fig. 1: Demonstrated workflow for Illumina's 16S rDNA protocol on the ep*Motion*. The grey boxes show submethods processed on the ep*Motion*. Steps highlighted in green are performed off-deck. PCR amplifications were performed using the Mastercycler X50 from Eppendorf. In alignment with the protocol, workflows are compartmented into logical units ending at safe-stopping points. Detailed run times and consumable usage are shown next to each submethod.

Experimental design

Libraries were generated using an automated version of an Illumina® protocol [2]. The automated protocol is split into 2 submethods (Fig. 1), each ending at a safe stopping point. All amplifications were performed off-deck on a Mastercycler® X50 from Eppendorf. For the initial experimental setup an input of 12.5 ng genomic *Escherichia coli* MG1655 genomic DNA (ATCC® 700926D5™) was used. 24 samples were prepared per run. For each library, the quality control (library size distribution and quantification) was performed using an Agilent® 2100 Bioanalyzer® DNA 1000 kit. For the second part of the experiment, a mock community of 20 Strain Staggered Mix Genomic Material (ATCC MSA1003™) was used to compare 16 libraries prepared on the ep*Motion* vs. 8 manually prepared libraries.

To complete 24 samples for the automated run, we included a control set of 4 *E. coli* samples and 4 non-template controls. Since the controls showed good results, sequencing of the pooled libraries was performed on an Illumina MiSeq® System using paired-end mode (2x150 bp). Data was analyzed using Illumina's BaseSpace® 16S Metagenomics app.

Results and discussion

Establishing 16S rDNA method using E. coli genomic DNA

Initially we sought to demonstrate the functionality of our approach by amplifying the V3–V4 region of 16S rDNA from *E. coli*. We investigated the variability and yield across different 24 replicates of the same input DNA. The samples clustered tightly with a median amplicon size of 657 bp (CV 0.5 %) and median yield of 218 nM (CV 10.9 %), without primer dimers, suggesting efficient PCR setups and bead purifications.

A factor of potentially high negative relevance to metagenomic studies is cross contamination. To test this, we designed and executed a library preparation run with an alternating set of *E. coli* DNA template and non-template controls (NTC). The resulting gel electrophoresis did not reveal any amplicons in the NTC, but only some primer dimers which did not show in the DNA samples (Fig. 2).

Investigating strain to strain reproducibility using a 20-strain mock community

We thus sought to apply the method established above to test the reproducibility of detecting metagenomic diversity. To this end, we prepared 16 replicates of a 20-strain mock community on the ep*Motion*. For comparison we performed the same experiment on 8 replicates manually. The resulting 24 samples were pooled and sequenced on a 2 x 150 bp MiSeq run. An operational taxonomic unit (OTU) classification was performed

Easy Automation of Metagenomic Library Preparation with the ep*Motion*® 5073m NGS Solution

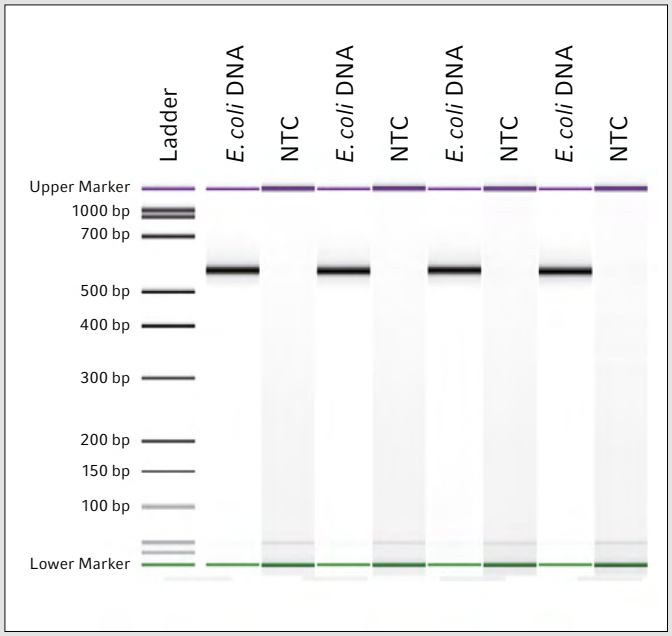

Fig. 2: Automated library construction with alternating *E. coli* and non-template controls confirms absence of cross contamination.

on the resulting files using Illumina's 16S BaseSpace app to check whether the constituent genii of the mock community could be reidentified.

The number of reads obtained from this experiment was high enough for all samples not to affect the % of reads assigned to genus, which was stable averaging at 87.1 %. To assess potential differences in the community calls for manually and automatically prepared samples, a principal coordinate analysis (PCoA) was performed on genus level (Fig. 3). The PCoA showed a very tight clustering of the samples suggesting little to no variation between the manual library preparation and the automated procedure for the assignment of genii. The results shown above qualify the automated method as delivering sequencing libraries similar or better than manually prepared ones.

Conclusion

The increased demand for high-quality NGS libraries in laboratories has necessitated the development of robust, automated methods for library preparation. This Application Note demonstrates the successful and reproducible automation of 16S rDNA amplicon libraries on the ep*Motion* 5073m NGS solution from Eppendorf and equal performance as the manual protocol.

Literature

[1] Knight, R. *et al.* Best practices for analyzing microbiomes. *Nature Microbiology Review* 2018; 16:410-422.

[2] Illumina. *16S Metagenomic Sequencing Library Preparation Guide.* Part # 15044223 Rev. B

Download the full version of this Application Note at www.eppendorf.com/appnote420

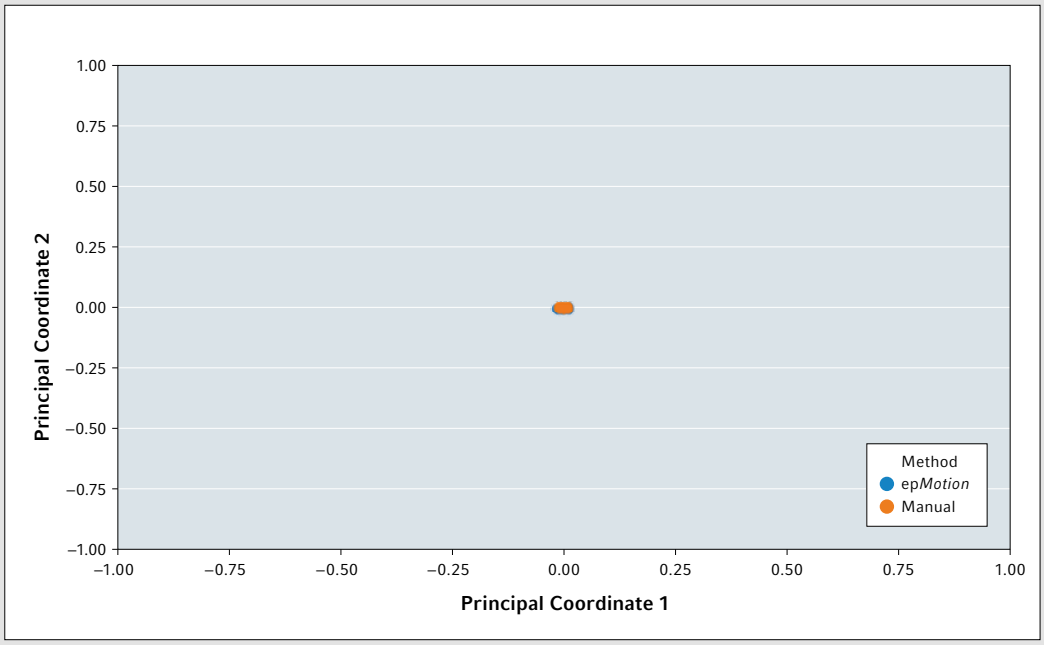

Fig. 3: PCoA on the OTU classification of 24 replicates of the mock community. The principal coordinates are color coded per sample type for the 8 samples prepared manually (orange) and the 16 samples prepared on the ep*Motion* 5073m NGS solution. This scatterplot shows a Principal Coordinate Analysis (PCoA) of the normalized relative abundance of all samples. The PCoA measures differences in the distribution of taxonomic classifications between samples at the genus level. The genus level shows tight taxonomic clustering of results.

Reliable and Robust Animal-Component-Free hMSC-BM Expansion on Ready-to-Use CCCadvanced® FN1 motifs Surface

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NADINE MELLIES, EPPENDORF AG, HAMBURG, GERMANY

Abstract

In the last decade, human mesenchymal stem cells (hMSCs) have generated increasing scientific interest. Prior to their use as a powerful tool for research applications, hMSCs must be expanded without losing their phenotypic properties. This requires stable and completely defined hMSC culture systems consisting of a combination of growth surface and culture medium. Made up of RGD-derived motifs supporting cell attachment, the CCCadvanced FN1 motifs surface from Eppendorf represents a completely synthetic surface for hMSC cultivation in animal-component-free culture conditions. This surface combines convenience with reliable hMSC cultivation: the ready-to-use consumable significantly reduces labor time and effort for scientists while offering a fully synthetic growth surface with a high level of consistency during long-term hMSC expansion.

Introduction

hMSCs consist in a heterogeneous population of multipotent cells isolated from various tissues [1].

Their specific properties such as mesodermal differentiation potential, immunomodulation and secretion of anti-inflammatory molecules make them a promising stem cell population in various basic and applied research applications [2].

Present at relatively low abundance in their tissue of origin, hMSCs require a robust *in vitro* expansion process to obtain sufficient high-quality cell numbers. Traditionally, hMSCs are expanded *in vitro* in presence of serum on a tissue culture-treated (TCT) surface.

Nevertheless, the common use of animal-derived materials such as serum presents several drawbacks [3]. In the absence of serum proteins, hMSCs require additional cell adhesion-promoting coating on the culture surface, but frequently used coatings of biological origin are inherently complex and non-defined, which might impact experimental reproducibility. The FN1 motifs surface is made up of synthetic RGD-derived motifs, specifically designed to mimic the cell attachment site of native extracellular matrix proteins such as fibronectin. Used in combination with synthetic culture medium and dissociation solution, this surface represents an effective synthetic alternative to biological coatings. Being ready-to-use, it constitutes a real improvement for researchers, reducing labor time and effort while offering a better lot-to-lot consistency and reliable performances in comparison to self-coating solutions. Here we show that the FN1 motifs surface is highly suitable for successful short-term and long-term hMSC-BM expansion in combination with various xeno-free media.*

Results and discussion

Efficient short-term hMSC-BM expansion in various xeno-free culture media

The FN1 motifs surface supports efficient hMSC-BM growth in combination with different xeno-free (XF) culture media (Fig. 1). In a traditional serum-containing culture system, hMSCs adhere and proliferate similarly on the FN1 motifs surface and on TCT. On both surfaces, cells exhibit their typical fibroblast-like morphology. In absence of serum, hMSCs present difficulties to adhere and proliferate on the TCT surface, suggesting the requirement of additional cell adhesion-promoting coating.

By contrast, the FN1 motifs surface efficiently supports hMSC attachment and growth, whatever the media tested. Cells show elongated spindle-shaped cell morphology classically observed when expanded in XF culture conditions [4].

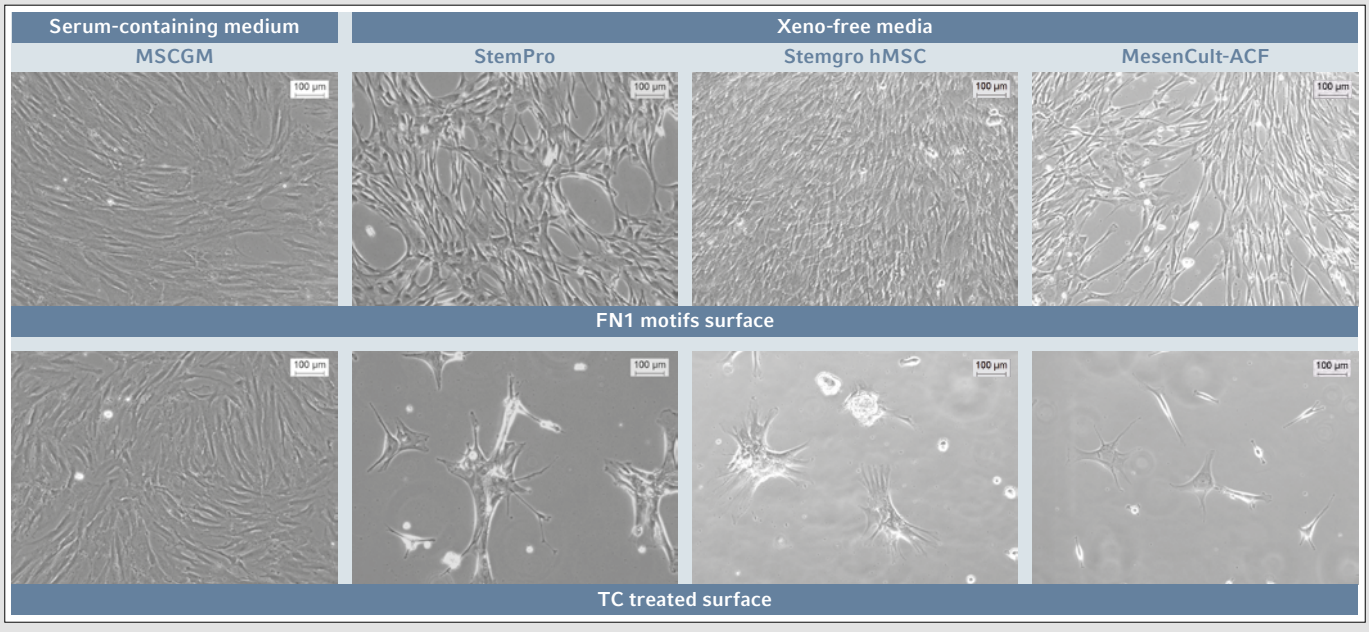
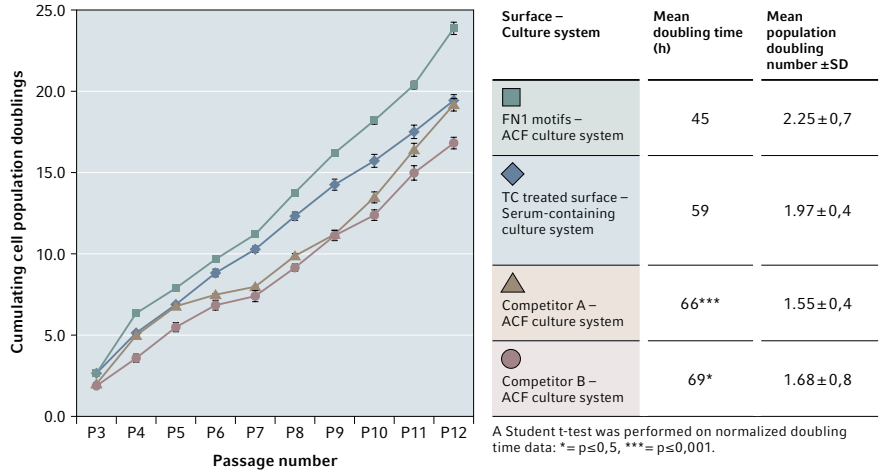


Fig. 1: hMSC-BM morphology after short-term expansion on CCCadvanced FN1 motifs surface in different culture media

Reliable and Robust Animal-Component-Free hMSC-BM Expansion on Ready-to-Use CCCadvanced® FN1 motifs Surface



Surface – Culture system	Mean doubling time (h)	Mean population doubling number ±SD
FN1 motifs – ACF culture system	45	2.25±0,7
TC treated surface – Serum-containing culture system	59	1.97±0,4
Competitor A – ACF culture system	66***	1.55±0,4
Competitor B – ACF culture system	69*	1.68±0,8

A Student t-test was performed on normalized doubling time data: *= p≤0,5, ***= p≤0,001.

Fig. 2: hMSC proliferation rate during long-term expansion in different animal-component-free culture systems

Robust long-term hMSC-BM expansion in a completely defined, synthetic culture system

To confirm that the FN1 motifs surface supports long-term hMSC-BM expansion in a completely synthetic culture system without impacting cell quality, cells were maintained during 10 successive passages on this surface. In parallel, cells were cultured on two other synthetic surfaces (ready-to-use Competitor A and self-coated Competitor B). As a reference, cells were expanded in a traditional culture system (TCT, serum-containing medium and Trypsin/EDTA). The proliferation rates show that the FN1 motifs surface supports robust and stable hMSC proliferation through the entire culture period (Fig. 2). As compared to the other experimental conditions, cells expanded on the FN1 motifs surface present a significantly faster proliferation rate with short doubling time and high population doubling number.

To confirm the characteristic hMSC multipotency maintenance after long-term expansion on the FN1 motifs surface in a synthetic culture system, the ability of expanded cells to differentiate *in vitro* into osteogenic, adipogenic and chondrogenic lineages was assessed.

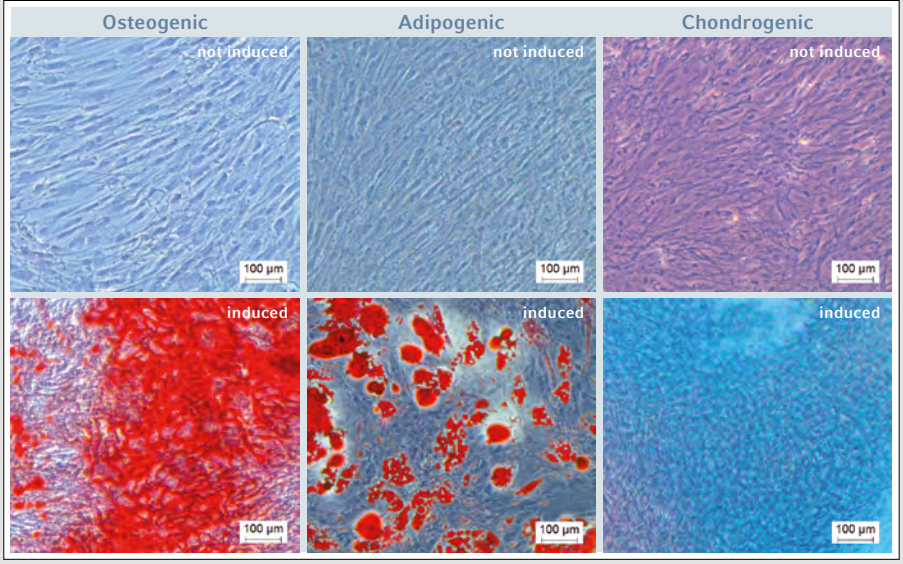


Fig. 3: Multi-lineage differentiation potential of hMSC-BM after long-term expansion on the CCCadvanced FN1 motifs surface in an animal-component-free environment

As suggested by positive specific staining shown in Fig. 3, after successive passages on the FN1 motifs surface hMSC-BM preserve a robust multipotency-associated differentiation potential. In parallel, the hMSC-specific immunophenotype was analysed by flow cytometry analysis (data not shown). Results confirm that even after long-term expansion on the FN1 motifs surface, hMSCs remain positive for the expression of mesenchymal markers and negative for hematopoietic lineage markers.

Conclusion

The ready-to-use CCCadvanced FN1 motifs surface from Eppendorf efficiently supports long-term hMSC-BM expansion in a completely defined, synthetic culture system. All through the expansion process, hMSCs maintain a stable and robust proliferation rate and typical hMSC morphology. Furthermore, after successive passages on the FN1 motifs surface in an ACF culture system, cells retain their typical marker expression profile as well as their multi-lineage mesodermal differentiation potential.

The suitability of the FN1 motifs surface to support efficient hMSC-BM proliferation in combination with different commercial XF culture media has been evaluated with success, facilitating the establishment of a defined environment for hMSC cultivation.

*Please refer to **www.eppendorf.com/appnote390** for detailed materials and methods.

Literature

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Long-Range PCR Optimization with the Mastercycler® X50 from Eppendorf

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Abstract

There is a strong association between the *Helicobacter suis* infection and gastric diseases in animals and humans. The *H. suis* genome sequences can contribute to the understanding of these pathogens' virulence mechanism. Here, we succeeded in the long-range PCR amplification of the genome of non-culturable *H. suis* strains using the Mastercycler X50 from Eppendorf prior to nanopore sequencing.

Introduction

Helicobacter pylori (*H. pylori*), is a Gram-negative microaerophilic bacterium usually found in the stomach and typically associated with various gastroduodenal diseases such as chronic gastritis and gastric ulcers. Infection with *H. pylori* has also been linked to risk of developing stomach cancer [1, 2]. Other than *H. pylori*, several other *Helicobacter* species (collectively called *H. heilmannii*-like organisms [HHLOs] including *H. suis* [type 1 *H. heilmannii*], with *H. suis* being the most prevalent gastric non-*H. pylori Helicobacter* species in humans) are also associated with multiple gastric pathological changes [3, 4]. While *H. heilmannii* gastritis is much rarer than *H. pylori* gastritis, both *H. heilmannii* and *H. suis* infection has been associated with a high rate of gastric mucosa-associated lymphoid tissue (MALT) lymphoma [4, 5, 6].

Diagnostic tests for *Helicobacter* infection include urease test (RUT), urea breath test (UBT), *in vitro* culture, serological and immunohistochemical assays. However, these tests were originally developed and optimized according to *H. pylori* characteristics, vastly reducing sensitivity and specificity of these tests in the detection of HHLOs, especially for human targets.

Not only does differentiating immunohistochemical assays lack HHLO-specific antibodies and antigens, HHLOs typically populate the mucus layer above the surface and foveolar epithelial cells, further limiting the suitability of these methods in diagnosing HHLO infection from biopsies. Furthermore, *in vitro* culture of HHLOs by traditional *H. pylori* culture techniques was found to be highly difficult. Hence, there is a crucial need in developing a sensitive, easy, reliable, and cost-effective method for the detection of these HHLOs in patient biopsy specimens.

Genetic diagnosis by polymerase chain reaction (PCR) is specific, robust, inexpensive, and does not require high technical expertise to operate, making it a highly suitable method for simple clinical diagnosis. This Application Note reports the establishment of an end-point PCR-based method using a *H. suis*-specific primer pair for detecting *H. suis* in gastric biopsy specimens.

Materials and methods

Uncultivable *Helicobacter suis* strains were individually maintained in the stomachs of C57BL/6 mice by repeated inoculations of gastric mucosal homogenates from infected to uninfected mice at intervals of around six months.

Total DNA was extracted from gastric biopsy and mucosa homogenate specimens using QIAGEN® DNeasy tissue kit.

For the preparation of gastric mucosa homogenate, the mouse's stomach was cut along the greater curvature from esophagus through proximal duodenum. The gastric mucosa was mashed by placing it between two ground glass slides, then homogenized with phosphate buffered saline (PBS). The final DNA samples were stored at –20 °C until further use. The following primers were used for the amplification of a 10 kb fragment including an outer membrane protein gene:

Forward Primer

5'-ATAAAGCCCATGAATTCTTAGGCATGCGTGCTCTG-3'

Reverse Primer

5'-TATTCAAGGAAAGTCCCTGGAGAAACTCCAGAGAC-3'

Extracted DNA was used as template for the long-range PCR protocol with KOD FX DNA Polymerase (Toyobo®). PCR reaction master mix was prepared in 0.2 mL PCR tubes using 2x PCR Buffer, 2 mM dNTP mix, 5 µM of each primer, 10 ng of DNA template, and 1 U of DNA polymerase. The final 50 µL volume per reaction was run in Mastercycler X50s according to the settings and cycling conditions in Table 1.

The PCR products were cleaned using the phenol extraction and ethanol precipitation method prior to gel electrophoresis. PCR products were detected via 0.6 % agarose gel electrophoresis using a DNA stain with ethidium bromide and visualized using the FAS-III system (Toyobo).

Long-Range PCR Optimization with the Mastercycler® X50 from Eppendorf

Header (Eppendorf settings)	Lid	105°C
	TSP/ESP	ON
	Lid auto-off	ON
	Temperature mode	Fast
Initial Denaturation		94°C/2 min
Cycles 5x	Denaturation	98°C/10 s
	Annealing + Elongation	74°C/5 min
Cycles 5x	Denaturation	98°C/10 s
	Annealing + Elongation	72°C/5 min
Cycles 5x	Denaturation	98°C/10 s
	Annealing + Elongation	70°C/5 min
Cycles 20x	Denaturation	98°C/10 s
	Annealing + Elongation	68°C/5 min
Post-Cycle Elongation		68°C/10 min
Storage	Hold	22°C

Table 1: PCR cycling conditions and Mastercycler X50s settings for the amplification of 10 kb gene fragment

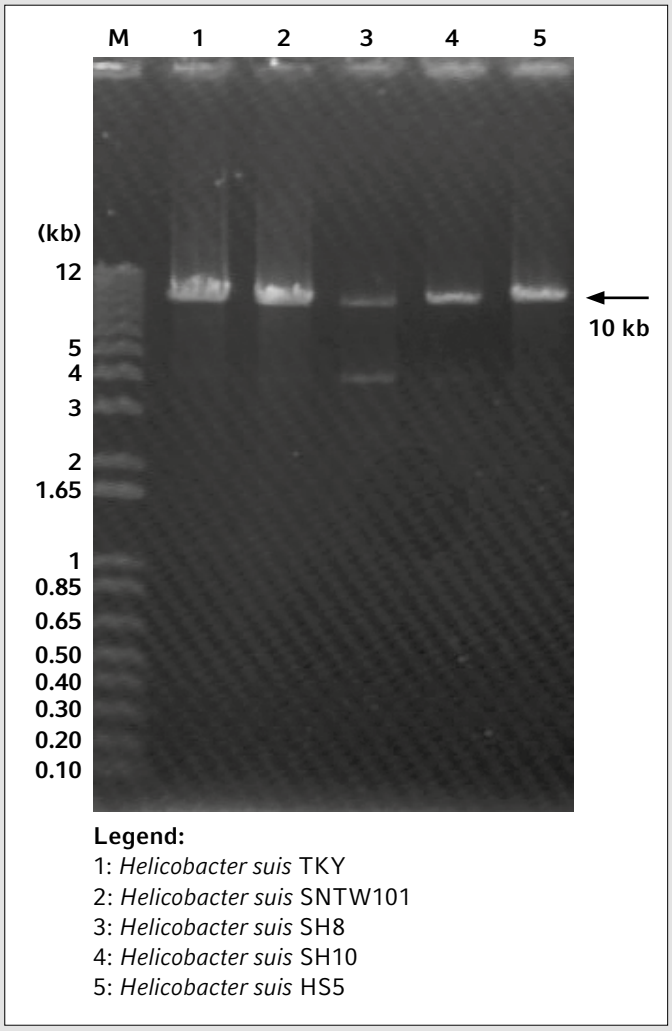

Fig. 1: Long-range amplification of five different *H. suis* strains

Results and discussion

To date, there is a dearth of established genetic-based methods in the diagnosis of HHLOs from human gastric biopsies. The most recent one was published by Blaecher *et al.* (2017) [7], which was a probe-based real-time PCR (RT-PCR) method and reported to exhibit a high degree of diagnostic specificity and analytical sensitivity.

In this paper, we report a robust detection method using end-point PCR technique with a *H. suis*-specific primer pair. Fig. 1 shows that a highly specific 10 kb long PCR product was amplified using the touch-down PCR strategy. This PCR system successfully amplifies DNA from all different strains of *H. suis* isolated from inoculated C57BL/6 mice.

Conclusion

The methods described above can be implemented for detection or isolation of *H. suis* from human gastric biopsies.

Literature

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JAN-HENDRIK BEBERMEIER, EPPENDORF AG

Ice Age for Your Samples?

Sample storage is easy. All you have to do is place your sample containers in the ultra-low temperature (ULT) freezer. However, even this simple step involves aspects that are not typically part of any training or university program, but which are nevertheless important for real life in the laboratory. Visit our knowledge database "Eppendorf Solutions". Here you will find answers to questions about sample storage, as well as content on topics including centrifugation, amplification, cell culture, liquid handling, and photometry.

Many, if not most, biological samples will eventually end up in an ultra-low temperature freezer at –80 °C. But have you ever calculated the combined value of all the samples in your freezer? In an instrument with a capacity of 50,000 samples, the value of your samples, along with the time that you spent generating them, may easily add up to 500,000 EUR.

ULT freezers: more complex than meets the eye

At first glance, these voluminous giants impress through their size and weight, but definitely not through complicated handling. One opens the door, looks for an empty space and places the samples inside. Just close the door, and – done! Done? Well, sometimes there are questions that are not answered by the operating manual (not everyone reads it), the advice of competent colleagues ("Just freeze the sample!"), the textbook (which does not even mention freezers), or the sales rep ("How many instruments do you require?"). Especially when it comes to the selection of a new ultra-low temperature freezer, users find themselves confronted by catchphrases and questions, including:

> Do I really have to read rules, e.g. (EU) No 517/2014 or the California Senate Bill 32, in order to understand whether I should choose CFC, HFC, or HC as a coolant?
> How is water-cooling going to achieve –80 °C?
> What, actually, is the purpose of those coffin-like ULT chest freezers like the ones in the basement?
> Why is it always warm by the freezer? Wouldn't it be better if it were cold?
> How can we improve on our "freestyle" sample storage system?
> Will I be able to find my sample in less than five minutes in the ultra-low temperature freezer?
> What is the meaning of "digital storage" of my samples – is there a computer inside the freezer?
> Will I have to hire a cryptologist to decipher a sample label?

You find these (partly tongue-in-cheek) questions intriguing because you are a scientist or a laboratory technician.

Your research makes you curious. You always want to know and understand everything. Statements such as: "This is the way we've always done it", or a shrug, accompanied by "It is what it is – get used to it", do not really satisfy you.

Achieving clarity for informed decisions

We at Eppendorf believe in the power of science, and we share your enthusiasm. For many decades now, Eppendorf has been developing solutions to problems in order to support scientists in achieving reliable and reproducible results. Countless working hours of engineers, chemists, molecular biologists, biotechnology experts, and other colleagues continue to flow into the development of our products. In this way, we have been able to gather a large pool of valuable knowledge and experience in the areas of Liquid Handling, Cell Handling, and Sample Handling. We are happy to pass this knowledge along.

At www.eppendorf.com/handling-solutions you will find clear answers to many of your questions.

Tip: https://bit.ly/2m3IXBW is the direct link to the topic of storage at –80 °C!

We invite you to contact us with any topic about which you would like to learn more.

GUEST AUTHOR: GERALD VALLENTIN, LEIPZIG, GERMANY

48 Years with Eppendorf: A Lab Technician Remembers

I was innocent, but full of anticipation when, in 1972, I entered the biochemical laboratory as a brand-new apprentice in the field of medicine at the University of Leipzig. Fascinating – all these unfamiliar instruments and chemicals, the white lab coat (of course), and those smells: hydrogen sulfide and mercaptoethanol, mixed in with the scent of rat cage and the vapors of solvents.

The profession of laboratory technician seemed interesting, instructive, and exciting. This impression was to be confirmed from the very beginning. Initially, however, there were some surprises. At that time, students in the German Democratic Republic (GDR) viewed everything coming from the "West" with some suspicion. And there it was: an instrument by "Eppendorf Gerätebau Netheler und Hinz GmbH" from Hamburg!

Fan from the very beginning

In the seventies, the Eppendorf spectral line photometer became my favorite instrument and most important tool. Coinciding with the start of my career, the analytical field experienced a transition from "bucket chemistry" toward microliter-technology. In our area, too, glass pipettes and mouth pipetting gave way to novel modern air-cushion piston pipettes – from Eppendorf (Fig. 1). Attempts at copying these pipettes failed, and the copies were not popular.

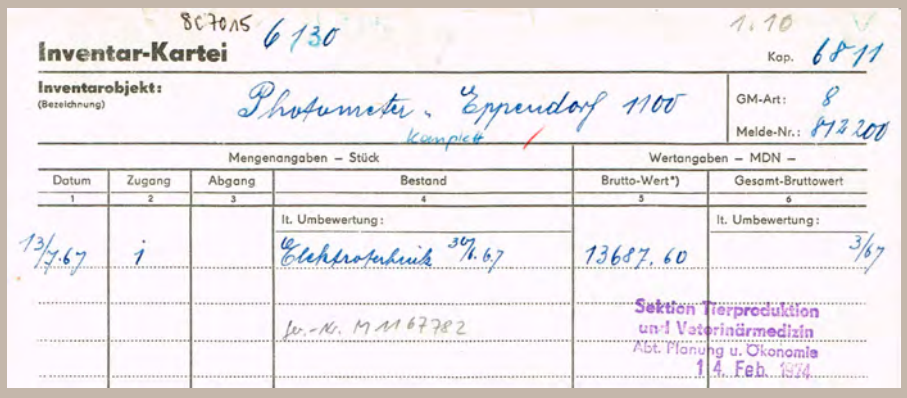
At the same time, the equipment and accessories needed to use the novel Eppendorf reaction tubes were available: rack, shaker, and heating block, as well as the automatic cuvette exchanger for the Eppendorf Photometer and the Eppendorf Thermostat used to control the temperature of the cuvette holder.

Good methods for enzymatic analyses, for example, by Boehringer Mannheim, were available. Over the years, we measured enzymes and substrate concentrations of thousands of blood and organ samples – with the help of analog (light) displays and lots of writing and calculating, in order to determine, for example, changes in extinction per unit of time. How lucky we were to work under such excellent conditions.

Reunion with old friends

After completing my apprenticeship and studies, I joined the Institute of Veterinary Medicine at the university, and in my new position, I was also responsible for the maintenance and repair of our instruments, as well as for the acquisition of spare parts. I was delighted to come across the familiar Eppendorf Photometer in 1980! This instrument, however, was much older – it had been purchased in 1964, and its casing appeared old-fashioned – anthracite-colored and with rounded corners. The purchase price is noted on the inventory card (Fig. 2): 13,687.60 Mark of the GDR. This instrument, which even had fluorescence mea-

Fig. 1: Gerald Vallentin 1979 with modern air-cushion piston-stroke pipette and the Eppendorf Photometer



Inventar-Kartei

Kap. 6811

Inventarobjekt: (Bezeichnung) Photometer „Eppendorf 1100" komplett

GM-Art: 8

Melde-Nr.: 872200

Mengenangaben – Stück				Wertangaben – MDN –	
Datum	Zugang	Abgang	Bestand	Brutto-Wert*)	Gesamt-Bruttowert
1	2	3	4	5	6
			lt. Umbewertung:		lt. Umbewertung:
13/7.67	1		Elektrotechnik 30/1.67	13687.60	3/67
					Sektion Tierproduktion und Veterinärmedizin Abt. Planung u. Ökonomie 14. Feb. 1974
			lf.-Nr. M1167782		

Fig. 2: Inventory card of the Eppendorf Photometer 1100

Fig. 3: Gerald Vallentin on February 25, 2019 with "historic" Eppendorf pipette

surement capabilities, accompanied me into the early 1990s. We measured large series of various endpoint-methods, which was made possible by the Eppendorf suction technology, in combination with the flow-through cuvette. In addition, plasma hormones ACTH and 11-OHCS were captured via fluorescence following processing and derivatization, and acetylcholinesterase activity was analyzed.

Since replacement and expendable parts were import products that came from the "West", the procurement of these parts had to be considered and submitted early, i.e. at least three years in advance. As late as for the year 1990, I had ordered a new mercury vapor lamp and a new photo-multiplier, which were promptly delivered.

After the fall of the Wall, the institute purchased a complete enzyme measurement workstation in 1990. A significant relief, as measured values were now calculated digitally, and the workstation came with cuvette changer, small computer, and a printer. The old photometer, however, remained by my side, and it continued to serve as a display and practice instrument in the education of students and laboratory technicians. There was really no better way to demonstrate the function of a spectral photometer.

Farewell to working life

It is with some regret that after 48 years, I will bid farewell to active employment in early 2020. The laboratory instruments which I had cared for all these years will follow me into retirement – who would be able to use or repair them today? During the scrapping process, I had the idea to offer the old photometer to the company Eppendorf for their museum. This was when I was asked to tell my little story.

And now please take a look at the pipette in Fig. 3. It is the same instrument as the one in the photo above that was taken 40 years ago! Back in the 1970s and today: shapely, elegant, and functional as always. I wish I could say the same for myself …

This proves it: Eppendorf stands for quality! To preclude any misunderstanding: we now work with the most modern equipment. Our Eppendorf instruments, including our benchtop centrifuge, thermocycler, and shaker, as well as our manual and electronic pipettes, are indispensable tools of daily laboratory practice. It is so easy to be content with everything.

With best regards to Eppendorf, and to laboratory colleagues near and far.

Gerald Vallentin

Diploma Engineer, Medical Laboratory Technology, Technical Staff at the Institute of Physiological Chemistry, Faculty of Veterinary Medicine, University of Leipzig

Tip

Keep Your Pipetting Results Reproducible!

Pipettes and dispensers are, depending on their individual use, subjected to different levels of wear, which will inevitably impact precision and accuracy. Only regular, thorough maintenance and calibration can assure the reliability of these precision instruments and therefore the trust in the quality of the results generated.

Worldwide, Eppendorf offers you, either directly or through regional partners, a comprehensive ISO 8655-compliant maintenance and calibration service in ISO 17025 accredited pipetting service laboratories. Highly qualified technicians with more than 200 hours of training utilize calibration software that is validated in accordance with ISO/IEC 17025:2017-11. Depending on the type of pipette, between 10 and 720 data points are generated, either following manufacturer's specifications or in accordance with ISO 8655, using single or multichannel balances, respectively. In addition, a detailed report is issued.

We offer this service for Eppendorf pipettes as well as for more than 4,500 models by other manufacturers. Take advantage of our experience and expertise to keep your pipettes and dispensers in the best possible condition – for reliable, reproducible results.

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BERRIT HOFF, EPPENDORF AG

Eppendorf Award Turns 25 Years

In 2020 Eppendorf will celebrate its 75th anniversary. In the same year, the Eppendorf Award for Young European Investigators will turn 25 years. Since 1995 this prize, which is endowed with a prize money of 20,000 EUR, is granted annually for outstanding contributions to biomedical research in Europe. It is presented in partnership with the scientific journal *Nature*®.

Eppendorf promotes young talents

The Eppendorf Award for Young European Investigators was established on the occasion of Eppendorf's 50th anniversary in 1995 by Dr. Heinrich Netheler, one of the two company founders. The prize was intended to symbolize the close links between Eppendorf and the field of biomedicine and to promote independent and creative research of early career scientists up to 35 years in Europe. Over the last 25 years, the Award has built a prestigious reputation with a long list of renowned awardees.

Independent from the start

From the very beginning, both the application process as well as the winner's selection have been characterized by their independence. Firstly, there is no nomination by third persons; candidates apply on their own initiative.

Secondly, neither Eppendorf nor *Nature* have any influence on the selection of the winner. "When I was approached by the owners of Eppendorf to set up a jury for the Eppendorf Young Investigator Award, I accepted with no hesitation", said the former Jury Chairman Prof. Ernst-Ludwig Winnacker [1].

However, Winnacker imposed two conditions: the jury should be free to select award winners solely on the basis of excellence, with no influence from Eppendorf; and that the award should be restricted to young investigators working in European laboratories.

Special anniversary, special celebration

Eppendorf will celebrate the special anniversary of the Eppendorf Award with the *Young European Investigators Conference* on June 25, 2020 at the EMBL Advanced Training Centre in Heidelberg, Germany. At this event, we will be welcoming back a great number of high-caliber Award Alumni, including the first winner ever – Prof. Steve Jackson from the United Kingdom – to talk about their science and careers and to mingle with the conference participants.

The scientific program is organized by former winners Dr. Simon Boulton (The Francis Crick Institute, United Kingdom) and Dr. Óscar Fernández-Capetillo (CNIO, Spain & Karolinska Institute, Sweden).

The free one-day conference is open to all scientists in biomedical research. The event includes networking coffee breaks and lunch as well as a gala buffet in the evening. It will be rounded off with a talk from the newly minted 2020 Award winner

In 2020 Eppendorf will turn 75 years. Festivities include the 25th anniversary of the Eppendorf Award for Young European Investigators which will be celebrated with the Young European Investigators Conference 2020 (see page 13).

(winner's name to be published in March 2020). The deadline for registration is April 30, 2020 (capacities are limited).

Jury Chairman Prof. Reinhard Jahn of the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany: "I am really excited about the meeting which shows that the science done by previous winners of the Eppendorf Award is indeed outstanding!"

[1] 5th Anniversary publication, Eppendorf AG

More information on the conference at www.eppendorf.com/award/25years

More information on the Award at www.eppendorf.com/award

Presented in partnership with nature

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Young European Investigators Conference 2020

FREE Conference: June 25, 2020 / EMBL Advanced Training Centre, Heidelberg, Germany

The Eppendorf Award for Young European Investigators is granted annually to an early career scientist for outstanding contributions to biomedical research. It has been awarded, in partnership with *Nature*, since 1995.

In 2020, we will celebrate 25 years of this prestigious prize with an event welcoming back Award Alumni to talk about their science and careers. The conference will be rounded off with a talk from the newly minted 2020 Award Winner.

This free one-day conference is open to all scientists in biomedical research. Event includes networking coffee breaks and lunch, and a gala dinner in the evening. Mingle with our Alumni!

Register for free at:
www.eppendorf.com/award/25years

Be inspired by award-winning researchers!

Scientific Program Organizers

Simon Boulton
The Francis Crick Institute • United Kingdom

Óscar Fernández-Capetillo
CNIO • Spain
Karolinska Institute • Sweden

Speakers*

Andrea Ablasser
EPFL • Switzerland

Dario Alessi
University of Dundee • United Kingdom

Silvia Arber
University of Basel & FMI • Switzerland

Mónica Bettencourt-Dias
Instituto Gulbenkian de Ciência • Portugal

Patrick Cramer
Max Planck Institute for Biophysical Chemistry • Germany

Thomas Helleday
University of Sheffield • United Kingdom

Steve Jackson
Gurdon Institute • United Kingdom

Ben Lehner
CRG • Spain

Elizabeth Murchison
University of Cambridge • United Kingdom

Jean Pieters
University of Basel • Switzerland

Luca Scorrano
University of Padova – VIMM • Italy

Michael Wegner
University of Erlangen-Nürnberg • Germany

Georg Winter
CeMM • Austria

Thomas Wollert
Institut Pasteur • France

*Status: August 2019

25 Years

For more information and to register visit:
www.eppendorf.com/award/25years

Tip

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CAROLYN TAUBERT & BERRIT HOFF, EPPENDORF AG

Welcome to Hamburg: Johannes Kohl and Georg Winter

Memories of Hamburg: Johannes Kohl with his personalized pipette

The German scientist Johannes Kohl, Ph.D., visited Eppendorf in spring of 2019. Johannes won the 2018 *Eppendorf & Science Prize for Neurobiology* for his work carried out at Harvard University on the neural mechanisms which underlie how parent mice take care of their offspring. Now in his own lab at The Francis Crick Institute in London, Johannes plans to investigate how pregnancy, stress, sleep, or hunger affect information processing to trigger a specific behavior in mice.

After visiting our production facilities Johannes said, "It was fantastic to see the inner workings of Eppendorf and to meet so many interesting people during my stay. I especially enjoyed touring the production facilities and was very impressed by the attention to detail and the degree of engineering that goes into every single pipette. I returned to the UK with an even higher appreciation for such precision equipment in my lab."

www.eppendorf.com/prize

Georg Winter with Eva van Pelt (Member of the Management Board, Chief Commercial Officer)

A few weeks later we welcomed Dr. Georg Winter of the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria. Georg won the *Eppendorf Award for Young European Investigators* 2019. He received the € 20,000 prize for his work in innovating a generalizable solution to targeted protein degradation *in vivo*. Targeted protein degradation is a new and radically different method in drug development. Georg Winter and his team are pursuing this new therapeutic paradigm toward the ultimate goal of degrading disease-relevant proteins that are thus far deemed "undruggable".

Like Johannes Kohl Georg enjoyed his short trip to Hamburg and the insights he could get while talking to many Eppendorf colleagues from different areas: "I have learnt a lot during my stay and gained many new impressions. It was very exciting to see how Eppendorf products are made."

www.eppendorf.com/award

Win a New Pipetting System

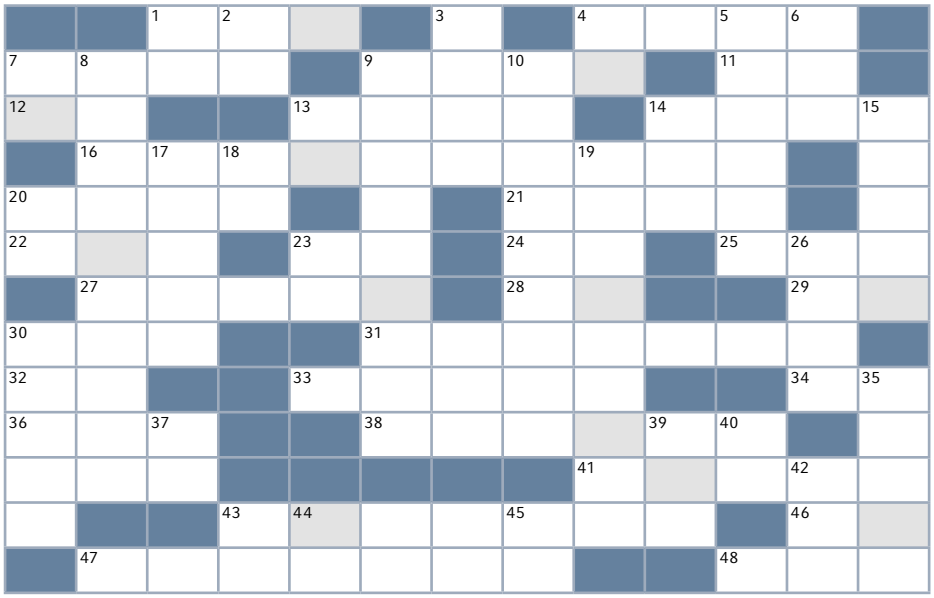
The solution of the prize competition of BioNews 50 was "Eppendorf Conical Tubes Amber". The main prize, a pack with 3 pipettes, went to Robert Barthel in France. Congratulations!

Good luck in our new competition!

Simply arrange all letters in the light gray boxes of the crossword in the correct order. Send us the solution until **June 30, 2020**.

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

All correct answers will be considered for a prize. 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. 54.



1st to 3rd Prize:

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

4th to 6th Prize:

1 Amazon® Voucher worth 50.00 EUR

7th to 15th Prize:

500 bonus epPoints® each

(epPoints registration required)

ACROSS

1 To correct or set right
4 Agricultural property
7 Short journey
9 Contribution in social media
11 Noble gas with a funny impact on one's voice when inhaled (abbrev.)
12 Caracas is its capital (ISO country code)
13 Wife of a raja
14 Paste made from soy beans
16 Group of people of about the same age
20 He developed a technique for staining bacteria
21 Function achieved with Ctrl + Z (or Command-Z)
22 Tetrapods have four (sing.)
23 10^{-3} m (abbrev.)
24 Landlocked country in South-Central Asia (ISO country code)
25 Ocean
27 Place to slalom
28 Post-transition metal (chemical symbol)
29 U.S. state, part of New England (abbrev.)
30 Utilize, employ
31 Victory, success
32 Transition metal used e.g. in corrosion-resistant alloys (chemical symbol)
33 Invented Robinson Crusoe (last name)
34 All correct (abbrev.)
36 Has an electrical charge
38 Athlete
41 Herd animal with black-and-white striped coat
43 Such a cycler is needed for PCR
46 Spanish article
47 Usual strike day for the climate (pl.)
48 Female given name

DOWN

1 Tampere and Turku are major cities in this country (ISO country code)
2 Internet protocol (abbrev.)
3 This Lisa's portrait is on display at the Louvre Museum
4 0.3048 m (abbrev.)
5 Nose-horned mammals (short form, pl.)
6 Spanish for month
7 Mass medium (abbrev.)
8 Backward movement
9 Measurable characteristic
10 Circumstances in a given moment
13 Chemical symbol for element 75
14 Subculture in Great Britain in the early 60ies
15 Official robe
17 Large bird of prey
18 10^{-9} m (abbrev.)
19 Infectious disease
20 10^{9} L (abbrev.)
23 Musical term for moderately softly (abbrev.)
26 Between Delta and Foxtrot
30 Alliance, league, association
35 Crazy for eukalyptus
37 Second part of the Christian Bible (abbrev.)
39 Snakelike fish
40 Chemical symbol for element 37
42 Color of fresh blood or a ripe tomato
43 "I love you" in Italian: … amo
44 Hard disk, high density, or heavy duty (abbrev.)
45 Chronic disease of the nervous system (abbrev.)

Solution hint for prize competition of BioNews No. 52:

	H			E						L

Send us the solution until **June 30, 2020**, via e-mail to **bionews@eppendorf.de**, or participate online at **www.eppendorf.com/bn-service**.