

DNA Purification from Blood Samples with the Thermo Scientific KingFisher Pure DNA Blood Kit

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Goal

This technical note describes genomic DNA (gDNA) purification from 200 μ L blood samples with the Thermo Scientific™ KingFisher™ Duo and Thermo Scientific™ KingFisher™ Flex. These magnetic particle instruments offer an efficient solution for purifying gDNA from 200 μ L to 1 mL of fresh or frozen blood with minimal hands-on work. In addition, gDNA can be purified from buffy coat or bone marrow samples. The KingFisher Flex instrument enables the automated purification of 96 samples in a high-throughput format, while the KingFisher Duo, a lower throughput instrument, is available for up to 12 samples per run. Both instruments are suitable for large volume sample preparation, processing volumes up to 5 mL.

Introduction

gDNA purification from blood samples is a very common sample preparation step for various applications. The Thermo Scientific™ KingFisher™ Pure DNA Blood Kit together with the KingFisher magnetic particle processors offer an efficient solution for purifying gDNA from 200 μ L to 1 mL of fresh or frozen blood with minimal hands-on work. In addition, gDNA can be purified from buffy coat or bone marrow samples. The KingFisher Flex instrument enables the automated purification of 96 samples in a high-throughput format, while the KingFisher Duo, a lower throughput instrument, is available for up to 12 samples per run. Both instruments are suitable for large volume sample preparation, processing volumes up to 5 mL.

Materials and Methods

The gDNA was isolated from 200 μ L of fresh human blood using the KingFisher Pure DNA Blood Kit (Cat. No. 98010196 and 98010496) according to the instruction manual. The elution volume for the KingFisher Duo was 100 μ L and 150 μ L for the KingFisher Flex. The KingFisher Pure DNA Blood Kit together with the KingFisher Flex was compared to 3 magnetic bead kits from different manufacturers. DNA was purified from 200 μ L of fresh blood and eluted according to the instruction manuals.

Results

The average yield and quality of purified gDNA using the KingFisher Pure DNA Blood Kit with the KingFisher Flex and KingFisher Duo are listed in Table 1.



Table 1. Typical DNA yields and purities from fresh blood samples* treated with EDTA or citrate.

Instrument	Blood sample	Typical DNA yield	Purity by absorbance 260/280 nm
KingFisher Flex 96 and KingFisher Duo 12	200 μ L	3 - 9 μ g	1.7 - 1.9
KingFisher Flex 24 and KingFisher Duo 6	1 mL	20 - 45 μ g	

* DNA yields obtained from individual blood samples vary due to the number of white blood cells in each sample.

Blood samples from 32 individuals were used for purification in the KingFisher Flex. From each sample triplicates were pipetted into adjacent wells, making a total of 96 samples on the plate. After purification, samples were pipetted onto an agarose gel (Figure 1). The gel image demonstrates that DNA purification was reproducible and highly specific, with no RNA co-purification (triplicate samples performed equally well).

In comparison to magnetic bead kits from 3 other manufacturers, the KingFisher Pure DNA Blood Kit delivered a higher yield, as shown by the agarose gel image of the DNA eluates (Figure 3).

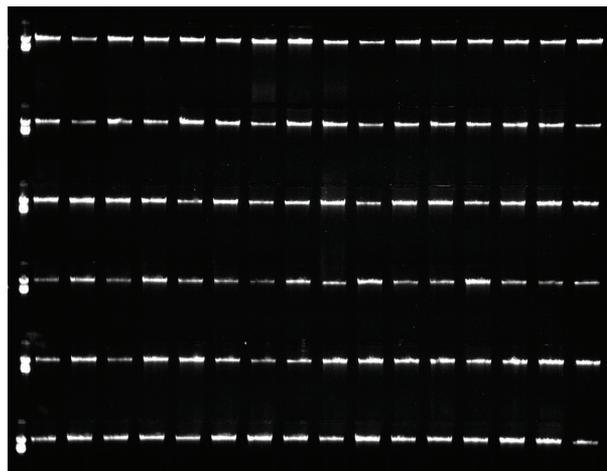


Figure 1. Triplicates from 32 blood samples were purified in the KingFisher Flex. DNA eluates were run on an agarose gel.

The gDNA eluates were used for allelic discrimination with the Thermo Scientific™ PikoReal™ 96 instrument using Thermo Scientific™ DyNAmo™ Genotyping Master Mix, GPR174 primers and TaqMan probes. The allelic discrimination graph is shown in Figure 2.

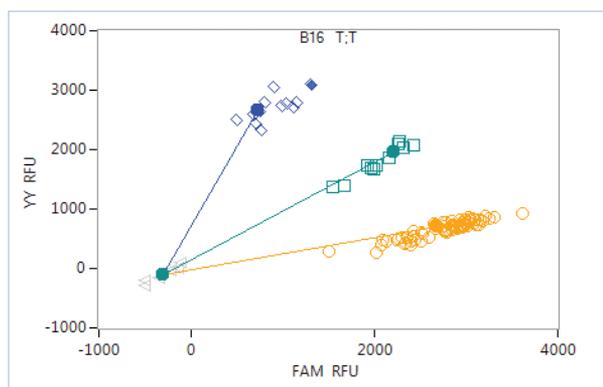


Figure 2. Allelic discrimination graph. Blue diamonds represent TT homozygotes, green squares are heterozygotes and yellow circles represent AA homozygotes.

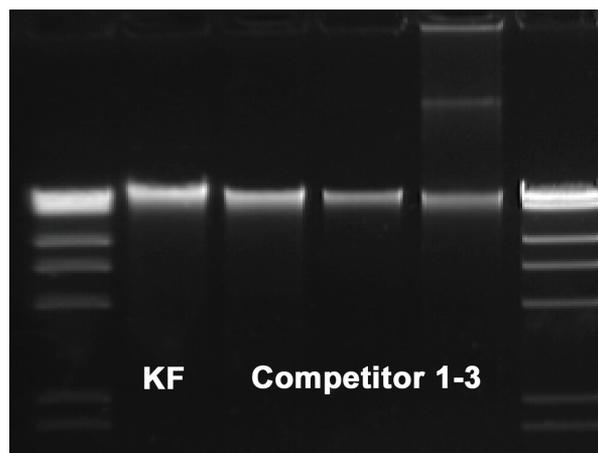


Figure 3. Agarose gel image of gDNA extracted with the KingFisher Pure DNA Blood Kit (KF) and kits from 3 other manufacturers.

Conclusion

Excellent gDNA yields of up to 9 µg were purified from 200 µL of blood. The purified gDNA was of high quality - free of proteins, salt and other inhibitors. gDNA purification of 96 blood samples took less than an hour with the KingFisher Flex. This optimized combination of kit reagents, plastic consumables, Thermo Scientific™ BindIt™ Software and patented magnetic particle handling makes an exceptional system for purifying gDNA at high yields and optimal purity.

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