

Unparalleled Nucleic Acid Purification and Downstream Analyses Using Thermo Scientific KingFisher Magnetic Particle Processor and PikoReal Real Time PCR System

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Overview

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

Methods: Thermo Scientific KingFisher system including magnetic particle processor and nucleic acid purification kits, Thermo Scientific PikoReal Real Time PCR and Multiskan GO spectrophotometer.

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. Additionally, real-time PCR with the 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. KingFisher Flex is high-throughput instrument enabling purification from up to 96 samples per run. The processing volume is 20 µl-5000 µl, depending on the flexible format of the instrument. 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.

FIGURE 1. Thermo Scientific Instruments used in the Molecular Biology Workflow



Methods

Sample Handling

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).

Nucleic Acid 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.

Reagent Dispensing

Multidrop Combi was used for dispensing purification reagents (lysis buffer, binding buffer, washing buffers and elution buffer) into 96 deepwell and shallow 96 KF plates before the KingFisher Flex extraction.

Nucleic Acid Quantity and Quality

Multiskan GO with 384-well plate or Thermo Scientific µDrop plate 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. (Figure 2A and 2B)

The gDNA eluates were run on the agarose gel and the Agilent 2100 Bioanalyzer (Agilent Technologies) was used with total RNA eluates to evaluate the integrity of nucleic acids.

FIGURE 2A. Multiskan GO with 384-well plate



FIGURE 2B. µDrop plate for small volume absorbance measurement



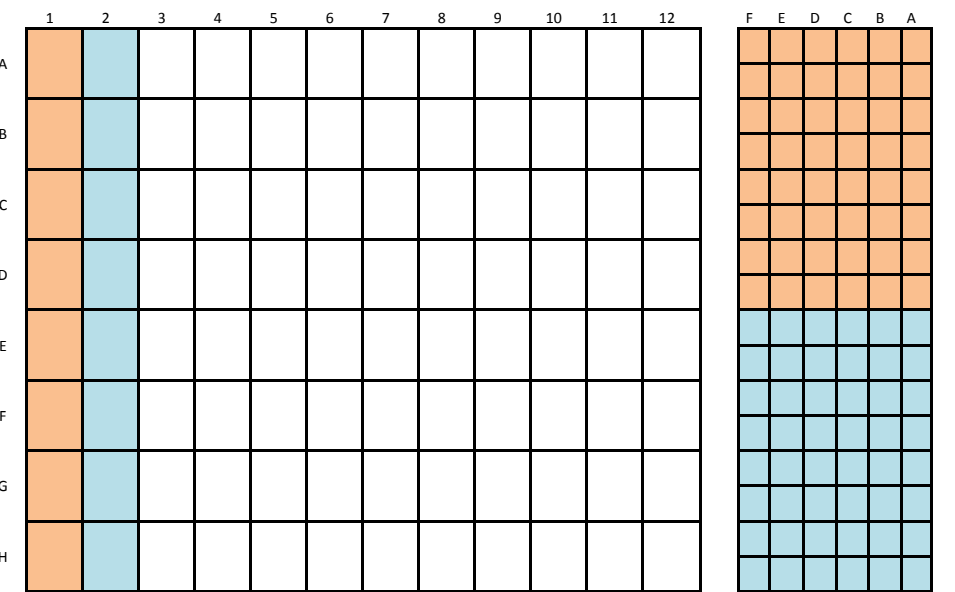
PikoReal qPCR Analysis

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

FIGURE 3A. PikoReal 96 Real Time PCR system



FIGURE 3B. Pipetting order from the KingFisher 96 KF elution plate to the Piko 96-well plate



Allelic Discrimination

The gDNA eluates from the KingFisher purification were used for allelic discrimination test by using PikoReal 96 instrument. Thermo Scientific DyNamo SNP Genotyping Master Mix, GPR174 F/R primers, FAM and YakimaYellow labeled TaqMan probes 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. Gene expression was performed on the PikoReal 96 instrument with Thermo Scientific Solaris qPCR Gene Expression Master Mix and pre-designed Solaris assays targeting GADPH and PPIH genes.

Results

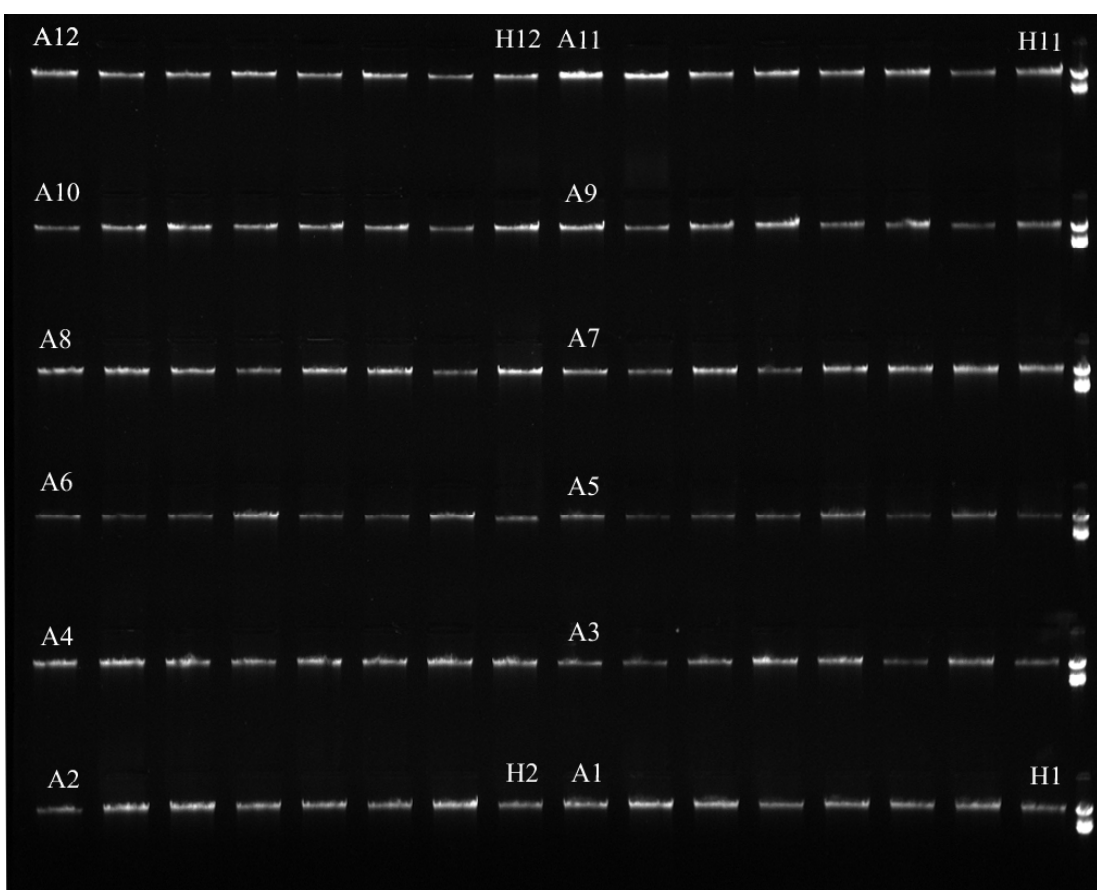
Genomic DNA Purification

The KingFisher magnetic particle purification system generated uniform gDNA results from parallel blood samples (Table 1). 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 4).

TABLE 1. Reproducibility of the yield (ng/µl) of gDNA purified from 3 parallel blood samples from 32 donors

Row\ Column	1	2	3	4	5	6	7	8	9	10	11	12
A	46,3	42,0	39,3	53,6	60,4	54,6	24,3	34,8	23,3	42,8	31,3	25,0
B	27,5	28,2	23,8	21,5	49,3	22,0	41,6	47,9	40,6	43,7	41,1	40,3
C	43,6	43,4	44,3	38,1	50,7	48,3	16,5	23,5	16,5	29,7	29,8	25,8
D	46,7	34,5	33,4	39,8	42,0	36,7	36,7	23,5	33,5	37,6	32,9	30,1
E	46,7	41,0	39,8	29,6	21,2	23,1	31,7	49,4	40,7	28,7	32,4	34,0
F	36,0	46,0	40,7	46,9	48,7	34,2	23,7	24,1	27,5	36,8	38,1	52,9
G	60,3	66,7	32,5	41,8	21,0	37,9	21,2	25,5	20,6	56,1	55,9	53,8
H	70,0	60,7	44,9	24,7	25,9	39,9	37,1	22,4	20,2	34,9	31,8	37,6

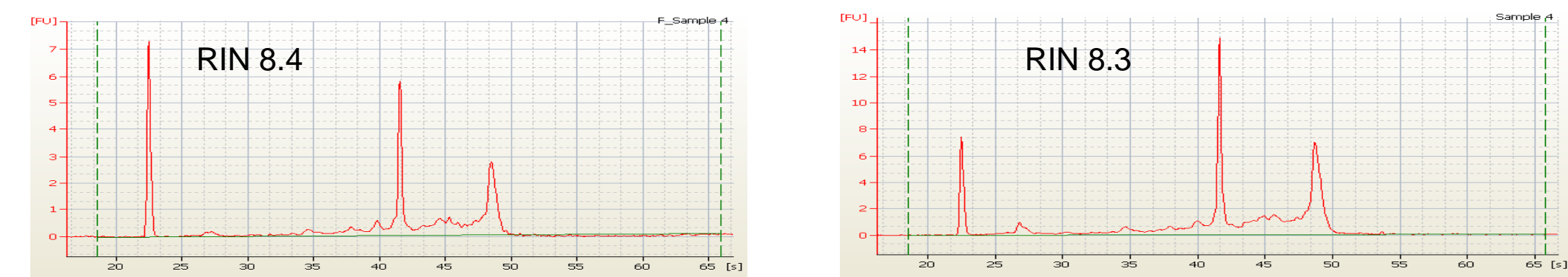
FIGURE 4. Agarose gel image of gDNA extracted from Blood with KingFisher Flex



Total RNA Purification

Total RNA extracted from blood with KingFisher magnetic particle purification system generated good quality RNA for downstream analysis. (Figure 5.)

FIGURE 5. RNA integrity analysis of total RNA from blood purified with KingFisher Flex



Allelic Discrimination

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

FIGURE 6A. Allelic discrimination graph. Blue diamonds represent TT homozygotes, green squares are heterozygotes and yellow circles represent AA homozygotes

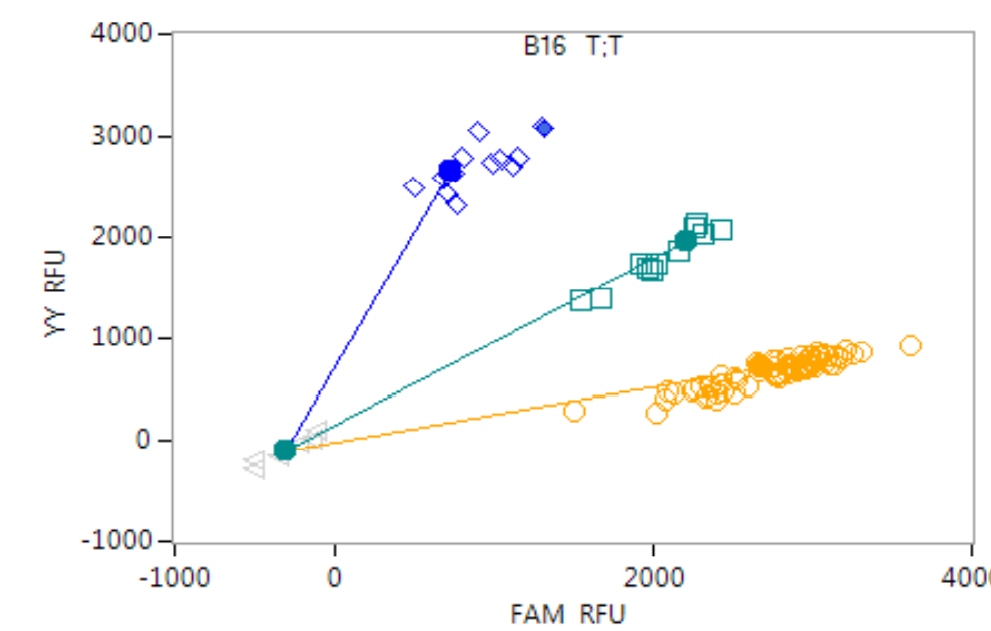
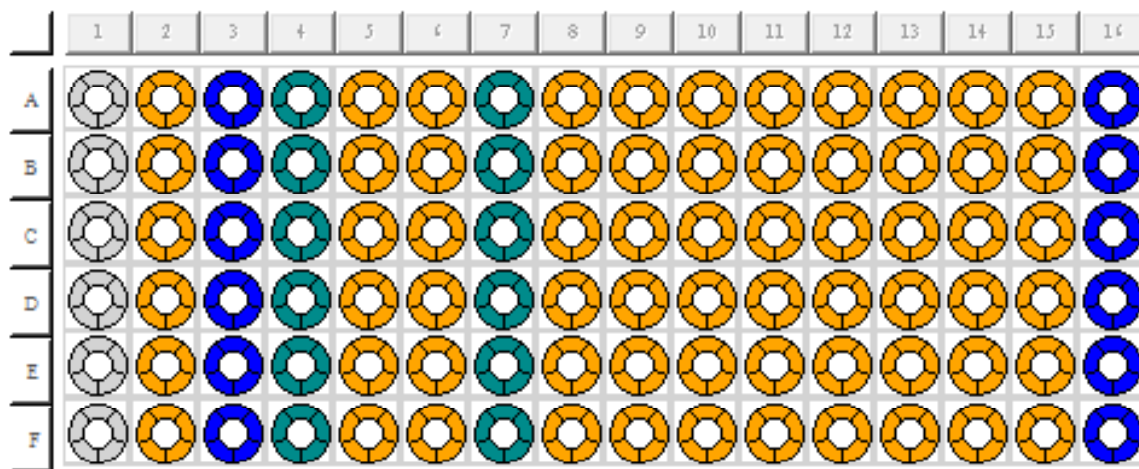


FIGURE 6B. Allelic discrimination plate grid. Visualization with color of called genotypes on the Piko 96 plate



Gene Expression

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

FIGURE 7A. Gene expression amplification graph. Reference gene (GADPH) in red and target gene in blue (PPIH)

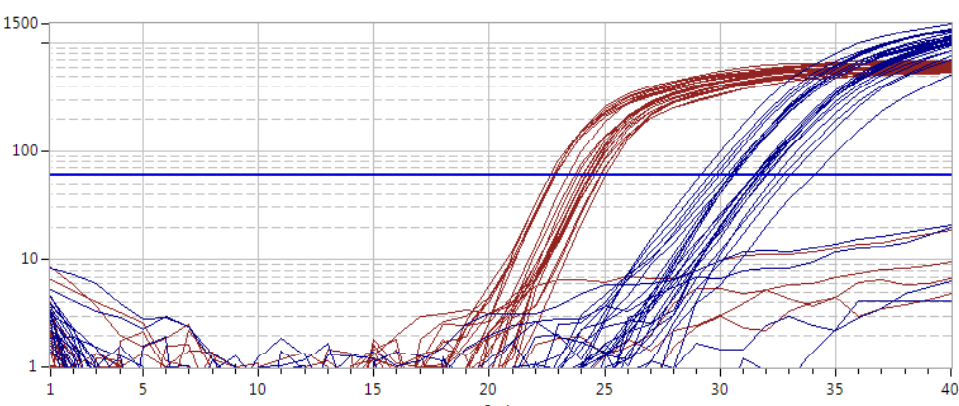
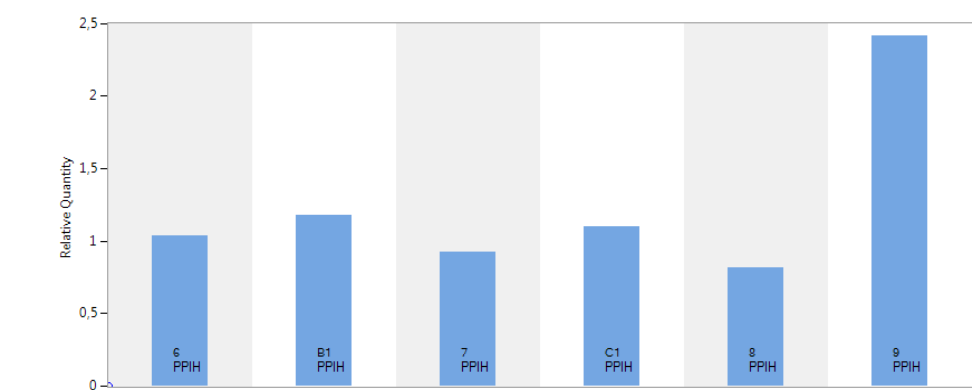


FIGURE 7B. Gene expression, Relative quantity of the target gene (PPIH)



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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