BIO NEWS
No. 51 – 2019

eppendorf

New Pipetting System: 384. Ready. Set. Pipette!

> The next level: Eppendorf Conical Tubes 25 mL
> NGS sample preparation: why automation is worth it
> Digitize your –80 °C treasures!


Is That Really DNA in Your Tube? Comparative Analysis of UV-Absorbing Leachables in Micro-Test Tubes

Application Notes

Comparative analysis of UV-absorbing leachables in micro test tubes · Production of hiPSC-derived cortical neurospheres in the DASbox® Mini Bioreactor System · etc.

Dear Readers,

Do you enjoy pipetting into 384-well-plates? We have asked users in the lab and their unanimous opinion is that manual pipetting in this format is time-consuming and cumbersome. Do the tips fit? Will I manage to fill the plate without making a mistake? Will I be able to concentrate sufficiently on 384 tiny wells? Forget about these worries; from now on manual pipetting into 384-well plates is child's play. With our new pipetting system, one minute is all you need to fill a 384-well plate in an effortless, safe, and reproducible manner. Read more on pages 4–6.

News from the world of Eppendorf Tubes®: Eppendorf Tubes 5.0 mL, which have been successfully introduced some time ago, are now also available amber-colored for light-sensitive samples and as DNA LoBind or Protein LoBind tubes for maximum sample recovery. Additionally, we are now also taking the field of conical tubes to the next level by launching the new Eppendorf Conical Tubes 25 mL. This new type of tube bridges the gap within the traditional conical tubes of 15 mL and 50 mL. Available with either screw cap or innovative, patented snap cap, the Eppendorf Conical Tubes 25 mL offer tangible benefits such as efficient contamination prevention, excellent growth of bacteria, less plastic waste, and space-saving storage. More on page 8.

As we all know, it's sometimes the small things that make a big impact! Try our new bioprocess accessories for improved handling (page 13).

Many other reports and four new Application Notes on diverse topics round off this 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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Berrit Hoff (Editor-in-Chief), Dr. Hanaë König, Dr. Tanja Musiol, Natascha Weiß

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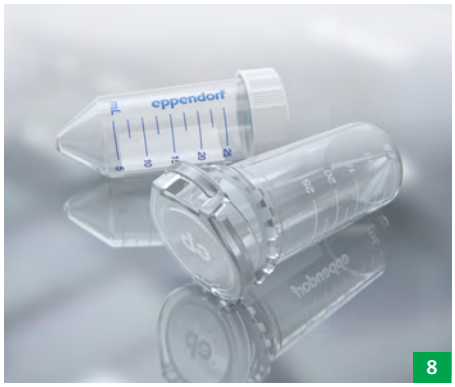

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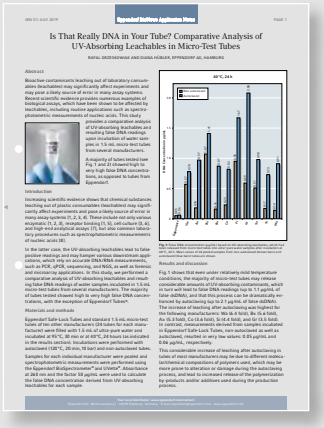
Eppendorf BioNews Application Notes


Is That Really DNA in Your Tube? Comparative Analysis of UV-Absorbing Leachables in Micro-Test Tubes

SAMIRA SCHROEDER, EPPENDORF AG

New Pipetting System: 384. Ready. Set. Pipette!

Do you enjoy pipetting into 384-well plates? We have asked users in the lab. Manual pipetting in this format is time-consuming and cumbersome. Do the tips fit? Will I manage to fill the plate without making a mistake? Will I be able to concentrate sufficiently on 384 tiny wells? Read on and learn how easy and efficient manual pipetting into 384 wells can be! One minute is all you need. Effortless. Safe and reproducible.

Alternate well pipetting using 8 and 12-channel pipettes? There is an easier way

When throughput increases and volumes decrease, the situation calls for appropriate and efficient solutions. Frequently, the decision to switch from 96 to 384-well plates is not an easy one. No wonder, as "alternate well pipetting" using 8 and 12-channel pipettes is both cumbersome and prone to error. Do you need to increase your throughput, establish automated processes, or are you looking for a backup system for your automation? The search is over – with the new Eppendorf manual pipetting system, optimized for 384-well plates, you have found what you were looking for.

Filling 384 wells in just one minute

The new system is based on the new Eppendorf Research® plus (mechanical) and Eppendorf Xplorer® plus (electronic) 16 and 24-channel pipettes in 20 µL and 100 µL volumes (Fig. 1). This means, depending on your personal preference, you can utilize a system for processing either rows (24 channels) or columns (16 channels) as shown in Fig. 2.

In any case, equipped with the ability to start 16 or even 24 reactions simultaneously, you will increase the reproducibility of your results. Test users have reported that even co-workers with little practice were able to fill an entire 384-well plate easily within a mere minute when using these multichannel pipettes.

Putting an end to tips that do not reliably fit

The new system also includes the pipette tips epT.I.P.S.® 384 and ep Dualfilter T.I.P.S.® 384, which were optimized to fit the cones of the new multichannel pipettes as well as the 384-well format.

Fig. 1: NEW! 24 and 16-channel pipettes and epT.I.P.S. 384

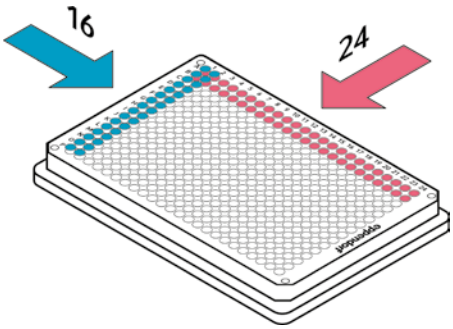


Fig. 2: Pipette entire rows (24 channels) or columns (16 channels) in a single step

The innovative "SOFTattach" technology (Fig. 3) not only guarantees an exceptionally reliable tip fit – at the same time, the tip attachment forces were successfully reduced by an additional 40 % per cone.

Their creativity and a lot of love for even the smallest of technical details enabled the developers at Eppendorf to master this technological balancing act of providing the highest possible security while minimizing operational forces. Learn more in our article on page 6.

Reliable coaxiality for super easy sample transfer

The individual wells of a 384-well plate may be small, but the new slim and fine tips are a perfect match. In order to ensure that liquids can be maneuvered simultaneously and flawlessly across 24 wells, the tips were designed to feature an optimal length as well as extraordinary coaxiality. Test customers were full of praise: "Both the shape and the alignment are optimally adapted to meet the special challenges that come with working with 384-well plates."

SOFTattach technology: innovation for a lasting, reliable tip fit

The epT.I.P.S. 384 are the first tips to feature elastic forming grooves. The resulting elasticity allows the tips to stretch during attachment – to the exact extent necessary that enables a reliable tip fit while reducing operating forces to a minimum.

Our test customers have confirmed that this system now even allows secure dispensing across multiple plates without loosening of the tips – without the use of O-rings!

More information

For more information, please visit www.eppendorf.com/ready-set-pipette If you are interested, please feel free to sign up for a product demo.

Tip: Eppendorf also provides 384-well plates. More information at www.eppendorf.com/plates

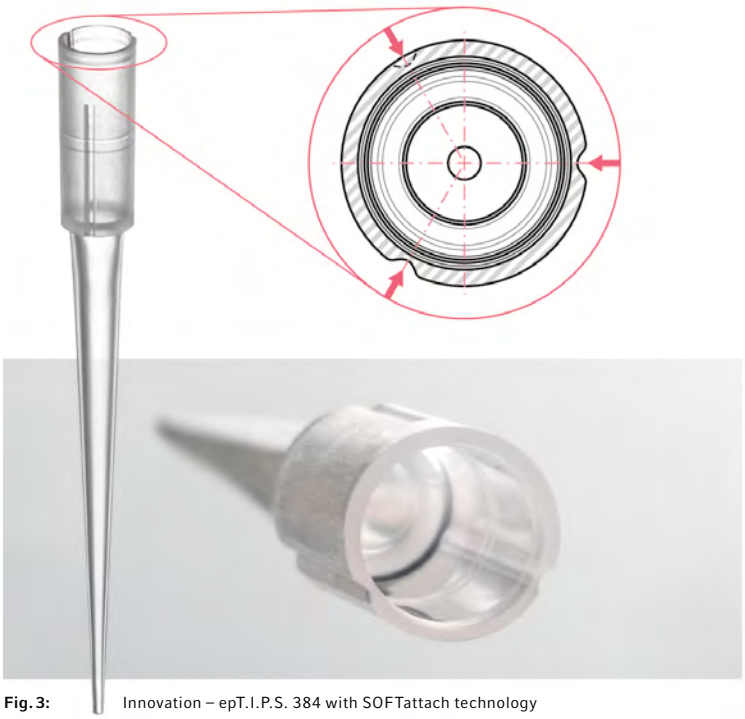


Fig. 3: Innovation – epT.I.P.S. 384 with SOFTattach technology

Tip

Ergonomics: Always Focusing on Your Needs

Some years ago, Eppendorf introduced the PhysioCare Concept® to sustainably support the health of our customers. Our philosophy since then has been to continuously listen to scientists' needs while challenging the status quo of existing laboratory tools.

With the new system for 384-well plates, we have combined proven concepts with essential innovations. Well-known and proven features of Eppendorf pipettes such as the **spring-loaded tip cone** and **SOFTeject** have been enhanced by the completely new **SOFTattach** tip technology (see text on the left).

The combination of these three special features ensure greater user safety and ergonomics during pipetting:

> Tip attachment – even and reliable

> Tip ejection – smooth and easy

> Reduced operating forces

> Perfect tip coaxiality

More information at www.eppendorf.com/physiocare

PhysioCare concept

BERRIT HOFF, EPPENDORF AG

Creativity and the Love for Even the Smallest of Technical Details

384. Ready. Set. Pipette! This slogan for the new pipetting system has been officially adopted. With the new system consisting of multichannel pipettes and new pipette tips, the tiny, delicate wells of 384-well plates can be handled efficiently, ergonomically, and safely. Read here about the creativity and love for detail that brought a technically extremely challenging project to life.

Until now, manual pipetting into 384-well plates has been considered an onerous and unpopular task. "We had heard it from our customers, and our own work in the laboratory, too, has provided us with first-hand experience", explains Dr. Samira Schroeder (Global Marketing Manager Liquid Handling). "Especially 'alternate well pipetting', using either an 8 or a 12-channel pipette, is no picnic, and it carries a high risk of error. On the other hand, the trend towards smaller sample volumes and high throughput cannot be ignored."

What could have been more logical than to develop a system that would allow the filling of entire rows or columns of 24 or 16 wells – depending on personal preference – quickly and easily in one single step?

"The new system consists of 16 and 24-channel pipettes, complete with custom pipette tips – the epT.I.P.S.® 384", explains plastics specialist and developer Hanna Rethwisch. "It was our explicit goal during the development of the new pipette tips to reduce the application forces by 40 % per cone in order to design a 16 or 24-channel system that is easy to work with and therefore attractive to the operator."

From left to right: Dr. Samira Schroeder, Hanna Rethwisch, and Maren Leonhardt

Reducing the application forces and at the same time achieving a secure tip fit was a challenge. "The more flexible a pipette tip, the better it will fit." The solution that Hanna Rethwisch has developed together with the tip molding experts and mold makers at Eppendorf Polymere GmbH, our plastics production plant, is based on elastic forming grooves", explains Samira Schroeder. "This is a truly innovative technology, and we have emphasized its usefulness to the customer by adding the name 'SOFTattach'."

The epT.I.P.S. 384 represent the successful implementation of one of the most sophisticated pipette tip projects thus far. Hanna Rethwisch: "The tip molding experts at Eppendorf Polymere have once again proven their extraordinary technical competency, and they have also provided important input into the geometry of the tips. In my opinion, the new epT.I.P.S. 384 pipette tips of the 20 µL size represent a true masterpiece created by our colleagues."

"This pipette tip possesses a long drawn-out tip, extraordinary coaxiality, and a reduced wall thickness in the area of the exit opening", explains developer Maren Leonhardt. "The tip is less stiff, which simplifies gel loading. It is easy to access the very small gel wells without damaging them. Even when dispensing liquid against the wall of a plate well, only minimal force is transferred to the cone – i.e., the tips will remain firmly attached."

Conclusion

The colleagues are delighted: "Through team work and continuous exchange with our tip molding specialists as well as with the pipette designers, we have achieved our goal."

With the new pipetting system and existing instruments, Eppendorf offers its customers a complete solution for the processing of 384-well plates, including centrifugation, temperature control and mixing, as well as PCR.

JAN-HENDRIK BEBERMEIER, EPPENDORF AG

Perfect Mixing Can Be That Easy

Eppendorf offers a wide range of high-quality, durable, and dependable temperature control and mixing instruments. And we never stop adjusting our instruments and accessories to your increasing or changing demands, for more throughput and flexibility in your lab.

Missing a second incubation temperature for your Eppendorf ThermoMixer®?

The new Eppendorf SmartExtender™ is a comfortable incubation tool and easy to use add-on to your existing Eppendorf ThermoMixer® C, ThermoMixer F, or Eppendorf ThermoStat™ C. Its special highlight: It can be used in parallel to every Eppendorf SmartBlock™ and can incubate up to 12 x 1.5 mL tubes.

> Up to 12 × 1.5 mL tubes for higher throughput and higher flexibility

> Active heating in a temperature range from 3 °C above room temperature to 110 °C

> Heating function independent from SmartBlock

> Firmware update needed; see www.eppendorf.com/software-downloads

www.eppendorf.com/thermomixer

Facing irregular mixing results?

The MixMate® from Eppendorf is an outstandingly comfortable and flexible high-speed mixer even for the most challenging mixing tasks. Its 2D Mix-Control technology (planar mixing orbit without vertical movement) ensures controlled mixing and reproducible results in seconds. Anti-spill technology allows for controlled mixing movements without lid wetting and cross-contamination.

You can order your MixMate basic device with the tube holder that fits your purposes best.

High tube flexibility

> 5 + 1 possibilites to mix: 5 tube holders for tubes from 0.2 mL to 50 mL + 1 integrated "Vortex" function

> You already have a MixMate in your lab? Expand its performance with our latest tube holders* for 8 x 5/15 mL and 4 x 25/50 mL.

*Can be inserted directly – with no extra software update required

Tip

Inside Cell Culture Newsletter for Professionals

You are experienced in cell culture and want to keep improving your knowledge? Maybe you are also looking for better resources that support your teaching and training? Then our new Eppendorf "Inside Cell Culture" newsletter is just perfect for you.

Get regular information about:

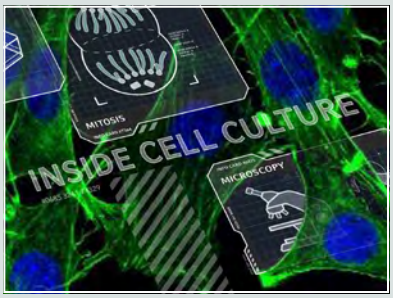
> Tips and tricks to improve your daily work

> Free videos, downloads, posters, and more

> Access to advanced educational webinars

> Teaching support resources

> Upcoming events and trainings



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BRIGITTE KLOSE, EPPENDORF AG

Eppendorf Conical Tubes 25 mL

When sample volumes higher than 15 mL, but much lower than 50 mL, must be prepared, centrifuged, mixed, or stored, researchers often have no choice but to use traditional conical screw cap tubes with 15 mL or 50 mL volume. This dilemma is now resolved by the new Eppendorf Conical Tubes 25 mL with either screw cap or innovative snap cap.

With the new Eppendorf Conical Tubes 25 mL, we take the field of conical tubes to the next level. This new tube bridges a significant gap within available traditional conical tubes. Its diameter corresponds to that of 50 mL conical tubes and ensures straightforward integration into existing workflows across a variety of applications by using appropriate adapters.

Lower height, greater benefits

Thanks to the appr. 20 % lower tube height, the insertion depth of pipettes into the tube is much shorter compared to 15 mL or 50 mL conical tubes. This prevents contamination and enables easier recovery of samples. Additionally, the tubes fit into 3.5-inch storage boxes and freezer racks thus saving valuable storage space.

Screw cap or snap cap

The 25 mL conical tube is available with screw cap or with patented* *g*-safe® snap cap – an innovative, patented cap solution within the field of conical tubes. It allows easy single-handed operation and supports an optimized and faster handling of sample solutions, especially in multistep protocols. Both cap variants provide centrifugation stability up to 17.000 x *g*.

Eppendorf Conical Tubes 25 mL

> Compact medium size tube of 25 mL allows easy one-handed operation.

> Excellent growth of bacterial cultures: in comparison to 15 mL tubes, better aeration and higher plasmid DNA yields have been achieved.

> For cell and tissue lysis, the improved handling benefits reduce the risk of contamination.

> In particular the snap cap version is very convenient and safe for cell culture applications as well as downstream extractions that involve centrifugation or organic solvent steps.

Find more information at www.eppendorf.com/tubes

*EP 2 965 816 A1, EP 2 654 958 A1

News

Novelties in the 5.0 mL Tube Family

Available now: Eppendorf 5.0 mL screw cap tubes amber colored for light-sensitive samples and as DNA LoBind or Protein LoBind tubes for maximum sample recovery.

> Handling light-sensitive samples in non-transparent long tube formats is a frequent source of contamination. The new 5.0 mL screw cap tubes in amber combine the effective protection of your light-sensitive samples from energy-rich light in the lower wavelength range with a high degree of transparency. You can fully control the insertion depths of your pipette tip.

> Tubes with DNA LoBind or Protein LoBind surfaces are ideal for use in applications where concentrations tend to be small and sample recovery is vital for assay results. A special, two-component polymer mix creates a surface that guarantees optimal recovery rates and leads to more stable concentrations as well as to more reproducible and reliable experimental results.

Check out www.eppendorf.com/tubes for best performance for your DNA, protein, or light-sensitive samples.

Is That Really DNA in Your Tube? Comparative Analysis of UV-Absorbing Leachables in Micro Test Tubes

RAFAL GRZESKOWIAK AND DIANA HÜBLER, EPPENDORF AG, HAMBURG, GERMANY

Abstract

Bioactive contaminants leaching out of laboratory consumables (leachables) may significantly affect experiments and may pose a likely source of error in many assay systems. Recent scientific evidence provides numerous examples of biological assays, which have been shown to be affected by leachables, including routine applications such as spectrophotometric measurements of nucleic acids. This study provides a comparative analysis of UV-absorbing leachables and resulting false DNA readings upon incubation of water samples in 1.5 mL micro test tubes from several manufacturers.

A majority of tubes tested (see Fig. 1 and 2) showed high to very high false DNA concentrations, as opposed to tubes from Eppendorf.

Introduction

Increasing scientific evidence shows that chemical substances leaching out of plastic consumables (leachables) may significantly affect experiments and pose a likely source of error in many assay systems [1, 2, 3, 4]. These include not only various enzymatic [1, 2, 3], receptor binding [1, 5], cell culture [3, 6], and high-end analytical assays [7], but also common laboratory procedures such as spectrophotometric measurements of nucleic acids [8].

In the latter case, the UV-absorbing leachables lead to false positive readings and may hamper various downstream applications, which rely on accurate DNA/RNA measurements, such as PCR, qPCR, sequencing, and NGS, as well as forensic and microarray applications. In this study, we performed a comparative analysis of UV-absorbing leachables and resulting false DNA readings of water samples incubated in 1.5 mL micro test tubes from several manufacturers. The majority of tubes tested showed high to very high false DNA concentrations, with the exception of Eppendorf Tubes®.

Materials and methods

Eppendorf Safe-Lock Tubes and standard 1.5 mL micro test tubes of ten other manufacturers (24 tubes for each manufacturer) were filled with 1.5 mL of ultra-pure water and incubated at 95 °C, 30 min or at 40 °C, 24 hours (as indicated in the results section). Incubations were performed with autoclaved (120 °C, 20 min, 10 bar) and non-autoclaved tubes.

Samples for each individual manufacturer were pooled and spectrophotometric measurements were performed using the Eppendorf BioSpectrometer® and UVette®. Absorbance at 260 nm and the factor 50 µg/mL were used to calculate the false DNA concentration derived from UV-absorbing leachables for each sample.

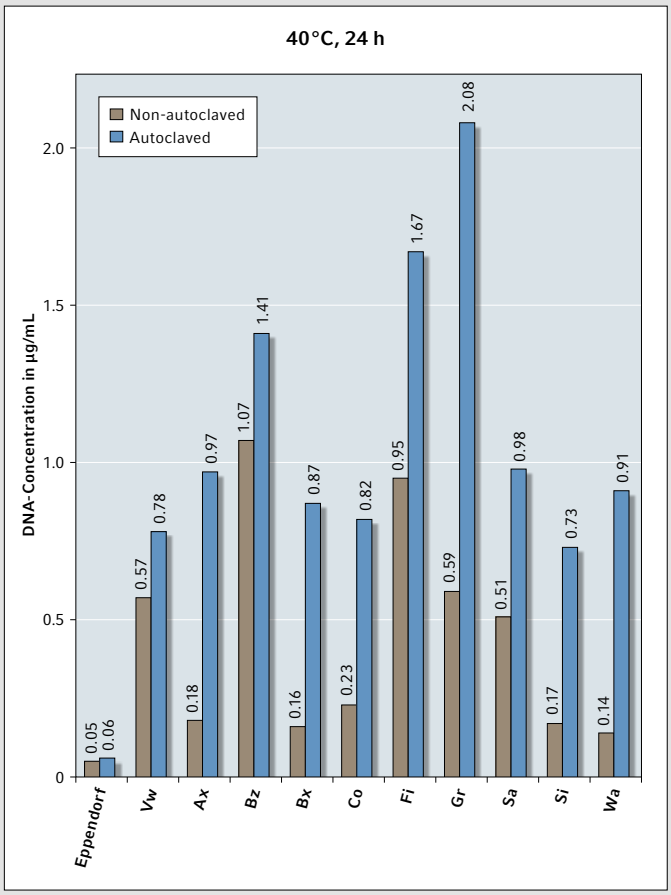


Fig. 1: False DNA concentration (µg/mL) based on UV-absorbing leachables, which had been released from micro test tubes into ultra-pure water samples after incubation at 40 °C, 24 h. Mean values of 24 pooled samples from non-autoclaved (brown bars) and autoclaved (blue bars) tubes are shown.

Results and discussion

Fig. 1 shows that even under relatively mild temperature conditions, the majority of micro test tubes may release considerable amounts of UV-absorbing contaminants, which in turn will lead to false DNA readings (up to 1.1 µg/mL of false dsDNA), and that this process can be dramatically enhanced by autoclaving (up to 2.1 µg/mL of false dsDNA). The increase of leaching after autoclaving was highest for the following manufacturers: Wa (6.4 fold), Bx (5.6 fold), Ax (5.3 fold), Co (3.6 fold), Si (4.4 fold), and Gr (3.5 fold). In contrast, measurements derived from samples incubated in Eppendorf Safe-Lock Tubes, non-autoclaved as well as autoclaved, resulted in very low values: 0.05 µg/mL and 0.06 µg/mL, respectively.

This considerable increase of leaching after autoclaving in tubes of most manufacturers may be due to different molecular/chemical compositions of polymers used, which may be more prone to alteration or damage during the autoclaving process, and lead to increased release of the polymerization by-products and/or additives used during the production process.

Is That Really DNA in Your Tube? Comparative Analysis of UV-Absorbing Leachables in Micro Test Tubes

Eppendorf Tubes are manufactured from highly pure polypropylene and neither plasticizers, slip agents, nor biocides are added during their production.

Thus far there have been no comprehensive studies published on influence of autoclaving on leaching. Since autoclaving is a very commonly used procedure, its effects on experiments should be therefore considered more cautiously in the general lab routine.

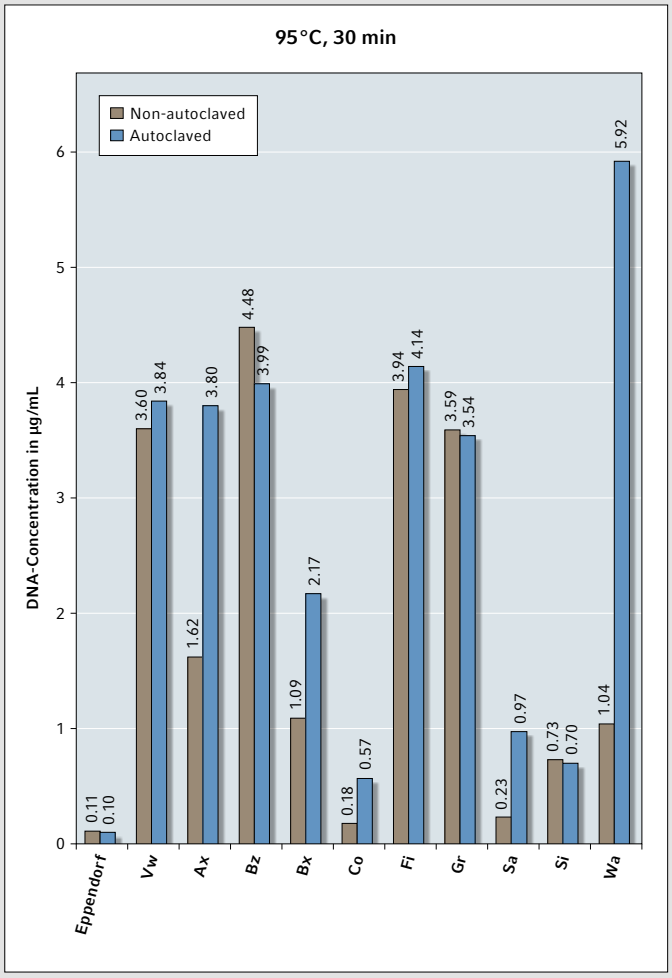


Fig. 2: False DNA concentration (µg/mL) based on UV-absorbing leachables, which had been released from micro test tubes into ultra-pure water samples after incubation at 95°C, 30 min. Mean values of 24 pooled samples from non-autoclaved (brown bars) and autoclaved (blue bars) tubes are shown.

As shown in Fig. 2, general levels of detected UV-absorbing leachables were further increased when water samples were incubated at 95 °C for 30 min. Even during this short time period, the average level of leaching (average for both autoclaved and non-autoclaved tubes) was considerable and reached up to 2.51 µg/mL of false dsDNA as compared to the average of 0.79 µg/mL of false dsDNA obtained after incubation at 40 °C for 24 h (3.17 fold increase).

This indicates that temperature is a critical factor, which influences the process of leaching and is in agreement with previous reports [8].

Similar to incubations at lower temperatures, by far the lowest values were obtained for water samples incubated in Eppendorf Safe-Lock Tubes (Fig. 2), thus significantly reducing the risk of leachable artifacts and false readings of nucleic acids.

Conclusion

In summary, the majority of tubes tested showed high to very high levels of UV-absorbing leachables in water samples incubated at 40 °C and 95 °C. The leachable levels were further considerably increased by the autoclaving process. UV-absorbing leachables were shown to cause false positive readings of nucleic acid and may therefore hamper various downstream applications, which rely on accurate DNA, RNA or nucleotide measurements, such as PCR, qPCR, sequencing, and NGS, as well as forensic and microarray applications. The Eppendorf Tubes showed consistently very low leaching values under all experimental conditions, and irrespective of the autoclaving process and therefore significantly reduce the risk of false spectrophotometric measurements.

Literature

[1] McDonald GR, Hudson AL, Dunn SM, You H, Baker GB, Whittal RM, Martin JW, Jha A, Edmondson DE, Holt A. Bioactive contaminants leach from disposable laboratory plasticware. *Science* 2008; 322(5903):917.

[2] McDonald GR, Kozuska JL, Holt A. Bioactive Leachates from Lab Plastics. *G.I.T. Laboratory Journal* 2009; 9-10: 2-4.

[3] 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.

[4] Grzeskowiak R, Gerke N. Leachables: Minimizing the Influence of Plastic Consumables on the Laboratory Workflows. *White Paper 026*; www.eppendorf.com/wp26

[5] Watson J, Greenough EB, Leet JE, Ford MJ, Drexler DM, Belcastro 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.

[6] Lee TW, Tumanov S, Villas-Bôas SG, Montgomery JM, Birch NP. Chemicals eluting from disposable plastic syringes and syringe filters alter neurite growth, axogenesis and the microtubule cytoskeleton in cultured hippocampal neurons. *J Neurochem* 2015; 133(1):53-65.

[7] Schauer KL, *et. al.* Mass Spectrometry Contamination from Tinuvin 770, a Common Additive in Laboratory Plastics. *Journal of Biomolecular Techniques* July 2013; 24(2):57-61.

[8] Lewis LK, Robson M, Vecherkina Y, Ji C, Beall G. Interference with spectrophotometric analysis of nucleic acids and proteins by leaching of chemicals from plastic tubes. *Biotechniques* 2010; 48(4):297-302.

Production of Human Induced Pluripotent Stem Cell-Derived Cortical Neurospheres in the DASbox® Mini Bioreactor System

ULRIKE RASCHE, EPPENDORF AG BIOPROCESS CENTER, JÜLICH, GERMANY

Introduction

There is substantial interest in the scientific community to use human induced pluripotent stem cell (hiPSC)-derived neural cells to study basic mechanisms of brain development, neuronal function, and drug-induced effects. *In vitro*-generated brain organoids are promising models, because they show a remarkable degree of maturation and can be kept in culture for up to 20 months [1].

Many established protocols for organoid production under static conditions require a considerable amount of manual lab work, which limits their scalability. In addition, there is a high degree of inter-organoid heterogeneity caused by stochastic effects during the initial formation of the organoids.

These factors make current organoid technologies unsuitable for the development of standardized models or assays. Cultivation in stirred-tank bioreactors allows the tight regulation of process parameters and therefore improves homogeneity of neural spheroid cultures [2, 3]. It also allows scale-up to produce sufficient cell numbers for novel pharmacological testing devices such as Multi-Organ-Chip systems [4]. Here, researchers at TissUse GmbH (Berlin, Germany) demonstrate the potential of stirred-tank bioreactors for the generation of hiPSC-derived neurospheres. They obtained 2 x 10^8 cells in a single DASbox Mini Bioreactor. Results from three independent bioprocesses demonstrate the reproducibility of the neural differentiation protocol.

Materials and methods

The researchers at TissUse® performed hiPSC spheroid cultivation and neuronal differentiation in a DASbox Mini Bioreactor System (Fig. 1). The culture was inoculated with 2.5 x 10^5 StemUse101 single cells/mL. The protocols for initial spheroid formation and neural differentiation are described in [5].

Results

hiPSC spheroids

The hiPSCs rapidly formed spheroids in the bioreactor during the first 24 hours of culture and increased in size until day five of cultivation to an average diameter of 136.5 µm.

Fig. 1: DASbox Mini Bioreactor System

Parameter	Device/setpoint
Bioprocess system	DASbox Mini Bioreactor System
Vessel	DASbox Mini Bioreactor; coated with siliconizing agent Sigmacote® (Sigma-Aldrich®, USA) before starting the process to prevet cell adhesion
Agitation	8-blade impeller with 60° pitch; 80 rpm
Temperature	37°C
Dissolved oxygen (DO)	19 %; maintained through gassing with 5 % CO_2/variable percentage of O_2 at 3 sL/h
Sampling	Submerged tube
Medium addition	Non-submerged tube connected to system's pump
Medium withdrawal	Submerged tube equipped with 10 µm porous filter connected to system's pump. The filter allows removal of single cells while retaining spheroids in the vessel

Table 1: Overview of process parameters and setpoints

After five days, hiPSCs expanded fivefold up to a total of 1.58 x 10^8 cells with a viability of 86 %. Expression of pluripotency marker genes was not affected by 3D cultivation in the bioreactor, as shown by flow cytometric measurement of the hiPSC markers SSEA-5, TRA-1-60, SOX-2 and OCT-3/4 at day 0 and 5 of hiPSC spheroid formation in the bioreactor. The early differentiation marker SSEA-1 was not expressed at any measured time point.

Cortical neurospheres

After 5 days of spheroid formation from single hiPSCs, the researchers induced the differentiation to cortical neurospheres. The medium composition of the feed medium was changed at multiple time points of the differentiation process and the medium was stepwise exchanged using the system's pumps, which led to a gradual shift of the medium composition from neural adaptation medium to neural induction medium and wash out of the small molecule inhibitors. After the medium transition phase, spheroid size increased up to an average diameter of 230 µm. After 21 days, the cell number increased twofold up to a total of 2 x 10^8 cells with a viability of 82 %. The total number of neuronal cells in the adult human brain is roughly 100 x 10^9.

Production of Human Induced Pluripotent Stem Cell-Derived Cortical Neurospheres in the DASbox® Mini Bioreactor System

In its Multi-Organ-Chip systems, TissUse usually uses a scaling factor of 1:100,000, which results in a cell number of 1 x 10^6 cells per neuronal model. With one bioreactor run, 200 neuronal models can be generated, which is sufficient even for large-scale experiments.

Marker gene expression

To assess the efficiency of the neural induction protocol, the ΔΔcT mRNA fold-change of selected marker genes, normalized to the housekeeper TBP and compared to hiPSC spheroids, was measured at the end of the neural induction (Fig. 2A).

The pluripotency marker genes NANOG and OCT3/4 were downregulated and gene expression of the neuronal stem cell marker gene Nestin (NES) increased in all three cultivations. The cortical development markers OTX1, TBR1 and SATB2 were upregulated, reflecting the cortical identity of the spheroids [3].

Multiple pan-neuronal markers such as MAP2, TUBB3, Synaptophysin (SYP), Enolase-2 (ENO2), and the more specific neuronal markers SLC6A4 (serotonergic neurons) and TH (dopaminergic neurons) were also upregulated, indicating the differentiation of neural stem cells into neuronal progenitor cells. Staining of cryosections revealed an even distribution of cells positive for the neural stem cell markers Nestin and PAX6, indicating a homogeneous differentiation of the spheroids (Fig. 2B).

Conclusion

The DASbox Mini Bioreactor System enabled efficient hiPSC spheroid formation and subsequent neural induction. Neurospheres generated in three independent bioprocesses showed comparable expression patterns on mRNA and protein level. Bioreactor technology is capable of generating a sufficient number of cells for applications like personalized drug testing in microphysiological systems. If higher cell numbers are needed for future applications, upscaling is possible by parallel operation of multiple bioreactors or by using larger vessels.

Download the full application note at
www.eppendorf.com/appnote364

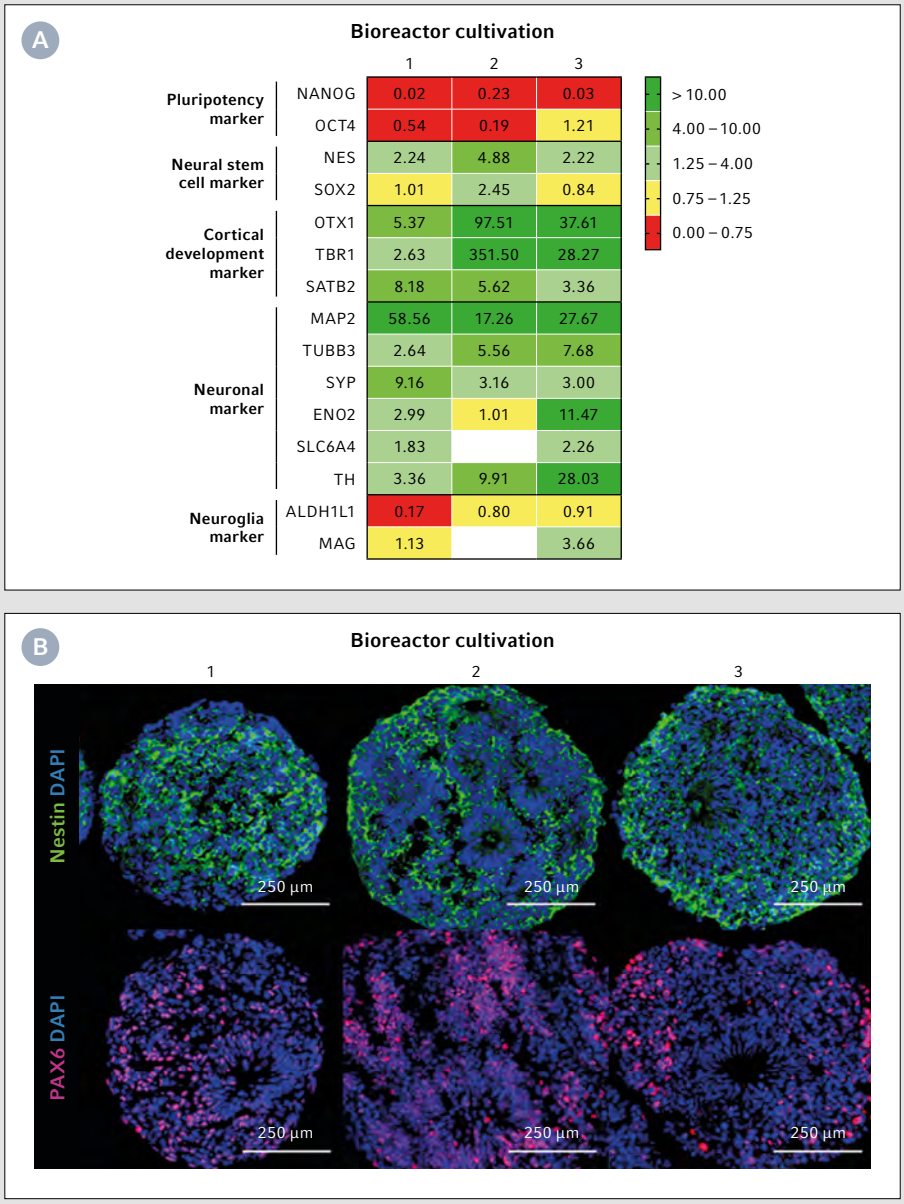


Fig. 2A: Fold-change mRNA expression of neurospheres at the endpoint of three independent bioprocesses (day 22–32), compared to hiPSC spheroids. When no expression was measured in the hiPSC samples no fold change could be calculated and fields were left blank. **2B:** Expression of neural stem cell marker PAX6 and Nestin. Scale: 250 µm.

Literature

[1] Sloan SA, *et al. Neuron.* 2017;95(4):779-790. e6. doi:10.1016/j.neuron.2017.07.035.

[2] Yan Y, *et al.* In: *Methods in Molecular Biology.* 2015:119-128. doi:10.1007/7651_2015_310.

[3] Rigamonti A, *et al. Stem Cell Reports.* 2016; 6(6): 993-1008. doi:10.1016/j.stemcr.2016.05.010.

[4] Marx U, *et al.* 2016; *Altex* 33.3: 272.

[5] Leopold Koenig, *et al.* Eppendorf Application Note 364, 2018.
www.eppendorf.com/appnote364

Mastercycler® Thermal Cyclers: Save Time and Cost in PCR

DUTT WAY WONG, JENNY LAU, YEE MIN KHOR, EPPENDORF ASIA PACIFIC REGIONAL CENTER, KUALA LUMPUR, MALAYSIA
ARORA PHANG, EPPENDORF AG, HAMBURG, GERMANY

Abstract

Thermal cyclers available in the market are of different ramp rates. One of the more obvious advantages of high ramp rates is faster completion of PCR, allowing higher work productivity. However, ramp rates alone do not contribute to faster PCR completion. This study compares the time taken to complete a 3-step and 2-step GC-rich PCR by five different thermal cyclers. Reported herein is also the cost saving aspect by reducing reagent volume. An ideal thermal cycler would allow users to save both time (faster PCR completion) as well as cost (reproducible results at lower volume) in reducing optimization effort and in subsequent routine runs.

Mastercycler® nexus GSX1 and nexus gradient from Eppendorf are found to be able to amplify difficult templates across a wide range of enzymes at high speed, high reproducibility, and low cost.

Introduction

Scientific advancement has made Polymerase Chain Reaction (PCR) work less time consuming and more cost effective. The gradient function has made possible testing of different temperatures in one single PCR.

Thermal cyclers with increasingly higher ramp rates are being introduced to the market one after another. Fast PCR consumables and fast PCR reagents are pushing the completion time of a PCR ever shorter. With certain difficult applications, however, such advancements elicit less excitement as it often comes with vastly higher cost.

Certain PCR amplification of GC-rich templates, such as the promoter region of a gene, requires higher melting temperatures due to the formation of secondary structures. The requirement for higher denaturation and annealing temperatures limits the advantage conferred by high cycler ramp rates, as the heat needs to be transferred to the liquid reagent. Hence, time saving strategies for this application generally comes in the form of combining annealing and extension steps (also known as 2-step PCR), cutting the time needed for transition between steps as the temperatures are closer to each other. Fortunately, ramp rates alone do not contribute to faster PCR completion. Efficient control of heating and cooling affects both the total run time as well as the performance of a thermal cycler, which is critical to producing reliable and reproducible results. The objective of this study is to investigate the amplification efficiency of different thermal cyclers in amplifying GC-rich templates.

Materials

We compared the differences between 3-step and 2-step PCR of the BAIP3 gene for five thermal cyclers (Table 1) in terms of amplification efficiency, reproducibility, PCR reagent cost, and total run time.

Time saving

The nature of GC-rich primers leads to non-specific binding to the template, often resulting in multiple products across different temperatures. Thus, a gradient function in a thermal cycler is important to test multiple temperatures in one run. Different thermal cyclers would operate using a different gradient technology or format (8 horizontal rows or 12 vertical columns across a 96-well plate block). Eppendorf thermal cyclers use the 12 vertical columns strategy, allowing users to concurrently test 12 different temperatures in each gradient mode.

This allows users to test more temperatures during optimization and hence eliminates the need to set up another run, which requires additional preparation and waiting time. Meanwhile, SimpliAmp requires four different PCR runs to provide 12 different comparable annealing temperatures.

Additionally, the total PCR run time can be reduced by combining the annealing and extension steps, thus reducing the time taken by the cycler to ramp up and down when transitioning between the two steps. As expected, 2-step PCR was completed in lesser time than 3-step PCR for all cyclers. However, the time difference between the two programs is highly dependent on the cycler in question (Fig. 1).

This is possibly due to several factors, such as how efficiently a thermal cycler handles the temperature transition between steps.

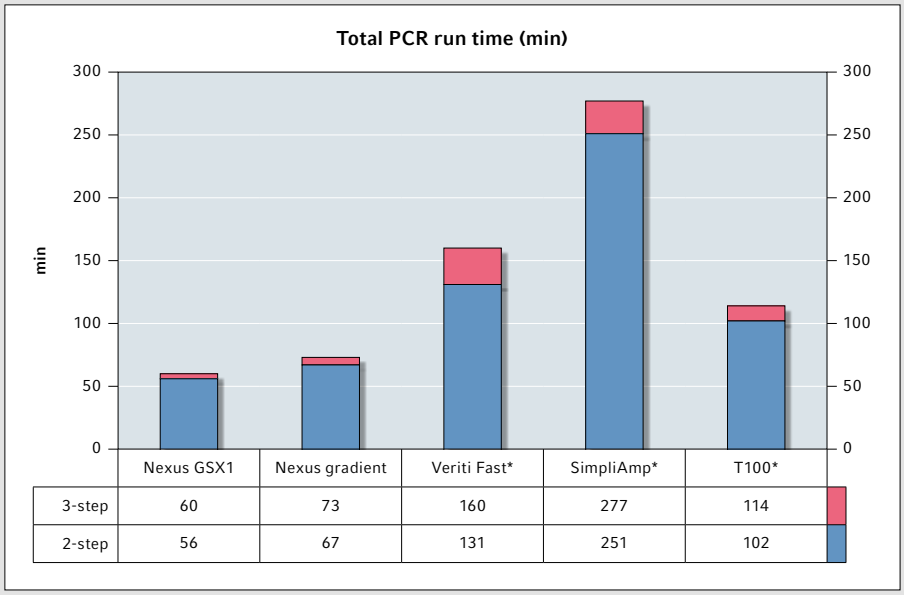
Additionally, fast run times are only important provided PCR reactions produce desired results, which might not be the case for certain cyclers at certain conditions (Fig. 2A, 2B).

For all enzymes and PCR strategies, Mastercycler nexus GSX1 and nexus gradient used the shortest time to successfully complete a PCR. This not only increases the productivity of a laboratory by freeing up the thermal cycler for more usage per day, it also increases sharing convenience between members of the same lab.

Thermocycler	Max. block heating rate
Mastercycler® nexus GSX1 (Eppendorf)	5 °C/s
Mastercycler® nexus gradient (Eppendorf)	3 °C/s
Veriti® Fast (Applied Biosystems®)	5 °C/s
SimpliAmp™ (Life Technologies®)	4 °C/s
T100 (Bio-Rad®)	4 °C/s

Tabe 1: List of thermal cyclers in this study

Mastercycler® Thermal Cyclers: Save Time and Cost in PCR



	Nexus GSX1	Nexus gradient	Veriti Fast*	SimpliAmp*	T100*
3-step	60	73	160	277	114
2-step	56	67	131	251	102

Fig. 1: Comparison of total run times between 3-step and 2-step PCR

**These thermal cyclers require more than a single PCR run due to the lower number of gradient temperatures possible per run. Hence, total run time stated here are cumulative of all runs required to give 12 annealing temperatures.*

Cost saving

Reagent costs can be reduced by using less specialized and lower-cost reagents in combination with common additives such as DMSO, as well as lower reagent volume per reaction. This study showed that only Mastercycler nexus GSX1 and nexus gradient maintained positive amplification with all 3 enzymes used, and even when the volume was reduced to 5 µL (Fig. 2A, 2B).

For many other cyclers, we observed random negative amplifications, which could be attributed to high evaporation rates especially at 5 µL reaction volumes, leading to partial or complete loss of reaction mix.

A thermal cycler that can reproducibly yield results at lower reaction volume enables users to save on reagent cost per reaction. A thermal cycler that is robust in producing results with various enzymes of differing price range would reduce user dependency on expensive enzyme formulations. Hence, taking all factors into consideration, resources-saving (cost, time, effort) needs to be balanced with quality of results (reproducibility) as final assessment of a thermal cycler quality.

Conclusion

The results showed that the Mastercycler nexus GSX1 and nexus gradient thermal cyclers from Eppendorf are robust in supporting amplification of GC-rich template in a wide range of enzymes, even at low reaction volume whilst allowing flexibility to choose between 3-step or 2-step protocols depending on the run. This makes them ideal thermal cyclers that allow users to save both time (faster PCR completion) as well as cost (reproducible results at lower volume) in reducing optimization effort and in subsequent routine runs.

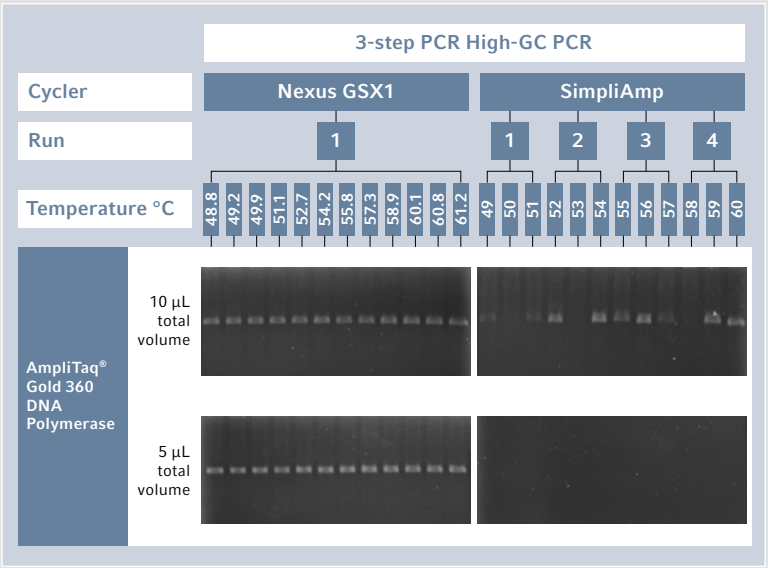


Fig. 2A: Amplification of BAIP3 with 3-step PCR**

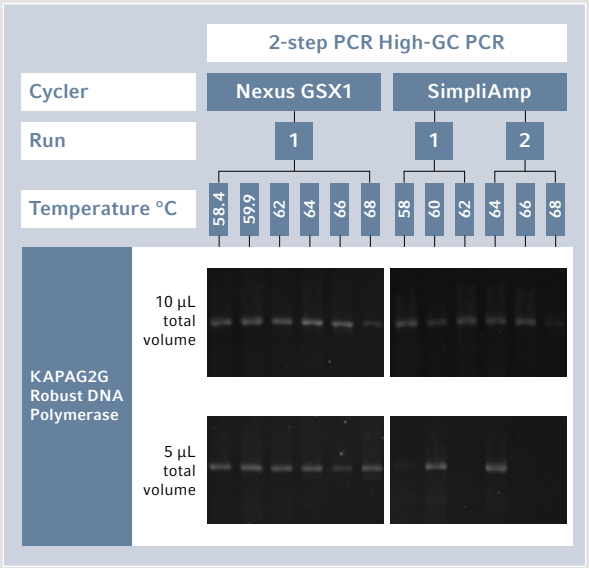


Fig. 2B: Amplification of BAIP3 with 2-step PCR**

**abbreviated; for full Application Note, please see www.eppendorf.com/appnote392

Intracytoplasmic Sperm Injection in the Mouse with the Eppendorf PiezoXpert®: How to Increase Oocyte Survival Rates After Injection

NUNO COSTA-BORGES, ENRIC MESTRES, IVETTE VANRELL, MARIA GARCÍA, GLORIA CALDERÓN, EMBRYOTOOLS SL, BARCELONA, SPAIN
SANDRA STOBRAWA, EPPENDORF AG, HAMBURG, GERMANY

Introduction

Intracytoplasmic sperm injection (ICSI) is a powerful technique that has been successfully used to inseminate metaphase II (MII) oocytes. While conventional ICSI technique based on the sperm injection into oocyte using a beveled microcapillary is very successful in humans, it has proven unsuccessful in the mouse.

The problem is mainly attributed to the fragility of the mouse oocyte with low cytoplasm viscosity and an oolemma much more elastic and sensitive than of human oocytes. Consequently, survival rates of mouse oocytes following the conventional ICSI procedure rarely exceed 50 %. To overcome these low survival rates, the piezo-actuated micromanipulation method is proposed, in which a piezo-electric effect propels a blunt end microcapillary forward in precise and rapid movements allowing an efficient penetration of membranes [1, 2].

This procedure is less traumatic than the conventional ICSI, and thus higher survival rates were described. However, many laboratories struggle with this technically demanding procedure. Beside general factors (e.g., room temperature, medium, etc.) the injection technique per se may contribute to the observed lack of consistent results in survival rates. In the following, a modified piezo-actuated ICSI procedure using the Eppendorf PiezoXpert is described, facilitating even less experienced operators to increase the survival rates up to around 100 %.

Materials and methods

Preparation of micromanipulation set-up

An ICSI micromanipulation workstation (e.g., 2 TransferMan® 4r, 2 CellTram® 4r Air/Oil, Eppendorf) to operate the microcapillaries for holding the oocyte and injecting the sperm is required.

A micromanipulation dish is prepared with small droplets of micromanipulation medium (e.g., Hepes-buffered and supplemented with 5 µg/mL cytochalasin B) as well as with droplets of 10% (v/v) PVP for performing the isolation of sperm heads. The ICSI microcapillaries used in piezo-actuated micromanipulation have typically a long blunt end tip (Piezo Drill Tip ICSI capillary, Eppendorf) and are to be backfilled with Fluorinert (FC-40, Sigma-Aldrich®, USA). The piezo impulse settings (intensity, speed, and pulse no.) usually depend on individual laboratory conditions and thus should be optimized and adjusted for your experiments.

In general, the penetration of the zona pellucida requires a stronger piezo impulse (e.g., intensity: 10–40, speed: 5–7, pulse: ∞, set in Channel A of the PiezoXpert) whereas the elastic oolemma should be treated with a softer impulse (e.g., intensity: 1–10, speed: 1–7, pulse: 1, set in Channel B).

The sperm immobilization and head/tail separation is performed at higher piezo impulses (e.g., intensity: 10–40; speed: 5–10, pulse: ∞).

Preparation of biological material

Mouse MII oocytes and mouse sperms are collected and prepared, as described elsewhere [4]. Matured oocytes are transferred to the droplets of micromanipulation medium and incubated at 37 °C for at least 5 min before starting the ICSI procedure.

For the isolation of the sperm heads, sperms should be transferred into a PVP droplet. A single, motile sperm should be selected and aspirated with the tail first into the ICSI microinjection capillary. Then, the sperm is positioned so that the neck is placed at the opening of the capillary to break the head from the tail by applying a few stronger piezo impulses.

Modified piezo-actuated ICSI with cytoplasm aspiration to reduce lysis

Having loaded the sperm heads into the ICSI capillary, both the holding and ICSI capillaries are moved to the droplet containing the oocytes. An oocyte is then fixed firmly at the holding capillary and the focus of the inverted microscope is aligned to the equatorial plane of the oocyte. The microinjection capillary loaded with the sperm heads is then placed in focus (Fig. 1A).

Afterwards, the zona pellucida is penetrated using the stronger piezo impulses stored at Channel A of the PiezoXpert (Fig. 1B–C).

A sperm head is then positioned close to the tip end of the ICSI capillary. The ICSI capillary is pushed into the oocyte almost up the opposite side of the oocyte, close to the holding pipette, making a deep indentation in the oocyte (Fig. 1D–E).

At this point, a small suction is applied into the ICSI capillary, a single soft piezo impulse is triggered (set in Channel B) to break the elastic oolemma, and then the sperm head is carefully ejected into the cytoplasm of the oocyte (Fig. 1F).

Afterwards, the ICSI capillary tip is rapidly withdrawn from the oocyte while aspirating at the right end of the indentation a bit of the oolemma into the capillary tip end (Fig. 1G).

By simultaneously withdrawing the micropipette and aspirating, it is possible to close the hole within the oolemma (Figure 1H–I).

Injected oocytes must then be washed thoroughly in culture medium droplets to eliminate the traces of the Hepes-buffer and cytochalasin B present in the manipulation medium and can then be cultured under appropriate conditions.

Results and discussion

The method described here was adapted from the original technique developed by Prof. Yanagimachi [1] with the intent to reduce the risk of cell lysis following piezo-actuated ICSI in the mouse. The penetration of the oolemma should be performed with a gentle piezo impulse, which just allows to succeed in drilling but not to stress the cell by lateral oscillations.

The closing of the oolemma by a short suction of this penetrated cell region

Intracytoplasmic Sperm Injection in the Mouse with the Eppendorf PiezoXpert®: How to Increase Oocyte Survival Rates After Injection

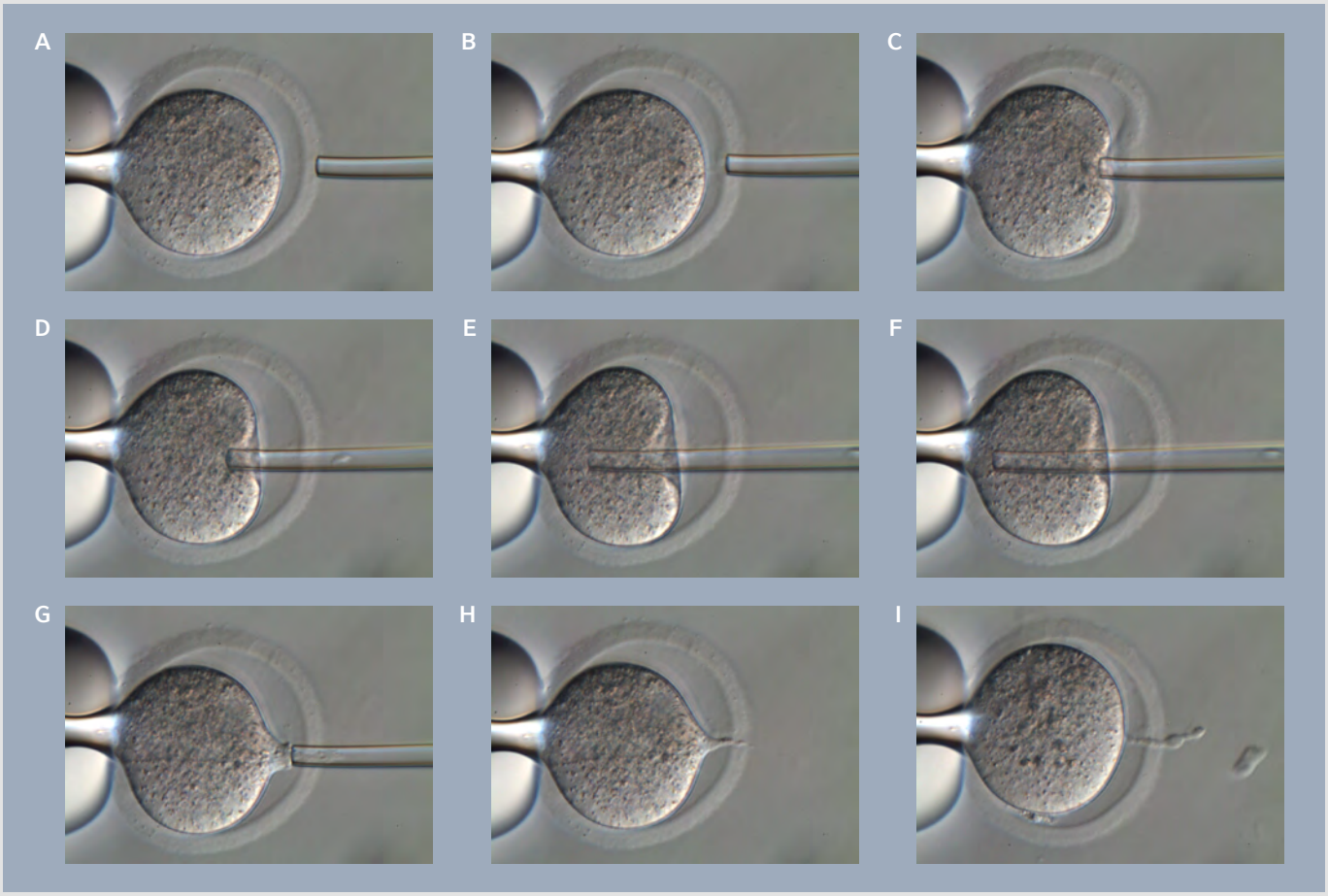


Fig. 1: Piezo-actuated mouse ICSI using the modified technique.

(A–C) The equatorial plane of the oocyte and the piezo ICSI capillary are aligned in microscopic focus. A stronger piezo impulse is applied to ICSI capillary to penetrate the zona pellucida. **(D–F)** The ICSI capillary, loaded with the sperm head, is then moved deep into the oocyte. Minimal suction is applied into the ICSI capillary and with a single soft piezo impulse the oolemma is penetrated and the sperm is released into the cytoplasm. **(G–I)** When withdrawing the ICSI capillary out of the injected oocyte, just at the right end of the indentation a short suction is applied into the capillary. This causes the penetrated oolemma to close.

into the ICSI capillary when just leaving the oocyte minimizes the risk of cell lysis. It is recommended to perform this procedure in a manipulation medium supplemented with cytochalasin B, as this microfilament disruptor agent relaxes the oocyte membrane and thus allows the membrane to seal easily immediately after the sperm was injected into the cytoplasm. With some practice and the optimal set-up and experimental conditions, this modified technique should enhance the survival rate of injected oocytes to nearly 100 %. The efficacy of the piezo drill is highly desirable, not only in ICSI procedures, but also for other challenging microinjection applications, such as somatic cell nuclear transfer or injection of highly-concentrated CRISPR complexes [2, 3].

Literature

[1] Kimura Y, Yanagimachi R. Intracytoplasmic sperm injection in the mouse. *Biol. Reprod.* 52, 709–720 (1995).

[2] Yoshida N, Perry AC. Piezo-actuated mouse intracytoplasmic sperm injection (ICSI). *Nat Protoc.* 2007; 2(2):296-304.

[3] Yang H, Wang H, Jaenisch R. Generating genetically modified mice using CRISPR/Cas-mediated genome engineering. *Nat Protoc.* 2014; 9(8): 1956-68

[4] Nagy A, Gerstenstein M, Vintersten K, Behringer B. Manipulating the Mouse Embryo: *A Laboratory Manual* (Third Edition). Cold Spring Harbor Laboratory Press; 2003.

FLORIAN BUNDIS, EPPENDORF AG

Expanded Application Range for Large Benchtop Centrifuges

Until now, the use of the popular 250 mL bottles has been limited to swing-bucket rotors with a maximum speed of slightly more than 5,000 x *g* and a capacity of four bottles per run. The new fixed-angle rotor FA-6x250 allows simultaneous centrifugation of six 250 mL bottles at speeds exceeding 15,000 x *g* – thus considerably expanding the application spectrum of the large refrigerated benchtop systems by Eppendorf, Centrifuge 5910 R and Centrifuge 5920 R.

High-capacity fixed-angle rotor for a broad range of applications with max. speed of 15,054 x *g* (10,100 rpm)

Both centrifuges offer a comprehensive program of fixed-angle as well as swing-bucket rotors for vessels ranging from 0.5 mL to 1,000 mL in volume. They are therefore suitable for countless applications in many different types of workflow.

Cell harvests in bottles up to 1,000 mL, large scale DNA and RNA isolations, and Ficoll® gradient centrifugation for the purification of lymphocytes and monocytes, just to name a few of many areas of use.

To accommodate the popular 250 mL bottles, Eppendorf has now introduced the high-capacity fixed-angle rotor FA-6x250. This rotor expands the application spectrum of the large refrigerated benchtop centrifuges, which encompasses the harvesting of bacteria, algae, and yeast, as well as mammalian cells, in vessels up to 250 mL. Additional areas of application include the extraction of plasmid DNA and the clearing of lysates following cell lysis.

Fewer rotor changes

With a broad selection of twelve different adapters, cumbersome rotor changes within the workflow are reduced to a minimum. A wide variety of vessel types of different volumes can now be accommodated with ease by simply changing the adapter. In addition to the common 15 mL and 50 mL tubes, the adapters accommodate blood collection tubes, Oak Ridge centrifugation tubes, and vessels for flow cytometry (FACS). This simplifies handling considerably while saving valuable time.

More information at
www.eppendorf.com/next-benchmark

A broad selection of twelve different adapters for a wide variety of vessel types of different volumes

HANAË KÖNIG, EPPENDORF AG

NGS Sample Preparation: 5 Points Why Automation Is Worth It

High throughput sequencing, also known as Next Generation Sequencing (NGS), is a technique that is frequently employed in the laboratory. It generates large amounts of sequencing data about DNA or RNA, from a wide range of sample material, in a relatively short time. Depending on the method, sample preparation may take up to several days; with many individual steps that require a high level of concentration and pipetting experience. Read about five important points to consider during manual NGS sample preparation, and why it may be worth standardizing the entire process using an automated pipetting system such as the ep*Motion*® 5073m NGS.

Point 1: Precision and accuracy

In the case of mechanical pipettes, precision and accuracy largely depend on the operator. Correct pipetting technique is a prerequisite for obtaining reliable results. Since relevant training of laboratory staff is hardly ever provided, most people operate on the principle of "learning by doing". However, accurate performance of each liquid transfer and the right environmental conditions are essential to guarantee maximum dispensing precision and accuracy. For liquid aspiration, this includes holding the manual pipette in a vertical position and immersing the tip no deeper than 3 mm into the liquid, as well as wetting the inside of the pipette tip prior to aspiration. Dispensing ideally occurs by holding the pipette at a 45-degree angle. Following these guidelines consistently and without fail is often impossible in the face of another hectic day in the laboratory, and it is exhausting and time-consuming.

In contrast, standardized liquid transfers using automated pipetting systems are pre-programmed in such a way that optimal precision and accuracy are achieved consistently, even if different types of liquid are being transferred. Unlike manual pipetting, one does not have to worry about flawless pipetting technique when "difficult" liquids, such as ethanol or PCR Mastermix, are handled in an automated fashion.

Point 2: Reproducibility

Reproducibility is the deciding control parameter which determines the validity of laboratory results. This not only pertains to R&D laboratories – it is especially true for the biotechnology and the pharmaceutical industries. It must be demonstrated that an experimental procedure will yield the same results at least a second time; unfortunately, this often proves difficult. Each laboratory technician can tell you that certain complex experiments can only be reproduced by one member of the team. However, that member, too, may fail – depending on how he or she feels that day. Reasons such as quality variations between different batches of reagents may play a role, but it is a known fact that an individual's mood strongly influences the ability to concentrate as well as the physical performance level.

So, what happens if the person responsible for a particular experiment is unavailable – due to either vacation or illness, or because he or she left the laboratory? It often means re-establishing the experiment in its entirety – at considerable expenditure of time and effort. Results that are supported by an automated pipetting system, on the other hand, are easier to reproduce.

The performance and quality of automated processes remain consistent, regardless of the time of day or night. If automated pipetting systems are used to perform routine tasks, multiple team members will be able to take advantage, resulting in consistency and standardization of the pipetting procedure.

Even if laboratory staff carries a high workload, one will be able to rely on sound results as the automated pipetting system takes care of tasks that consist of multiple pipetting steps and therefore require a high level of concentration. The use of an automated pipetting system relieves laboratory staff while at the same time achieving quicker success in cases where reproducible results are needed.

Point 3: Documentation and security

Updating laboratory notebooks, reserving instruments, and archiving audit-relevant documents are onerous but inevitable – particularly in regulated environments. Electronic lab books, as well as the networking of laboratory instruments, can significantly simplify many documentation steps. Log books and protocols may be saved automatically and linked to their respective experiments. Last, but not least, new software solutions, such as VisioNize®, allow networking between individual laboratory instruments as well as remote monitoring via mobile devices.

In this way, notifications of run times, error messages, and the end of an experiment can be delivered via email. Especially in cases where not all instruments are located in the same room, a system of this nature allows for more relaxed work as laboratory workers are free to concentrate on other tasks. The automated pipetting system can handle a great part of the tedious documentation of an experiment while simultaneously freeing up valuable time during the workday in the laboratory.

Point 4: "Walk-away time"

Manual construction of DNA libraries is very labor-intensive and can take hours, or even several days, which, of course, includes incubation times. During such incubation times, one is free to focus on other tasks; however, it necessitates careful scheduling of daily activities. Experienced laboratory staff will easily fit a multitude of tasks into available time slots to complete all the steps at hand in as little time as possible.

If the construction of DNA libraries is entrusted to an instrument, one will gain additional walk-away time and, in turn, more flexibility in the planning of daily activities. Team members will be able to concentrate on new tasks while the automated pipetting station handles several steps, or even the entire workflow.

Point 5: Risk of injury

Laboratory hazards include more than just hazardous substances and sharp objects. Continuous strain on individual muscle groups during pipetting can also cause injury, chronic damage, and even the inability to work.

Pipetting with mechanical pipettes is one of the most frequent causes of chronic injuries such as carpal tunnel or Repetitive Strain Injury (RSI). Symptoms such as pain in the neck, shoulder, forearm, or hand, loss of grip strength, or sensations of numbness result from performing lengthy pipetting series and the ensuing continuous strain on the thumb flexor as well as muscles in the forearm and the upper arm.

Even more insidiously, these symptoms often appear years later and are almost impossible to treat. For these reasons, precautionary measures are the most effective way to prevent illnesses caused by excessive strain. If it makes sense, and if it is feasible, it is recommended to use either electronic pipettes or – in the case of highly repetitive work – switch to an automated pipetting system.

Conclusion

Leaving recurring pipetting tasks or lengthy pipetting series to an automated system brings many advantages that relieve users while offering safety and reliability.

Results become more precise and more reproducible, and experiments must be repeated less often. These aspects will not only delight the principal investigator, but the researchers themselves. In addition, reproducibility between different experiments will improve, and documentation of workflows is made easy. All of this will also satisfy the laboratory quality manager.

More information at
www.eppendorf.com/automation

JAN-HENDRIK BEBERMEIER, EPPENDORF AG

Digitize Your –80 °C Treasures!

Over many years you have collected hundreds, or even thousands of samples – samples that are the result of years of hard work, samples of high value. Sample storage at –80 °C should be safe and reliable, allowing samples to be easily identified, removed, and well maintained. Read here how to always stay in control of the content and performance of your freezer.

Have you ever estimated the value of the samples stored in your freezer? Can you tell how many hours, days, weeks, and months you have spent in the lab working on these samples?

More than 50,000 samples fit into large ultra-low temperature (ULT) freezers. The value of every single sample may differ – from simple buffers to high-value cell extracts, expensive enzymes, or very rare sample material. 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!

Safety for your frozen high-value samples

With the VisioNize® monitoring concept for ULT freezers, such as the CryoCube® F740hi and F740hiw, you can check freezer performance whenever you want. All temperature data and events are stored in the freezer control device of VisioNize.

Careful sample labeling is recommended and 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.

Printed labels on vessels may contain either plain writing or a barcode or QR code, or both. Whatever you decide, smart labeling is crucial for safe identification and, finally, for safe results.

Careful sample labeling and proper storage in freezer storage boxes is just the beginning. Additionally, you must keep accurate records on the content of your ultra-low temperature freezer. Many researchers still use spreadsheet-based software and sometimes even paper lists to keep track. Although this works, there are risks due to human error, damage, and misplacement.

In line with the PhysioCare® Concept for ergonomic and worry-free working conditions, we recommend using an intuitive, reliable sample management tool. Software solutions like eLABInventory will make your lab life more comfortable and more efficient.

Happy to share information and knowledge

Countless working hours of engineers, chemists, molecular biologists, biotechnology experts, and other colleagues flow into the development of Eppendorf products – thus creating a large pool of invaluable knowledge and experience in the fields of Liquid Handling, Cell Handling, and Sample Handling.

We are happy to share this expertise at www.eppendorf.com/handling-solutions. One of the latest additions includes contributions on sample storage at –80 °C.

ULRIKE RASCHE, EPPENDORF AG BIOPROCESS CENTER, JUELICH, GERMANY

Small Accessories – Big Impact

For many years, bioprocess specialists have been successfully using the parallel DASbox® Mini Bioreactor System for process development, and they love it for its capabilities. We at Eppendorf want to make our users' life easier and we listen hard to customer feedback. As a result, we now introduce new small accessories designed to provide an improved user experience.

In the course of a bioprocess, liquids are pumped into the bioreactor, perhaps to adjust the medium pH or feed the culture. Four feed lines per vessel are needed just to pump in acid and base for pH control, a feed solution, and an anti-foam agent. With several bioreactors running in parallel, the number multiplies. The result is a tangle of feed lines – spaghetti syndrome, as we like to call it. Dealing with this can be annoying and costs precious time.

Fight spaghetti syndrome

Small accessories help. Eppendorf introduces cable drillers for feed line routing (Fig.1).

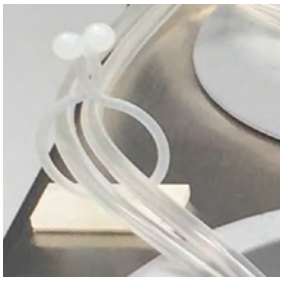


Fig. 1: Feed line routing using cable drillers

With adhesive tape underneath, they can be easily mounted onto the DASbox base unit. Cable drillers are included with every new DASbox Mini Bioreactor System and can also be ordered separately, in packs of 10, to retrofit existing systems. Get rid of your feed line chaos!

Don't get lost

To help operators to keep track of their feed lines, we improved the lines' labeling. A cable tie firmly attaches the new label to feed lines of any diameter. By tagging each end, the route of the feed line can easily be tracked, and it will be readily assigned to the correct pump head, which is labeled in the same way (Fig. 2). For all new DASbox systems, C-Flex feed lines are delivered pre-labeled. Labels are also available separately, so that you can tag additional lines yourself.

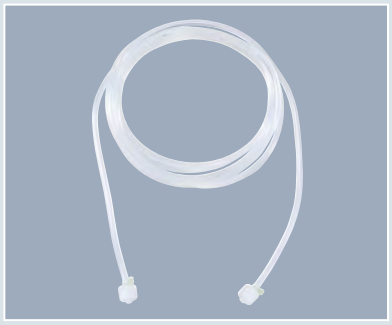
Firmly attach

During cleaning in place (see box), feed lines are connected to a waste bottle. To prevent overturning of the bottle, we now equip the DASbox bottle carrier with a spring clamp. An upgrade kit is available to retrofit existing systems.

Fig. 2: Feed line with new label, consisting of cable tie, identification plate, and adhesive label

Close-Up

Liquid Addition to the Bioreactor



Feed lines deliver liquids to the bioreactor using the system's integrated pumps. DASGIP® feed lines consist of three separate parts that are connected to each other: a feed line to connect the addition bottle and the pump; the pump head tubing in the middle inserted into the pump head; and another feed line to connect the pump to the bioreactor. Before starting a bioprocess, the researcher needs to ensure the sterility of the feed lines. Feed lines made of PTFE are "cleaned-in-place" with ethanol and NaOH, and subsequently rinsed with sterile water. Feed lines made of C-Flex can be autoclaved and are the preferred choice for cell culture applications.

Attention: Always check which sterilization method is suitable for the tubing material you are using.

The feed lines can be connected with the tubing attached to the bioreactor ports either with Luer lock connectors or by welding.

Feed line welding

> Avoids breaking the sterile barrier
> Simplifies handling, because it can be done outside a laminar airflow cabinet
> Weldable material needed
> Tubing which is not suited for welding needs to be extended with weldable connectors

Download Short Protocol 33 to read about welding of feed lines through weldable connectors. www.eppendorf.com/sp33

CAROLYN TAUBERT AND BERRIT HOFF, EPPENDORF AG

Eppendorf Prize Winners 2018/2019: Johannes Kohl & Georg Winter

Johannes Kohl

The German scientist Johannes Kohl, Ph.D. (The Francis Crick Institute, London, UK), won the 2018 *Eppendorf & Science Prize for Neurobiology* of 25,000 USD.

His work has revealed how a small population of genetically defined neurons controls the motor, motivational, hormonal, and social aspects of parental behavior in male and female mice. Previous work had implicated specific neurons in parenting located in the medial preoptic area (MPOA) of the hypothalamus (a brain area that shares common features with other vertebrates). However, it remained unclear how a small group of neurons could control such a complex social behavior. First, using anatomical techniques, Johannes Kohl revealed that these neurons form a hub in a complex, brain-wide parenting network. Subsequently, he used imaging approaches to visualize the activity of these MPOA neurons during parenting and manipulated their function in behaving animals. Together, these experiments revealed that MPOA neurons form subpopulations, each controlling different aspects of parenting.

www.eppendorf.com/prize

Georg Winter

The 2019 *Eppendorf Award for Young European Investigators*, endowed with € 20,000, went to Dr. Georg Winter of the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria.

The award is given for his pioneering work developing a method for targeting specific proteins for degradation using heterobifunctional chemical compounds to specifically recruit ubiquitin E3 ligases to the intended protein target for destruction. This powerful system enables targeting of previously undruggable targets and shows promise both in cells and *in vivo* in model systems as an emerging therapy. His work has led to a fury of excitement across pharmaceutical companies and has resulted in several patents; it holds promise to yield novel therapies for cancer and other diseases of unmet need.

www.eppendorf.com/award

Win a New Pipetting System

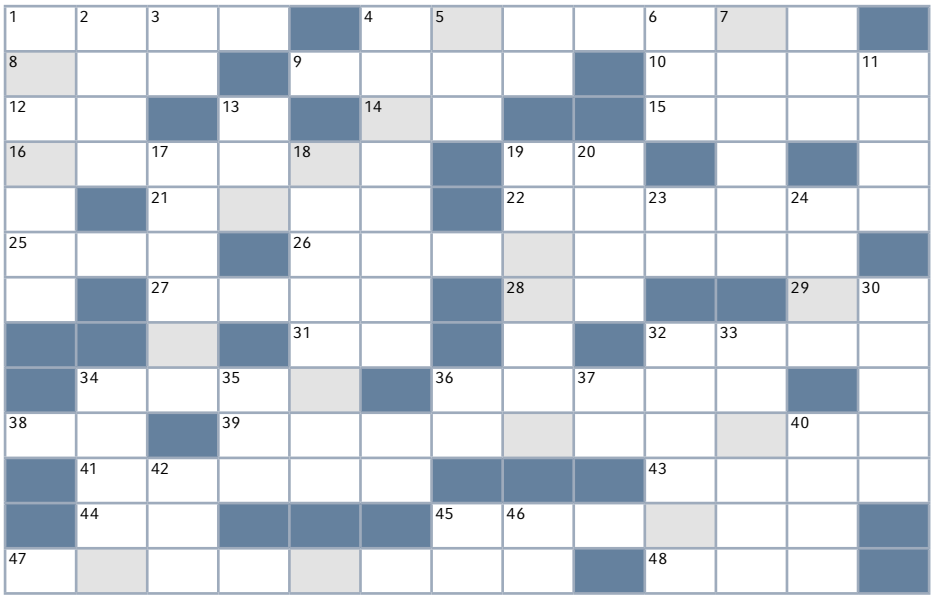
The solution of our anniversary prize competition of BioNews 49 was "VisioNize System". The five main prize winners were happy about a personalized pipette each: Julie Challant (France), Steven Murfitt (United Kingdom), Silke Frenz (Germany), Dhanesh Appu (India), and Thomas Giersch (Australia).

Good luck in our new competition!

Simply arrange all letters in the light gray boxes of the crossword in the correct order. Send us the solution until **October 31, 2019**.

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

All correct answers will be considered for a prize. Winners will be notified in writing. Cash payment of the prize is not possible. No recourse to legal action. The judges' decision is final. Eppendorf employees and their families may not participate. The winner of the first prize will be published in issue No. 53.



1st to 3rd Prize:

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

4th to 6th Prize:

1 Amazon® Voucher worth 50.00 EUR

7th to 15th Prize:

400 bonus epPoints® each

(epPoints registration required)

ACROSS

1 Violent, explosive anger
4 Growing of microorganisms or cells in nutrient medium
8 Message indicating a mistake or fault (abbrev.)
9 Filling 384 of this is now easier and faster than ever
10 Loves Kermit the Frog (part of name)
12 Complements DC
14 The largest city and capital is Vilnius (ISO country code)
15 Mamma Mia! Still on the radio …
16 Surname of Coco
19 Famous for cheese, chocolate, watches, and more (ISO country code)
21 Message, written work
22 Egg cell
25 To make a choice or decision
26 To convey from one place to another
27 Field, sphere
28 Branch that deals with the use of computers and telecommunication (abbrev.)
29 Nordic island country where they pay with ISK (ISO country code)
31 1×10^{-9} m (abbrev.)
32 Smallest unit of an element
34 Country of north-central Africa
36 Generic term for spaghetti, capellini, maccheroni, and more
38 Value that specifies how acidic or basic a water-based solution is
39 Periodically sent publication or mail
41 Informal short for introduction
43 L' …, c'est moi!
44 French article (definite, masc.)
45 Receives a signal and responds to it
47 Characterized by fluency and persuasiveness
48 Highest position or rank

DOWN

1 Vessel used to carry out bioprocesses
2 Marble-faced landmark in London
3 Also know as "Hellas" (ISO country code)
4 Supports your manual microinjection
5 Freezer for ultra-low temperatures (abbrev.)
6 Actress starring in Pulp Fiction (first name)
7 Type of beef steak
11 Japanese rice wine
13 Single entity or unit
17 Combined with "soft", it forms a new tip technology
18 A simply smart tool
19 Coneshaped
20 Organism on/in which another organism lives
23 Radioactive chemical element discovered in California (abbrev.)
24 Group of three
30 Bright, mentally quick
32 To affirm the correctness or truth
33 Body decoration
34 Cool down, relax (verb)
35 Small social insect
36 post scriptum or thermoplastic polymer
37 Native country of 15 across (ISO country code)
40 Doc Holliday's buddy (surname)
42 Greek prefix for "new"
45 Dakar is its executive capital (ISO country code)
46 Extra-Terrestrial

Solution hint for prize competition of BioNews No. 51:

				D			E				U			U			

Send us the solution until **October 31, 2019**, via e-mail to **bionews@eppendorf.de**, or participate online at **www.eppendorf.com/bn-service**.

SOMETIMES THE GRASS REALLY IS GREENER SOMEPLACE NEW.

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