

# Reproducible liquid handling for the molecular biology workflow

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## Overview

**Purpose:** Evaluation of the Thermo Scientific high throughput molecular biology workflow for determination of gender and Rhesus B factor from human blood

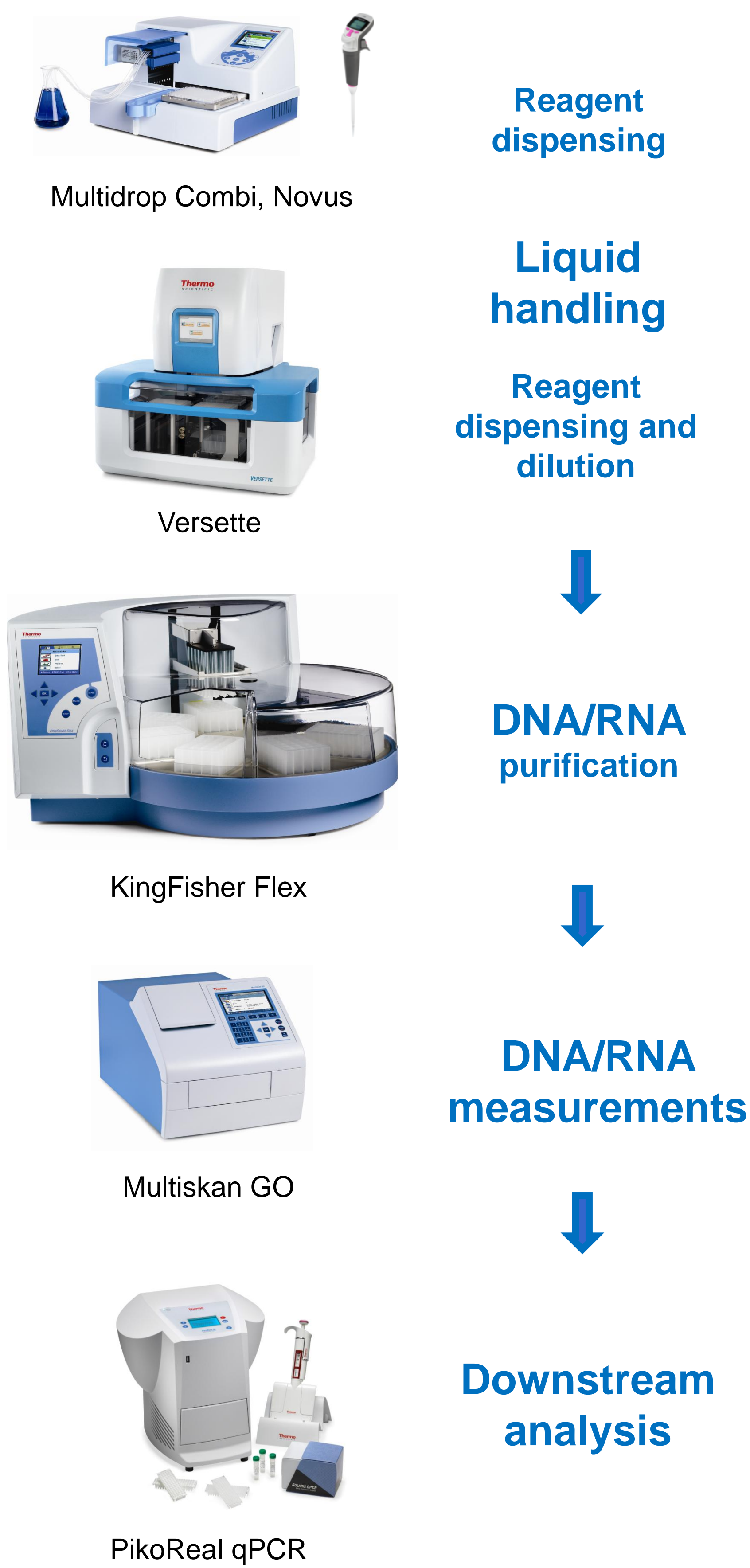
**Methods:** Thermo Scientific automated liquid handling instruments, Multidrop Combi, Versette and Multidrop Combi nL in the DNA purification with the KingFisher system, QC with Multiskan GO spectrophotometer and data analysis using PikoReal Real Time PCR system

**Results:** The automated liquid handling with KingFisher Flex constitutes an exceptional high-throughput purification system for obtaining excellent yield and purity of DNA. Real-time PCR with the user-friendly PikoReal showed sensitive detection of genes of interest

## 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 which 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 high-throughput instrument enabling purification from 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 wide volume range. By using Versette Automated Liquid Handler the transferring and dilution of samples and other liquid handling tasks are fast and simple to perform. For downstream analyses Thermo Scientific PikoReal Real Time PCR 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

### 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.

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 Thermo Scientific Novus electronic pipette step function.

TABLE 1. Reagent dispensing for KingFisher process

Process step	Plate type	Content	Reagent volume
Wash 1_1	Microtiter deep well 96 plate (DW96)	Wash Buffer 1	1000 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

### 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 H<sub>2</sub>O onto the measurement UV-plate and transferring 10 µl of gDNA from the KF96 elution plate using Versette 96-channel pipetting head (Figure 2).

FIGURE 2. Versette Automated Liquid Handler 96-channel pipetting head



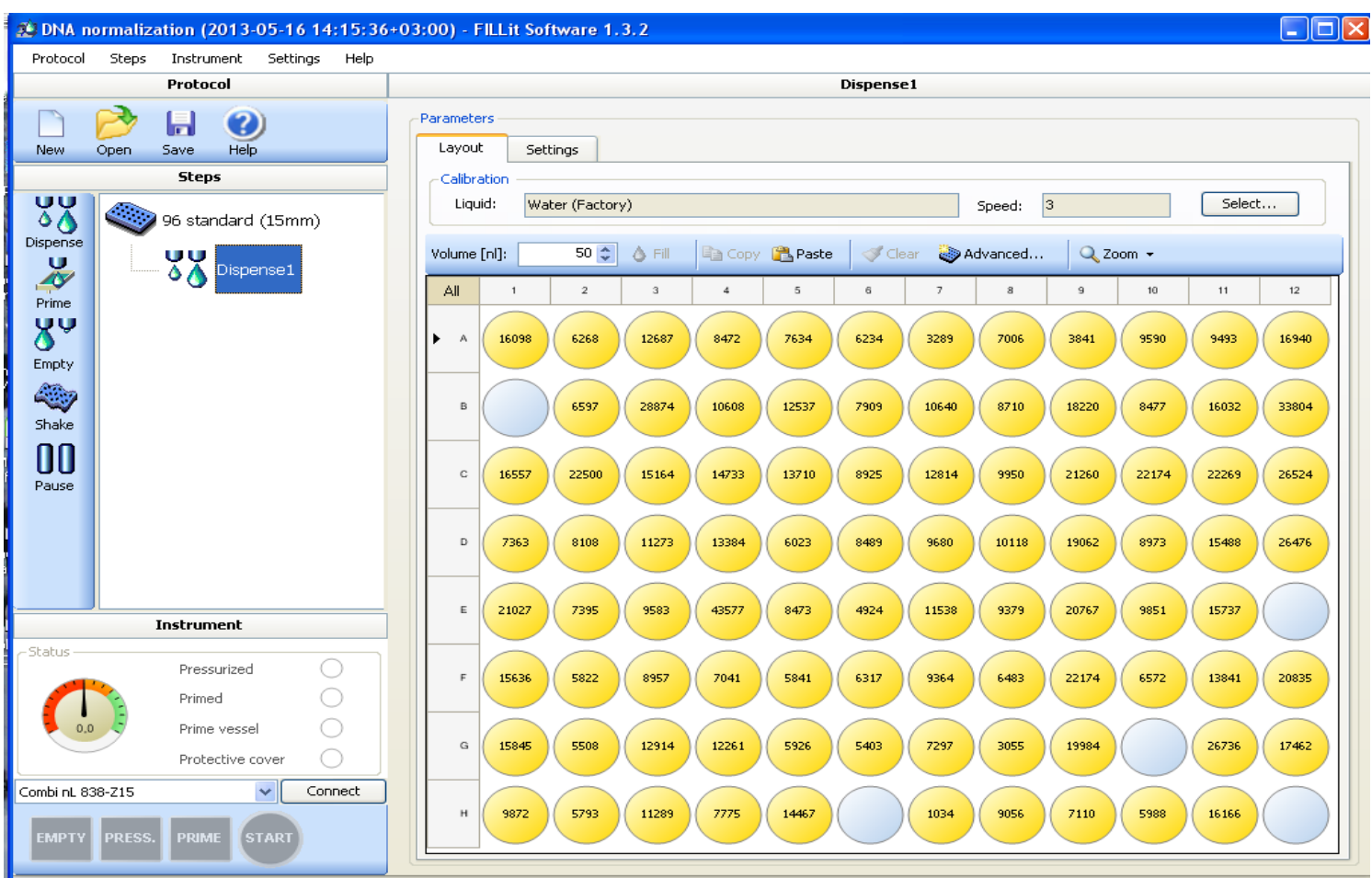
### Nucleic acid Quantity and Quality

Multiskan GO with 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 exporting the data.

### DNA normalization for Real Time PCR Assay

The optimal gDNA template concentration, 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 H<sub>2</sub>O 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 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. Thermo Scientific DyNAmo ColorFlash SYBR Green qPCR Kit was used for the Chromosome Y and Thermo Scientific DyNAmo ColorFlash Probe qPCR Kit with TaqMan ROX probe was used for the Rhesus D factor.

Thermo Scientific 96-well Piko PCR plates were used for performing PCR reactions with the PikoReal 96 Real Time PCR instrument. The data generated with PikoReal qPCR instrument was analysed with the PikoReal Software 2.1.

## Results

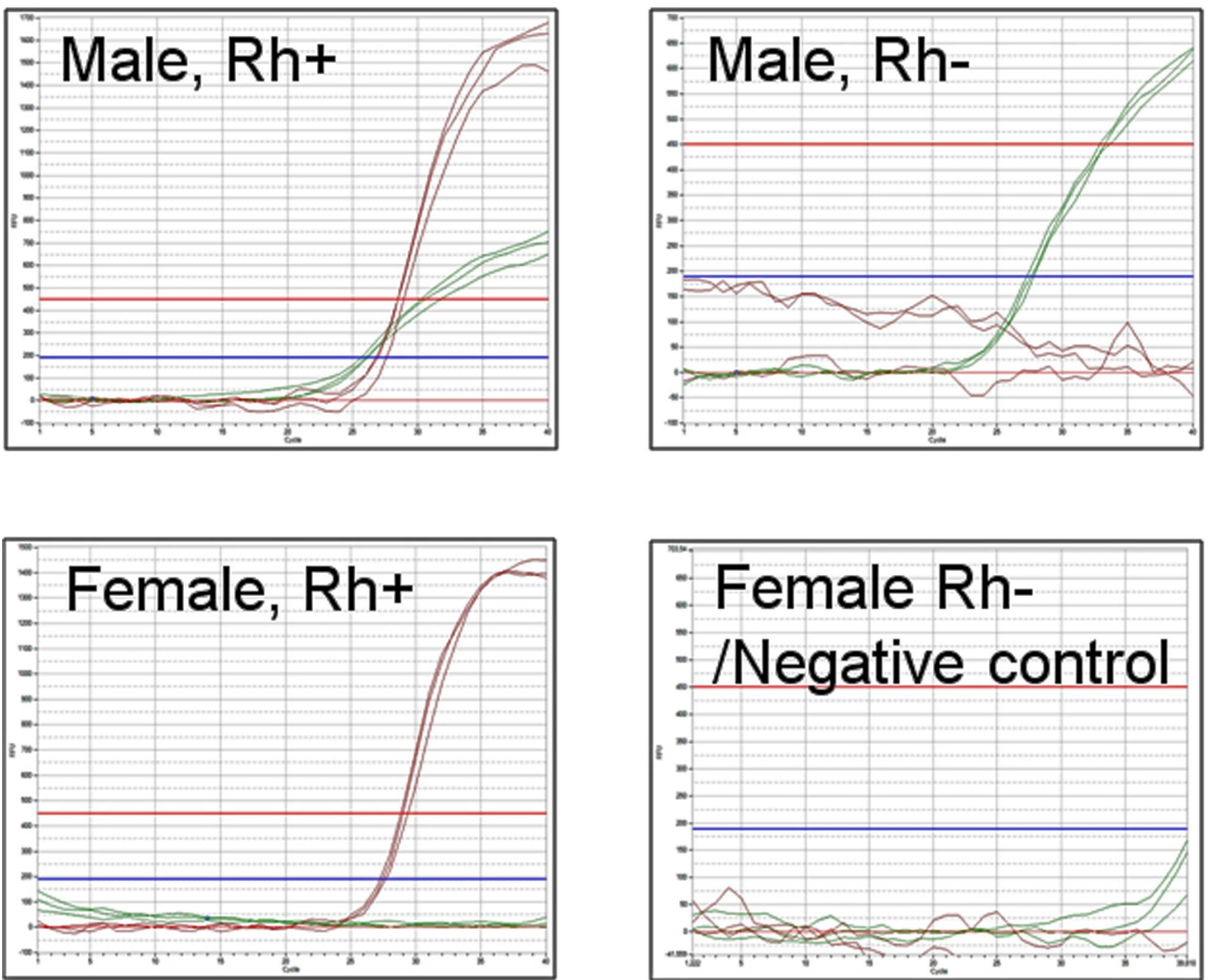
### Genomic DNA Purification

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

- Combining automated liquid handling with the KingFisher Flex offers fast and reproducible plate filling and dilution of samples
- KingFisher magnetic particle purification system generates excellent quality gDNA and total RNA from blood
- Versatile photometric applications from variable volumes are easily analyzed with Multiskan GO
- PikoReal Real Time PCR System provides high performance with reduced bench space

## References

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



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