

eppendorf

# BIO NEWS

No. 34 – 2011



## Exclusive: the many faces of Captain Eppi!

- Eppendorf Eporator®: your bacteria's favorite
- Eppendorf Xplorer®: interactive e-learning
- Two new refrigerated microcentrifuges



Comparison of the efficiencies of electroporation and traditional chemical transformation ·  
Use of LoBind Tubes to prepare and store standard panels for real-time PCR absolute quantifications · etc.



# Dear Reader!

*Sultans, avatars, kings and soccer fans – the imagination with which the participants at the 16th Eppendorf International Sales Meeting decorated and dressed their Captain Eppis was impressive. So impressive indeed, that we are presenting the results to our BioNews readers on the cover of this edition. In addition, you may admire all Captain Eppis online at [www.facebook.com/eppendorf](http://www.facebook.com/eppendorf).*

*Apart from two new refrigerated microcentrifuges (5418 R and 5424 R), we are presenting you additional rotors and accessories for our Centrifuges 5804/5810. Further, we invite you to meet epBlue ID (a novel barcode module for the epBlue software) and the Eporator which transforms your bacteria fast, safely and with high efficiency.*

*Our electronic pipette Pipette Xplorer shows an impressive spectrum of features and is nevertheless so easy to operate (page 6). On page 7 you can read an interview with the Xplorer's product designer in which he speaks about his design philosophy.*

*Apart from further news and product solutions for a vast variety of applications, the insert, as always, provides you with detailed Application Notes. And, at the end of the magazine, you will find our popular prize competition with great prizes.*

*Enjoy reading!*

Your BioNews editorial team

## IMPRINT

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## Innovation

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PETER SCHREINER, EPPENDORF AG

# More performance – more safety!

Starting January 2011, Eppendorf will offer additional rotors and accessories for the Centrifuges 5804/5804 R (R = refrigerated) and 5810/5810 R, providing for larger capacity and aerosol-tight centrifugation of plates and tubes. Centrifuges 5810/5810 R benefit from 25 % higher maximum capacity (4 x 500 mL instead of the 4 x 400 mL in the current version), as well as 40 % higher capacity when using 50 mL conical tubes.

The new certified aerosol-tight rotors for tubes and plates provide safety against hazardous samples and thus enable the centrifuges to be used for an even wider range of applications.

## **New! Aerosol-tight fixed angle rotor FA-45-6-30**

- 6 x 15/50 mL conical tubes or various Oak Ridge formats
- Max. 20,130 x g in Centrifuge 5810 R
- Extremely light weight (only 3.2 kg) allowing for easy handling



## **New! Aerosol-tight plate rotor A-2-DWP-AT**

- For use in Centrifuge 5810/5810 R only
- 2 x 4 MTP
- Max. rcf: 3,486 x g



*Please note that a software upgrade will be necessary for older centrifuges when using rotor FA-45-6-30 or rotor A-2-DWP-AT. Please contact your local Eppendorf organization or dealer for further information.*

## **New! Buckets for high throughput**

- For use with rotor A-4-81
- Max. 28 x 50 mL conical tubes
- Adapter for 15 mL conical tubes



Centrifuges 5804/5804 R and 5810/5810 R • Ref. no. 185



### **Tip**

## **Performance you can count on**

Eppendorf Centrifuges 5804/5804 R and 5810/5810 R with their renowned quality and reliability offer you cost efficient solutions for your medium to high-throughput applications – now and in the future.

Whether your applications require spinning many tubes at a time or centrifugation of larger volume tubes at high speed, these multipurpose centrifuges with their large variety of rotors and adapters cover virtually any application in tubes, bottles and microplates.

Additional information may be found in the brochure (see ref. no.) or at [www.eppendorf.com/centrifugation](http://www.eppendorf.com/centrifugation).

PETER SCHREINER, EPPENDORF AG

# The new coolness

The popular Eppendorf microcentrifuges 5418 and 5424 are now available as refrigerated options. The new refrigerated 18-place and 24-place microcentrifuges 5418 R and 5424 R are designed in accordance with Eppendorf's Silence | Speed | Simplicity™ philosophy for centrifugation. With their small footprint, exceptionally quiet operation and the FastTemp function for quick pre-cooling, the 5418 R and 5424 R set new standards for refrigerated microcentrifuges.

Both design and operating concept of these instruments are based on in-depth ergonomic studies, making everyday routines faster and easier. The familiar blue rotary knobs allow quick, intuitive parameter setting. The low access height enables easy insertion and removal of rotor and samples.

Both models include a color coded, aerosol-tight rotor as standard equipment – for increased safety in the laboratory. However, even when operated without the rotor lid, both centrifuges run incredibly quietly.

Centrifuge 5418 R is the ideal entry-level model for routine molecular applications. Centrifuge 5424 R, on the other hand, convinces through power and versatility. There are four rotors to choose from, including a special Kit-Rotor™ (for spin columns) with an extra high rim to support open tube lids during centrifugation.

## Would you like to know more?

You can order your personal copy of our new centrifuge brochure by using the reference number denoted below.

Detailed information is also available at [www.eppendorf.com/centrifuges](http://www.eppendorf.com/centrifuges).

### New! Centrifuge 5418 R

Your entry-level model – ideal for routine molecular applications.



- 18 places for 1.5/2.0 mL tubes
- Max. 16,873 x g (14,000 rpm)
- Temperature range 0°C to +40°C
- Maintains a constant +4°C at maximum speed
- FastTemp function for quick pre-cooling of the centrifuge
- Aerosol-tight QuickLock™ rotor lid for quick locking of the rotor

Centrifuge 5418 R • Ref. no. 236

### New! Centrifuge 5424 R

For users who desire a powerful and versatile microcentrifuge.



- 24 places for 1.5/2.0 mL tubes
- Max. 21,130 x g (15,000 rpm)
- Temperature range -10°C to +40°C
- Maintains a constant +4°C at maximum speed
- Especially quick FastTemp function for quick pre-cooling of the centrifuge, e. g. from ~21 °C to 4 °C in only 8 minutes!
- Available with rotary knobs or easy to clean keypad
- Choice of 4 rotors

Centrifuge 5424 R • Ref. no. 235

JANINE JACOBI, EPPENDORF AG

# Interactive e-learning

The new electronic pipette Eppendorf Xplorer® is synonymous with an intuitive operating concept, ergonomics and reproducibility. Even the inexperienced user will master handling in less than 3 minutes, as confirmed by a study conducted at the Institute of Human Factors Engineering at the Technical University of Darmstadt, Germany\*.

Via a new interactive e-learning program you can now directly experience the advantages and possibilities offered by the Xplorer, the applications it covers and, most importantly, its well thought-out and simple operation.

## Your Xplorer quick entry!

The new, clearly laid out e-learning training program may be found at [www.eppendorf.com/xplorer-start](http://www.eppendorf.com/xplorer-start) under "Interactive Demonstration". Here, you will find the following contents:

## The pipette in detail

- Product benefits
- Key Features with in-depth information
- Technical data
- Delivery package
- Accessories

## Operation

- Application examples (loading gels, pipetting and mixing, adjustment for ethanol 75%)
- Handling (interactive demonstration of the individual modes)

## "FAQs": Frequently asked questions

- Questions on the device and operation
- Questions on accessories
- Questions on the applications

## Downloads

This chapter contains useful documents for downloading, e.g. the operating manual, technical data, speed tables, information about spare parts, and much more.

## Conclusion

No matter whether you already are the happy owner of an Xplorer, or whether you are currently contemplating its acquisition – our e-learning program is your all-around-carefree information package with all valuable information about the new Eppendorf Xplorer family of pipettes.

*\*please also refer to Application Note p. 5–6*

## News

## Fit with epServices

**Any questions about Eppendorf products, protocols or applications?**

**Our Application Support will help you!**

To provide the highest standard of support for our customers, product experts in all Eppendorf organizations are available to assist you. In close cooperation with our global team of Application Specialists they are happy to help you with advice and assistance for all kinds of product and application questions.

Please contact your local Eppendorf organization or our global Eppendorf Support Centers:

### Europe, International:

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### Asia Pacific:

Tel. +60 3 8023 6869

E-Mail: [support\\_asiapacific@eppendorf.com](mailto:support_asiapacific@eppendorf.com)

**epservices**  
for premium performance

In addition, a whole range of useful Application Notes, FAQs and other support documents is available in the Support section of all local Eppendorf websites and at

[www.eppendorf.com/support](http://www.eppendorf.com/support).



Interactive e-learning at  
[www.eppendorf.com/xplorer-start](http://www.eppendorf.com/xplorer-start)

Eppendorf Xplorer® • Ref. no. 233

JANINE JACOBI, EPPENDORF AG

# Design as key to success

Norbert Koop, born in 1961, founded his studio KOOP INDUSTRIAL DESIGN in 1991. Since 1997 he has been col-



laborating with the Eppendorf AG. Numerous Eppendorf products designed by Koop and his team have been recognized inter-

nationally and were awarded prizes such as the red dot design award, the iF Award or the Good Design Award; the "red dot design award – best of the best 2010" for the electronic pipette Eppendorf Xplorer® being the highlight of Norbert Koop's design success for Eppendorf products.

***How did you manage to design a product which is distinctly different from a manual pipette in such a way that it is nonetheless immediately recognizable as an Eppendorf pipette?***

One of the most important criteria was the creation of outstanding ergonomics. During the development phase of the manual pipette Research plus we conducted extensive studies in order to obtain a handle of optimal form. The result: a profile featuring a characteristic vertical line which is further emphasized by a colored stripe. The geometry of the profile, the Eppendorf handle grooves as well as the hand rest, have been transferred to the Xplorer. Combined with the Eppendorf colors and typeface, these features suffice to make both pipettes immediately recognizable as Eppendorf products. The lower and multichannel parts adapted from the Research plus complete the picture.

***Which rules do you follow when designing products belonging to a product family?***

Without rules, even the best design is bound to fail. This is particularly true if, in addition to all other aspects under consideration, the product is a part of a larger collection.

Encompassing design development can only be successful with the help of a guideline. The Product Design Manual, developed by us in collaboration with Eppendorf, names design goals, as well as the means for their realization. Different design features define membership of individual products in product families, such as centrifuges or pipettes. Colors and typefaces are also strictly defined. Naturally, not all design features can be applied to all Eppendorf products simultaneously. After all, a MixMate is different from a Multipipette! That being said, all Eppendorf products share one feature: a "smiling line" gives them a friendly face.

***How would you describe your design philosophy for Eppendorf products?***

Functionality and handling are the major aspects which influence the design of an Eppendorf product. Many parameters may be determined with certain accuracy by employing measurements, tests and user surveys. However, one aspect of functionality is not as easily defined: emotionality. It is responsible for the user's initial emotional response when laying eyes on his/her Eppendorf pipette and which subsequently is manifested when the instrument is handled.

The goal of our design efforts is that the user will perceive the Eppendorf product as a friend and helper who performs his tasks in a reliable and highly precise fashion, while at the same time never pushing into the foreground.



**red dot design award**  
best of the best 2010



Eppendorf Xplorer®

Design: Norbert Koop, Stefan Espenhahn; KOOP INDUSTRIAL DESIGN, Hamburg; [www.koopdesign.de](http://www.koopdesign.de)

Eppendorf Xplorer® • Ref. no. 233



BRIGITTE KLOSE &amp; TANJA MUSIOL, EPPENDORF AG

# An ideal system!

**Eppendorf epT.I.P.S.® LoRetention, the Eppendorf BioPhotometer™ plus and the UVette® comprise an ideal system for contamination-free sample recovery following simple and fast photometric concentration determination.**

## Perfect sample recovery

The excellent sample recovery results obtained with the epT.I.P.S. LoRetention pipette tips are easily and effectively illustrated via photometric absorption tests (please also refer to Application Notes p. 1–2).

epT.I.P.S. LoRetention minimize loss of valuable sample material due to their ultra hydrophobic, extremely homogeneous surface. Therefore they are an important tool for applications involving detergent-based solutions (especially in very small volumes).

## For example: “Genomics”

A possible area of application is the field of genomics. Concentration determination via photometry often stands at the very beginning of the process chain. When quartz cuvettes are used, carry-over contamination or sample degeneration by RNases or DNases can never be completely ruled out. The individually packaged UV/VIS plastic cuvettes from Eppendorf, the UVettes, are available in certified PCR clean quality (free from DNases, RNases and PCR inhibitors) just like the epT.I.P.S.

LoRetention. This quality is especially well suited for sample recovery [1], as well as for valuable samples and samples of low concentration.

## Immediately available for subsequent applications

The photometric determination of sample concentration itself can be performed very easily and fast in the BioPhotometer plus. 32 pre-programmed routine methods enable fast and reliable sample analysis. Directly following measurement, the samples may be used in subsequent applications such as PCR and real-time PCR, with the added advantage of complete correspondence between the measured concentration and the concentration used. Furthermore, no valuable sample is lost [2]. Due to their product properties, the epT.I.P.S. LoRetention pipette tips are especially well suited for the preparation of the master mix.

## Conclusion

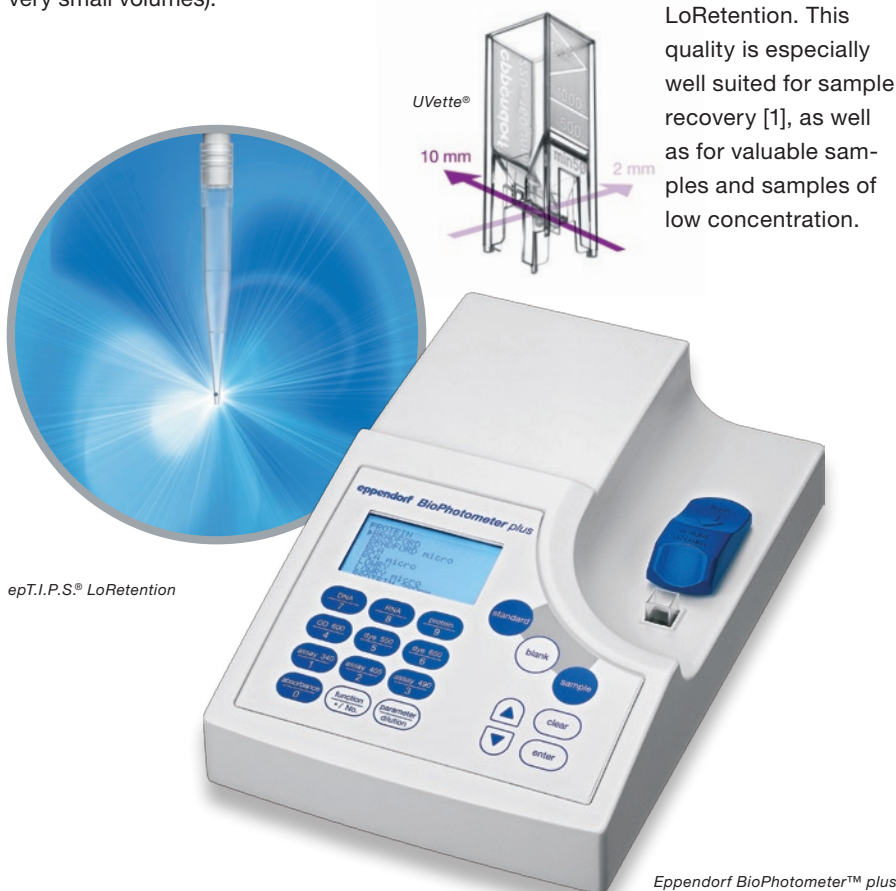
The combination of epT.I.P.S. LoRetention, the BioPhotometer plus and the UVette allow for simple, fast and contamination-free determination of the concentration of your sample – while at the same time providing for maximum sample recovery.

## Literature

[1] Application Note 228: Reproducible photometric determination of DNA concentrations using the Eppendorf UVette® in the Eppendorf BioPhotometer™ plus

[2] Application Note 229: PCR and real-time PCR experiments performed with DNA samples which have undergone multiple measurements in the UVette®, using the Eppendorf BioPhotometer™ plus

Both Application Notes can be found at [www.eppendorf.com/applications](http://www.eppendorf.com/applications).



epT.I.P.S.® LoRetention

Eppendorf BioPhotometer™ plus

epT.I.P.S.® LoRetention • Ref.no. 225  
Eppendorf BioPhotometer™ plus • Ref.no. 221  
UVette® • Ref.no. 108



# Comparison of low retention pipette tips via simple and fast absorbance measurements in the BioPhotometer™ plus

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Foong Teng Lu, Eppendorf Asia Pacific Headquarters, Kuala Lumpur, Malaysia

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## Introduction

In comparison with water, the surface tension of detergent solutions is low. For this reason, such solutions do not drip well off plastics like polypropylene; instead, wetting will occur. Thus, following dispensing, a liquid film remains on the surface of the pipette tip. Since most detergent-containing solutions in the laboratory are transparent and dye-free, the film remaining inside the tip is hardly visible. Hence, neither the loss of sample, nor the diminished accuracy and precision, are noticed during the pipetting process.



Fig. 1: Residues of liquid in a standard pipette tip (left) and in Eppendorf epT.I.P.S. LoRetention pipette tip (right)

Using colored solutions, normally invisible drops and films, which remain inside the pipette tip, may be made visible (Fig. 1). Often, it is already visible to the naked eye during the pipetting process, if the chosen tip type is suitable for a given solution. Smaller differences may be brought to light by absorbance measurements in a photometer. In addition, repeat measurements enable the calculation of pipetting precision.

This Technical Report compares the Eppendorf epT.I.P.S. LoRetention pipette tips to Standard Eppendorf epT.I.P.S., as well as to “low retention” tips made by other manufacturers via an absorbance test in the BioPhotometer plus. These experiments demonstrate how the properties of pipette tips may be made visible during the use of detergent-containing solutions using a simple procedure.

## Materials and methods

For these experiments, sterile Standard ep Dualfilter T.I.P.S. (Eppendorf) and ep Dualfilter T.I.P.S. LoRetention (Eppendorf) of the 200 µL size were tested, as well as seven “low retention” tips from other manufacturers. The electronic pipette Eppendorf Research pro 20-300 µL was used to pipet the samples. This electronic pipette was chosen to exclude all manual influence on the pipetting process, such as varying pipetting speed, thus ensuring the highest possible reproducibility of the results [1].

The programs and the chosen options [2] of the Research pro are listed in table 1.

A 0.1% Triton® X-100 solution, dyed with Brilliant Blue FCF, was used as the test solution. The absorption measurements were performed in the Eppendorf BioPhotometer plus using the Eppendorf UVette. The method “Absorbance” was chosen at 595 nm [3].

First, 100 µL distilled water were pipetted into a UVette using the Standard ep Dualfilter T.I.P.S. (Program 1), thus defining the blank value in the BioPhotometer plus. Subsequently, using a pipette tip to be tested, 100 µL of the colored detergent solution were aspirated and dispensed back into the same tube by pipetting against the inside wall (Program 2). In order to avoid re-aspiration of the liquid from the tube wall, the following dispensing technique was performed using the Research pro pipette: The dispensing button was continually depressed during the dispensing of the detergent solution; only after the tip was removed from the solution, it was released [2]. Subsequently, the remaining liquid was rinsed from the pipette tip into the water which had previously been placed into the UVette (Program 3).

### Program 1: Pipetting of 100 µL water into the UVette

Aspiration and dispensing speed: **Highest**

Option: **Standard (comparable to a manual pipette)**

### Program 2: Aspiration and dispensing of 100 µL detergent solution

Aspiration and dispensing speed: **Lowest**

Option: **Standard (comparable to a manual pipette)**

### Program 3: Rinsing of the residual liquid into the water inside the UVette (100 µL)

Aspiration and dispensing speed: **Lowest**

Option: **Rinse (RNS) = Threefold rinsing and mixing**

Table 1: Programming of the Eppendorf Research pro

## Comparison of low retention pipette tips via simple and fast absorbance measurements in the BioPhotometer™ plus

The UVette was measured in the BioPhotometer plus at a path length of 10 mm. The absorbance value represents the amount of detergent solution remaining inside the pipette tip. 10 values were obtained for each type of tip, from which the averages and standard deviations were calculated.

### Results and discussion

Figure 2 shows the results of the absorbance measurements. The higher the absorbance, the more residual liquid remained inside the pipette tip. ep Dualfilter T.I.P.S. LoRetention achieved the lowest absorbance values and the smallest standard deviation. These tips retained only small amounts of liquid, while at the same time achieving high reproducibility. All other “low retention” tips tested yielded considerably higher absorbance values, i. e. more residual liquid inside the tip.

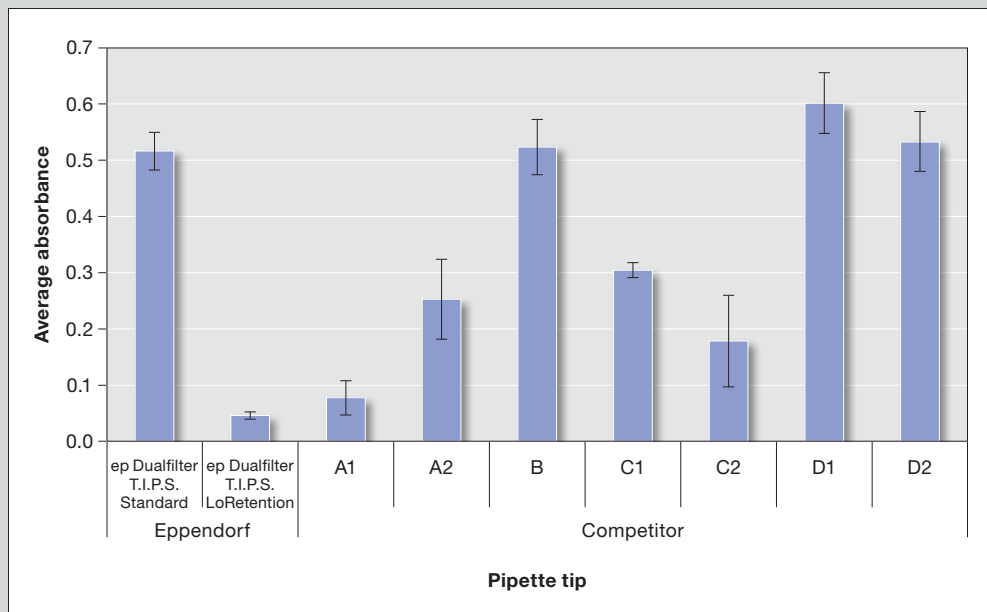
Tip A1's absorbance values are 1.7 times as high, whereas those of tip D1 are 13 times higher.

Tip A1's absorbance values are 1.7 times as high, whereas those of tip D1 are 13 times higher.

The absorbance values of Tips B, D1 and D2 are at a level similar to that of Eppendorf Standard ep Dualfilter T.I.P.S., i. e. tips without “low retention” properties. These tips do not offer an advantage during pipetting of this detergent-containing solution compared to standard tips.

Tips A2 and C2 produced particularly high standard deviations at relatively low absorbance. In these cases, the amount of residual liquid inside the tip varied considerably between individual measurements, which was already visible to the naked eye.

Major variations between tips present a source of error during the pipetting process.



**Fig. 2:** Average absorbance values per tip type. Values were calculated from 10 individual measurements performed in the BioPhotometer plus. The error bars represent the standard deviations.

The results demonstrate that absorbance measurements performed in the BioPhotometer plus are able to distinguish differences in the flow properties of pipette tips. By performing ten individual measurements, the homogeneity of a particular type of tip (for example, from one tip box) may also be determined using this fast and simple test system. Please note, however, that this experimental design is not suitable for calibration of dispensing systems in accordance with EN ISO 8655 [4].

### Conclusions

Within this report, a test was demonstrated which provides a fast and simple measurement of the extent to which colored residual liquids are retained inside a given pipette tip. This test enables the user to determine whether a certain pipette tip is suitable for the solution on hand. The experiments performed here verify that the Eppendorf ep Dualfilter T.I.P.S. LoRetention have the lowest wetting values, thus providing superior flow behavior compared to “low retention” tips made by other manufacturers. Due to minimized sample

loss and high tip homogeneity when using Eppendorf ep Dualfilter T.I.P.S. LoRetention for pipetting detergent-containing solutions, sophisticated experiments may be performed in a reproducible and reliable fashion.

### Literature

[1] Application Note 92: Comparison of pipetting behavior of the electronic pipette Eppendorf Research® pro and the manual pipette Eppendorf Research® via an enzyme linked immunosorbent assay (ELISA)

[2] User manual Research pro

[3] User manual BioPhotometer plus

[4] Eppendorf SOP - Standard Operating Procedure for Pipettes

(1-4 are available for download at [www.eppendorf.com](http://www.eppendorf.com) in the section Support & epServices.)

### Readers' service

ep Dualfilter T.I.P.S.® LoRetention • Ref. no. 225  
 ep Dualfilter T.I.P.S.® • Ref. no. 212  
 Eppendorf BioPhotometer™ plus • Ref. no. 221  
 UVette® • Ref. no. 108  
 Eppendorf Research® pro • Ref. no. 115

# Use of Eppendorf LoBind® Tubes to consistently prepare and store standard panels for real-time PCR absolute quantifications

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## Introduction

The so-called absolute quantification in real-time PCR enables to determine DNA concentrations of samples by running standards in parallel. However, this requires a strict setup of the PCR reaction and rigorous standard panel preparation in order to obtain reliable results [1]. Standard panels serve as a basis for the calibration curve by plotting  $C_t$  values of the standards against  $\log_{10}$  of corresponding amounts of DNA. The slope of this curve reflects the amplification efficiency and can be determined as follows:

$$\text{Equation 1: Efficiency} = 10^{-1/\text{slope}} - 1$$

Optimized PCR, being an exponential reaction at least in the beginning, with a theoretical maximum efficiency of 1.00 (or 100 %) means that the amplicon quantity doubles at each cycle. Moreover, the calibration curve also allows the user to determine template concentration of samples from  $C_t$  values which were determined experimentally.

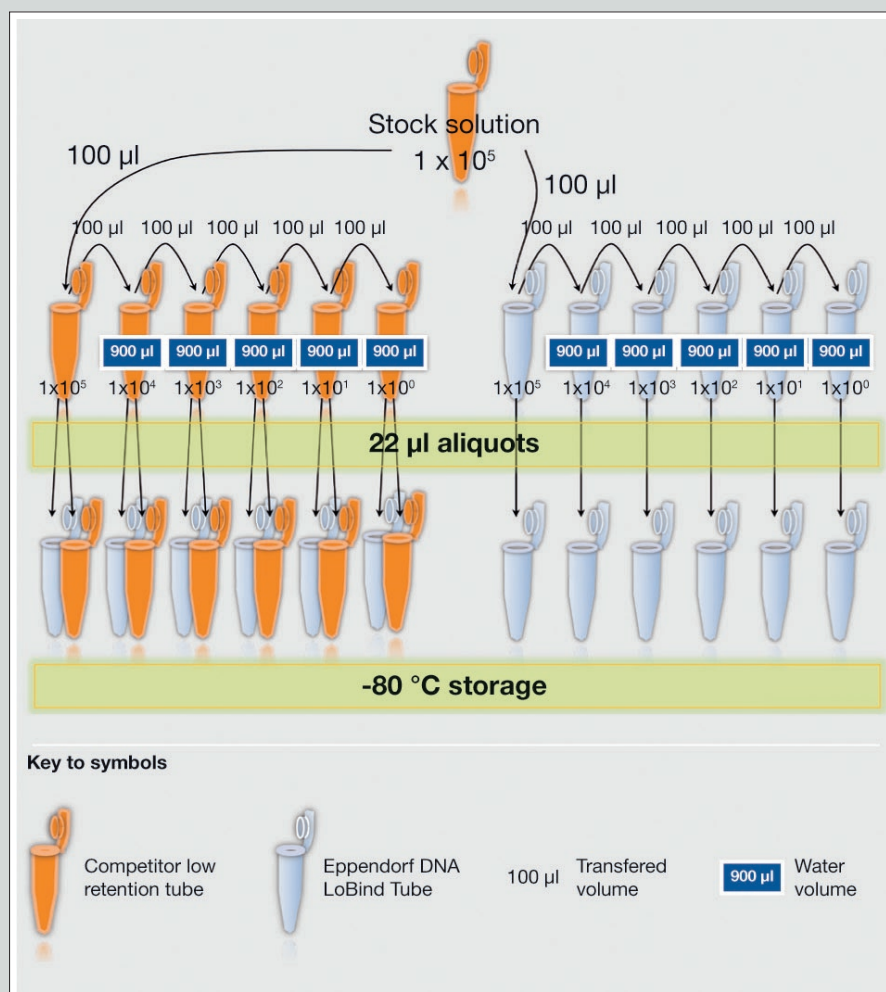
In this context, standard panel preparation appears as a crucial step in the correct assessment of amplification efficiency and sample quantification. However, the preparation of a good panel, which should range over several orders of magnitude of dilution ( $\log_{10}$  steps), is often challenging [1]. Dealing with very low concentrations of nucleic acid such as 10 copies of genomic DNA per 10  $\mu\text{L}$ , the loss of DNA on vessel surfaces will be amplified at each dilution step and will be particularly significant for the most diluted standards. This leads to a DNA concentration actually lower than expected.

Accordingly, the observed amplification efficiency will be significantly reduced because the initial DNA quantity is overestimated, leading to misinterpretation of the results. The following experiment will describe how the choice of laboratory vessels influences the apparent real-time PCR efficiency by preparing serial dilution of standard panels and/or storing them in Eppendorf DNA LoBind or competitor low retention tubes.

## Materials and methods

### Nucleic acid purification

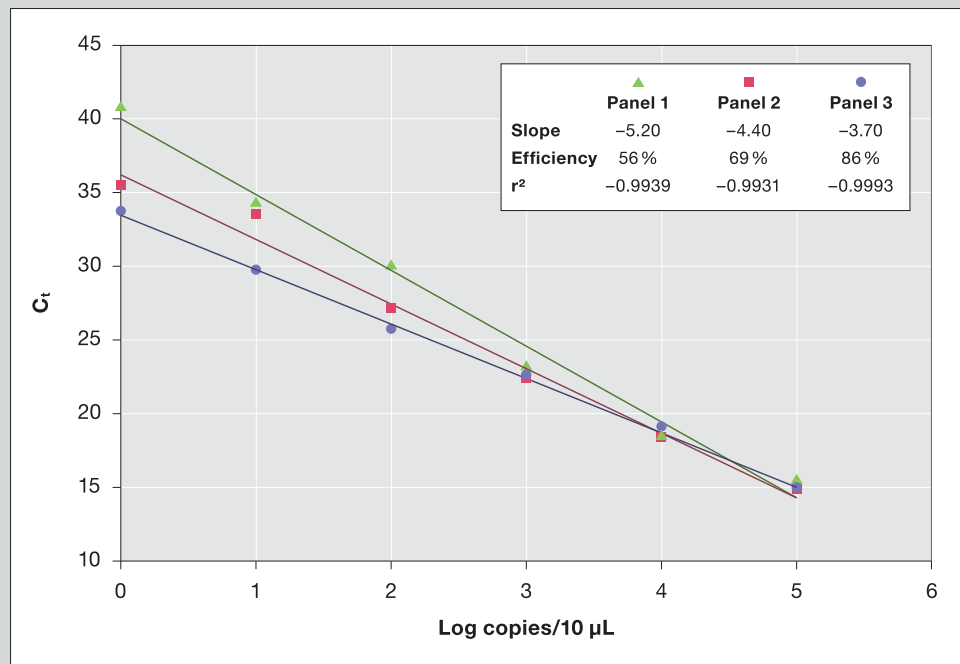
Genomic DNA from lymphoblastoid T-cell line 8E5 (NIH; ATCC 8993) was extracted from 200  $\mu\text{L}$  of cell suspension using the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Elution was made in 200  $\mu\text{L}$  of water to obtain a stock solution at  $1 \times 10^5$  copies of genomic DNA per 10  $\mu\text{L}$ .



**Fig. 1:** Flowchart of genomic DNA standard assay panels preparation. DNA stock solution was serial 10-fold diluted in either competitor low retention or Eppendorf DNA LoBind tubes. 22  $\mu\text{L}$  aliquots of each dilution were stored in either competitor low retention or Eppendorf DNA LoBind tubes. Aliquots were stored at  $-80^\circ\text{C}$  for 24 h before real-time PCR assay.



## Use of Eppendorf LoBind® Tubes to consistently prepare and store standard panels for real-time PCR absolute quantifications



**Fig. 2:** Standard curves obtained for each panel.  $C_t$  versus  $\log_{10}$  of expected DNA concentration was plotted and slopes were obtained after linear regression. PCR efficiencies are derived from slope values.

### Serial 10-fold dilutions

Either Eppendorf DNA LoBind 1.5 mL tubes or competitor low retention tubes were used. 100  $\mu$ L of genomic DNA solution was transferred into a tube containing 900  $\mu$ L of water. The tube was then vortexed for 1 min and served as start solution for the next dilution step. Then, each dilution was aliquotted by 22  $\mu$ L and stored at  $-80^\circ\text{C}$  for 24 h (Fig. 1).

### Quantification of genomic DNA target

Target gene (ALB) was quantified by real-time PCR. The forward primer was 5'-GCTGTCATCTCTGTGGGCTGT-3', the reverse primer was 5'-AACTCATGGAGCTGCTGGTT-3' and the probe was 5'-FAM CCTGTCATGCCCCACACA-AATCTCTCC TAMRA-3' [2]. Real-time PCR was carried out on a LightCycler® instrument using the LightCycler® FastStart® DNA Master HybProbe® kit (Roche Applied Science, Basle, Switzerland) [3]. Thermocycling conditions consisted of an initial step (95  $^\circ\text{C}$  for 10 min) and 45 cycles of denaturation

(95  $^\circ\text{C}$  for 10 s) and an annealing and polymerization step (60  $^\circ\text{C}$  for 30 s). All three standard panels were assayed in the same experiment.

### Standard curve

$C_t$  values were plotted versus the  $\log_{10}$ -transformed expected concentration of the target gene. Linear regression was applied to each scatterplot and slope was used to determine apparent amplification efficiency using equation 1.

### Results and discussion

Fig. 2 shows the standard curves obtained for each panel. Panels 1 and 2 originated from the same serial dilution carried out in competitor low retention tubes. Panel 1 was stored aliquotted in competitor tubes and panel 2 in Eppendorf DNA LoBind tubes.

Panel 3 was both diluted and stored in Eppendorf DNA LoBind tubes. Apparent efficiencies were 56 % for panel 1, 69 % for panel 2 and 86 % for panel 3 and  $C_t$  values at  $10^0$  copies/10  $\mu$ L were 41.00, 35.44 and 33.65, respectively.

As these three standard curves were obtained in the same experiment, it is highly likely that the differences observed are due to a loss of genomic DNA. This loss can occur both during dilution preparations, leading to a decrease by 17 % in apparent efficiency, and during storage giving an additional 13 % decrease.

The  $C_t$  values are also consistent with DNA adsorption on tubes as the maximum effect is observed for lowest DNA concentration (up to 7  $C_t$ s of difference at  $10^0$  copies/10  $\mu$ L).

These results highlight an obvious effect of the vessel quality in the accuracy of standard panel preparation. In a more general extent, they reveal how

much DNA adsorption can become a major point when dealing with low concentrations.

### Literature

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### Readers' service

Eppendorf LoBind® Tubes • Ref.no. 184

# The electronic pipette Eppendorf Xplorer® – Intuitive Handling

Kornelia Ewald, Eppendorf AG

## Introduction

For users it is extremely important that new products should, among other things, feature ease of operation. This requires that the special needs and characteristics of the user groups within a specific application field are recognized and implemented in the development of the new product. In the case of the new electronic pipette Xplorer, this was ensured, among other things, by an operating study which was conducted during the development phase in conjunction with the Institute of Human Factors Engineering at the Technical University of Darmstadt, Germany.

## Ease of operation

Intuitive pipette operation is a basic requirement for clear and easy handling. Each element of an electronic pipette must be designed to enable new users to master operation within a very short time. The new electronic pipette Eppendorf Xplorer meets these requirements and provides optimal support during pipetting tasks. The Eppendorf Xplorer pipette features a unique multifunctional rocker, which has been developed to enable precise control of liquid aspiration and dispensing operations. The size of the rocker allows for comfortable operation regardless of the size of the user's hand and for both left and right-handed use (Fig. 1).

The rocker is based on the straightforward "up is up and down is down™" principle. This ensures that users always retain complete control over the piston motion. The rocker is pressed up in order to aspirate liquid and down to dispense liquid. The identical operating philosophy between the rocker of the Xplorer and the control button of a conventional manual pipette (slide control button up = aspiration, press control button down = dispensing) facilitates the changeover between a manual and an electronic pipetting system.



Fig. 1: Intuitive operation of the main operating elements

## Operating modes

The different operating modes available in the Xplorer are selected with the selection wheel. The desired function is simply set by turning the wheel accordingly. This means no more getting lost in submenus. Two softkeys and the rocker are available for editing the desired parameters. A help function can be called up at any time. The clear color display always shows all the adjustable parameters.

Operating modes that can be selected:

- Ads** – Automatic dispensing
- Dis** – Dispensing
- Pip** – Pipetting
- P/M** – Pipetting and Mixing
- Man** – Manual pipetting
- Opt** – Options
- Off** – Power off

The dispensing function is suitable for, e.g., an ELISA for filling a microtest plate. This requires many repetitive steps. A more efficient approach to completing the application is to use the "Automatic dispensing" function. In this case, the liquid is dispensed "automatically", with the rocker pressed down, in a preselected interval.

To achieve greater dispensing precision of liquids which have a tendency to foam, or highly viscous solutions, the "Pipetting" function offers the possibility of performing additional blow-outs.

The "Pipetting and Mixing" function is recommended, e.g., for pipetting very small volumes. For a dispensing volume < 10 µL, it is recommended to rinse it into the respective reaction liquid. This can be done by automatically starting a mixing movement after the liquid has been dispensed. Both the mixing volume and the mixing cycles are defined before. An application for this would be, e.g., the addition of DNA to a PCR master mix.

"Manual pipetting" is suitable for the pipetting of supernatants, for measuring an unknown amount of liquid, for titration or for loading gels. The display will then show the volume contained in the pipette tip.

The loading of gels demands an extremely high level of concentration while working. Even the slightest irregular thumb movement when dispensing the sample with manual pipettes will mean that the sample will partly flow out of the gel pocket.

The excellent synchronization of the Xplorer motor as well as the dispensing speed, which can be adjusted in 8 levels, ensure precise sample dispensing. The blow-out can be deactivated if required in order to prevent liquid from being accidentally blown out of the gel pocket.

The "Options" function can be used to make general settings, such as the sound level, the display brightness, adjustments to a specific medium, and for making further settings.

The "Off" function switches off the pipette. This helps to greatly reduce the discharging of the rechargeable battery.

## The electronic pipette Eppendorf Xplorer® – Intuitive Handling

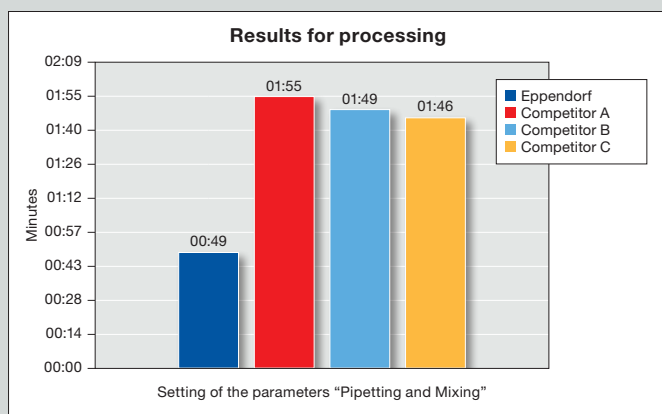


Fig. 2: Time required to set the parameters for "Pipetting and Mixing"

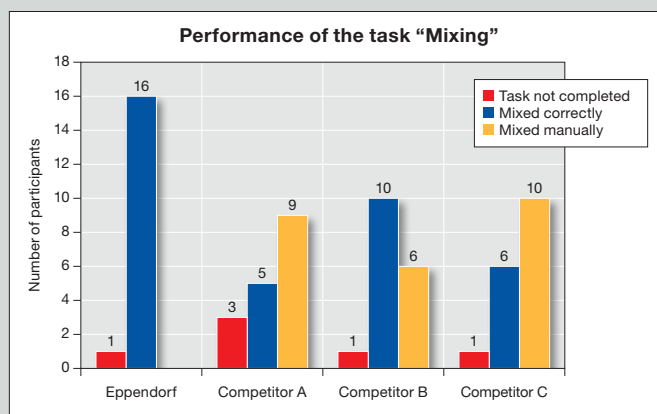


Fig. 3: 17 participants used each pipette for the task "Mixing"

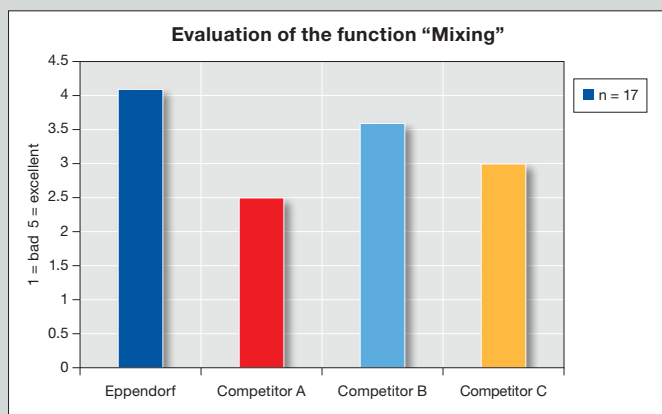


Fig. 4: Subjective evaluation of the function "Mixing"

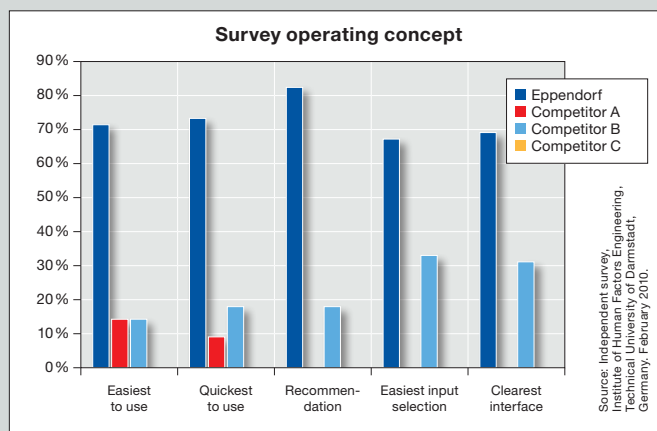


Fig. 5: Results of the study regarding the operating concept of the electronic pipettes

### Study on operating concept (Fig. 2–5)

During the development of the electronic Eppendorf Xplorer pipette, the Institute of Human Factors Engineering at the Technical University of Darmstadt, Germany, conducted a study on how easy it is to learn how to use the pipette. This study included, e.g., the time required to learn how to operate the Xplorer in comparison with different competitor pipettes. Results showed that inexperienced users were able to master Xplorer operation in less than 3 min.

The test subjects performed the task described below:

- Step 1:** 1000 µL bromophenol blue solution was placed into a container.
- Step 2:** 90 µL glycerol solution was added.
- Step 3:** The solutions were mixed until the blue solution had turned into a clear yellow solution.

At the end of the mixing process the pipette tip should be clear and free of any glycerol residue.

This experimental design is comparable to tasks where a small volume of a "critical" liquid is to be dispensed carefully into a larger volume. At the same time, the liquids are to be mixed thoroughly following dispensing. Due to its higher density glycerol is forced to the bottom, where it remains. Therefore, mixing of both solutions needs to be achieved via a separate mixing process. During the mixing process, residues from the tip are completely rinsed into the solution (e.g., DNA sample into PCR master mix).

The Xplorer's advantages, especially during the complex task of "Mixing", are obvious. The function "Mixing" was difficult or impossible to find when us-

ing competitors' pipettes due to unclear menu structures.

The study rated as extremely positive both the programming of the individual parameters and the clear presentation of these parameters in the display.

From among the tested pipettes, the Xplorer was found to be the pipette which was the easiest and quickest to operate as well as the pipette offering the best input options and the best clarity. This is an important factor when using electronic pipettes compared to manual pipettes in the performance of daily routine tasks.

**Readers' service**  
Eppendorf Xplorer® • Ref. no. 233



# Comparison of the transformation efficiencies achieved with electroporation vs. traditional chemical transformation

Stefanie Topp, Eppendorf Instrumente GmbH, Hamburg, Germany  
Ilka Schneider, Eppendorf AG, Hamburg, Germany

## Abstract

In order to compare the transformation efficiency (TE) of *Escherichia coli* (*E. coli*) achieved with the classic heat shock method with that achieved with electroporation using the Eppendorf Eporator, chemically competent bacteria and electrocompetent variants from the same manufacturer were tested. Transformation was carried out in accordance with the manufacturer's protocol in order to achieve the best possible TE.

According to the manufacturer's specifications, the TE of chemically competent *E. coli* is  $1-3 \times 10^9$ , and  $1-3 \times 10^{10}$  per  $\mu\text{g}$  DNA for electrocompetent *E. coli*. The results obtained were consistent with these values. Thus, electroporation using the Eporator yielded a 10 times higher TE than chemical transformation.

## Introduction

Transformation of bacteria is a routine task in many biochemistry and molecular biology laboratories. This method is employed in order to amplify recombinant DNA in bacteria. Often, chemical transformation, renowned for its costeffectiveness and reliability, is chosen. However, this method, which is also known as heat shock method or calcium chloride method [1], is very time consuming and labor intensive, and it does not always yield a sufficiently high number of positive bacterial colonies.

In comparison with the chemical method, electrical transformation [2, 3] has the potential to yield a ten times higher transformation rate while saving time. Using this method, bacteria are subjected to an electric pulse (1,000–2,500 V for several milliseconds), in order to permeabilize the membrane. At this stage, it is important to ensure that the bacterial medium is of low conductivity, as with

most commercial instruments, the possibility of short circuit ("arcing") exists. This problem often leads to researchers resorting to the traditional method despite the availability of an electroporation device. In contrast, the Eppendorf Eporator is equipped with a special safety feature which prevents the occurrence of a short circuit ("arcing").



Fig.1: Eppendorf Eporator®

This Application Note will demonstrate that the Eporator (Fig. 1) enables a higher transformation efficiency compared to the classic method.

In order to compare the transformation efficiency of chemically competent *E. coli* with that of electrocompetent *E. coli*, both variants were purchased as DH 5-alpha from the same manufacturer (New England Biolabs, [NEB], Frankfurt, Germany). Transformation was performed by adhering to the manufacturer's protocols in order to optimize the ability to compare the two variants. The results were evaluated by comparing the TE.

## Materials and methods

### Media and bacteria

- NEB 5 alpha Competent *E. coli* (High Efficiency) (NEB)
- NEB 5 alpha Electrocompetent *E. coli* (NEB)
- SOC Outgrowth Medium (NEB)
- pUC 19 Control DNA 10 pg/ $\mu\text{L}$  (NEB)
- LB-Agar (Roth, Karlsruhe, Germany)
- Ampicillin 100  $\mu\text{L}$  (Roth)

### Instruments

- Thermomixer comfort\* (Eppendorf, Hamburg, Germany)
- Eporator with electroporation cuvettes, 1 mm gap width (Eppendorf)
- Incubator Heraeus type VT 5042 EK (Heraeus, Hanau, Germany)
- Waterbath GFL type 1086 (GFL, Burgwedel, Germany)

### Methods

- Heat shock method (calcium chloride method):

Transformation of chemically competent *E. coli* was performed in strict adherence to the manufacturer's instructions. One aliquot of *E. coli* was thawed on ice, and 40  $\mu\text{L}$  of cells were transferred to a

\*US/CAN: Thermomixer R

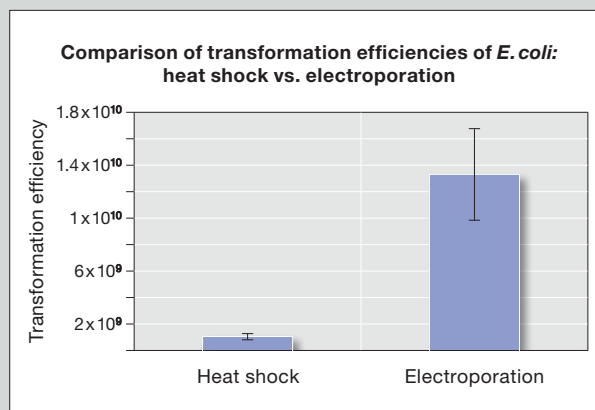


Fig.2: Transformation efficiencies following chemical transformation (heat shock) and electroporation. Mean values and standard deviations are shown.

## Comparison of the transformation efficiencies achieved with electroporation vs. traditional chemical transformation

pre-cooled 1.5 mL Safe-Lock tube containing 1  $\mu$ L pUC (10 pg/ $\mu$ L). Following 30 min on ice, a heat shock step was performed for 30 s in a 42 °C water bath. The cells were then immediately incubated on ice for 5 min.

Following this incubation, 960  $\mu$ L of room temperature SOC medium were added, and the mixture was transferred to a 2 mL Eppendorf tube. The cells were then agitated for 60 min at 37 °C and 400 rpm in the Thermomixer comfort, and subsequently 10  $\mu$ L of the *E. coli* were diluted with 90  $\mu$ L SOC and plated on a pre-warmed LB-Agar plate supplemented with 100  $\mu$ g/mL ampicillin.

The plate was incubated over night at 37 °C in the incubator. After 24 h, the colonies were counted and the TE was calculated.

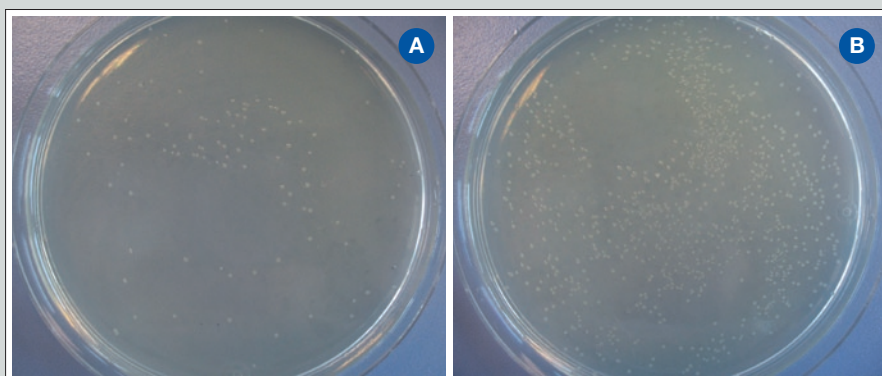
### • Electroporation using the Eporator:

Transformation of electrocompetent *E. coli* was performed in strict adherence to the manufacturer's instructions. One aliquot of each *E. coli* was thawed on ice, and 40  $\mu$ L of cells were transferred to a pre-cooled 1.5 mL Eppendorf Safe-Lock tube containing 1  $\mu$ L pUC (10 pg/ $\mu$ L).

The mixture was transferred to a pre-cooled electroporation cuvette (1 mm gap width), and the electroporation was carried out immediately at 1,700 V. The Eporator performs an exponentially declining pulse with a defined time constant of 5 ms.

Immediately following the pulse, 960  $\mu$ L of SOC medium, pre-warmed to 37 °C, were added and the mixture was transferred to a 2 mL Eppendorf tube and mixed at 37 °C and 400 rpm for 60 min in the Thermomixer comfort. 5  $\mu$ L of the *E. coli* were diluted with 95  $\mu$ L SOC and plated on a pre-warmed LB-agar plate supplemented with 100  $\mu$ g/mL ampicillin.

All further treatments were performed as described for the chemically competent cells.



**Fig. 3:** Comparison of overnight cultures following heat shock transformation (A) and electroporation (B). Plate A represents a two-fold higher amount of plasmid DNA.

Heat shock of chemically competent <i>E. coli</i>		
Sample	Number of colonies	TE
1	133	$1.33 \times 10^9$
2	105	$1.05 \times 10^9$
3	87	$8.70 \times 10^8$
4	89	$8.90 \times 10^8$
5	138	$1.38 \times 10^9$

**Table 1:** TE using chemical transformation by heat shock. Results of the 5 individual experiments are shown. The TE is calculated as number of colonies per microgram DNA.

Electroporation of electrocompetent <i>E. coli</i>		
Sample	Number of colonies	TE
1	446	$8.92 \times 10^9$
2	780	$1.56 \times 10^{10}$
3	890	$1.78 \times 10^{10}$
4	500	$1.00 \times 10^{10}$
5	731	$1.46 \times 10^{10}$

**Table 2:** TE using electroporation. Results of the 5 individual experiments are shown. The TE is calculated as number of colonies per microgram DNA.

## Results and discussion

Each experiment was performed five times; the results are listed in tables 1 and 2. The TE is defined as number of colonies (transformants) per microgram of DNA used. The amount of pUC 19 DNA used in these experiments was 10 pg.

In case of the classical heat shock method this amount was diluted to a total volume of 1 mL with SOC medium, of which 10  $\mu$ L were plated. Hence, an equivalent of 0.1 pg of DNA were plated on each agar plate after chemical transformation. After electroporation, 5  $\mu$ L of the cell suspension was plated which corresponds to an equivalent of 0.05 pg DNA.

The mean values and coefficient of variation (CV) (Fig. 2), as well as the percent coefficient of variation (% CV), were calculated from the measured values. The mean values result in a TE of  $1.34 \times 10^{10}$  when using the Eppendorf Eporator and a tenfold lower efficiency of  $1.1 \times 10^9$  when performing the classic heat shock method. The variation between individual experiments was comparable at 25 % for the Eppendorf Eporator and 20 % for the heat shock method (% CV).

These results fall within the range of variation to be expected for biological systems. The results show that the Eppendorf Eporator delivers consistently high transformation rates which fall within the range outlined by the manufacturer of the competent cells.

## Literature

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- [3] Taketo A. DNA transfection of *Escherichia coli* by electroporation. *Biochim Biophys Acta* 1988; 949:318-324

### Readers' service

Eppendorf Thermomixer®/ThermoStat plus Family  
• Ref. no. 123  
Eppendorf Eporator® • Ref. no. 239

HEIDE NIESALLA, EPPENDORF AG

# Your bacteria's favorite

## New: Eppendorf Eporator®

The new Eppendorf Eporator offers a fast, simple and safe option for the transformation of bacteria, yeasts and other microorganisms with DNA/RNA.

When bacteria or yeast strains are exposed to short high voltage pulses, macromolecules such as plasmid DNA can diffuse into the cell via temporary pores in the cell membrane. The results are highly reproducible, and in comparison with chemical methods, electroporation yields up to ten times higher transformation efficiency.

Transformation of bacteria for the purpose of recombinant DNA amplification is often performed using chemical methods. Even though this option is reliable and cost-efficient, it is very time-consuming, and transformation efficiencies can be low. The new Eppendorf Eporator saves valuable time, while simultaneously yielding considerably higher transformation rates. Two new program buttons, designed for storage and recall of most commonly used parameters, along with the simple one-button operation, guarantee intuitive operation for fast and

safe sample handling. The Eporator is so easy to use that even beginners can start electroporating right away. State-of-the-art electronics and an integrated electroporation chamber offer highest safety for the user and minimize the risk of sample loss. Eppendorf offers electroporation cuvettes with three different gap widths.

Due to its compact design, the Eporator may be transported effortlessly within the lab and stored during times when it is not in use. To facilitate GLP compliant documentation of experiments, the Eporator is equipped with a USB port for simple data export.

## Conclusion

The Eppendorf Eporator is the ideal system for electroporation of bacteria and yeasts. At long last, electroporation is fun – and not just for you: treat your bacteria and yeast with something special – let them experience the new Eppendorf Eporator!

For more details, please order the Eporator brochure by using the reference number denoted below or visit

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to receive your personal bacterium. This is also the place to find information about the Eppendorf Eporator, as well as application protocols for electroporation of different strains of bacteria and yeasts.



Eppendorf Eporator® • Ref. no. 239



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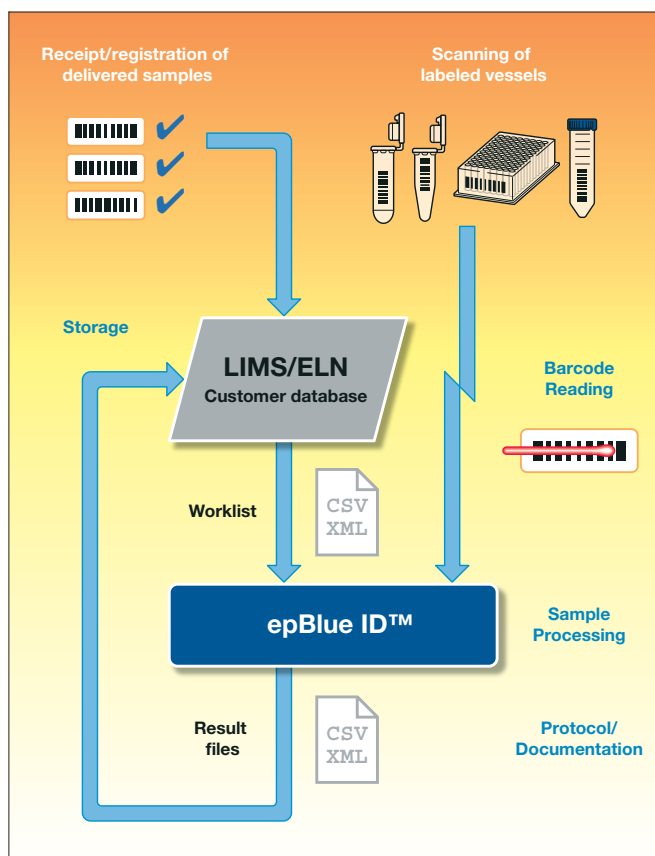
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## News

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BERRIT HOFF &amp; CAROLYN POWELL, EPPENDORF AG

# Research prize winners visit Eppendorf AG



Kerri Smith (*Nature*) interviewing Óscar Fernández-Capetillo for the Award Podcast.

**Dr. Óscar Fernández-Capetillo** (Spanish National Cancer Research Centre, Madrid, Spain), winner 2009 of the *Eppendorf Award for Young European Investigators* visited Eppendorf headquarters in Hamburg in August 2010.

There he gave a talk about his research about the effects of a type of endogenous DNA damage known as replicative stress (RS) on mammalian ageing. RS is intrinsically associated with DNA replication and prevented mainly by the ATR kinase. By developing a murine model of the Seckel syndrome characterized by a severe deficiency in ATR, he and his group were able to show high levels of RS during embryogenesis can accelerate ageing later in life. Their results support that RS, particularly *in utero*, contributes to the onset of ageing in postnatal life, this being balanced by the activities of checkpoint proteins such as ATR or p53.

While in Hamburg, Dr. Fernández-Capetillo also met with Kerri Smith (Podcast Editor of *Nature* Publishing Group, London) for an interview for a *Nature* podcast on the Eppendorf Award. You can listen to it at [www.eppendorf.com/awardpodcast](http://www.eppendorf.com/awardpodcast).



The 15,000 Euro *Eppendorf Award for Young European Investigators* is presented in partnership with *Nature*.

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Richard Benton (left) receives a Research plus pipette from Axel Jahns, Ph.D., Eppendorf AG.

Also in summer of 2010, Eppendorf had the pleasure of welcoming **Richard Benton, Ph.D.** (Center for Integrative Genomics, University of Lausanne, Switzerland) 2009 winner of the *Eppendorf & Science Prize for Neurobiology*.

On this occasion Dr. Benton gave a talk about his groundbreaking work on the fruit fly *Drosophila melanogaster*. Dr. Benton has shown that insects have invented unusual receptors to detect smells. By targeting these molecules with specific chemical inhibitors, it may be possible to control the odor-evoked behaviors of insects that transmit human diseases such as malaria. As a reminder of his visit to Hamburg Dr. Benton was presented with Eppendorf's new Research plus pipette.



The international US\$ 25,000 *Eppendorf and Science Prize for Neurobiology* is awarded jointly by Eppendorf and the journal *Science*.

For more information visit [www.eppendorf.com/prize](http://www.eppendorf.com/prize).

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# Energy efficient cold storage

## **Independent study on New Brunswick freezers**

In a case-study by the Higher Education Environmental Performance Improvement (HEEPI) the University of Newcastle implemented a cost and carbon-saving initiative focusing on cold storage as the target area.

36 freezers more than ten years old were replaced mostly with New Brunswick Scientific (NBS) -86 °C U725-G freezers. Data showed the old freezers cost up to £ 2,000 a year per unit to run whereas the NBS energy efficient freezers cost only £ 527.00. Annual energy consumption was reduced from 9,349 kWh to 5,548 kWh.

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hydrocarbon-based ultra-low temperature freezers on the market\*. NBS was delighted to have been chosen as the University of Newcastle's preferred manufacturer and to have the energy efficiency of their 'green' freezers recognized in an independent study.

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## **Views**

*"This was an excellent opportunity for the University to make immediate financial and carbon savings. This programme was relatively simple to administer, provided a range of benefits, and was popular with research teams."*

(CLARE ROGERS, ESTATE SUPPORT SERVICE DIRECTOR)

*"This was a fantastic initiative that benefited all, with a reduction in overall long term costs to the University, and a number of new reliable freezers for the Institute that are environmentally friendly with lowered running costs. It has also set an important benchmark for all future freezer purchases within the University."*

(BOB NICHOLSON, TECHNICAL MANAGER, INSTITUTE FOR AGEING AND HEALTH)

*"The exercise showed just how much energy is used in older freezers and fridges compared to new ones – a threefold difference in the worst case! There's also a big difference in energy efficiency between the best and worst models available today."*

(CARA TABAKU, ASSISTANT ENERGY MANAGER)



\*Correct at time of publication of the study: August 2010



# Prize competition

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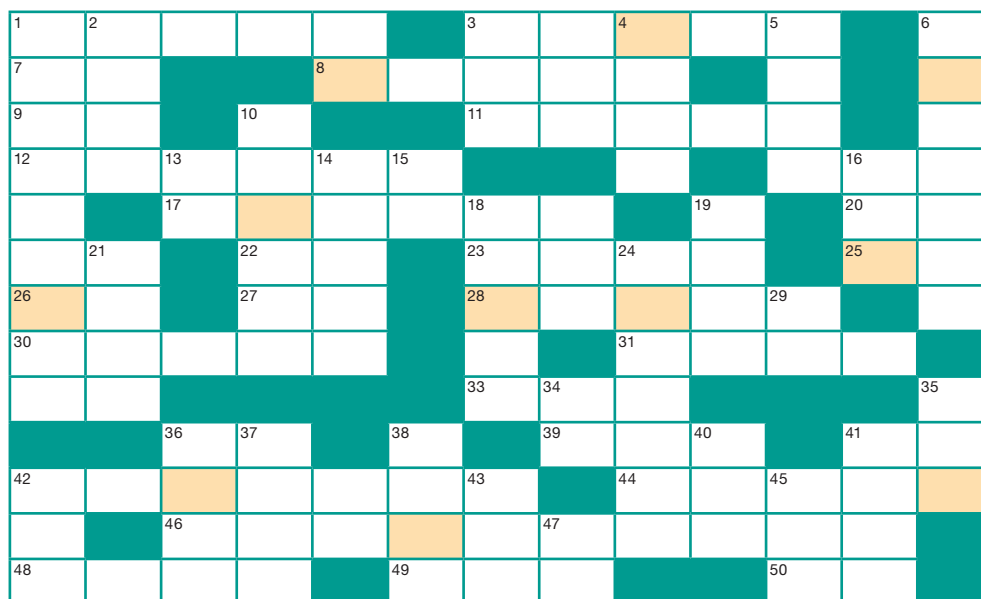
The solution of the prize competition of BioNews No. 32 was "Research plus". Sandra Gibson (University of Pittsburgh, USA) won the first prize, a Research plus 8-channel pipette.

## Have fun in our new crossword!

How to find out the solution: Simply arrange the letters in the yellow fields of the crossword in the correct order. Send us the solution until **30th June 2011**.

You can either use the reply fax (p.15), send us an e-mail to [bionews@eppendorf.de](mailto:bionews@eppendorf.de), or participate online at [www.eppendorf.com/bn-service](http://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. 36.



## 1st prize:

1 Eppendorf  
 Xplorer® Pipette  
 5–100 µL

## 2nd to 5th prize:

1 MP3 player each

## 6th to 15th prize:

200 bonus  
 ep-points each

### ACROSS

- 1 Relationship between two numbers
- 3 Fast, quick
- 7 Chemical symbol for erbium
- 8 Perfect, excellent
- 9 Chemical symbol for tantalum
- 11 Classic genre in literature
- 12 This plus p.m. means 23.00 h
- 17 To create, draft
- 20 ISO country code of Turkey
- 22 Till tomorrow (internet slang)
- 23 Molten rock
- 25 Marking that confirms a product's compliance with EU legislation
- 26 ISO country code of Iceland
- 27 Chemical symbol for tellurium

- 28 Honor, prize
- 30 Mammal, not at all afraid of water
- 31 Mother
- 33 Single Nucleotide Polymorphism
- 36 ISO country code of Spain
- 39 Movable cover
- 41 Chemical symbol for lithium
- 42 With two polarities (adj.)
- 44 Robbing from the rich and giving to the poor (first name)
- 46 Especially recommended in conjunction with 42 down and "plus"
- 48 US state
- 49 Large Australian bird
- 50 Chemical symbol for sodium

### DOWN

- 1 If this is low you get max. sample recovery
- 2 Lake between Kazakhstan and Uzbekistan
- 3 The color of 24 down's most favorite drink
- 4 Drawing, diagram or map
- 5 Having two like parts
- 6 Learn how to handle it in less than 3 min
- 10 UV/VIS plastic cuvette
- 13 This Mr. was a talking horse
- 14 First name (female)
- 15 Transition metal (abbrev.)
- 16 And so on
- 18 Amorphous (non-crystalline) solid material
- 19 Gross weight minus net weight

- 21 Italian city in the Piedmont
- 24 Count Dracula is one of them (sing.)
- 29 Whether Diabetes mellitus or Depeche Mode – keep it short
- 34 ISO country code of the Netherlands
- 35 Personal numeric password
- 36 The one and only Captain ...
- 37 Manhattan district
- 38 Fixed ratio between two things
- 40 Small spot
- 41 Currency of Turkey
- 42 Prefix that complements 46 across
- 43 Read-only memory
- 45 First name (male)
- 47 ISO country code of Mauritius

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<input type="radio"/>	225	ep T.I.P.S.® LoRetention		
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<input type="radio"/>	239	Eppendorf Eporator®	<b>I have suggestions/topics for the next BioNews:</b>	
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**Solution of BioNews No. 34 prize competition:**

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Closing date: 30th June 2011 (Go to [www.eppendorf.com/bn-service](http://www.eppendorf.com/bn-service) to participate online!)



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