Technical Note:
TN-PSKFKitBlood_0211

Flexible purification of DNA from variable volume blood samples with the Thermo Scientific KingFisher Blood DNA Kit

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- *Efficient purification of genomic DNA*
- *Isolation from fresh or frozen blood and buffy coat*
- *Flexible DNA isolation from 1-96 samples*
- *Variable sample volume up to 3 ml*
- *DNA yields up to 130 µg*

Introduction

The Thermo Scientific KingFisher Blood DNA Kit together with the KingFisher® magnetic particle processor offers an efficient solution for purifying genomic DNA (gDNA) from fresh or frozen blood and buffy coat with minimal hands-on work. The Thermo Scientific KingFisher Flex instrument enables automation of high-throughput or large - volume sample preparation, while the lower throughput instrument, the Thermo Scientific KingFisher mL, is ideal for up to 15 samples at a time. The optimized combination of kit reagents, plastic consumables, Thermo Scientific BindIt Software and patented magnetic particle handling constitutes an exceptional purification system for obtaining a high yield and optimal purity of DNA. This technical note describes the excellent quality of the rapid method for gDNA purification from variable volumes of blood samples.

Material and Methods

KingFisher Flex 96

The gDNA was isolated from 250 µl of fresh and frozen human blood using the KingFisher Blood DNA Kit (Cat. No. 97010196) according to the instruction manual. The purification protocol was optimized for the KingFisher Flex with the BindIt® Software 3.1. The protocol "KF_BloodDNA_Flex96" was run on the KingFisher Flex. The elution volume was 150 µl.

KingFisher Flex 24

The gDNA was isolated from 3 ml of fresh and 1.5 ml of frozen human blood using the KingFisher Blood DNA Kit according to the instruction manual. The protocol "KF_BloodDNA_Flex24" was run on the KingFisher Flex. The elution volume was 500 µl.

Walk-away protocol

For the walk-away process, the Flex 24 protocol was modified for 1 ml blood samples by using BindIt Software. The lysis step of the modified protocol included the KingFisher Magnetic Beads and Binding Buffer from the beginning of the step (Figure 1). The modified protocol was run on the KingFisher Flex 24 and the elution volume was 500 µl.

Comparisons

The KingFisher Blood DNA Kit was compared to three competitive methods or instruments. The competitors comprised a spin column method, a magnetic particle purification kit combined to a semi-automated instrument and a purification automate with sealed reagent cartridges. DNA was purified from 200–250 µl of blood and eluted according to the instruction manuals.

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

Instrument	Blood sample (fresh or frozen blood)	Typical DNA yield	Purity by absorbance 260/280 nm
KingFisher Flex 96 and KingFisher mL	250 µl	3 –10 µg	1.7 – 2.0
KingFisher Flex 24	3 ml	90 –130 µg	

* DNA yields obtained from blood samples vary individually due to the number of white blood cells.

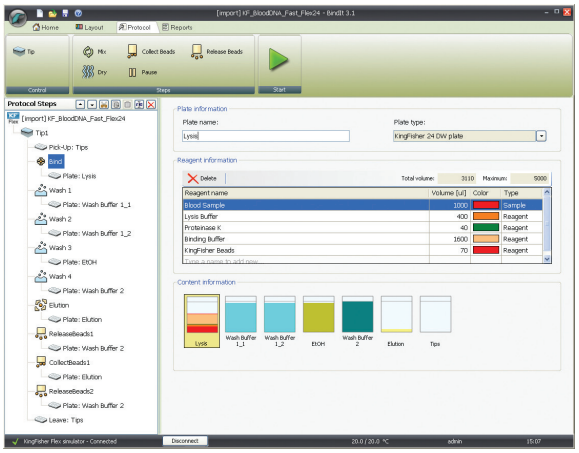


Figure 1. Lysis and binding of the magnetic beads by BindIt Software 3.1 for the walk-away process.

Results

The results of the gDNA purification by using the KingFisher Blood DNA Kit with the KingFisher Flex and KingFisher mL are listed in Table 1. The quality of DNA was controlled by measuring the UV absorption spectrum at 220–340 nm. The shape of the absorption curve and ratios between the absorbance 260/280 nm and 260/230 nm show an excellent purity of the gDNA (Figure 2).

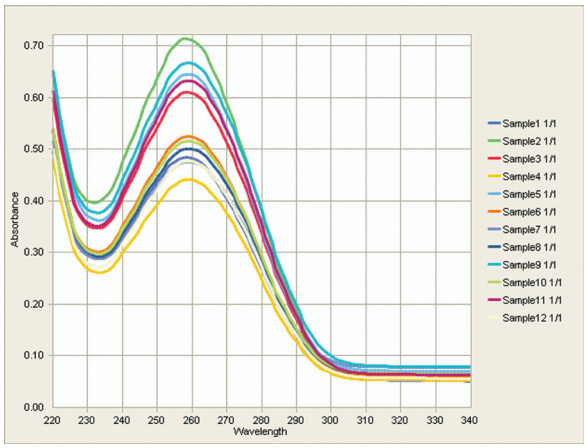
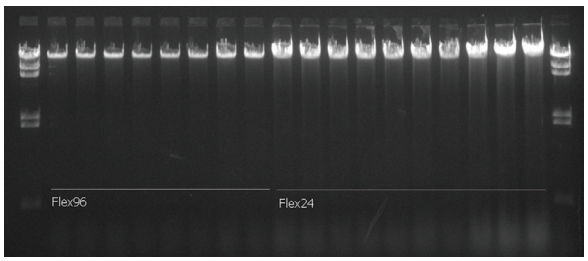


Figure 2. Absorption spectra (220–340 nm) of twelve DNA isolates extracted from 250 µl of fresh blood with the KingFisher Flex.

The purification of DNA from different volumes of frozen blood resulted in very good quality and quantity of gDNA for any secondary applications. Figure 3 shows purified gDNA on agarose gel and the PCR performed from these isolates.

a)



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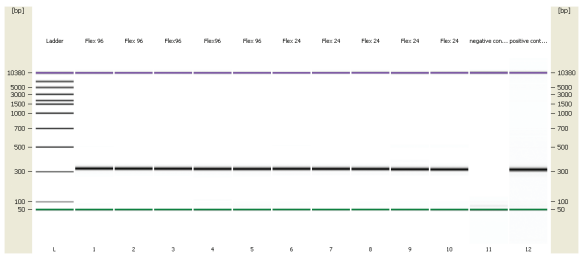

FIGURE 3. DNA purified from frozen blood samples. a) gDNA isolated from 250 µl of blood with the KingFisher Flex 96 and from 1.5 ml of blood with the KingFisher Flex 24. b) PCR performed from frozen blood DNA isolates.

With the KingFisher Flex 24-well format, it is possible to use 3 ml of blood to reach up to 130 µg of gDNA. Experiments of the walk-away protocol, with even less hands-on work, show that the starting volume of blood should be reduced to 1 ml to achieve sufficient washing of DNA and the best purity. The gDNA quality was excellent for downstream applications but the yields were lower with the walk-away protocol, as was expected (Figure 4).

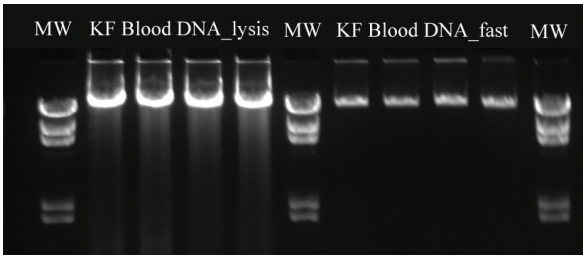

FIGURE 4. DNA purified from 3 ml of blood by lysing samples according to the kit instructions (KF Blood DNA_lysis) and from 1 ml of blood by using the walk-away protocol including the magnetic beads and the Binding Buffer from the beginning of the process (KF Blood DNA_fast).

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 5 represents the agarose gel image of the DNA elutions from tested purification systems.

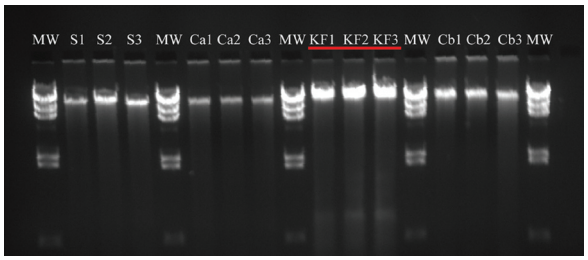

FIGURE 5. In the comparison between different purification processes, the highest yield of gDNA was obtained with the KingFisher Blood DNA Kit (S1 = spin column, Ca = competitor magnetic particle kit, KF = KingFisher, Cb = competitor purification automate).

Conclusions

- **Excellent DNA yields up to 130 µg from 3 ml of blood**
- **High-quality DNA free of proteins, salt and other inhibitors**
- **DNA purification of 96 blood samples in less than an hour**
- **Optimized process including the purification kit, instrument and software**
- **Semi-automated process saves time with minimal hands-on work**

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