

Automated PCR Product Purification using Thermo Scientific Matrix PlateMate 2x3

Alberta Colakovic, B.S.

Applications Laboratories, Thermo Fisher Scientific, Hudson, NH USA

Abstract

Polymerase Chain Reaction (PCR) is a method for purifying amplified DNA to isolate a DNA sample from nucleotides, primers, and buffer additives which comprise the PCR reaction mixture. These components are necessary to amplify DNA by several orders of magnitude starting from a minute starting sample. In this study, the Thermo Scientific Matrix PlateMate 2x3 is used with a vacuum manifold accessory and NucleoFast 96 PCR Clean-up Kit from Macherey-Nagel to perform the automation of PCR purification.

The PlateMate® 2x3 is a robust and efficient automated pipetting station designed to perform rapid liquid handling operations with high precision and accuracy (Figure 1).

In order to determine procedure efficiency, purified and unpurified samples were run side by side on an agarose ethidium-bromide gel for comparison. Experimental results demonstrate that the automated purification of 96 individual samples can be accomplished in approximately 12 minutes with an 82 % sample recovery yield.

Introduction

PCR product purification is a common technique for isolating DNA samples from the undesired components of the PCR reaction mixture (i.e. nucleotides, primer oligonucleotides, and buffer additives like Betain, DMSO, and Q-solution). For a majority of applications, PCR reaction products must undergo purification before further manipulation can be attempted. During the course of this experiment, a PCR product is purified via vacuum filtration through the ultra filtration membrane of the NucleoFast 96 PCR Plate mounted on the PlateMate 2x3. PCR product purification is routinely executed for DNA cloning, sequencing, microarray spotting and hybridization assays among a wide range of other applications. For example purified DNA is used for paternity testing, and legal investigations where forensics laboratories receive minute amounts of DNA that has to be amplified and then meticulously purified for ensuing proper analysis.

The NucleoFast 96 PCR purification system filters contaminants through the membrane while the DNA binds the membrane reversibly. A DNA sample can then be recovered by re-suspending in low-salt buffer (RB buffer) or distilled water. Traditional



Figure 1: Image of the Thermo Scientific Matrix PlateMate 2x3 deck. Note: Labware on deck does not represent the experimental set-up.

PCR product purification is complicated to perform, is non-automation friendly, and involves ethanol precipitation. In addition to the multiple process steps of the method which produce throughput hindrance, ethanol residue can be present in purified samples. Consequently, residual liquid contamination can interfere with applications downstream unless samples undergo further purification steps. The purification procedure used in this study, alternatively, involves only three steps, a DNA binding, DNA recovery, and an optional wash steps, drastically reducing execution

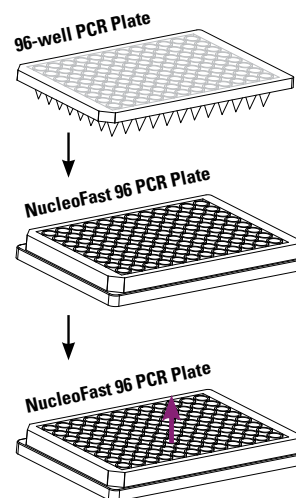


Figure 2: This figure shows the steps of PCR production purification on the NucleoFast 96 PCR Plate. (Top) PCR sample is transferred into (Middle) NucleoFast 96 PCR Plate and filtered through via vacuum filtration. (Bottom) Pure DNA is recovered from the NucleoFast plate by re-suspending membrane-bound DNA in a low salt buffer or water.

Key Words

- Thermo Scientific Matrix PlateMate 2x3
- PCR
- DNA
- DNA Purification
- Vacuum Manifold
- Filtration

time and sample loss. The results of this study demonstrate that the PlateMate 2x3 is an efficient and robust instrument on which to perform PCR product purification.

Materials

1. Thermo Scientific Matrix PlateMate 2x3 (Item No. 801-10001)
2. Thermo Scientific Matrix D.A.R.Ts Tips, 96-format, 300 µl (Item No. 5516)
3. Thermo Scientific Matrix Air Displacement Pipetting Head, 96 Channel, 5.0-300 µl (Item No. 501-20001)
4. Maxim Biotech, Inc. Human Beta-Actin Primer Set Kit (Cat# SP-10033)
5. Novagen® Human Genomic DNA Conc. 234 µl/mL (Item No. 69237)
6. DNA High Range Marker
7. Orange Dye
8. Thermo Scientific Matrix PlateMate 2x3 Plate Risers
9. Macherey-Nagel NucleoFast 96 PCR Cleaning Kit (Item No. 743500.4)
10. Thermo Scientific 96-well Polystyrene, Clear, Flat Bottom Microplates (Item No.4915)
11. Thermo Scientific 96-well Polystyrene, Clear, V Bottom Microplate (Item No. 4913)
12. Thermo Scientific Single Channel Manual Pipette (Item No. 1039 and 1932)
13. PCR Thermocycler
14. Sterile 96-well PCR Plate
15. Thermo Scientific Microplate Assay Sealing Tape (Item No. 4417)
16. Millipore™ MultiScreen HTS Vacuum Manifold (Item No. 301-50024)
17. 2% agarose (ethidium bromide) gels
18. E-gel unit
19. Quant-iT™ PicoGreen® dsDNA Assay Kit, Broad Range (Item No. Q33130)

Methods

PCR Sample Preparation:

PCR samples were prepared from the Maxim Biotech, Inc. Human Beta-Actin Primer Set Kit. Kit components were mixed according to kit user manual instructions. Human genomic DNA (Novagen) was diluted to 2 µg/mL in sterile water and 10 µl of the diluted DNA was added to 40 µl PCR (Beta-Actin 474 base pairs) mix per plate well. The total volume per PCR plate sample well was 50 µl. The PCR plate was then placed into the Thermo cycler for 2.5 hours, after which the DNA sample was amplified by several thousand folds.

Thermo Scientific Matrix PlateMate 2x3 Set-Up with Manifold:

The Thermo Scientific ControlMate Software settings were changed for the vacuum manifold deck set-up. Under the *Add-Ins* menu *Change Pipettor or Tips* was selected and then *Select Pipettor or Tips* was chosen. On this screen the *Deck Layout* option has a scroll-down menu with a variety of instrument options, and the *PlateMate 2x3 with Vacuum Manifolds* option was selected to conveniently adjust pipetting head height. Plate risers were placed on deck positions 1, 2 and 4 and the vacuum manifold was placed onto position 3. To increase time savings and daily throughput an additional vacuum manifold and PCR plate can be placed onto positions 5 and 6 on the PlateMate 2x3 deck. This set-up will provide an additional time savings of 2-3 minutes compared to executing two separate PCR plate purifications.

Table 1: Tip Height Adjustments

- Deck Position 1:** PCR Plate
- Deck Position 2:** Wash Buffer Plate
- Deck Position 3:** Vacuum Manifold with NucleoFast 96 PCR Plate
- Deck Position 4:** Elution (RB) Buffer Plate

Deck Position	Dispense Height (1/100 mm)
Deck Position 1: PCR Plate	9575 1/100 mm
Deck Position 2 and 3: 96-Well Plates/Reagent Reservoirs with Buffers	9225 1/100 mm
Deck Position 4: NucleoFast 96 PCR Plate on Vacuum Manifold	7425 1/100 mm

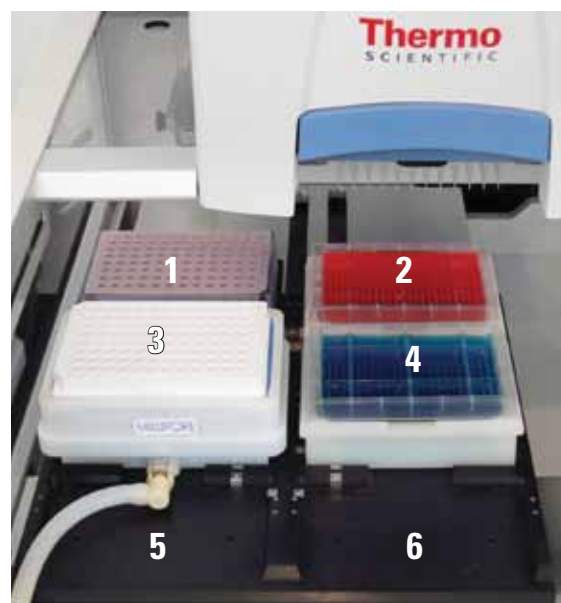


Figure 3: The PlateMate 2x3 stage deck set-up. (Note: Dyed liquids are used to represent buffers for clear distinction but these are not the actual buffers used). Deck positions 1, 2, and 4 are fitted with plate risers for height compatibility with the vacuum manifold. For the purpose of this study, only deck positions 1-4 were used. Deck positions 5 and 6 were not necessary for the set-up.

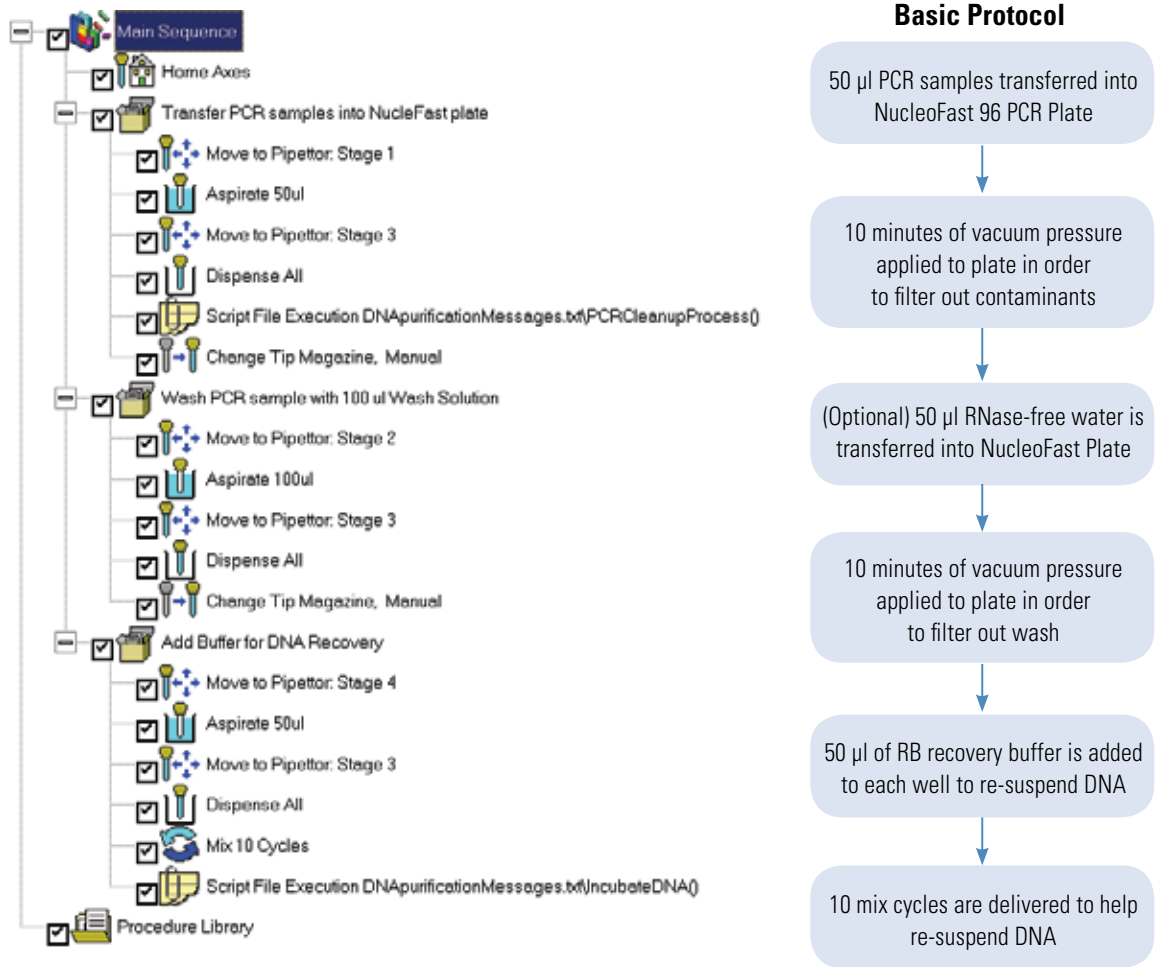


Figure 4: The ControlMate program created for PCR product purification by the Thermo Scientific Matrix PlateMate 2x3 liquid handling station. The script files execute messages about the protocol steps; these however, are not essential to the program. Message commands were displayed to enable the easy comprehension of each step of the protocol.

Protocol

1. 50 µl of freshly amplified PCR product was transferred from Deck position 1 into the NucleoFast 96 PCR Plate on Deck position 3.
2. Unpurified DNA was then vacuum filtered for 10 minutes until wells were completely dry.
3. 50 µl of RNase-free water was transferred from Deck position 2 into the NucleoFast 96 PCR Plate on Deck position 3. Again, vacuum pressure was applied for 10 minutes and the wash solution was filtered through the membrane until wells were dry.
4. 50 µl of re-suspension RB buffer was transferred from Deck position 4 into the NucleoFast 96 PCR plate. The PlateMate 2x3 delivered 10 mixing cycles to re-suspend the DNA sample in each well.

In order to determine the DNA yield resulting from the purification, the purified fraction was visually compared with the unpurified fraction by agarose gel electrophoresis. The PicoGreen DNA

quantification kit (Quant-iT) was used to determine % CV and % recovery. 2% agarose ethidium bromide gels were used to run samples. The first and last gel lane contained 20 µl of low and high range markers respectively and lanes 2 and 3 contained the positive and negative controls. The positive control was amplified from a 2 µg/ml human genomic DNA initial concentration, whereas the other DNA samples were diluted to 400 ng/ml. 20 µl of purified and unpurified samples were placed into each gel lane for electrophoresis.

Automated Versus Manual Purification:

Automated purification of 8 samples was conducted following the above procedure. For the purpose of comparison, liquid transfers were conducted manually using an 8-channel manual pipette. Manual liquid transfers and mixing steps required an additional 30 seconds to complete per row without the optional wash step. In total this would add to at least 2 extra minutes for 12 rows or a full 96-well plate.

Table 2: Purification of Samples

Purification Method	Execution Time for 96 Samples (without wash)	Execution Time for 96 Samples (with wash)	%CV	Absorbance OD (260 nm)	% Recovery
Automated	12 minutes	22 minutes	7%	0.207	82%
Manual	14 minutes	25 minutes	7%	0.208	82%

Table 3: Time Savings for Increasing No. PCR Plates: Automated vs. Manual Methods

No. of PCR Plates Purified	Automated vs. Manual Method (with wash)	Automated vs. Manual Method (without wash)
1	3min	2 min
4	12 min	8 min
8	24 min	16 min
12	36 min	24 min
16	48 min	32 min
20	60 min	40 min
25	75 minutes	50 minutes

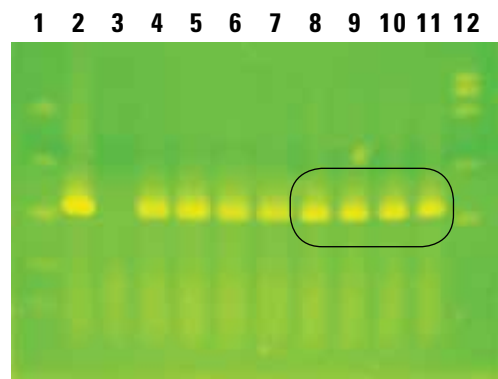


Figure 6: Analysis of purified and unpurified PCR products by gel electrophoresis. 50 µl of PCR product samples of 474 bp fragment were purified by the PlateMate 2x3. The purification procedure took 12 minutes to complete. Purified samples were compared with unpurified samples. Each lane contained 20 µl of each sample. Lanes 4-7 represent unpurified samples and lanes 8-11 are purified.

Results

The results of this study show that automated purification can be performed using the PlateMate 2x3 platform. This system produces consistent, reliable data. Manual purification takes approximately 2 minutes longer to perform than the automated method and the time gap broadens even more if the optional wash step is included. Table 2 shows the increased potential time savings that can be attained as throughput and capacity

increases in the laboratory. A researcher has the potential to save over one hour per day when processing approximately 25 purification plates. More important than time savings is the elimination of variability associated with manual sample preparation. Manual operation has a greater potential to negatively influence results due to inconsistency, and unaccountable variation from human error, unlike process automation which circumvents these drawbacks.

The gel analysis of purified versus unpurified samples visibly shows that the purified sample bands are more defined than the unpurified samples which exhibit less distinct bands with blurred borders. This was anticipated, as PCR reaction contaminants i.e. dNTPs, primers and buffer additives were not separated from the PCR products via purification.

Conclusion

Experimental results indicate that process automation positively effects result consistency. Automating PCR purification helps decrease hands-on involvement and user-intervention during program execution while providing increased throughput, time savings and walk-away capability.

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North America

+1 800 345 0206
matrix.info@thermofisher.com

Europe

+44 (0) 161 486 2110
matrix.eu.info@thermofisher.com

Asia

matrix.ap.info@thermofisher.com

www.thermo.com/matrix

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