

The 10 μ L Dispensing Tool Ensures Highly Accurate and Precise Sub-microliter Volume Pipetting on epMotion[®] 5073I

Eric Gancarek¹, Dominik Schneider², Sandrine Hamels¹

¹ Eppendorf Application Technologies S.A., Namur, Belgium

² Eppendorf AG, Hamburg, Germany

Abstract

This Application Note shows the possibility to automate quantitative Polymerase Chain Reaction (qPCR) setup involving small reaction volumes by using the epMotion[®] 10 μ L dispensing tool. For PCR setup, a sample volume of 0.2 μ L was used in a total reaction volume of 5 μ L. The qPCR performances were evaluated by two sensitive qPCR assays.

It was demonstrated that a low volume qPCR setup can be successfully automated on the epMotion 5073I. Excellent accuracy and reproducibility were obtained for both assays.

The use of the epMotion workstation is an excellent solution to increase the consistency and efficiency of small volume dispensing by removing the human and day-to-day variability. In addition, researchers will save time and money by eliminating repetitive work and by reducing the use of costly reagents, respectively.



Introduction

Nowadays, laboratory processes are becoming more and more complex driving the need for assay miniaturization. The tendency towards assay miniaturization is present in a lot of different applications. An example is the screening of compounds to discover new drugs in the pharmaceutical industry [1]. Indeed, the ability to perform primary screening assays in high-density micro-well plates at volumes of

1–2 μ L will accelerate the early stages of drug discovery and reduce costs. Another field is in molecular biological research using the quantitative real-time Polymerase Chain Reaction (qPCR) technology. However, qPCR is an expensive technology. One way to decrease the costs is to reduce reagent volumes.

In addition to reducing reagent costs, miniaturization offers many other advantages. In applications such as forensic analysis, the volume of extracted DNA available for qPCR is often limited [2]. A miniaturized assay allows achieving the qPCR even with a low amount of DNA. Another advantage is the possibility to run more experiments with the same amount of biological sample leading to better result interpretation. Finally, by decreasing the volume, it is possible to increase the number of reactions performed in parallel and reduce the analysis time. In the past, low throughput qPCR systems were common in analytical labs. Currently, they are more and more replaced by 384-well instruments and even higher throughputs.

Despite the advantages, miniaturization asks for dispensing small liquid volumes accurately and precisely. Delivery of sub-microliter volumes is difficult to achieve and is a major obstacle to the implementation of miniaturized assays. One of the issues that all labs are facing is the human error. The smaller the dispensing volume, the greater the operator expertise should be. Additionally, in a miniaturized assay, it is usual to work with 384-well plates which also increases the risk of human error. Lastly, beside the human factor, environmental conditions such as small variations in labora-

tory temperature and humidity can have a significant effect on the correct handling of small volumes.

With so many variables affecting the dispensing process, choosing the proper solution for small volume handling is of the highest importance. One solution, especially when a large sample number is required to be processed in a short time, is automation. The capability of the *epMotion* liquid handling workstation to automate a qPCR assay from Master Mix preparation to 96-well PCR plate setup was already demonstrated [3-5]. By reducing human intervention and thanks to an accurate pipetting system, *epMotion* automated liquid handling systems provide high assay reproducibility without cross-contamination, ensuring reliable results.

The new 10 μL dispensing tool developed for the *epMotion* automated liquid handling systems allows the dispensing of volume as low as 200 nanoliter. The complete volume range covered by all *epMotion* dispensing tools is now extended from 0.2 μL to 1000 μL . The use of this new accessory ensures accurate and precise automated dispensing of sub-microliter volumes. The purpose of this Application Note is to demonstrate the efficiency of the 10 μL tool in a qPCR setup by using sample volumes as low as 200 nanoliter.

Materials and Methods

qPCR

- > Eppendorf twin.tec® real-time PCR plates 96, semi-skirted (Eppendorf, cat # 0030 132.505)
- > DNA LoBind Tubes, 1.5 mL (Eppendorf, cat # 0030 108.051)
- > HeatSealing PCR Film (Eppendorf, cat # 0030 127.838)
- > HeatSealer S200 (Eppendorf, cat # 5392 000.005)
- > Mastercycler® ep realplex (Eppendorf)
- > Water, Sterile, Nuclease-free, Biotechnology Grade (VWR®, cat # E476)

qPCR Assay 1

- > Kapa SYBR® Fast qPCR Kit (Kapa Biosystems, cat # KK4602)
- > Specific primers (Eurogentec®)
- > Lambda Phage DNA (Sigma-Aldrich®, cat # D3779)

Real-time qPCR assay developed for Lambda phage DNA is composed of two target specific primers (Primer 1: 5'-CGC ACA GGA ACT GAA GAA TG-3'; Primer 2: 5'-CCG TCG AGA ATA CTG GCA AT-3').

Each reaction was carried out in a total volume of 5 μL containing 4.8 μL of qPCR MasterMix Plus as well as 300 nM of each specific primer, water and 0.2 μL Lambda phage DNA. The Lambda DNA standard was serially diluted manually in nuclease free water. The mix was subjected to the following thermal conditions: 95 °C for 3 minutes, followed by 40 cycles of 95 °C for 5 seconds and 60 °C for 25 seconds.

qPCR Assay 2

- > Kapa Library quantification kit for Illumina® sequencing platforms (Kapa Biosystems, cat # 4824).

Kapa Library quantification kit for Illumina sequencing platforms contains the Master Mix, the Primer Mix (Primer 1: 5'-AAT GAT ACG GCG ACC ACC GA-3'; Primer 2: 5'-CAA GCA GAA GAC GGC ATA CGA-3') and ready-to-use DNA standards. Each reaction was carried out in a total volume of 5 μL containing 4.8 μL of Kapa SYBR Fast qPCR Master Mix containing Primer Premix and water. As template DNA, 0.2 μL DNA standards were used. The mix was subjected to the following thermal conditions: 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 30 seconds and 60 °C for 45 seconds.

Automation

- > epMotion 5073I for automated PCR set-up, CleanCap, system incl. Eppendorf MultiCon, epBlue™ software and LH assistant, keyboard, mouse, waste box, 100 – 240 V $\pm 10\%/50 - 60 \text{ Hz} \pm 5\%$ (Eppendorf, cat # 5073 000.612)
- > TS10 single-channel dispensing tool (Eppendorf, cat # 5280 000.100)
- > epT.I.P.S.® Motion 10 μL PCR-clean, with filter, sterile (Eppendorf, cat # 0030 015.193)
- > Thermoadapter Frosty (Eppendorf, cat # 5075 789.000)
- > Thermoblock for PCR plates, 96-well (Eppendorf, cat # 5075 766.000)
- > Eppendorf twin.tec® PCR plates, 96-well, LoBind, semi-skirted (Eppendorf, cat # 5075 129.504)

For each qPCR assay, a method dedicated to the qPCR reaction setup has been programmed. Master Mixes and samples are provided in an Eppendorf twin.tec PCR plates, 96-well, LoBind, semi-skirted. 4.8 μL of the final Master Mix is firstly dispensed into the real-time PCR 96-well plates followed by the addition of 0.2 μL DNA template. Negative controls without template were included. Before starting a program, the epMotion surfaces and tools were cleaned using a DNA decontamination solution and treated with UV-light for 15 minutes. The worktable of epMotion 5073I instrument is equipped as on figure 1.

Results and Discussion

The automation of the qPCR setup using the 10 μL dispensing tool and the epMotion 5073I was compared to the manual setup. For a direct comparison, the manual and automated setups were performed on a same day using the same qPCR plate.

Assay Efficiency

To evaluate the dispensing efficiency of the epMotion 5073I liquid handling system equipped with the 10 μL dispensing tool, a very sensitive qPCR assay, the Kapa SYBR Fast qPCR Kit,

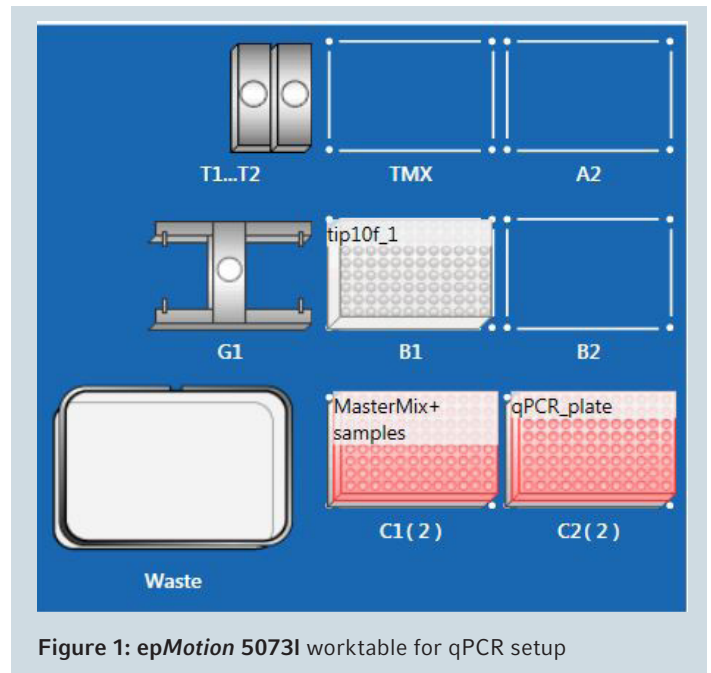


Figure 1: epMotion 5073I worktable for qPCR setup

has been selected. Performances were demonstrated by generating a standard concentration curve of Lambda DNA either manually or by using the epMotion 5073I with the 10 μL dispensing tool. The curves shown in figure 2A correspond to a series of 10-fold dilutions of a target from 10^6 copies to 10 copies.

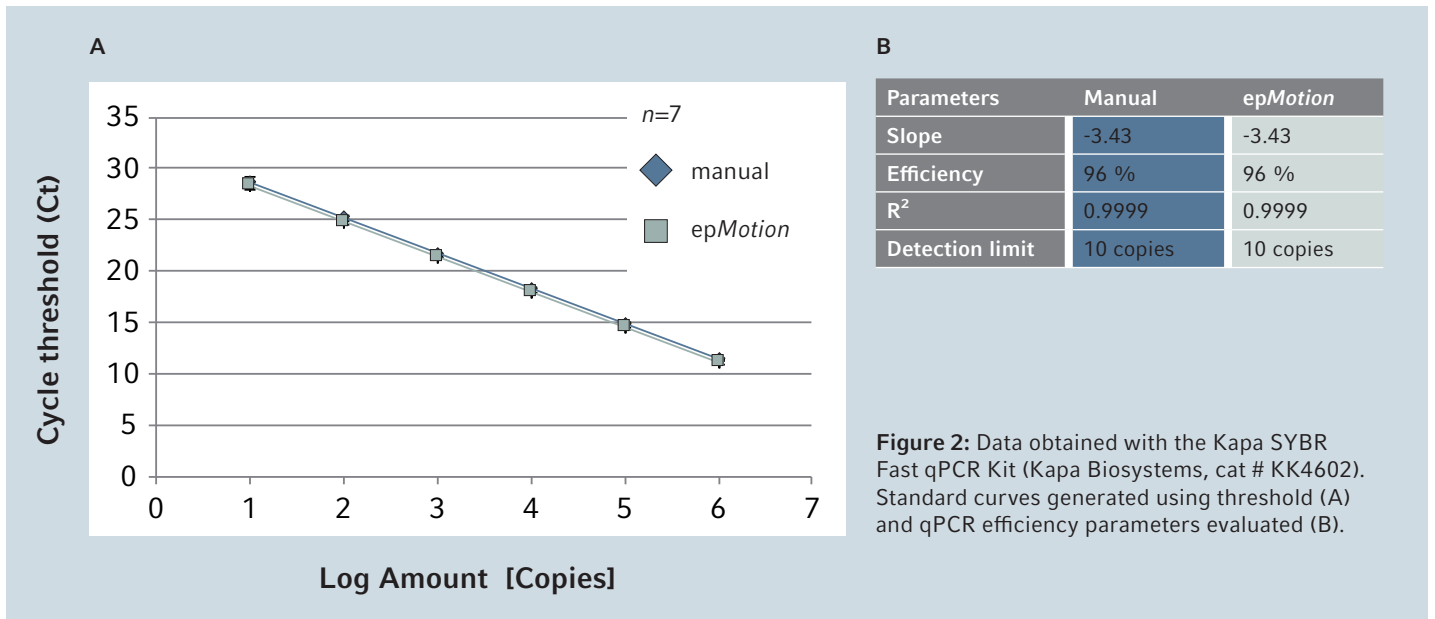


Figure 2: Data obtained with the Kapa SYBR Fast qPCR Kit (Kapa Biosystems, cat # KK4602). Standard curves generated using threshold (A) and qPCR efficiency parameters evaluated (B).

Parameters used to determine the assay efficiency are the dynamic range, the R² value and the detection limit (figure 2B). A slope of -3.43 reflects an efficiency of 96% while a R² value above 0.9999 indicates the good curve linearity and provides confidence in correlating two values. Finally, the assay sensitivity is ensured as 10 copies of Lambda DNA can be detected. As demonstrated by all parameters evaluated, assay efficiency is preserved when the qPCR setup is automated on an epMotion equipped with a 10 µL dispensing tool.

A second qPCR assay was used to confirm those results. The Kapa Library Quantification kit provides all reagents needed for the quantification of Illumina libraries by qPCR.

The efficiency was tested on a standard curve using the six ready-to-use standards included in the kit. The standards are supplied in a buffered solution to ensure stability of the diluted DNA and to reduce DNA adsorption to plastic. As the physical properties of this buffered solution were different from water, adjusting the dispensing parameters was a prerequisite for accurate pipetting of 0.2 µL DNA standards for this assay. Optimization could be achieved easily by modification of the liquid type options. Results obtained with this second assay, are shown in figures 3A and 3B, confirming that, thanks to the 10 µL dispensing tool, the qPCR assay efficiency is preserved when the automated setup involves handling of very low volumes (0.2 µL).

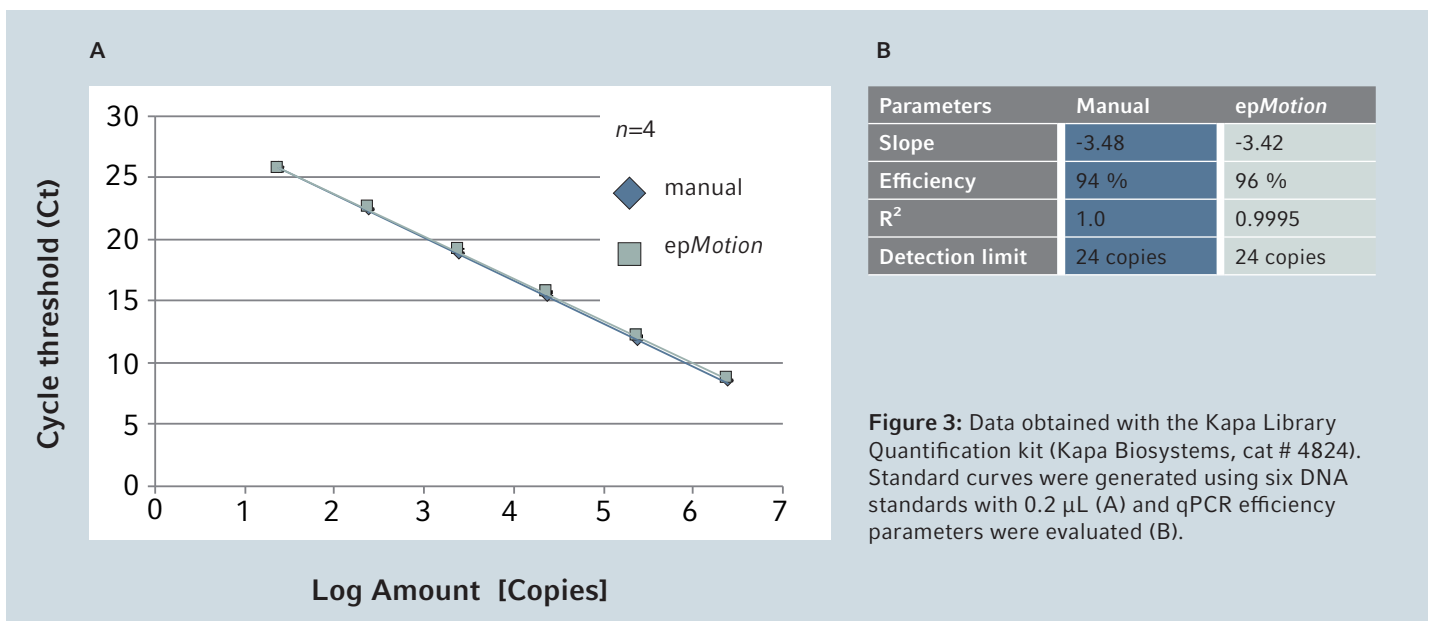
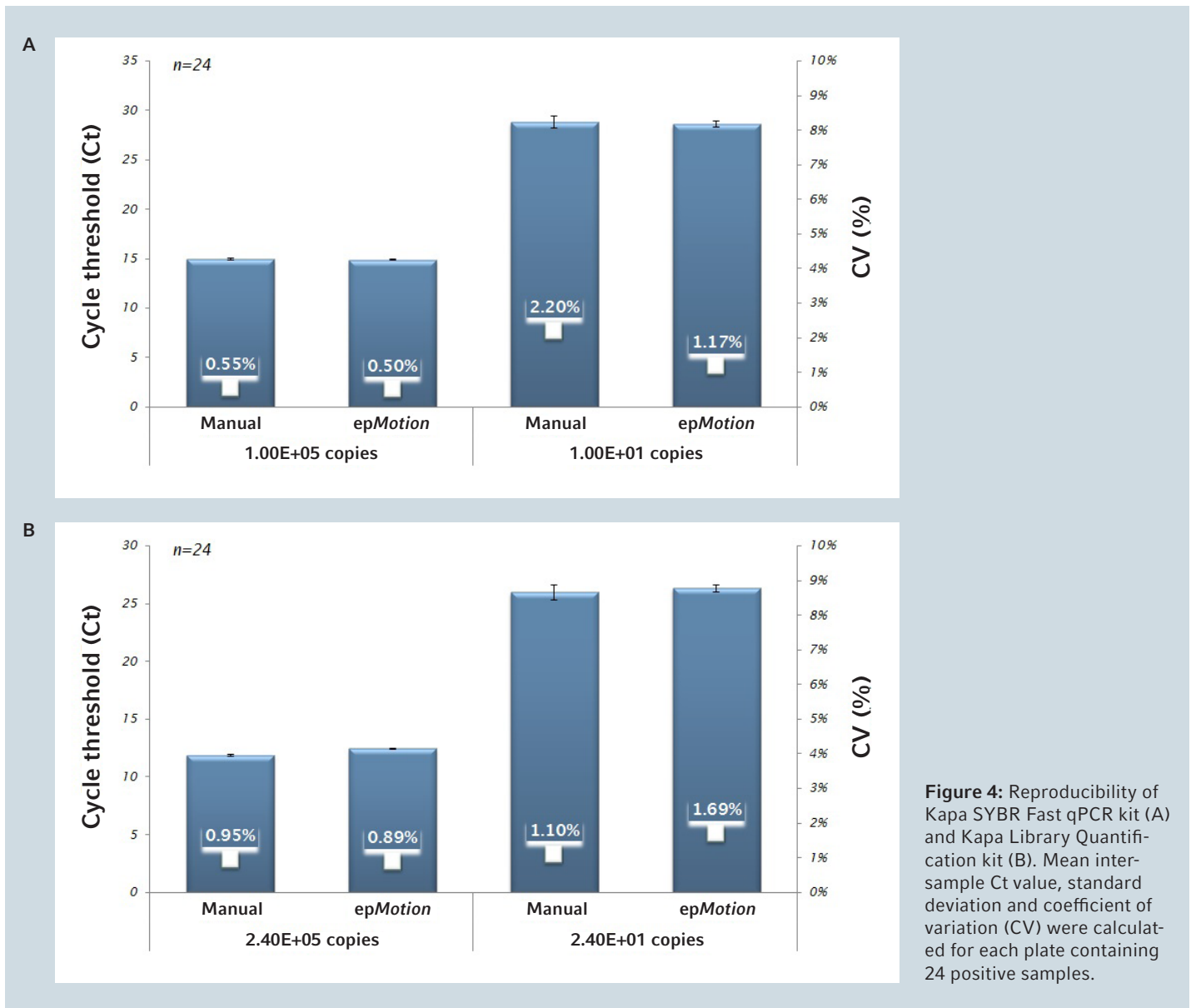


Figure 3: Data obtained with the Kapa Library Quantification kit (Kapa Biosystems, cat # 4824). Standard curves were generated using six DNA standards with 0.2 µL (A) and qPCR efficiency parameters were evaluated (B).

Reproducibility

Reproducibility is a key component of real-time PCR assay reliability. The assay reproducibility when an *epMotion* 5073I is used for qPCR setup was assessed on qPCR assays, using the Kapa SYBR Fast Kit and the Kapa Library Quantification kit, by comparing results generated from 24 positive samples containing a low and high number of DNA copies per qPCR reaction. As illustrated on figure 4, whatever the qPCR assay evaluated, the mean Ct value is very consistent for a

defined amount of target for the automated setups as well as for the manual setups. Reproducibility of a qPCR involving volume manipulation as small as 0.2 µL is ensured when qPCR setup is automated on an *epMotion* equipped with the 10 µL dispensing tool. The coefficient of variation does not exceed 0.50% and 0.89% for high copies number and 1.17% and 1.69% for low copy numbers for Kapa SYBR Fast qPCR Kit and Kapa Library Quantification kit, respectively.



Conclusion

In this Application Note, the capability of the *epMotion* 5073I liquid handling system to automate a complete qPCR assay setup involving very low volumes such as 0.2 μL was demonstrated. The assay efficiency and reproducibility were successfully assessed on two different qPCR assays. Results obtained with the automated qPCR setup were highly accurate and reproducible yielding data similar to a manual preparation. The qPCR, a highly sensitive application, was used in this Application Note to demonstrate the robustness of the 10 μL *epMotion* dispensing tool. Dispensing of sub-microliter

volumes can be extended to a large variety of applications requiring handling of small volumes such as protein applications, compound screening or cell-based assays.

These results clearly indicate that the *epMotion* automated liquid handling system equipped with the 10 μL dispensing tool is an excellent solution for scientists interested in automation of miniaturized assays. *epMotion* automated workstations provide a high assay reproducibility ensuring reliable results by reducing human error and by providing an accurate pipetting performance.

Literature

- [1] Hertzberg R. P. and Pope A. J. High-throughput screening: new technology for the 21st century. *Current Opinion in Chemical Biology* 2000; 4:445–451.
- [2] Proff C., Rothschild M.A. and Schneider P.M. Low volume PCR (LV-PCR) for STR typing of forensic casework samples. *International Congress Series* 1288 2006; 645– 647
- [3] Gancarek E., Vanbellinthen B., Hamels S. and Art M. Efficient qPCR Setup Without Cross Contamination Using the *epMotion* Family of Automated Liquid Handling Systems; Eppendorf Application Note 368; 2016368
- [4] Desneux J. and Pourcher A. M. Comparison of DNA extraction kits and modification of DNA elution procedure for the quantitation of subdominant bacteria from piggery effluents with real-time PCR. *MicrobiologyOpen* 2014; 3(4): 437–445
- [5] Zhang H. and Zhu G. Quantitative RT-PCR assay for high-throughput screening (HTS) of drugs against the growth of *Cryptosporidium parvum* in vitro. *Front Microbiol.* 2015; 6: 991

Ordering information

Description	Order no. International	Order no. North America
epMotion® 5073I for automated PCR set-up, CleanCap, system incl. Eppendorf MultiCon, epBlue™ software and LH assistant, keyboard, mouse, waste box, 100 – 240 V ±10 %/50 – 60 Hz ±5 %	5073 000.612	5073007511
TS10 single-channel dispensing tool	5280 000.100	5280000100
epT.I.P.S.® Motion 10 µL Filter, sterile	0030 015.193	0030015193
Thermoadapter Frosty	5075 789.000	960002300
Thermoblock for PCR plates, 96-well	5075 766.000	960002083
Eppendorf twin.tec® PCR plates, 96-well, LoBind, semi-skirted	0030 129.504	0030129504
Eppendorf twin.tec® real-time PCR plates 96, semi-skirted	0030 132.530	951022043
DNA LoBind Tubes, 1.5 mL	0030 108.051	022431021
HeatSealing PCR Film	0030 127.838	0030127838
HeatSealer S200	5392 000.005	5392000013

Your local distributor: www.eppendorf.com/contact

Eppendorf AG · Barkhausenweg 1 · 22339 Hamburg · Germany

eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com

Sigma-Aldrich® is a trademark of Sigma-Aldrich Co., LLC., USA. Eurogentec® is registered trademarks of Eurogentec S.A., Belgium. SYBR® is a registered trademark of Life Technologies Corporation or their respective owners. Illumina® is registered trademarks of Illumina, Inc., USA. Tween® is a trademark of ICI Americas, inc., USA. VWR® is a registered trademark of VWR International, Inc., USA. Eppendorf®, the Eppendorf Brand Design, epMotion®, epT.I.P.S.®, Mastercycler® and twin.tec® are registered trademarks of Eppendorf AG, Germany. epBlue™ is a trademark of Eppendorf AG, Germany. All rights reserved, including graphics and images. Copyright © 2017 by Eppendorf AG.