eppendorf

APPLICATION NOTE No. 129

Eppendorf MixMate – Experimental evidence of controlled mixing, using a PCR-based chessboard assay

Abstract

The Eppendorf MixMate was developed to enable small volumes of 5 µl – 2 ml to be mixed quickly and under controlled conditions in micro plates or micro test tubes. The results of the chessboard assay performed herein prove that even at high mixing speeds and long mixing time, there is no cross-contamination between the wells of a PCR plate when this is mixed on MixMate without the wells being sealed. The innovative 2DMix-Control technology of MixMate accordingly improves reproducibility of experimental conditions and saves time by eliminating unnecessary centrifugation steps after mixing.

Figure 1: Icon for 2DMix-Control technology

Introduction

The cutting-edge progress of technological development over recent years has led to laboratories working with smaller and smaller volumes. Whereas yesterday they were still using 0.5 ml PCR tubes, now they are using 96- or 384-well PCR plates. Whereas only a few years ago, 10 ml tubes were still being used for bacterial cultures, now deepwell plates are the norm. However, these new formats and their special geometries, together with the smaller and smaller reaction volumes involved, do place very specific demands on efficient mixing that conventional mixers cannot fully satisfy (1). Recent experiments have shown that mixing speed is not the sole criterion for efficient mixing of small volumes in small tubes. It is far more the case that the perfect mixing process demands optimized interaction of parameters such as speed, type of mixing movement (e.g., orbital), mixing radius and smooth running. This latest information about mixing small volumes was taken into account in developing the Eppendorf MixMate. The term "2DMix-Control" (Figure 1) covers all the properties of the MixMate that contribute to rapid and controlled mixing of even the smallest volumes. The optimized properties of the MixMate act on the three categories described below.

1) Generating adequate mixing energy: MixMate's orbital mixing movement at a high mixing speed (max. 3,000 rpm) and a relatively large mixing radius (1.5 mm) generates enough mixing energy (analogous to g-force in centrifuges) to mix even the smallest volumes efficiently.

2) Minimized loss of mixing energy: During MixMates's development, special attention was paid to reducing inherent vibration. Its smooth operation combined with its reliable stability ensures that all the mixing energy is available for mixing and not wasted through transmission of vibrations to the device or its surroundings.

3) Controlled transmission of energy from the device to the sample: Minimizing vertical movements enable a plane, 2-dimensional (2D) mixing stroke. This, in conjunction with the reliable plate holder, prevents uncontrolled chaotic motion of liquid and ensures a secure fit of all formats even at high mixing frequency.

MixMate's planar mixing without vertical movement, extreme running smoothness and great sturdiness allows it to control the movement of the liquid inside the tubes. This controlled mixing movement (anti-spill technology) prevents spilling or lid wetting in standard laboratory applications, thereby eliminating time-consuming centrifugation steps after mixing, as well as preventing cross-contamination between individual wells.
The experiments described in this Application Note are intended to provide experimental evidence of controlled mixing. In order to prove that mixing with the MixMate does not lead to cross-contamination between different wells in a PCR plate, a so-called chessboard assay (2) was performed. To this end, template DNA or H2O as a negative control were added to alternate 50 µl PCR preparations in a 96-well PCR plate. The PCR plate was then mixed for 10 min in the MixMate without the wells being sealed at the recommended mixing speed of 1,650 rpm (settings correspond to the MixMate's preprogrammed soft key for 96-well PCR plates: PCR 96/0.5). Subsequent to the PCR, PCR products were detected in agarose gels to allow potential cross-contamination to be detected.
A tenth volume of the experimental preparations was then analyzed qualitatively in a 0.9 % agarose gel using ethidium bromide staining and DNA marker (100 bp DNA ladder, BioCat, Heidelberg, Germany).

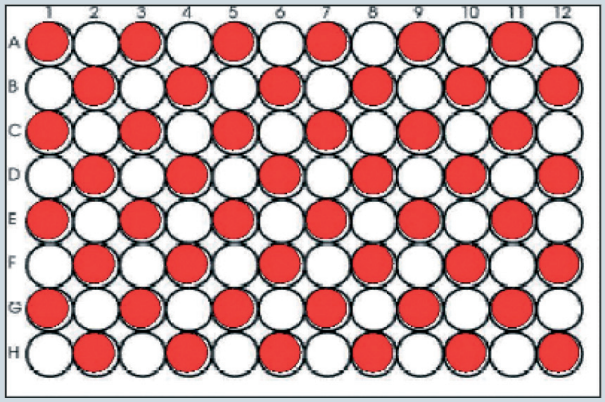

Figure 2: Pipetting diagram of the chessboard assay: Template DNA (red) or water for the negative controls (white) were added to alternate wells of a 96-well PCR plate.

Materials and Methods

"Chessboard assay" PCR setup

50 µl hot start PCR reaction preparations for amplifying a 535 bp fragment of the human ß globin gene (HBB), consisting of Eppendorf HotMasterMix and in each case 0.2 µM primer (sense: GGTTGGCCAATCTACTCCCAGG; antisense: GCTCACTCAGTGTGGCAAAG), were pipetted into Eppendorf twin.tec PCR Plates 96. To detect potential cross-contamination, either genomic DNA (final concentration 1 ng/µl, Roche, Penzberg, Germany) or H2O as a negative control were pipetted into alternate wells as shown in Figure 2.

Practice of the patented polymerase chain reaction (PCR) process requires a license. The Eppendorf® Thermal Cycler in an Authorized Thermal Cycler and may be used with PCR licenses available from Applied Biosystems. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents.

Mixing the PCR preparations

To provide evidence that the MixMate can mix the samples under controlled conditions and without wetting the lids, the readypipetted chessboard assay was mixed without sealing (i.e., with open wells) for 10 min at 1,650 rpm (soft key PCR 96/0.5). The plates for amplification were then subsequently.

PCR amplification and detection

PCR amplification was performed in a Mastercycler® ep gradient S thermocycler* (Eppendorf) in accordance with the following temperature profile: initial denaturing for 2 min at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at 60 °C and 30 s at 65 °C. Following addition of 6 µl 10x DNA Gel Loading Buffer (Eppendorf) to each well, the preparations were thoroughly mixed in the MixMate for 5 s at 1,650 rpm.

Results

To provide evidence of the anti-spill technology of the Eppendorf MixMate, PCR preparations pipetted in the pattern of a chessboard with and without template DNA were mixed before amplification in a 96-well PCR plate without the wells being sealed first. Although the MixMate requires just a few seconds to mix PCR preparations completely, the plates were mixed for10 min at 1,650 rpm without being sealed to demonstrate controlled mixing.
As can be clearly seen from Figure 3, there is no cross-contamination or false-positive PCR signal in the negative controls (preparations without template DNA) as a result of the plate being mixed without being sealed, despite the relatively large PCR volume of 50 µl. Experiments performed to determine the sensitivity of the PCR system used showed that cross-contamination with less than 0.5 µl is enough to lead to a clearly visible PCR band (data not shown).
To further demonstrate controlled mixing, the mixing stroke of the MixMate was photographed in time-lapse (Figure 4). The pictures show that the sample liquid moves orbital within the wells of the PCR plate without uncontrolled chaotic movements.

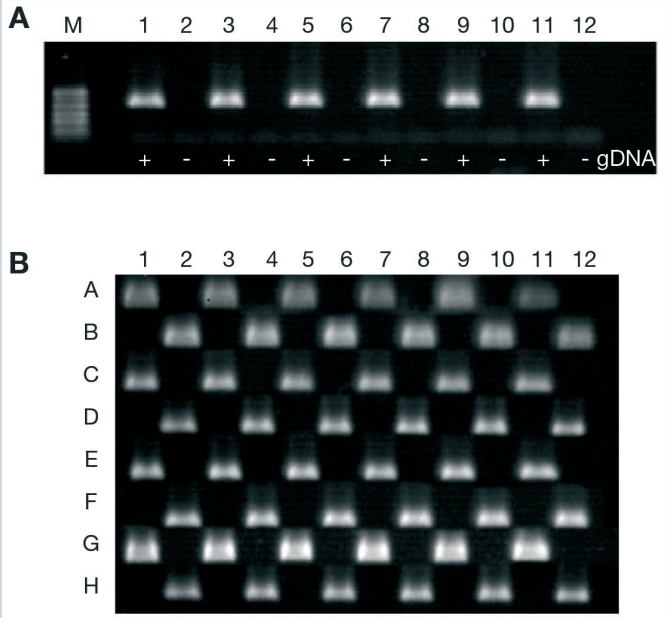

Figure 3: Amplification of 535 bp fragments of the human ß-globin gene (HBB): Template DNA (+) or water (–) were added to the PCR preparations in alternate wells of a 96-well PCR plate, as shown in Figure 1, and the plate was then mixed on the MixMate for 10 min at 1,650 rpm without being sealed. For the actual amplification process, the plates were then sealed with adhesive film. Despite open mixing on the MixMate, no cross-contamination was observed in any of the negative controls.
A: Illustration of the 12 reaction products from Row C of the 96-well PCR plate separated by gel electrophoresis.
B: Illustration of all 96 reaction products. To improve the illustration, the PCR signals of the individual gel images have been extracted and merged to match plate layout.

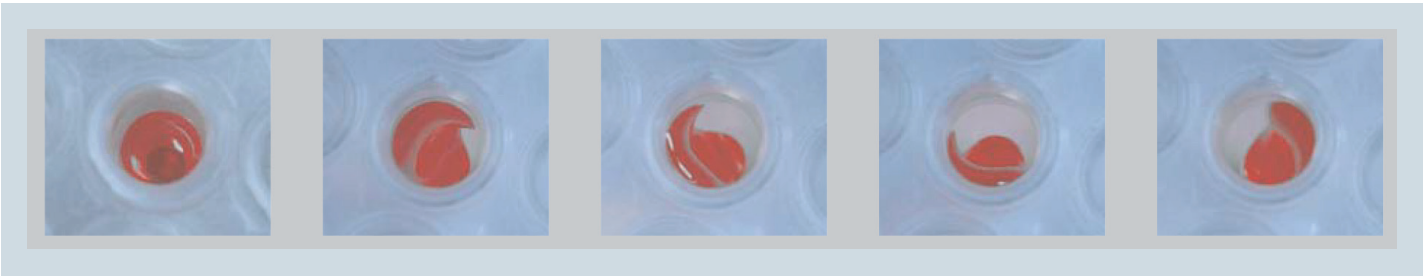

Figure 4: Time-lapse photography of controlled mixing with the Eppendorf MixMate: One well of a skirted Eppendorf twin.tec PCR Plate 96 filled with 75 µl water with dye Ponceau 4R is shown without mixing (left photo) and at 1,650 rpm mixing speed (4 time-lapse photos). Due to the 2DMix-Control technology, the liquid is forced into an orbital flow without chaotic movements, which enables controlled mixing without wetting the lid.

Discussion

The data presented in this Application Note show that the MixMate is capable of controlling the mixing movement of liquids so as to prevent uncontrolled splashing and consequently contamination of adjacent wells. Compared to conventional mixers, the MixMate has the advantage that samples no longer need centrifuging down after mixing, saving working steps and consequently time. Even in PCR applications, in which PCR preparations are frequently mixed by being aspirated and dispensed several times, it is possible to use the MixMate to save time, reduce additional consumption of pipette tips and avoid the risk of contamination due to formation of aerosols when pipetting.
The innovative 2DMix-Control technology accordingly facilitates reproducible results, an important prerequisite for PCR setup, resuspension of pellets, reporter gene assays, luciferase assays and ELISA assays, for example, as well as in colorimetric quantification of proteins.
While it is possible to control the mixing movement with the MixMate, the risk of wet lids is, in principle, also dependent on factors like tube geometry, mixing speed, filling volume and the sample material used. To simplify the setting of the ideal mixing parameters given this large number of variables, the MixMate has five soft keys with preprogrammed parameters that guarantee fast and controlled mixing in standard applications in a variety of plate and tube formats.
All in all, the option of controlled mixing and the thorough mixing in seconds of even tricky sample materials up to 96-well and 384-well plates (1) mean that the Eppendorf MixMate ensures maximum reproducibility for the experimental conditions and saves time because centrifugation steps are no longer required.

Literature

[1. Osterhoff C, Mueller P, Borrmann L. Comparison of mixing performance in 96- and 384-well plates of Eppendorf MixMate and competitor devices. Eppendorf Application Note 130, 2006.
2. Apostel F. Automated PCR setup in the 384-well format without cross-contamination with the Liquid Handling Workstation epMotion 5070. Eppendorf BioNews Application Notes 2003; 20:1–2.Eppendorf Application Note 400.

Ordering information

Description	Order no. International	Order no. North America
MixMate, includes 3 tube holder; PCR 96, 0.5 ml and 1.5/2.0 ml		
230 V, 50-60 Hz	5353000510	
110 V, 50-60 Hz	5353000529	2231000804
twin.tec PCR Plate 96, skirted (clear wells), Clear frame, 25 pcs.	5353000510	951020401
twin.tec PCR Plate 96, semi-skirted (clear wells), Clear frame, 25 pcs.	0030 128.575	951020303
PCR Film (self-adhesive), 100 pcs.	0030127781	0030127781

Your local distributor: www.eppendorf.com/contact
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