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Extracting DNA from all forensic samples with KingFisher® technologies

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Abstract. Many of the forensic DNA extraction methods appeared to have limitations, such as inability to remove inhibitors of PCR reaction, or appeared to be time-consuming by needing too many steps and multiple tube transfers (which increase the risk of contaminations by exogenous DNA). We report here that by using technology based on paramagnetic silica particles which bind DNA in presence of chaotrophic salts, we are able to standardize and validate less kinds of DNA extraction methods for forensic samples. These new extraction methods allow us to reduce significantly number of steps, reagents, additional procedures, and time. Moreover, it allows us to avoid the classical and quite dangerous organic extraction method for most of forensic samples. Finally, these methods seem to give better quality of DNA in a shorter time.

Keywords: Forensic; Human DNA; STR

1. Introduction

In the field of forensic science, a wide range of biological samples are encountered. This leads to the use of as many different DNA extraction protocols. By now, several DNA extraction methods have been developed by the forensic community, but many of them appeared to have limitations such as inability to remove inhibitors of PCR reaction [1], or to be time-consuming by needing too many steps and multiple tube transfers which increase the risk of contaminations by exogenous DNA [1]. By using KingFisher® technologies, we were able to standardize and validate DNA extraction methods for almost all forensic samples. Thus, samples are treated with just one protocol [2] which allow us to (1) avoid the quite dangerous organic extraction method (e.g., phenol/chloroform method), (2) reduce significantly number of steps, reagents, or additional procedures (e.g., Centricon®), (3) reduce cost of experiments, and (4) save time.

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2. Methodology

Several forensic-like samples (home-made samples hairs, oral cotton swabs, gloves, cigarette butts, nails, etc.) were tested to improve the new extraction method using buffers from the Genomic DNA Purification Kit associated to the KingFisher® mL Automate [2]. Incubation steps were simplified to obtain only four kinds of incubation steps for all forensic samples (no incubation, 2 and 5 h or overnight [12 h] incubation time at 56 °C). Results show that quality and quantity of DNA were good enough to perform multiplex PCR with conventional kits (e.g., AmpFLSTR® SGM Plus® PCR Amplification Kit, Applied Biosystems). Thus, we were able to obtain only 6 different extraction protocols instead of the 10 which were routinely used in our lab. Encouraged by the results, we enlarged samples types to all kind of real forensic samples. Only fragments of clothes remain treated by classical techniques because of the size of those fragments incompatible with an extraction on the KingFisher® mL Automate [3].

Table 1
Summary of protocols used with and without KingFisher technologies

Samples		Steps					
		S	L	E 1	P/C 1	E 2	P/C 2
Blood		GFX Kit (Amersham)					
Nails, cotton swab			Buffer A	PCI	Centricon 30	Chelex 20%	μcon 100
Oral cotton swab/histobrush			Chelex 10%				
Cigarette butt			Chelex 20% Over Night				μcon 100
Blood stain			Buffer A	PCI	Centricon 30	Chelex 20%	μcon 100
Hair			Buffer D	PCI	(a) or (b)		
Human tissue[1] and clothes			Buffer A	PCI			
Sperm stain	UP	Buffer B		PCI		(c) or (d)	
	LP		Buffer C				
Blood, fresh human tissue[1] Nails, cotton swab, oral cotton swab/histobrush, cigarette butt, blood stain		KF ml					μcon 100*

1 = Muscle, fresh flesh; Buffer A = NaCl, Tris–HCl, EDTA, DTT, PK; Buffer B = PBS 1X, SDS, PK; Buffer C = PBS 1X, Sarcosyl, EDTA, DTT, PK; Buffer D = NaCl, Tris–HCl, EDTA, SDS, Urea, PK; KF ml = Protocol A (see user's manual); PCI = organic extraction method. *optional step. UP = upper phase, LP = lower phase. The different steps are defined as follows: W = washing, S = split L = lysis, E = extraction and P/C = purification/concentration. (a) = without KingFisher technologies (KF): Cleamix (TALENT); (b) with KF: KF ml; (c) = without KF: PC 1, E 2 and P/C 2 are Centricon 30, Chelex 20% and μcon 100, respectively; (d) with KF: PC 1, E 2 and P/C 2 are KF ml and an optional step with μcon 100.

3. Results

3.1. Standardization of extraction protocols

Table 1 shows how samples are treated with or without KingFisher® Technologies. It clearly appears that by using this new technologies, many protocols are simplified in number of steps, reagents or additional procedures. Moreover, for most of the forensic samples, it lets us avoid the use of quite dangerous organic extraction method.

3.2. Principle of automated magnetic particle processing

This method is based on paramagnetic silica particles which bind DNA in the presence of chaotrophic salts. Forensic samples are first incubated at 56 °C (2–12 h) in a lysis buffer with the addition (or not) of Protease K and/or DTT. Some samples—as fresh blood, muscle or oral histobrushes—do not need incubation step. DNA extracts are then purified on the KingFisher® mL Automate, according to the manufacturer's procedure [3].

3.3. Quantity and quality of extracted DNA

Total amount of extracted DNA is measured with AluQuant® Kit [4]. Results show that extractions with KingFisher® methods give DNA for all types of samples tried. Moreover, DNA amounts are less spread in wide ranges as classical extraction methods. Such kind of less spread ranges are also obtained for peak amplitudes (data not shown). Thus, whatever the type of samples analyzed, it appears that extractions with KingFisher® methods seem to give better DNA quality.

4. Discussion

Because forensic studies are taking a bigger place in courtyard investigations, it is crucial for laboratories to use modern technologies. Since its creation 15 years ago, CODGENE develops or adapts new techniques and technologies for the purpose of reducing time and cost of experiments with the same or better DNA quality. With the help of KingFisher® technologies [2], CODGENE was able to reduce extraction time by three! Thus, most samples give us results in less than 72 h. Actually, we are working to reduce the number of extraction methods to only three different protocols: one for hard human tissues (bones/teeth), a second one for sperm stains, and a third one for all other forensic samples. We also work to avoid all phenol/chloroform treatment and reduce steps in the way to have less tube transfers as possible.

References

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[3] Instructions for use of the Genomic DNA Purification Kit, ThermoLabsystems.
[4] AluQuant®, Human DNA Quantitation System on Centro LB 960 from Berthold.