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Short communication

Powerplex® ES versus Powerplex® S5—Casework testing of the new screening kit

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ABSTRACT

The new Powerplex® S5 Mini STR-System from Promega with the four provided STR loci D18S51, D8S1179, TH01 and FGA as well as the Amelogenin marker (PCR products ranging from 80 to 220 bp not considering the longer FGA fragments) is designed as a screening tool especially in difficult casework samples. To test its suitability we amplified highly degraded DNA from casework samples, which had shown no or only poor results in analyses with the Powerplex® ES kit, as well as artificially degraded DNA or DNA samples containing PCR inhibitors. Despite a tendency for allelic drop-ins in the amplification of highly degraded DNA the Powerplex® S5 kit was a reliable tool for the analysis of casework samples with degraded DNA which gave better results than the Powerplex® ES kit in 64% of analysed swabs. Furthermore, it was especially suitable for the investigation of formalin fixed tissue, tissue samples showing advanced putrefaction or telogen hair samples. However, there was no strict relation between positive Powerplex® S5 results and amplification success with the Powerplex® ES kit.

1. Introduction

Multiplex PCR systems based on short tandem repeats (STRs) are an accepted technology in forensic applications with PCR products ranging from 100 to 400 bp length resulting in full DNA profiles for a majority of high quality DNA samples from forensic casework. However, there are many problematic samples containing degraded DNA or minute amounts of good quality DNA, and in these cases DNA typing often gives only partial profiles or no results at all [1,2]. The low genetic information content in these difficult samples leads to a reduced discrimination power and possibly to random false-positive matches in DNA databases. Therefore, a variety of working groups have focussed on shorter amplicon lengths and developed new multiplex PCRs (for example [3–8]). Three of those "mini" multiplex approaches are commercially available: the BioPlex-11 [9], the AmpFlSTR® MiniFiler™ [10] and the Powerplex® S5. The last one is – according to the manufacturer – designed as a screening kit in forensic casework for problematic samples with degraded DNA or PCR inhibitors. Here, the question was whether the Powerplex® S5 kit can function as a pre-test to foresee the quality of the Powerplex® ES typing. Therefore, we tested the Powerplex® S5 kit in comparison to the Powerplex® ES kit which is routinely used in our laboratories for stain analysis regarding low DNA concentration, degraded DNA and DNA containing PCR inhibitors.

2. Methods

2.1. Stains and controls

The commercial standard 9948 male DNA and 9947A female DNAs were purchased from Promega (Mannheim, Germany). Both were serially diluted to a concentration of 1 ng–15 pg/μl for the sensitivity study. Four replicates were tested for each concentration of DNA and genotype results at each dilution were compared to the published genotype [11]. Genotyping failure was declared when no peaks were observed above the interpretational threshold of 50 relative fluorescent units (RFUs).

For further tests we used artificially prepared samples (from formalin fixed tissue (muscle tissue pieces of 1 cm^3), paraffin embedded tissue, putrefied tissue samples, hair, hand prints) and corresponding buccal swabs as well as 76 samples from routine casework investigations (swabs from a variety of surfaces) (examples in Table 1). Each sample was investigated at least twice.

2.2. DNA extraction

DNA extraction from buccal swabs was done using the Qiagen Blood Mini Kit (Qiagen, Hilden, Germany). DNA extraction from artificially prepared stains as well as casework samples was performed with a phenol/chloroform method as published by

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Table 1
Examples of amplification results of casework samples using the Powerplex® ES and Powerplex® S5 kits.

	PPES results[a]	PPS5 Amelo	PPS5 D18S51	PPS5 D8S1179	PPS5 TH01	PPS5 FGA	PPS5 results	PPS5 gain
Epithelial cells								
Hand print 1	4	a	c	a	c	a	3	2
Hand print 2	0	a	a	d	a	a	4	4
Hand print 3	3	a	a	a	c	a	4	3
Hand print 4	0	a	b,c	d	b,c	a	2	2
Hand print 5	1	a	a	d	c	a	4	3
Swab mug	1	a	a	a	a	a	5	5
Swab bottle 1	0	d	d	d	d	d	0	0
Swab bottle 2	0	a	a	a	a	a	5	5
Swab bottle 3	1	a	c	d	a	b	2	2
Swab bottle 4	3	a	a	a	a	a	5	3
Swab bottle 5	2	a	a	c	a	d	3	3
Swab bottle 6	0	a	d	d	d	d	1	1
Swab bottle 7	0	a	b,c	d	b,c	d	1	1
Swab bottle 8	0	d	d	d	d	d	0	0
Swab car door	0	a	d	d	a	a	3	3
Swab steering wheel	0	a	a	a	a	a	5	5
Swab gear lever	0	a	a	c	a	a	4	4
Swab screwdriver 1	1	a	a	c	a	b,c	3	3
Swab screwdriver 2	2	a	a	a	a	a	5	5
Handle of a hammer (swab)	6	a	a	a	a	a	5	2
towel	0	a	b	d	c	b	1	1
Putrefied tissue								
Aorta 1	2	a	a	d	a	a	4	3
Aorta 2	0	a	c	c	c	d	1	1
Liver 1	1	a	a	a	a	a	5	4
Liver 2	6	a	a	a	a	a	5	1
Kidney	5	a	a	a	a	a	5	2
Heart	0	a	a	a	a	a	5	5
Lung	0	a	d	d	d	d	1	1
Spleen	5	a	a	a	a	a	5	2
Bones								
Jaw-bone	3	a	a	a	a	a	5	4
Paraffin embedded tissue								
9 years[b]	7	a	a	a	a	a	5	2
11 years 1	2	a	a	a	a	a	5	3
11 years 2	0	a	a	a	a	a	5	5
11 years 3	0	a	a	d	a	a	4	4

a, expected alleles; b, drop-out; c, drop-in; d, no results.
[a] Number of STR loci with reproducible results.
[b] Years in paraffin before DNA extraction.

DeSalle and Bonwich [12] with slight modifications or with the Invisorb Spin Tissue Mini kit (Invitek, Berlin, Germany) for putrefied tissue samples.

2.3. DNA quantification of nuclear DNA using real time PCR

For DNA quantification a TaqMan® *MGB* fluorescent *real time PCR* assay was chosen amplifying a 98 bp fragment of the nuclear telomerase gene (forward primer: 3′-ggc aca cgt ggc ttt tcg-5′ and reverse primer: 3′-ggt gaa cct cgt aag ttt atg caa-5′, DNA Probe: NED-acg-tcg-agt-gga-cac-g-MGB). PCR was performed using a standard buffer and an Immolase polymerase (both Bioline, Luckenwalde, Germany) at a concentration of 1 U/25 μl reaction mix. The concentrations of the primers, magnesium chloride, and dNTPs were 0.08 mM, 1.5 mM, and 0.2 mM per dNTP, respectively. PCR was carried out on an ABI 7300 Real Time PCR System (Applied Biosystems) according to the following program: 5 min 95 °C as initial step for hot start activation, 60 s 94 °C denaturation, 1 min 60 °C annealing, 1 min 72 °C extension for 40 cycles. Quantification of nuclear DNA was done using the associated 7300 system software and comparison of DNA fluorescence from the unknown samples to standards containing known amounts of DNA (Promega) from 100 ng down to 10 pg. Every amplification was done in triplicates to ensure reproducibility and reliability.

2.4. Models for PCR inhibition and DNA degradation

An inhibition study was performed by adding diluted humic acid (Sigma–Aldrich) to the PCR to obtain different final concentrations in the PCR (2.5, 5, 10, 30 and 50 ng/μl).

Degraded DNA was produced as follows: a total of 800 pg DNA was digested at 37 °C with 0.4 U DNase I (Promega) in a final volume of 80 μl. At predefined time points (0, 0.5, 1, 2, 5, 8 and 10 min) 10 μl were removed and added to 1 μl stop solution (provided with DNase I). Enzyme inactivation was performed for 10 min at 65 °C according to the manufacturer's instructions. The grade of degradation was verified by a polyacrylamide electrophoresis with silver staining.

2.5. Mixture study

Mixture studies of two DNA samples with no overlapping alleles in the four STRs of the Powerplex® S5 kit were performed with a total of 1 ng DNA template. The PCRs contained 500 pg (1:1), 125 pg (1:7), 91 pg (1:10), and 50 pg (1:19) of the minor component.

2.6. Amplification and electrophoresis

The amplification protocols for Powerplex® S5 kit and Powerplex® ES kit followed the manufacturer's instructions with a