Technical Note:
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High-throughput purification of DNA with the Thermo Scientific KingFisher Cell and Tissue DNA Kit

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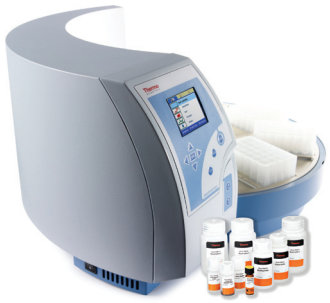

- *Efficient purification of genomic DNA*
- *Purification from different sample materials*
- *Flexible DNA isolation from 1-96 samples*
- *Excellent yield and quality of purified DNA*

Introduction

The Thermo Scientific KingFisher Cell and Tissue DNA Kit is developed for the purification of DNA from tissues and cultured cells or bacteria with KingFisher® magnetic particle processors. The maximum sample volume for the purification is 1 x 10^7 cultured cells, 20 mg of tissue or 1 ml of bacterial culture. Also FFPE samples can be used after the samples have been deparaffinized. The purified DNA is of high quality and free of protein, nucleases and other contaminants or inhibitors. This technical note describes DNA purification from different sample materials; from HeLa-S3 cells, several mouse internal organ samples, and mouse ear and tail samples – typical for genotyping.

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.

Material and Methods

The DNA purification process was performed with the KingFisher Cell and Tissue DNA Kit (Cat. No. 97030196) and the KingFisher Flex. The purification was conducted according to the instruction manual. DNA was purified from frozen HeLa-S3 cells and from frozen mouse liver, kidney, spleen, ear and tail samples. Different processing times for lysing of the tissues were tested. DNA was eluted in 150 µl of the Elution Buffer, but the volume can be adjusted. Optimized Thermo Scientific BindIt Software 3.1 protocols for KingFisher Kits with the Thermo Scientific KingFisher Flex and KingFisher mL are available online, see www.thermoscientific.com/kingfisher.

TABLE 1.
Examples of purified DNA yields from different cell or tissue samples.

Sample	Sample input	Typical yield
HeLa-S3 cells	1 x 10^6	6–12 µg
Mouse tissue samples		
Ear punch	One punch, ~0.2 cm diameter	10–17 µg
Tail sample	0.1–0.2 cm	5–10 µg
Liver	15 mg	20–30 µg
Kidney	15 mg	20–35 µg
Spleen	20 mg	70–130 µg

Lysing the samples

Efficient lysis is important in order to gain a good yield of high quality DNA. The requirements of the lysis time are usually different due to the various structures of the cells and tissues. Also, in case of cell lysis, the step should be done thoroughly, although the actual time the step takes is short. After the addition of the Lysis Buffer to the HeLa-S3 cells, the samples were efficiently mixed by pipetting up and down and vortexed for 30 seconds until the viscosity of the samples was lost, and incubated at 70°C for 15 minutes. The mouse tissue samples were lysed in the Lysis Buffer at 56°C from 15 minutes up to overnight, and then centrifuged shortly to clear the lysate from cell debris. The samples lysed in the KingFisher Flex continued directly to the purification process without a centrifugation step. Different time points for the lysis were 15 minutes (performed in the instrument), 1 h, 4.5 h and overnight (~20 h). The approximate time for the purification process was 25 minutes in the KingFisher Flex after lysis.

Results

The DNA purification process begins with lysing or homogenization of the samples. The shortest time used for lysis with the KingFisher Cell and Tissue DNA Kit in the experiments discussed in this note was 15 minutes. It was a sufficient time to lyse the HeLa-S3 cells outside the instrument. In addition, lysis of ear and kidney samples in the KingFisher Flex for 15 minutes was efficient and an

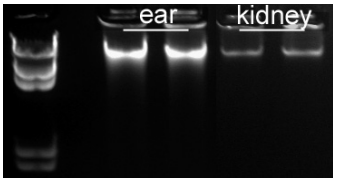

Figure 1. Purification of DNA from ear punches and 10 mg of kidney samples after lysing the samples for 15 minutes in the KingFisher Flex.

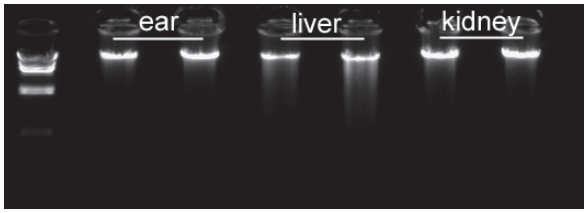

Figure 2. Purification was performed simultaneously from mouse ear punches and from 10 mg of liver or kidney samples after lysing the samples for 4.5 h.

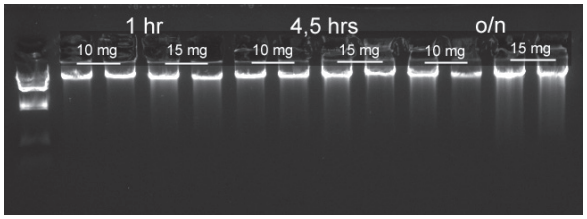

Figure 3. 10 mg or 15 mg samples of mouse kidney were lysed for 1 h, 4.5 h or overnight (o/n), followed by the purification of DNA. The DNA yields depended on the lysis time and the amount of the tissue, but from all of the samples the purified DNA showed an excellent yield and ratio.

adequate yield was gained for PCR reactions (Figure 1). However, beginning the purification process directly without clearing the lysate by centrifuging the samples might slightly reduce the expected DNA yield.

The cleared lysates of ready-lysed samples were transferred to the KingFisher Flex together with the Binding Buffer and KingFisher Magnetic Beads. During this step, DNA binds to the beads in the presence of a chaotropic salt. The following wash steps 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 and used in downstream applications. The purification process can be performed at room temperature, excluding the lysis step. Additionally, heating of the elution step increases the DNA yield.

The KingFisher Cell and Tissue DNA Kit is suitable for various sample materials. Table 1 shows examples of the DNA yields that can be obtained with the kit from different cells and tissues. DNA purified from mouse ear samples, typical samples in genotyping of mice, or liver and kidney samples are shown in the Figure 2, indicating a good yield of DNA.

In Figure 3, 10 mg and 15 mg of mouse kidney samples were lysed between an hour and overnight before DNA purification. Even after 1 hour of lysis, the DNA yield was excellent for downstream applications.

The effects of the amount of sample material were compared with the purification of DNA from a different quantity of HeLa-S3 cells. The results indicate the correspondence of purified DNA yield with the number of cells in the sample, as expected (Table 2). Even with the lowest amount of tested cells, approximately 15,000 cells, the purification process was efficient and the PCR was successful. Figure 4 shows the PCR performed from DNA isolates from 50,000 and 100,000 HeLa-S3 cells.

Conclusions

- **The KingFisher Cell and Tissue DNA Kit offers efficient and fast DNA purification with a wide variety of sample materials.**
- **Different sample materials require varied 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. Additionally, with some sample materials, the hands-on time can be shortened if the lysis is performed in the KingFisher instrument**
- **Purified DNA is suitable for direct use in different downstream applications, such as PCR and restriction analysis.**

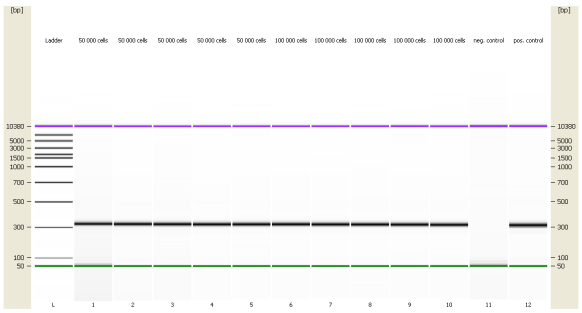

Figure 4. PCR from DNA isolates purified from 50,000 and 100,000 HeLa-S3 cells.

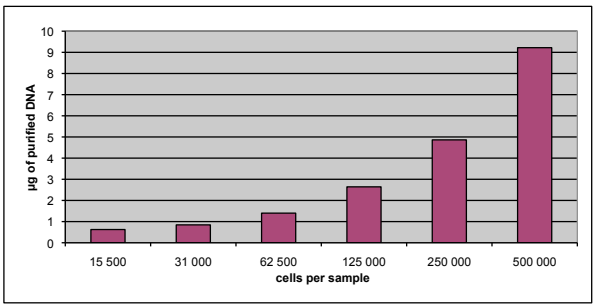

Table 2. Dilution series from HeLa-S3 cells indicate that the purified DNA yield correlated with the quantity of cells used in the samples.

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