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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. © 2009 Elsevier Ireland Ltd. All rights reserved.

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 AmpF/STR[®] MiniFilerTM [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/ μ l 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 cm³), 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.

Epithelial cells </th <th></th> <th>PPES results^a</th> <th>PPS5 Amelo</th> <th>PPS5 D18S51</th> <th>PPS5 D8S1179</th> <th>PPS5 TH01</th> <th>PPS5 FGA</th> <th>PPS5 results</th> <th>PPS5 gain</th>		PPES results ^a	PPS5 Amelo	PPS5 D18S51	PPS5 D8S1179	PPS5 TH01	PPS5 FGA	PPS5 results	PPS5 gain
\dot{h} and print 14acacaa32Hand print 20aaaaaaaa44Hand print 33aab,cdb,ca22Hand print 40ab,cdb,ca22Hand print 51aaaaaa33Swab bott 10ddddd000Swab bott 20aaaaaaaa33Swab bott 61aa<	Epithelial cells								
Hand print 20aa <th< td=""><td>Hand print 1</td><td>4</td><td>a</td><td>с</td><td>a</td><td>с</td><td>a</td><td>3</td><td>2</td></th<>	Hand print 1	4	a	с	a	с	a	3	2
Hand print 33aaaaacaa43Hand print 40abca43Hand print 51aadca43Swab botte1aaaaaa55Swab botte 10aaaaaaa55Swab botte 20aaa <td< td=""><td>Hand print 2</td><td>0</td><td>a</td><td>a</td><td>d</td><td>a</td><td>а</td><td>4</td><td>4</td></td<>	Hand print 2	0	a	a	d	a	а	4	4
Hand print 40abcdbca22Hand print 51aaadca43Swab nug1aaaaaa55Swab botte 10ddddd000Swab botte 20aaaaaa55Swab botte 31acdaaa33Swab botte 43aaaaaaa33Swab botte 52aaaddd111Swab botte 60ab,cdddd111Swab botte 70ab,cdddd000Swab botte 80ddddddddd11Swab botte 80aa<	Hand print 3	3	a	a	a	с	a	4	3
Hand print 51aa <th< td=""><td>Hand print 4</td><td>0</td><td>a</td><td>b,c</td><td>d</td><td>b,c</td><td>a</td><td>2</td><td>2</td></th<>	Hand print 4	0	a	b,c	d	b,c	a	2	2
Swab bottle 11aaaaaaaab5Swab bottle 20aaaaaaab22Swab bottle 31acdab22Swab bottle 43aaaaab22Swab bottle 52aaaadd33Swab bottle 60aaaaddd11Swab bottle 70ab,cddddd00Swab bottle 80dddddddda33Swab bottle 70ab,cddd	Hand print 5	1	a	a	d	с	a	4	3
Swab bottle 10ddddddd00Swab bottle 20aaaaaa55Swab bottle 31acdaa53Swab bottle 52aaaaaa33Swab bottle 50adddd11Swab bottle 60abcddd11Swab bottle 80ddddd000Swab bottle 80dddddd000Swab bottle 80ddddddd000Swab screwdriver 11aaaaaaa333Swab screwdriver 11aaaaaaa333Swab screwdriver 11aaaaaaa333Swab screwdriver 11aaaaaaa333Swab screwdriver 11aaaaaaa333Swab screwdriver 11aaaaaaaaaaaaaaaaa </td <td>Swab mug</td> <td>1</td> <td>a</td> <td>a</td> <td>a</td> <td>a</td> <td>a</td> <td>5</td> <td>5</td>	Swab mug	1	a	a	a	a	a	5	5
Swab bottle 20aaaaaaab22Swab bottle 31aaaaaab22Swab bottle 43aaaaaaaa33Swab bottle 52aaacadd33Swab bottle 60ab,cddd11Swab bottle 70ab,cddd00Swab bottle 80dddddd33Swab bottle 70aaaaaaa33Swab stret door0aa <td>Swab bottle 1</td> <td>0</td> <td>d</td> <td>d</td> <td>d</td> <td>d</td> <td>d</td> <td>0</td> <td>0</td>	Swab bottle 1	0	d	d	d	d	d	0	0
Swab bottle 31acdaab22Swab bottle 43aa<	Swab bottle 2	0	a	a	a	a	a	5	5
Swab bottle 43aa <t< td=""><td>Swab bottle 3</td><td>1</td><td>a</td><td>с</td><td>d</td><td>a</td><td>b</td><td>2</td><td>2</td></t<>	Swab bottle 3	1	a	с	d	a	b	2	2
Swab bottle 52aaacadd33Swab bottle 60addddd11Swab bottle 70abcddd000Swab bottle 80dddddd000Swab bottle 80aadddaa333Swab stereing wheel0aaaaaa3333Swab stereing wheel0aaaaaaa444Swab sterewdriver 11aaacaaa522Swab sterewdriver 22aaaaaaa52211aaa <td>Swab bottle 4</td> <td>3</td> <td>a</td> <td>a</td> <td>a</td> <td>a</td> <td>a</td> <td>5</td> <td>3</td>	Swab bottle 4	3	a	a	a	a	a	5	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 stering wheel 0 a a a a a 3 3 Swab stering wheel 0 a a a a a a 3 3 Swab screwdriver 1 1 a a a a a a 3 3 Swab screwdriver 2 2 a a a a a a 3 3 Handle of a hammer (swab) 6 a	Swab bottle 5	2	a	a	с	a	d	3	3
Swab bottle 70ab,cdb,cd11Swab bottle 80dddddd000Swab cat door0aaaaaa3333Swab steering wheel0aaaaaa444Swab steering wheel0aaacaa444Swab screwdriver 11aacaa3333Swab screwdriver 22aaaaaaa553Handle of a hammer (swab)6aaaaaaa35211Putrefied tissueaaaaaa333 <td< td=""><td>Swab bottle 6</td><td>0</td><td>a</td><td>d</td><td>d</td><td>d</td><td>d</td><td>1</td><td>1</td></td<>	Swab bottle 6	0	a	d	d	d	d	1	1
Swab bottle 80ddddddd00Swab at door0aaddaaa33Swab at cering wheel0aaacaa44Swab sterewdriver 11aacab,c33Swab sterewdriver 22aaaaaa233Swab sterewdriver 20aaaaaa52towel0abdcb11Putrefied tissueaaaa33Swab at	Swab bottle 7	0	a	b,c	d	b,c	d	1	1
Swab car door0aadaaaaa33Swab stering wheel0aaaaaaa55Swab gear lever0aaacaa44Swab screwdriver 11aacab,c33Swab screwdriver 22aaaaaa55Handle of a hammer (swab)6aaaaaaa52Vetrefied tissue0abdcb111Arta 12aaaaaaa43Liver 11aaaaaaa51Liver 26aaaaaaa51Kidhey5aaaaaaa52Lung0addddd111Spleen5aaaaaaa52Bones	Swab bottle 8	0	d	d	d	d	d	0	0
Swab steering wheel0aa<	Swab car door	0	a	d	d	a	a	3	3
Swab gear lever0aaacaaa44Swab screwdriver 11aaacab,c33Swab screwdriver 22aaaaaa55Handle of ahammer (swab)6aaaaaa52Putrefied tissue0abdcb11Putrefied tissue0accd11Aorta 20accd11Liver 11aaaaaa54Liver 26aaaaaa52Heart0aaaaaa52Lung0adddd11Spleen5aaaaa52BonesJav-bone3aaaaaa54Paraffin embedded tissueJav-bone7aaaaaaaa5211 years 12aaaaaaaa523	Swab steering wheel	0	a	a	a	a	a	5	5
Swab screwdriver 1 1 a a c a b,c 3 3 Swab screwdriver 2 2 a	Swab gear lever	0	a	a	с	a	a	4	4
Swab screwdriver 22aaaaaaaaabbaaa </td <td>Swab screwdriver 1</td> <td>1</td> <td>a</td> <td>a</td> <td>с</td> <td>a</td> <td>b,c</td> <td>3</td> <td>3</td>	Swab screwdriver 1	1	a	a	с	a	b,c	3	3
Handle of a hammer (swab)6aaaaaaaaabaaaabaa	Swab screwdriver 2	2	a	a	a	a	a	5	5
towel0abdcb11Putrefied tissueAorta 12aadaa43Aorta 20acccd11Liver 11aaaaaa54Liver 26aaaaaa51Kidney5aaaaa55Lung0addd11Spleen5aaaaa55BonesJaw-bone3aaaaaa54Paraffin embedded tissue9 years ^b 7aaaaaaa5211 years 12aaaaaaaa53	Handle of a hammer (swab)	6	a	a	a	a	a	5	2
Putrefied tissue Aorta 1 2 a a d a a 4 3 Aorta 1 2 a a d a a a 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 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 2 Lung 5 a a a a a 5 2 Bones Jaw-bone 3 a a a a 5 4 Paraffin embedded tissue - - - - - - - 9 years 7 </td <td>towel</td> <td>0</td> <td>a</td> <td>b</td> <td>d</td> <td>с</td> <td>b</td> <td>1</td> <td>1</td>	towel	0	a	b	d	с	b	1	1
Aorta 12aadaa43Aorta 20acccd11Liver 11aaaaaa54Liver 26aaaaaa51Kidney5aaaaaa52Heart0aaaaa55Lung0addd11Spleen5aaaaa52BonesJaw-bone3aaaaa54Paraffin embedded tissue9 years ^b 7aaaaaaa5211 years 12aaaaaaa52	Putrefied tissue								
Aorta 20acccd11Liver 11aaaaaaa54Liver 26aaaaaaa51Kidney5aaaaaa52Heart0aaaaa55Lung0addd11Spleen5aaaaa52BonesJaw-bone3aaaaa54Paraffin embedded tissue9 years ^b 7aaaaaa5211 years 12aaaaaaa52	Aorta 1	2	a	a	d	a	a	4	3
Liver 11aaaaaaaabfLiver 26aaaaaaaaaaf1Kidney5aaaaaaaa52Heart0aaaaaaa55Lung0adddd11Spleen5aaaaa52BonesJaw-bone3aaaaa54Paraffin embedded tissue9 years ^b 7aaaaaaa5211 years 12aaaaaaa53	Aorta 2	0	a	с	с	с	d	1	1
Liver 26aaaaaaaab1Kidney5aaaaaaa52Heart0aaaaaaa55Lung0adddd11Spleen5aaaaa52BonesJaw-bone3aaaaa54Paraffin embedded tissue9 years ^b 7aaaaaa5211 years 12aaaaaaa53	Liver 1	1	a	a	a	a	a	5	4
Kidney5aaaaaaa52Heart0aaaaaaa55Lung0adddd11Spleen5aaaaa52BonesJaw-bone3aaaaa54Paraffin embedded tissue9 years ^b 7aaaaaa5211 years 12aaaaaaa53	Liver 2	6	a	a	a	a	a	5	1
Heart0aaaaaaa55Lung0adddd11Spleen5aaaaaa52BonesJaw-bone3aaaaa54Paraffin embedded tissue9 years ^b 7aaaaa5211 years 12aaaaaa53	Kidney	5	a	a	a	a	a	5	2
Lung Spleen0adddd11Spleen5aaaaaa52Bones Jaw-bone3aaaaaa54Paraffin embedded tissue 9 years7aaaaaa5211 years 12aaaaaaa53	Heart	0	a	a	a	a	a	5	5
Spleen5aaaaaa52Bones Jaw-bone3aaaaaa54Paraffin embedded tissue 9 years ^b 7aaaaaa5211 years 12aaaaaa53	Lung	0	a	d	d	d	d	1	1
Bones Jaw-bone3aaaaaa54Paraffin embedded tissue 9 years ^b 7aaaaa5211 years 12aaaaa53	Spleen	5	a	a	a	a	a	5	2
Jaw-bone 3 a a a a a a 5 4 Paraffin embedded tissue 9 years ^b 7 a a a a a a a 5 2 11 years 2 a a a a a a 5 3	Bones								
Paraffin embedded tissue9 years ^b 7aaaaa5211 years2aaaaa53	Jaw-bone	3	a	a	a	a	a	5	4
9 years ^b 7 a a a a a 5 2 11 years 1 2 a a a a a 5 3	Paraffin embedded tissue								
11 years 1 2 a a a a a a 5 3	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 2	0	a	a	a	a	a	5	5
11 years 3 0 a a d a a 4 4	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 $1 U/25 \mu l$ 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 $50 \text{ ng/}\mu\text{l}$).

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 μ l. At predefined time points (0, 0.5, 1, 2, 5, 8 and 10 min) 10 μ l were removed and added to 1 μ l 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

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