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Short Communication

A comparison of four methods for PCR inhibitor removal

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ABSTRACT

Biological samples collected from the crime scenes often contain some compounds that can inhibit the polymerase chain reaction (PCR). The removal of PCR inhibitors from the extracts prior to the PCR amplification is vital for successful forensic DNA typing. This paper aimed to evaluate the ability of four different methods (PowerClean® DNA Clean-Up kit, DNA IQ™ System, Phenol–Chloroform extraction and Chelex®-100 methods) to remove eight commonly encountered PCR inhibitors including: melanin, humic acid, collagen, bile salt, hematin, calcium ions, indigo and urea. Each of these PCR inhibitors was effectively removed by the PowerClean® DNA Clean-Up kit and DNA IQ™ System as demonstrated by generating more complete short tandem repeat (STR) profiles from the cleaned up inhibitor samples than from the raw inhibitor samples. The Phenol–Chloroform extraction and Chelex®-100 methods, however, could only remove some of eight PCR inhibitors. Our results demonstrated that the PowerClean® DNA Clean-Up kit and DNA IQ™ System were very effective for the removal of known PCR inhibitors that are routinely found in DNA extracts from forensic samples.

1. Introduction

Currently, in the field of forensic science, the polymerase chain reaction (PCR) technology is widely used in DNA analysis of forensic cases. However, biological samples collected from crime scenes often contain various PCR inhibitors that can interfere with the PCR amplification, and this makes forensic DNA testing face huge challenges. Therefore, the removal of PCR inhibitors from the extracts prior to the PCR amplification is vital for successful forensic DNA typing. To cope with the PCR inhibitors, a simple approach is to dilute the sample extracts containing PCR inhibitors, which may dilute out the PCR inhibitor in the hope that there is enough remaining DNA in the sample to still develop a full STR profile. Another strategy is to remove them at the stage of DNA extraction using silica, Chelex and Phenol–Chloroform based protocols [1–6] or before amplification by the means of DNA purification kits [7,8]. However, the relative efficiency and effectiveness of these processing methods have not been fully explored.

In this study, four different methods for PCR inhibitor removal, the PowerClean® DNA Clean-Up kit, DNA IQ™ System, Phenol–Chloroform extraction and Chelex®-100 methods, were evaluated with mock DNA extracts prepared by mixing the K562 DNA with varying concentrations of known PCR inhibitors: melanin, humic acid, collagen, bile salt, hematin, calcium ions, indigo and urea [9–16]. The ability of each method to remove these PCR inhibitors was evaluated by comparing the short tandem repeat (STR) results from the cleaned up inhibitor samples to the STR results from the raw inhibitor samples.

2. Materials and methods

2.1. Experimental materials

The following materials were used in this study: K562 DNA with the concentration of 766 μg/mL (Promega, DD2011), melanin (Sigma, #M8631), humic acid (Sigma, #53680), collagen solution (Sigma, #C8919), bile salt (Sigma, #B3426), hematin (Sigma, #H3281), calcium chloride (Sigma, #21059), indigo (Sigma, #229296), urea (Sigma, #U1250), PowerClean® DNA Clean-Up kit (MoBio, #12877), DNA IQ™ System (Promega, DC6700), Phenol–Chloroform solution (Sangon Biotech, RBR02620), Chelex®-100 (Bio-Rad, #143-2832), AmpFℓSTR® Identifiler® PCR Amplification kit (Applied Biosystems, #4322288) and AmpliTaq Gold® DNA Polymerase with the concentration of 5 U/μL (Applied Biosystems, #4398823).

2.2. Inhibitor preparation

Stock inhibitor solutions were prepared as follows: melanin (1 μg/μL), 10 mg in 100 μL 1 N sodium hydroxide with subsequent dilution in 10 mL sterile water; humic acid (1.5 μg/μL), 15 mg in

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11.25 μL 1 N sodium hydroxide with subsequent dilution in 10 mL sterile water; collagen solution (1 μg/μL), 0.1% solution in 0.1 M acetic acid; bile salt (35 μg/μL), 350 mg in 10 mL sterile water; hematin (1.99 μg/μL), 19.9 mg in 450 μL 1 N sodium hydroxide with subsequent dilution in 10 mL sterile water; calcium chloride solution (11.098 μg/μL), dilution in sterile water; indigo (78.66 μg/μL), 786.6 mg in 900 μL 1 N sodium hydroxide with subsequent dilution in 10 mL sterile water; urea (35 μg/μL), 350 mg in 10 mL sterile water.

2.3. Inhibitor concentration

A series of different concentrations of eight PCR inhibitor solutions were added to the K562 DNA and ensured the DNA with the concentration of 0.2 ng/μL. For the K562 DNA, total of 26 different alleles can be detected in 15 STR loci and a sex-typing marker amelogenin by using the AmpFℓSTR® Identifiler® PCR Amplification kit. For each of eight PCR inhibitors, the inhibitor concentration with which all of 26 alleles of the K562 DNA were just inhibited completely and no allele could be detected was identified as the working concentration. With this criterion, the working concentrations of eight PCR inhibitors were determined as follows: melanin, 55 ng/μL; humic acid, 85 ng/μL; collagen, 220 ng/μL; bile salts, 5 μg/μL; hematin, 133 ng/μL; calcium chloride, 1.998 μg/μL; indigo, 15.208 μg/μL; urea, 15.3 μg/μL.

In addition, in order to further evaluate the ability of four methods to remove these PCR inhibitors, 2× and 4× working concentration of each inhibitor was also tested.

2.4. Inhibitor removal

2.4.1. PowerClean® DNA Clean-Up kit

150 μL of the K562 DNA (0.2 ng/μL) mixed with eight PCR inhibitors respectively was cleaned-up according to the manufacturer's instructions (http://www.mobio.com/images/custom/file/protocol/12877-50.pdf), and several steps were slightly modified: in step 5 and step 8, the incubation time was extended to 10 min, and in step 17, 50 μL of PowerClean® DNA Solution 7 was used instead of 100 μL for DNA elution.

2.4.2. DNA IQ™ System

150 μL of the K562 DNA (0.2 ng/μL) mixed with eight PCR inhibitors respectively was processed according to the Promega protocol [17] except that 150 μL of lysis buffer was used. In addition, in the step of DNA elution, 50 μL of elution buffer was used.

2.4.3. Phenol–Chloroform extraction

450 μL of the K562 DNA (0.2 ng/μL) mixed with eight PCR inhibitors respectively was purified using the Phenol–Chloroform protocol [18]. Finally, the DNA pellet was re-dissolved in 50 μL of TE buffer.

2.4.4. Chelex®-100 method

150 μL of the K562 DNA (0.2 ng/μL) mixed with eight PCR inhibitors respectively was processed using 150 μL of 5% Chelex®-100 following Walsh et al. [19].

Table 1
The number of Identifiler alleles detected from the K562 DNA mixed with different concentrations of PCR inhibitors after processed using four methods. Samples were run in triplicate (n1, n2 and n3).

	1× Working concentration			2× Working concentration			4× Working concentration		
	n1	n2	n3	n1	n2	n3	n1	n2	n3
PowerClean® DNA Clean-Up kit									
Melanin	26	26	26	26	26	26	26	26	26
Humic acid	26	26	26	26	26	26	26	26	26
Collagen	26	26	26	26	26	26	26	26	26
Bile salt	26	26	26	26	26	26	26	26	26
Hematin	26	26	26	26	26	26	26	26	26
Calcium chloride	26	26	26	26	26	26	25	24	24
Indigo	25	25	25	16	12	14	0	3	1
Urea	26	26	26	26	26	26	26	26	26
DNA IQ™ System									
Melanin	26	26	26	26	26	26	26	26	26
Humic acid	26	26	26	26	26	26	26	26	26
Collagen	26	26	26	26	26	26	26	26	26
Bile salt	26	26	26	26	26	26	26	26	26
Hematin	26	26	26	26	26	26	26	26	26
Calcium chloride	26	26	26	26	26	26	26	26	26
Indigo	24	26	25	10	8	9	3	2	0
Urea	26	26	26	26	26	26	26	26	26
Phenol–Chloroform method									
Melanin	0	0	0	0	0	0	0	0	0
Humic acid	0	0	0	0	0	0	0	0	0
Collagen	0	0	0	0	0	0	0	0	0
Bile salt	26	26	26	26	26	26	26	26	26
Hematin	26	26	26	26	26	26	26	26	26
Calcium chloride	2	2	1	0	0	0	0	0	0
Indigo	25	26	26	20	20	22	0	0	0
Urea	26	26	26	26	26	26	26	26	26
Chelex®-100 method									
Melanin	0	0	0	0	0	0	0	0	0
Humic acid	0	0	0	0	0	0	0	0	0
Collagen	26	26	26	26	26	26	26	26	26
Bile salt	26	26	26	0	0	0	0	0	0
Hematin	0	0	0	0	0	0	0	0	0
Calcium chloride	22	25	24	0	1	0	0	0	0
Indigo	25	26	26	6	7	6	0	0	0
Urea	0	0	0	0	0	0	0	0	0