Technical Note:
TN-KF-OPTIMIZATION-0310

Optimization of the DNA Purification Protocol for the Thermo Scientific KingFisher Flex and BindIt 3.1 Software

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Overview

Purpose: Optimal quantity and quality of DNA with the magnetic particle based extraction technology
Method: Thermo Scientific KingFisher Flex magnetic particle processor and BindIt® 3.1 Software
Results: By simple optimization of the nucleic acid extraction protocol it is possible to achieve a great yield of high quality DNA

Introduction

Rapid and efficient isolation of nucleic acids from complex biological matrixes is an important step to get optimal starting material for various experiments. KingFisher®, the magnetic bead based automated purification system, provides a quick and easy solution to achieve reproducible results in purification of pure and intact DNA or RNA with minimal hands-on time. The KingFisher technology is based on magnetic rods transferring particles through the various purification phases – binding, mixing, washing and elution.

The KingFisher is an open and flexible system; the user can choose any available magnetic particle based purification kit suitable for the application. The purification protocol is created with the BindIt 3.1 Software enabling the user to optimize the purification conditions by taking into consideration the sample type and particles used in the extraction kit. The latest member of the KingFisher family is the KingFisher Flex that offers both high throughput and a wide range of processing volumes.

It is important to optimize the purification protocol to reach the optimal quantity and quality of nucleic acids with the magnetic particle based technology. All the purification steps require beads to separate effectively, and the mixing speeds are significant for optimal binding, efficient washing and active elution. In this technical note we present some guidelines for generating an ideal DNA purification protocol for KingFisher Flex. Different mixing combinations and the effect of heating in the elution were tested, and the quantity and quality of purifications were compared. Wide range of the processing volumes with the KingFisher Flex are possible because several different magnetic heads and plate formats are

Figure 1. KingFisher Flex magnetic rods

available. In this study we have used 96-deep well and 24-well magnet heads and tip combs. The DNA quantity was measured by reading the absorbance at 260 nm and the quality by analyzing the 260/280 nm ratio. The quality of DNA was also tested by an end-point PCR to control the presence of PCR inhibitors in the eluate.

The data shows that the optimization of the KingFisher Flex purification protocol enhances the quality and quantity of the extracted DNA. The protocol is easy to modify with the BindIt 3.1 Software, which is available for all the KingFisher instrument models.

Material and methods

A KingFisher Flex DNA purification protocol typically consists of cell lysis / DNA binding, several washing steps and DNA elution. All these purification steps were optimized for both the 96- and 24-deep well format. In the 96-well format the DNA extraction was performed from 200 µl of blood or ~6 µg of pure calf thymus DNA, and in the 24-well format from 1 mL of blood or ~35 µg of calf thymus DNA. The kits used in the experiments were InviMag® Blood DNA kit (Invitek, Germany) and BioSprint DNA Blood kit (Qiagen, Germany).

The absorbance of the eluates was measured with the Thermo Scientific NanoDrop 8000 and the DNA yield was calculated based on the measured 260 nm absorbance.

The protocols were optimized by changing one variable at a time. The mixing speed of one step was optimized by alternating the available mixing speeds while the rest of the protocol remained unchangeable. BindIt 3.1 Software (Fig. 2) was used for making modifications to the isolation protocols.

Results

Faster mixing speeds increase DNA yields for the DNA purification from blood. When binding, washing and elution were tested one variable at a time, the faster mixing speeds for all the steps resulted in better yield (Table 1). The mixing speed causes a bigger difference to the results with the KingFisher Flex 24-well format, because of the shaping of the tip and the well. The protocol parameters in the binding and elution steps affected primarily the total DNA yield. In the washing steps the SLOW mixing had an effect on the DNA quality. The purified DNA was analyzed with the PCR and

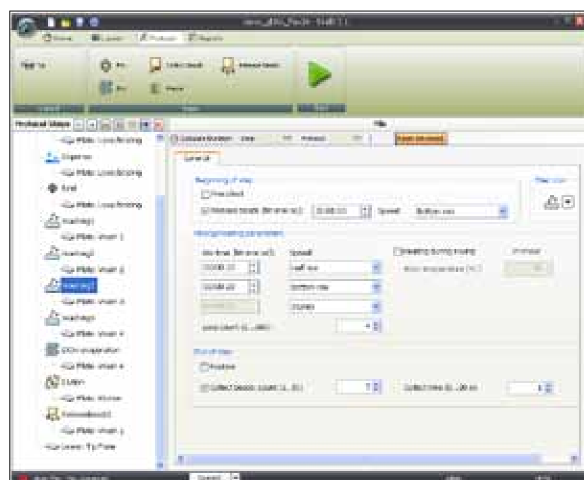
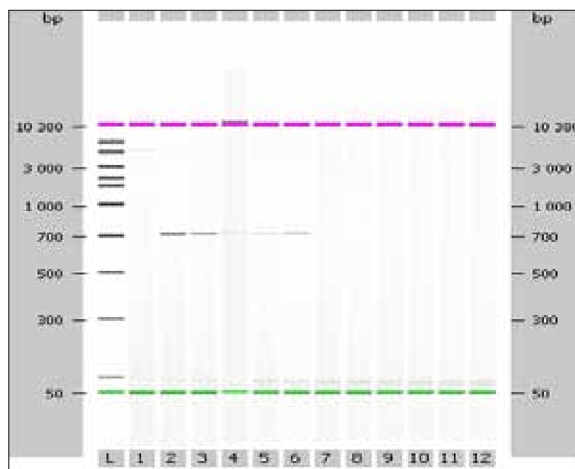
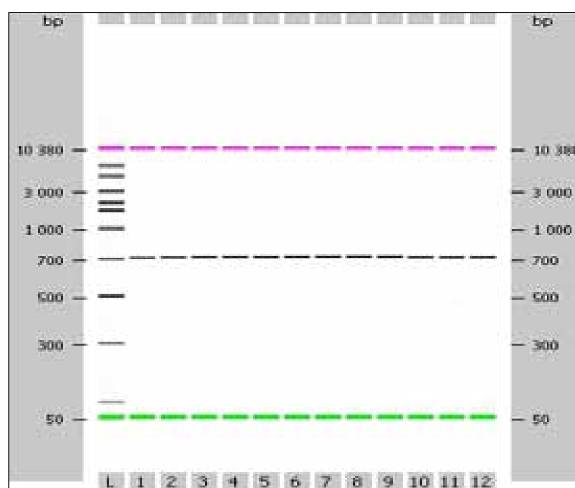


Figure 2. BindIt 3.1 Software parameters

- Easy step selection
- Mixing speed
- SLOW
- MEDIUM
- FAST
- HALF MIX
- BOTTOM MIX
- Mixing time
- Incubation without mixing
- Premix / Postmix
- Dry step
- Optional heating (10-115°C)
- Testing specific steps by "Disable step"
- Wide volume range





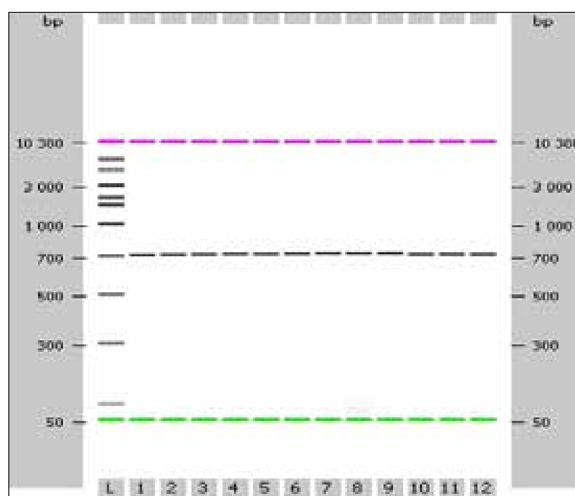


Figure 3. Effects of the Wash step mixing speed on the final DNA quality. PCR products of the elution on Express DNA 7500 LabChip (BIOTAQ INC.)

- DNA purification from human whole blood
- Wash step, mixing speed variables: SLOW, MEDIUM or FAST
- Bind and Elution steps, mixing speed: FAST
- PCR performed directly from the elution by using CD19 primers
- First eight samples from a 96-well format isolation and the last four from a 24-well format in each picture

the results show the remaining impurities affecting the secondary applications (Fig. 3). Table 1 shows the effects of different mixing speeds on the bind and elution steps and the effect of the heating and elution volume on the final DNA yield.

Conclusions

The different mixing speeds have a strong effect on the DNA purification with the KingFisher Flex magnetic particle processor. For the DNA purification it is important to mix the solutions efficiently to reach optimal binding between the DNA and the bead. Powerful washing is essential for the high purity of the DNA. Results of the SLOW mixing speed indicate impurities in the elution and inhibition of the enzymatic reaction due to inefficient washes. The slower mixing speeds are frequently preferred during the elution due to the DNA degradation, but according to our experiments the FAST mixing in the elution results in the best quantity and quality of DNA. The SLOW mixing speed is useful for the heated steps of the nucleic acid purification.

These test results correspond to the DNA purification. However, the same optimizing is not directly applicable to e.g. RNA and protein purification on the KingFisher Flex. HALF MIX, MEDIUM or SLOW mixing speeds are recommended for more sensitive bead-biomolecule complexes.

Summary

- **KingFisher Flex provides a rapid and reliable method for DNA isolation**
- **Easy adjustment of isolation protocols with BindIt 3.1 Software**
- **Open platform is simple to adapt for all available magnetic particle separation kits**

TABLE 1. Optimizing binding and elution of the DNA purification protocol

Bind step	SLOW	MEDIUM	FAST
Yield µg	1,65	3,63	3,66
%CV	11,45	6,05	5,7
A260/A280	1,8	1,9	1,87
%CV	1,72	1,76	1,38

Elution step	SLOW	MEDIUM	FAST	BOTTOM MIX
Yield µg	2,24	3,29	3,38	3,39
%CV	3	8,7	7,45	5,55
A260/A280	1,93	1,95	1,84	1,87
%CV	1,26	3,11	0,45	0,68

TABLE 2. BindIt 3.1 Software mixing speed recommendations for DNA purification with KingFisher Flex

SLOW	FAST	BOTTOM MIX	"MIXED MIXES"
• For long incubations • "Clean" sample material, e.g. serum MEDIUM • Good basic mixing • For longer washes • Easy-to-lyse samples, e.g. most bacterial cells	• Efficient mixing • Especially for samples with high amount of cells, e.g. blood • Good speed for rapid washes HALF MIX • Half length of the movement • Closer to the bottom of the plate (96-well format) • Efficient mixing for higher processing volumes: 96-well >700 µl, 24-well >3 mL	• Short length of the movement • Closer to the bottom of the plate (96-well format) • Efficient mixing for elution (especially 96-well format) • Breaks bead clumps	Former Fast Dual Mix = FAST 10s + BOTTOM MIX 10s, Loop x times for total mixing time Premix (Release beads), Short BOTTOM MIX or FAST for homogeneous mixture Postmix, Short BOTTOM MIX or FAST following e.g. incubation without mixing or SLOW mixing to homogenize the bead solution for efficient collection

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