

Reproducible liquid handling for the molecular biology workflow

SP&A Application Laboratory
Thermo Fisher Scientific, Vantaa, Finland

Goal

This application note shows how the Thermo Scientific™ high-throughput molecular biology workflow is efficient and effective for determining gender and Rhesus B factor from human blood.

Introduction

Automation of molecular biological experiments require several steps and instruments in the process, from sample to results. Fast but reproducible liquid handling will speed up the essential nucleic acid purification step that precedes common downstream assays, e.g., real-time PCR. DNA and RNA purification can be easily automated using magnetic particle technology and automated liquid handling instruments. Thermo Scientific KingFisher™ magnetic particle processors are fast and efficient purification systems offering benefits of automation such as consistent results and hands-free processing. KingFisher Flex is a high-throughput instrument enabling purification of up to 96 samples per run. Combining the KingFisher Flex with Thermo Scientific Multidrop™ Combi and Thermo Scientific Versette™ for reagent dispensing and dilution automates the process even further. Multidrop Combi is an easy-to-use bulk reagent dispenser with a wide volume range. By using the Versette Automated Liquid Handler the transfer and dilution of samples and other liquid handling tasks are fast and simple to perform. For downstream analyses the Thermo Scientific PikoReal™ Real Time PCR instrument offers a unique system with excellent thermal performance, high-sensitivity camera and five detection channels (Figure 1).



Multidrop Combi, Novus

Reagent dispensing

Liquid handling



Versette

Reagent dispensing and dilution



KingFisher Flex

DNA/RNA purification



Multiskan GO

DNA/RNA measurements



PikoReal qPCR

Downstream analysis

Figure 1. Thermo Scientific instruments used in the molecular biology workflow

Methods

Nucleic Acid Purification from Blood

KingFisher Flex 96 deep well magnetic particle processor was used together with the KingFisher Pure DNA Blood purification kit to perform the genomic DNA (gDNA) extraction from 200 µl EDTA blood samples according to kit instructions.

A Multidrop Combi reagent dispenser was used to fill the reagent plates for the KingFisher process according to Table 1. Magnetic beads were dispensed by using the Thermo Scientific Novus™ electronic pipette step function.

Process step	Plate type	Content	Reagent volume
Wash 1_1	Microtiter deep well 96-plate (DW96)	Wash Buffer 1	1,000 µl
Wash 1_2	DW96	Wash Buffer 1	800 µl
Wash 2	DW96	Wash Buffer 2	800 µl
Elution	KingFisher Flex 96 KF plate (KF96)	Elution Buffer	150 µl
Binding	DW96	Binding Buffer	400 µl

Table 1. Reagent dispensing for KingFisher process

DNA Dilution for the Absorbance Measurement

Versette automated liquid handler was used to transfer and dilute the gDNA eluate onto the 96-well low volume UV-plate for the DNA quantity and quality measurement. Dilution was performed by dispensing 30 µl of nuclease-free H₂O onto the measurement UV-plate and transferring 10 µl of gDNA from the KF96 elution plate using a Versette 96-channel pipetting head (Figure 2).

Figure 2. Versette automated liquid handler 96-channel pipetting head



Nucleic Acid Quantity and Quality

Multiskan GO with a 96-well low volume plate was used for DNA detection by measuring the absorbance at 260 nm (A₂₆₀). The quality of gDNA was calculated from the A₂₆₀/A₂₈₀ and A₂₆₀/A₂₃₀. Background at 320 nm was subtracted before calculations. Thermo Scientific SkanIt™ software for Multiskan GO was used to calculate the concentrations, and for exporting the data.

DNA Normalization for Real-Time PCR Assay

The optimal gDNA template concentration of 10 ng/µl for the following real-time PCR assay was achieved by diluting the gDNA automatically using Thermo Scientific Multidrop Combi nL together with the FILLit™ software. Volumes of nuclease-free H₂O needed for the dilutions were calculated based on the concentration measurement (Figure 3).

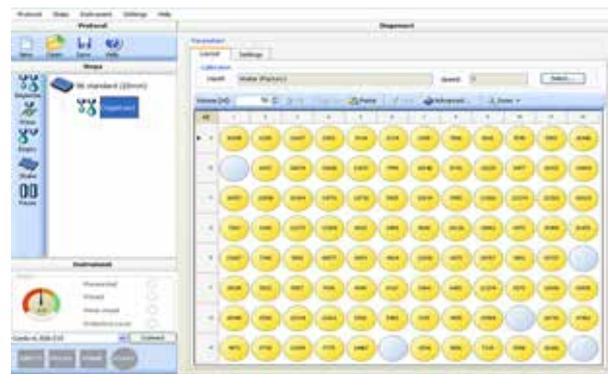


Figure 3. Dispensing order in the Thermo Scientific FILLit software for the DNA template normalization

Real Time PCR Analysis

Detection of Rhesus D factor and Y-chromosome was performed using the PikoReal Real Time PCR instrument with SYBR Green and TaqMan probes. Primer sequences are published by Al-Yatama, et. al, and probe sequences are confidential. A Thermo Scientific DyNAmo ColorFlash SYBR Green qPCR Kit was used for the chromosome Y, and a Thermo Scientific DyNAmo ColorFlash Probe qPCR Kit with a TaqMan ROX probe was used for the Rhesus D factor.

Thermo Scientific 96-well Piko PCR plates were used to perform PCR reactions with the PikoReal 96 Real Time PCR instrument. The data generated with the PikoReal qPCR instrument was analyzed using PikoReal Software 2.1.

Results

Genomic DNA Purification

The KingFisher magnetic particle purification system generated uniform gDNA results from parallel blood samples. The purity of gDNA was very good according to A₂₆₀/A₂₈₀ ratio of 1.8 +/- 0.1.

Real Time PCR Analysis

Results of Rhesus D factor and Y-chromosome detection were analysed with the PikoReal Software 2.1. Figure 4 shows the amplification graphs of the Rhesus factor (red curve) and the gender determination from Y-chromosome detection (green curve).

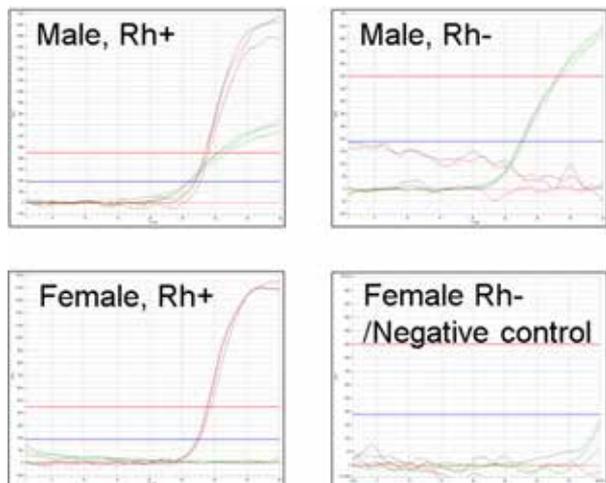


Figure 4. PikoReal amplification graphs showing results of all sample types within the experiment

Conclusion

This application note describes the methods for automating liquid handling steps in the DNA purification and analysis process. Determination of gender and Rhesus B factor from human blood samples is used as a practical example for the evaluation of the Thermo Scientific molecular biology workflow.

The Multidrop Combi reagent dispenser offers fast and reproducible plate filling prior to the DNA purification process with the KingFisher Flex instrument. Diluting purified samples for concentration measurement on the microplate format is effortlessly by using the Versette automated liquid handler to transfer the sample and diluter onto the measurement plate. DNA concentration values measured using Multiskan GO can be transferred to the FILLit software for normalizing the DNA template concentration using the Multidrop Combi nL. Normalizing the sample concentration allows using the same volume of variable yield samples for the analysis by PikoReal Real Time PCR System.

References

Al-Yatama, et.al. 2007. Medical Principles and Practice, 16(5):327-332.



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Europe:

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Belgium +32 2 482 30 30

Finland/Nordic +358 9 329 100

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Germany National Toll Free 08001-536 376

Germany International +49 6184 90 6940

Italy +39 02 95059 1

Netherlands +31 76 571 4440

Russia/CIS +7 (812) 703 42 15

Spain/Portugal +34 93 223 3154

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Asia:

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Japan +81 45 453 9220

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