Nucleic acid purification with magnetic particle processor prior to qPCR

Éva Tas, Ossian Saris, Marika Suomalainen and Sini Suomalainen
Thermo Fisher Scientific, Vantaa, Finland

Overview

Purpose: Evaluation of the Thermo Scientific high throughput molecular biology workflow for blood samples.

Methods: NA purification with the Thermo Scientific KingFisher system including magnetic particle processor and nucleic acid purification kits, QC with Multiskan GO spectrophotometer. Allelic discrimination and a gene expression study with the Thermo Scientific PikoReal Real Time PCR System.

Results: The KingFisher system constitutes an exceptional high-throughput purification system for obtaining excellent yield and purity of DNA and RNA with high-consistency of parallel samples. Real-time PCR with the user-friendly PikoReal showed sensitive detection of genes of interest.

Introduction

Nucleic acid purification is an essential step before many common downstream assays, including PCR and real-time PCR. DNA and RNA purification can be easily automated using magnetic particle technology. Thermo Scientific KingFisher magnetic particle processors are fast and efficient purification systems offering consistent results and hands-free time instead of laborious manual processing. For a medium-throughput laboratory, Thermo Scientific KingFisher Duo offers flexible system with wide processing volume range. KingFisher Flex is a high-throughput instrument enabling purification from up to 96 samples per run. Combining the KingFisher Flex with Thermo Scientific Multidrop Combi or Versette for reagent dispensing and Thermo Scientific Orbitor RS for microplate moving automates the process even further. For downstream analyses Thermo Scientific PikoReal qPCR instrument offers a unique system with excellent thermal performance, high sensitivity camera and five detection channels.

Methods

Sample Handling and Nucleic Acid Purification

Blood samples from 32 individuals were collected into EDTA containers. The samples were stored at +4°C and used within 24 hours (RNA purification) or 36 hours (DNA purification). KingFisher Flex magnetic particle processor was used together with the applicable purification kit to perform the genomic DNA (gDNA) and total RNA extractions from the EDTA blood samples. For each KingFisher Flex purification, three parallel aliquots (200 µl) of 32 blood samples were used in the deepwell 96-well format. The elution volume for gDNA was 150 µl and for RNA 75 µl.

Nucleic Acid Quantity and Quality

Multiskan GO with 384-well plate or Thermo Scientific µDrop plate (Figure 2A and 2B) was used for DNA and RNA detection by measuring the absorbance at 260 nm (A260). The quality of nucleic acid elution was calculated from the A260/A280 and A260/A230. Background at 320 nm was subtracted before calculations.

The gDNA eluates were run on an agarose gel and integrity of the total RNA samples was analyzed on the Agilent 2100 Bioanalyzer (Agilent Technologies).

FIGURE 2A. Thermo Scientific Multiskan GO with 384-well plate

PikoReal qPCR Analysis

Thermo Scientific 96-well Piko PCR plates were used for performing PCR reactions with the PikoReal 96 qPCR instrument (Figure 3A). PCR templates were transferred from KingFisher nucleic acid elution plates with Thermo Scientific Matrix Equalizer Pipette (refer to Figure 3B for our workflow recommendation). The data generated with PikoReal qPCR instrument was analyzed with the PikoReal Software 2.1.

FIGURE 3A. PikoReal 96 Real Time PCR system

Allelic Discrimination

The gDNA eluates from the KingFisher purification were used for allelic discrimination test by using PikoReal 96 instrument. Thermo Scientific DynaNo SNP Genotyping Master Mix (F-480), QPR174 F-R primers and TaqMan probes labeled with FAM and YakimaYellow were used for performing the PCR. The reference ID of the A/T mutation SNP is rs17251642.

Gene Expression

Total RNA eluates from the KingFisher purification was used in cDNA synthesis with Thermo Scientific Maxima cDNA Synthesis Kit (K1641). Gene expression was performed on the PikoReal 96 instrument with Thermo Scientific Solari qPCR Gene Expression Master Mix and pre-designed Solari assays targeting GADPH and PPIH genes.

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. The agarose gel image shows a clear band without any smear indicating intact gDNA (Figure 4A).

Total RNA Purification

Total RNA extracted from blood with KingFisher magnetic particle purification system generated good quality RNA for downstream analysis (Figure 4B).

FIGURE 4A. Agarose gel image of gDNA extracted from Blood with KingFisher Flex

Allelic Discrimination

PikoReal analysis of 32 samples were called as AA homozygotes 72%, heterozygotes 19% and TT homozygotes 9% (Figure 5).

Gene Expression

One out of six samples analyzed with PikoReal Software had 2.5-fold increased expression of PPIH gene (Figure 6).

FIGURE 5. Allelic discrimination graph. Blue diamonds represent TT homozygotes, green squares are heterozygotes and yellow circles represent AA homozygotes

FIGURE 6. Gene expression amplification graph. Reference gene (GADPH) in red and target gene in blue (PPIH)

Conclusion

– KingFisher magnetic particle purification system generates excellent quality gDNA and total RNA from blood
– Combining the KingFisher Flex with Multidrop Combi for reagent dispensing offers fast and reproducible plate filling
– Versatile photometric applications from variable volumes are easily analyzed with Multiskan GO
– PikoReal Real Time PCR System provides high performance with reduced bench space

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