# Unparalleled nucleic acid purification using Thermo Scientific KingFisher Flex and KingFisher Kits

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# Overview

**Purpose:** Evaluation of Thermo Scientific KingFisher Kits for DNA/RNA purification by using KingFisher Flex.

**Methods:** Different plant and animal samples were homogenized and used for DNA or RNA purification.

**Results:** KingFisher® Flex and KingFisher Kits produced excellent yield of high quality DNA and RNA from all the tested sample materials.

Blood DNA Kit	250 μl or 3 ml blood
Cell and Tissue DNA Kit	1 x 10 <sup>7</sup> cells, 20 mg of tissue or 1ml of cultured bacteria
Plant DNA Kit	20-50 mg of fresh or 4-10 mg of dried plant
Total RNA Kit	2 x 10 <sup>6</sup> cells or 20 mg of tissue
Viral NA Kit	200 µl of cell-free body fluid

# Introduction

KingFisher® magnetic particle processors together with the KingFisher Kits provide a remarkable alternative for manual nucleic acid isolation. The magnetic particle technology combines the speed and efficiency of automation with high quality purification capability.

The KingFisher Flex can process 96 samples during one run with working volume up to 1 ml, or 24 samples when the processing volume is increased up to 5 ml. The KingFisher technology uses magnetic rods to transfer particles through various purification phases of binding, mixing, washing and elution, offering a solution with minimized hands-on time.

KingFisher Kits accomplish the KingFisher Flex for DNA and RNA purification from different starting materials, such as blood, cultured cells or bacteria, tissues, cell-free body fluids and plant samples (Table 1). High purity and excellent yield of DNA and RNA, free of impurities and contaminants, are achieved by using KingFisher Kits. In addition, as an open system KingFisher Flex can extract wide variety of small molecules with specific magnetic beads.

To gain highest possible yield certain sample materials require preparation steps before the purification process can begin. Efficient homogenization or lysis step is required to break cellular structures and release nucleic acids.

## FIGURE 1. KingFisher Flex





KingFisher Blood DNA Kit can be used for genomic DNA purification from up to 3 ml of blood. Two different Bindlt protocols for large blood volumes, lysis and walk-away protocols, were tested by using KingFisher Flex 24-well format and PCR was performed from the purified DNA (Figure 3A). Results indicate high yield of DNA. Comparing the performance of the KingFisher Blood DNA Kit against three different DNA isolation procedures showed superiority of the KingFisher Blood DNA Kit purification process by using the kit together with the KingFisher Flex instrument. Figure 3B represents the agarose gel image of the DNA elutions from tested purification systems.

FIGURE 3. A) PCR performed from DNA purified from 3 ml of blood. Samples 1-9 are purified with walk-away protocol and samples 5-8 with normal protocol. Sample 9 is a negative control. B) Competitor comparison between KingFisher Flex and three competitors show excellent performance of KingFisher system. Sample volume was 250 µl. S1 = spin column, Ca = competitor magnetic particle kit, KF = KingFisher, Cb = competitor purification automate

# Methods

## Sample Preparation

Sample materials for nucleic acid purification included whole blood samples, buccal swabs, HeLa-S3 cells, mouse kidneys, and tobacco and rice leaves. Some of these samples required separate homogenization step in addition to the lysis step before purification began. Several samples also required cooling in liquid nitrogen before mechanical homogenization.

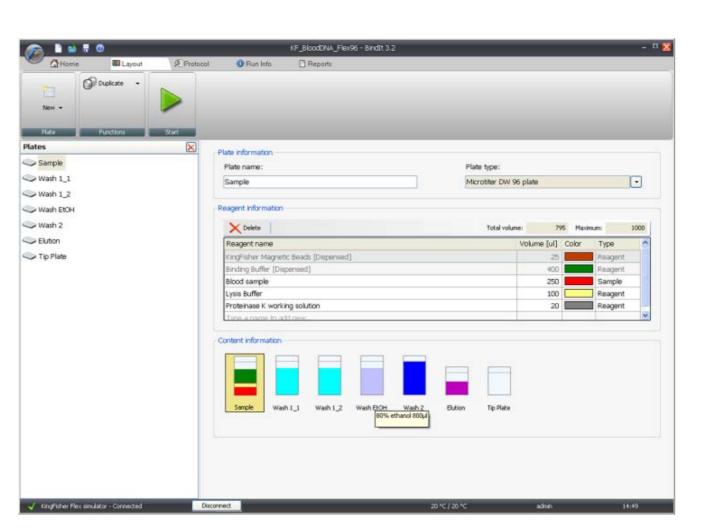
#### Purification process

Nucleic acids purification process was performed with KingFisher Flex or KingFisher Duo together with the KingFisher Kits. Five different KingFisher Kits were used; KingFisher Blood DNA Kit, KingFisher Cell and Tissue DNA Kit, KingFisher Total RNA Kit, KingFisher Plant DNA Kit and KingFisher Viral NA Kit. The protocols were created with new Bindlt 3.2 software (Figure 2).

The purity and yield of the DNA and RNA were analyzed with Thermo Scientific Multiskan GO. Total RNA and PCR products were run on an Agilent Bioanalyzer 2100 (Agilent Technologies).

## FIGURE 2. Example of a protocol for KingFisher Kits with Bindlt software 3.2.

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## Results

## KingFisher Blood DNA Kit

Ladder	Walk-away 1	Walk-away 2	Walk-away 3	Walk-away 4	Lysis 1	Lysis 2	Lysis 3	Lysis 4	negative co
1	1	2	3	4	5	6	7	8	9

MW S1 S2 S3 MW Ca1 Ca2 Ca3 MW KF1 KF2 KF3 MW Cb1 Cb2 Cb3 MW ------

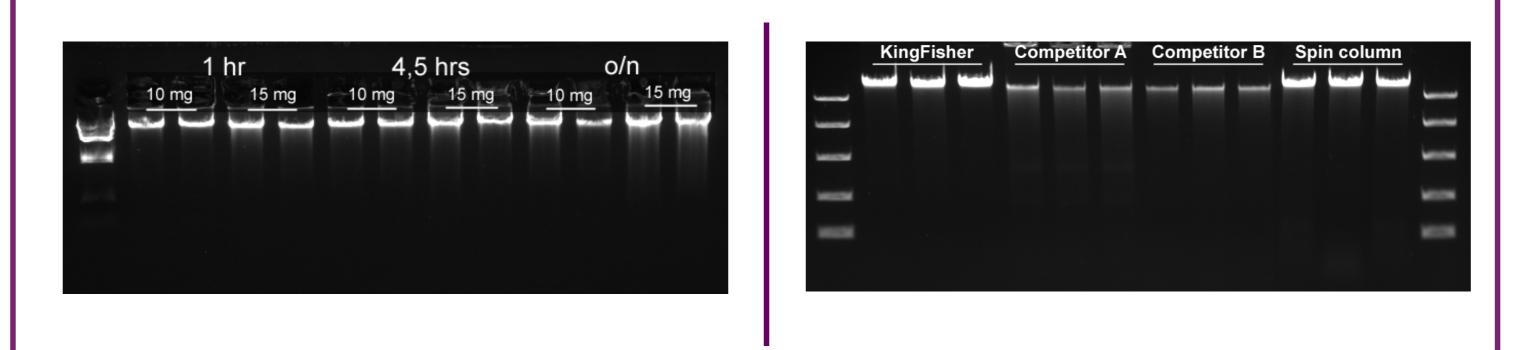
## KingFisher Cell and Tissue DNA Kit

DNA was purified from 10 mg or 15 mg of mouse kindey with KingFisher Cell and Tissue DNA Kit. Samples were lysed in the Lysis Buffer including Proteinase K for one hour, 4.5 hours or overnight. DNA yields depended on the lysis time and the amount of the tissue, but from all of the samples the purified DNA showed excellent yield and ratio, suitable for downstream analyses (Figure 4A).

The KingFisher Cell and Tissue DNA Kit with KingFisher Duo was compared with three different competitive methods. Two of the systems were magnetic bead purification processors and the third was a spin column kit. DNA was purified from 1 x 10<sup>6</sup> HeLa-S3 cells. KingFisher Cell and Tissue DNA Kit performed exquisitely in comparison with the competitors (Figure 4B).

DNA was purified from buccal swab samples and the quality and yield were controlled by qPCR performed with Thermo Scientific PikoReal (Figure 5).

# the KingFisher Duo and three competing purification systems.



#### FIGURE 5. The results from the qPCR indicate excellent yield and purity of DNA from buccal swabs.

	7000 -			
		Buccal sw	ab, KF Cell	& Tissue Kit
	6000 -	Sample	Cq	Quantity
		BS1	28,49	1981
		BS1	28,44	2045
	5000 -	Standard	26,23	10000
	5000	Standard	26,14	10000
	-	Standard	26,18	10000
	4000 -	Standard	29,55	1000
ΒFU		Standard	29,26	1000
œ	3000 - 2000 -	Standard	29,34	1000
		Standard	32,91	100
		Standard	32,83	100
		Standard	33,11	100
	1000 -			
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	1	5		10 1

#### KingFisher Total RNA Kit

KingFisher Total RNA Kit was used for purification of RNA from 5 x 10<sup>5</sup> HeLa-S3 cells. The results show consistency and high quality of RNA (Figures 6 and 7). The RNA integrity number (RIN) of the samples was 10, indicating that RNA was intact in all of the samples.

FIGURE 4. A) Mouse kidney was lysed for 1 h, 4.5 h or overnight (o/n), followed by purification of DNA in the KingFisher Flex. B) Competitor comparison between

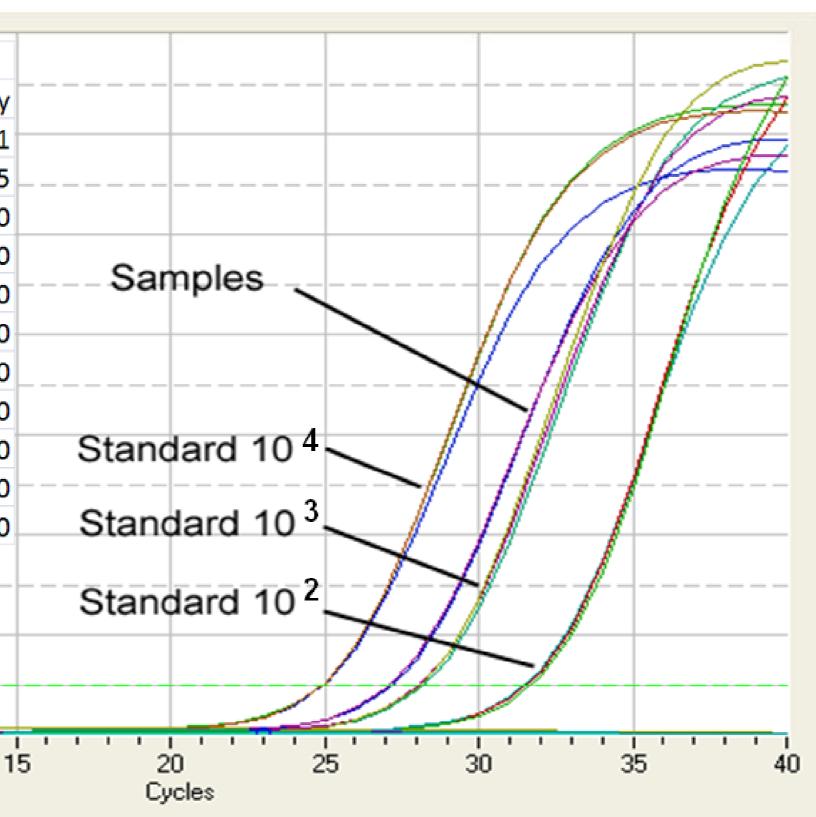


FIGURE 6. RNA purified with the KingFisher Duo (samples 1-6) or KingFisher Flex (samples 7-12).

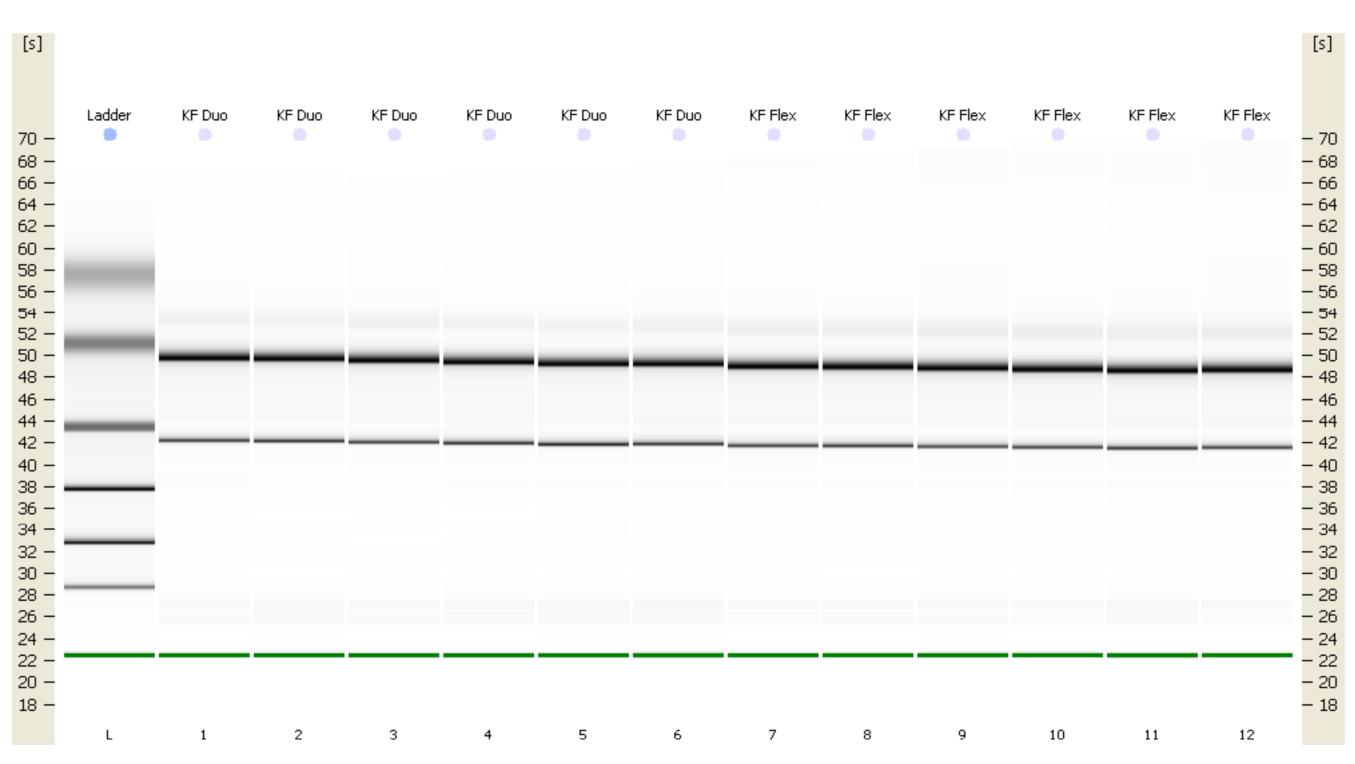
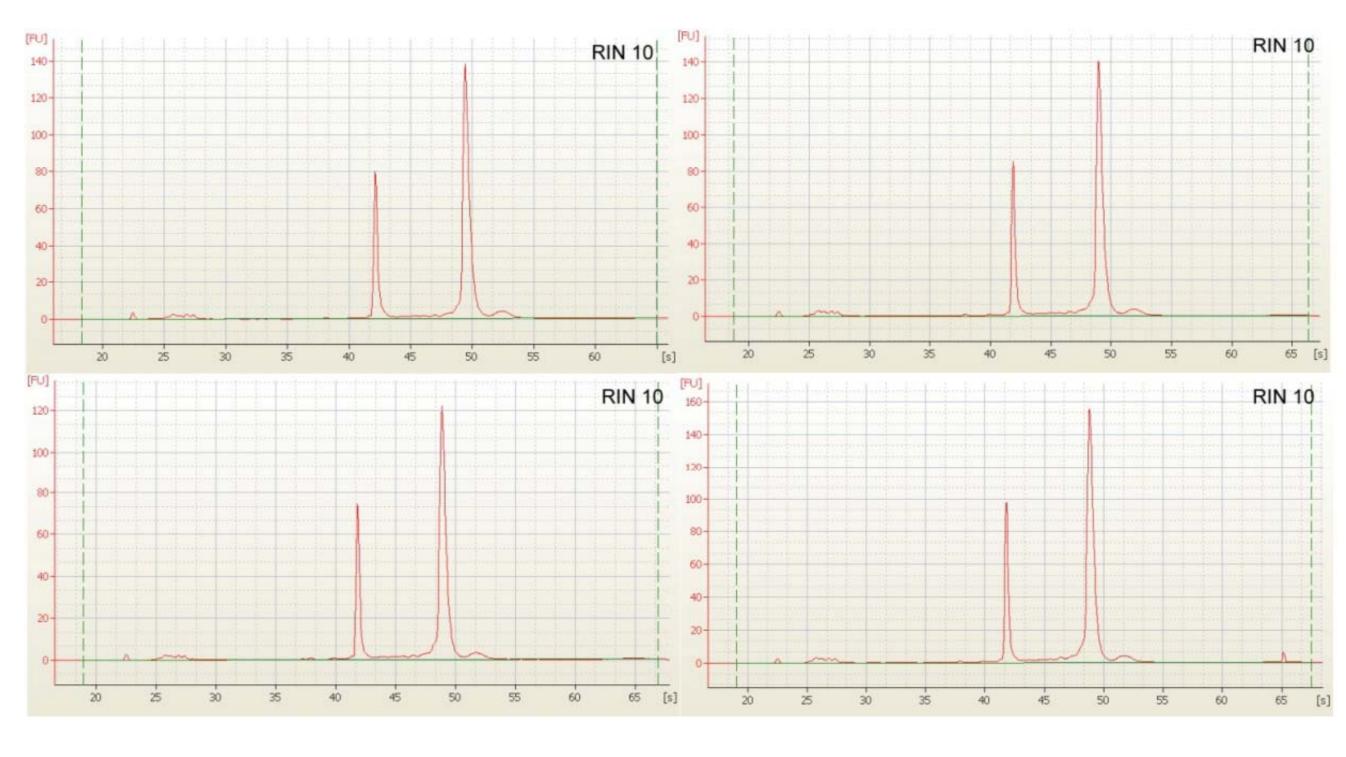


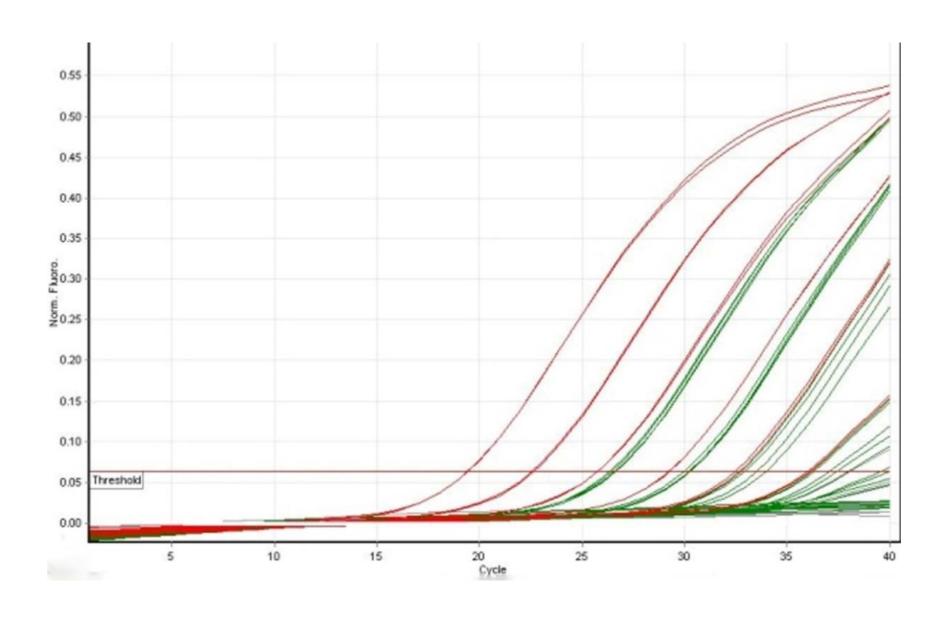
FIGURE 7. Electropherograms of four different RNA samples purified with KingFisher Flex show consistency and good quality of purified RNA.



#### KingFisher Viral NA Kit

KingFisher Viral NA Kit has excellent sensitivity for the purification of viral NA, which is often scarce in the sample. Viral NA of HCV was purified from serum samples with the KingFisher Flex and the quality of the samples was analyzed with qPCR (Figure 8). The samples showed excellent performance in the qPCR.

FIGURE 8. Purified RNA was used for rt-qPCR to analyze the quantity of the HCV in the serum samples. The red color indicates standard curves and the green color indicates the purified samples.





#### KingFisher Plant DNA Kit

Fresh rice and tobacco leaves were first frozen in liquid nitrogen and then the samples were homogenized with mortal, continuing keeping the samples under liquid nitrogen. 20 mg of homogenized sample per well were purified with KingFisher Plant DNA Kit and KingFisher Duo. DNA was used for PCR with universal primers. Results indicated that the performance of KingFisher Plant DNA Kit was excellent (Figure 9).

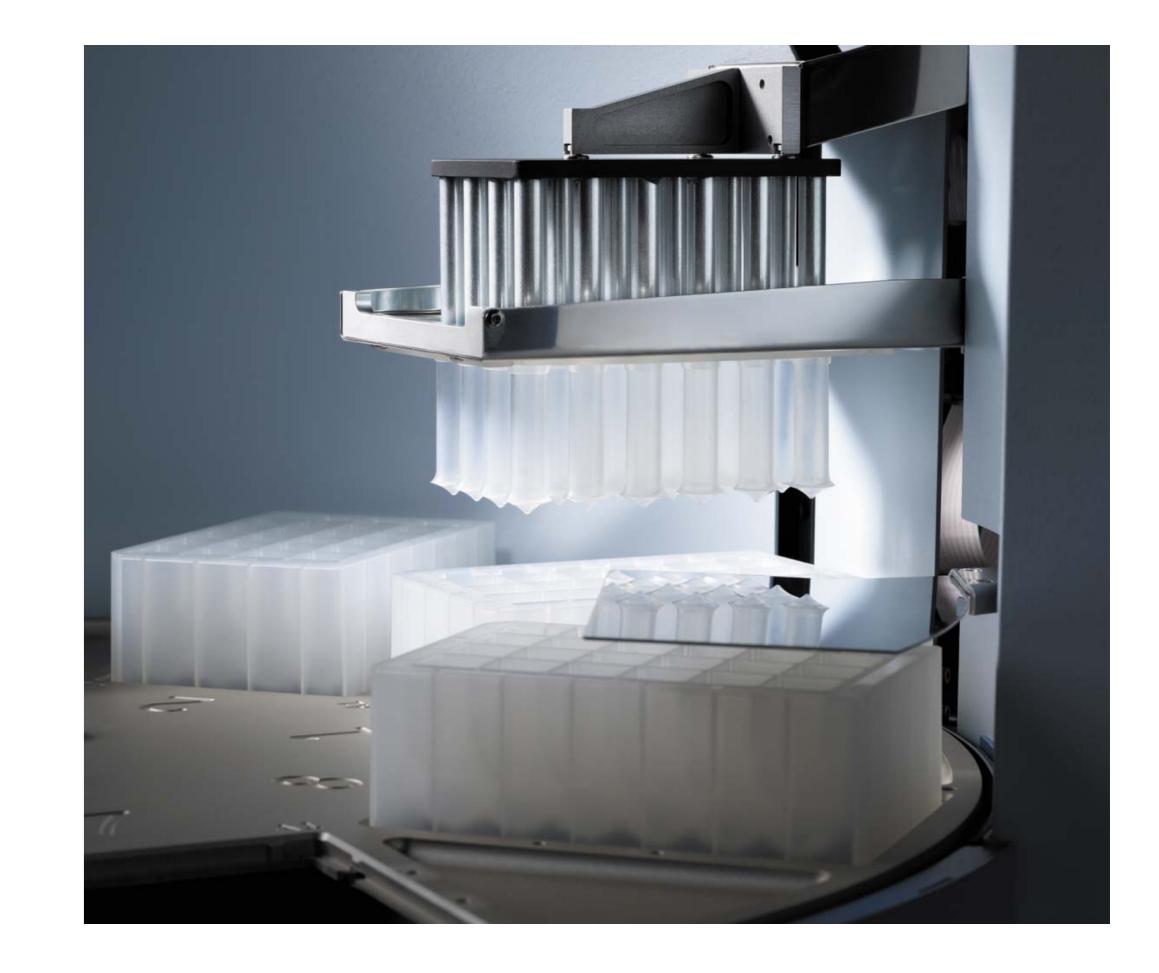
FIGURE 9. A) DNA purified from 20 mg of fresh rice sample. B) PCR from DNA purified from rice and tobacco leaves. Samples 1-5 are rice samples, 6-10 tobacco samples and 11-12 negative controls.

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## Conclusion

- Flexible KingFisher system with optimized nucleic acid purification kits
- KingFisher Flex offers processing positions for 1-96 samples for up to 5 ml volume
- Walk-away solution for high-throughput laboratories with KingFisher Flex
- Proven excellent performance and reproducibility
- Customized kits for wide variety of sample types



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