Purification of High-quality DNA with the Thermo Scientific KingFisher Cell and Tissue DNA Kit

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Technical Note

Goal

This technical note describes the purification of DNA from different sample materials with the Thermo Scientific™ KingFisher™ Cell and Tissue DNA Kit together with Thermo Scientific™ KingFisher™ magnetic particle processors. Samples included HeLa-S3 cells and various mouse internal organ samples, as well as mouse ear and tail samples – typically used in genotyping. The purified DNA is of high quality and free of protein, nucleases, and other contaminants or inhibitors.

Introduction

The KingFisher Cell and Tissue DNA Kit together with the KingFisher magnetic particle processors is an efficient solution for purifying DNA from different sample materials. The maximum sample volume for purification is 10^7 cultured cells, 20 mg of tissue or 1 mL of bacterial culture, and FFPE samples can also be used after the samples have been de-paraffinized. The obtained DNA yield depends on the sample material and the method of storage. The expected A_{260}/A_{280} ratio is usually 1.7–2.0.

Materials and Methods

The DNA purification process was performed with the KingFisher Cell and Tissue DNA Kit (Cat. No. 97030196 and 97032496) in conjunction with either the Thermo Scientific™ KingFisher™ Duo or Thermo Scientific™ KingFisher™ Flex. The purification was conducted in accordance with the instruction manuals. DNA was purified from frozen HeLa-S3 cells, human buccal swab samples, and from frozen and thawed mouse liver, kidney, spleen, ear and tail samples. Different processing times for lysing the tissues were tested. DNA was eluted into 100 µL or 150 µL of Elution Buffer, depending on the KingFisher processor, however the volume can be adjusted. For competitor comparison, two magnetic bead purification systems and a spin column kit were used in accordance with the respective instruction manuals. The purification protocols were optimized for both the KingFisher Duo and KingFisher Flex with Thermo Scientific™ BindIt™ Software 3.2.

Lysing the samples

Efficient lysis is an important step in order to gain a good yield of high-quality DNA. The requirements of the lysis time are usually different due to the structures of the cells and tissues. After the addition of the Lysis Buffer to the HeLa-S3 cells, the samples were efficiently mixed by pipetting up and down until the viscosity of the samples was lost, vortexed for 30 s, and incubated at 70 °C for 15 mins. The sample size varied from 50,000 to 1 x 10^6 HeLa-S3 cells. The mouse tissue samples were lysed in the Lysis Buffer at 56 °C for either 1 hour, 4.5 hours or overnight (~20 hours), and then centrifuged for a short time to clear the lysate of cell debris.

KingFisher process

The cleared lysates were then transferred to the KingFisher Duo or KingFisher Flex together with the Binding Buffer and Thermo Scientific™ KingFisher™ Magnetic Beads for the binding step, in which DNA binds to the beads. Wash steps then disposed of proteins, cell debris and other contaminants, while the DNA bound to the magnetic beads was transferred through the steps. The DNA was eluted into the Elution Buffer. In certain cases, e.g. cultured cells, it is also possible to carry out the lysis step beforehand in the KingFisher instrument. The purification process took approximately 25 mins.

Results

Table 1 shows examples of the DNA yields from different cell and tissue types. The agarose gel picture indicates high yield of DNA purified from mouse ear, liver and kidney samples (Figure 1). In Figure 2, 10 mg and 15 mg of mouse kidney samples were lysed for between 1 hour and overnight before DNA purification; even after 1 hour, the DNA yield was excellent for downstream applications.

Buccal swab samples were collected from human cheek with cotton swabs. The samples were lysed and the cotton swabs were removed before purification. The obtained DNA was then run in a Thermo Scientific™ PikoReal™ qPCR instrument. The results indicate that the purified DNA was of good quality and did not include inhibitors (Figure 3).

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Table 1. Examples of purified DNA yields.

Sample	Sample input	Typical yield
HeLa-S3 cells	1 x 10^6	6-8 µg
Mouse tissue samples		
Ear punch	One punch, ~0.2 cm diameter	6-17 µg
Tail sample	0.1-0.2 cm	2-5 µg
Liver	15 mg	20-30 µg
Kidney	15 mg	20-35 µg
Spleen	20 mg	80-160 µg

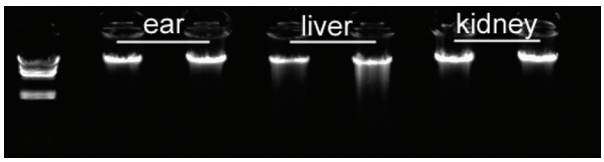

Figure 1. Purification was performed simultaneously from mouse ear punches and from 10 mg of liver or kidney samples after lysing the samples for 4.5 hours.

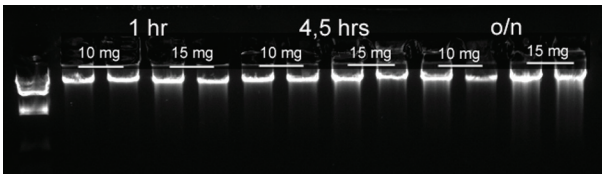

Figure 2. 10 mg or 15 mg of mouse kidney was lysed for 1 hour, 4.5 hours or overnight, followed by purification of DNA in the KingFisher Flex. The DNA yields depended on the lysis time and the amount of tissue, but from all of the samples the purified DNA showed excellent yield and ratio, suitable for downstream analyses.

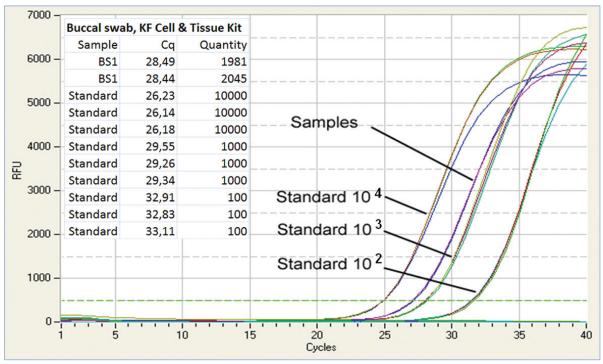

Figure 3. Results from the qPCR run with the PikoReal™ indicate excellent yield and purity of DNA from buccal swab samples.

Figure 4 shows the PCR attained from 50,000 and 100,000 HeLa-S3 cells. The comparison between the KingFisher Cell and Tissue DNA Kit in conjunction with the KingFisher Duo, two equivalent magnetic bead purification systems, and the spin column kit was performed using 1 x 10^6 HeLa-S3 cells. The results indicate that the KingFisher purification system ensures a higher DNA yield and a better A_{260}/A_{280} ratio than the competing magnetic bead systems and equivalent yield and ratio as the spin column system (Figure 5).

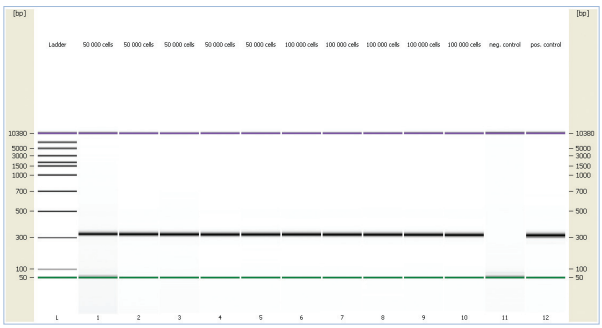

Figure 4. PCR from DNA purified from 50,000 (samples 1-5) and 100,000 (samples 6-10) HeLa-S3 cells. Negative control (sample 11) and positive control (sample 12).

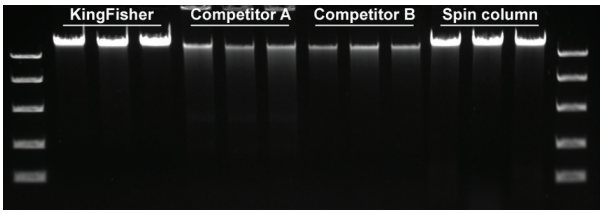

Figure 5. DNA was purified from 1 x 10^6 HeLa-S3 cells with four different competing methods, i.e. the KingFisher Cell and Tissue DNA Kit in conjunction with KingFisher Duo, two competitive magnetic bead systems, and the spin column kit. The KingFisher Cell and Tissue DNA Kit performed very well.

Conclusions

The KingFisher Cell and Tissue DNA Kit provides efficient and fast DNA purification from a wide variety of sample materials, performing very well in comparison to other magnetic bead systems and a spin column kit. Different sample materials require varying lysing times, but the duration of the process can also be optimized, depending on the available time for the purification and the requirements for the DNA yield. Purified DNA is suitable for direct use in different downstream applications, such as PCR and restriction analysis.

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