Further study on suitability of Profiler Plus in personal identification

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Abstract

This work reports a validation study on the AmpFlSTR Profiler Plus™ kit (PE/AB) on capillary electrophoresis (CE).

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Keywords: STR; Profiler Plus; Capillary electrophoresis; Validation study

1. Introduction

In order to evaluate the performance of AmpFlSTR Profiler Plus™ kit for forensic applications, sensitivity tests were carried out to establish the optimal amount of DNA required for interpretable and reliable profiles on capillary electrophoresis (CE). The next stage of the study verified the suitability and efficiency of multiloci analysis in practical forensic applications.

2. Materials and methods

Serial dilutions of the GM9947A cell line were performed, covering the whole range between 4 and 0 ng. The most significant casework samples reaching our laboratory over the last 2 years were selected; bloodstains aged from 2 to 16 years were also included (Table 1). DNA was quantified using the slot blot hybridization; the Microcon-30 procedure was used to purify DNA samples giving negative or partial profiles. Amplifi-
cation was carried out following the manufacturer’s recommendations and PCR products were electrophoresed on an ABI Prism 310 Genetic Analyzer (PE/AB).

3. Results and discussion

Results of sensitivity studies are listed in Table 2. As expected, the smallest and most homozygous loci appeared more sensitive to low amounts of DNA. Considering an arbitrary cut-off peak height threshold of 25 RFU, complete typing profiles were obtained from as little as 0.063 ng of DNA. Locus drop-out phenomenon was noted for DNA concentrations below this value, with the loss of the longer loci. Over 0.25 ng, all the loci were clearly detectable above the background, with peak heights >150 RFU, a conventional threshold limit accepted for scoring alleles. Unequal amplification of heterozygous

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extraction method</th>
<th>Number tested</th>
<th>Number with complete typing profile</th>
<th>Number with partial typing profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bloodstains—100% cotton</td>
<td>Ch, Ph</td>
<td>10</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>(aged from 2 to 16 years at room temperature)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bloodstains (carpet, fabric, fingernail, glasses, leather, marble, paper, plastic, tape, terrycloth, wood)</td>
<td>Ch, Ph</td>
<td>48</td>
<td>43</td>
<td>2</td>
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<tr>
<td>Buccal cell swab</td>
<td>Ch</td>
<td>18</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>Ch</td>
<td>2</td>
<td>2</td>
<td>-</td>
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<tr>
<td>Cigarette butt</td>
<td>Ch [1]</td>
<td>3</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Condom—outside surface</td>
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<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Fingernail</td>
<td>Ch</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fingernail—bitten off</td>
<td>Ch</td>
<td>1</td>
<td>Mixed profile</td>
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<tr>
<td>Glass</td>
<td>Ch</td>
<td>3</td>
<td>2</td>
<td>1</td>
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<tr>
<td>Hair (root)</td>
<td>Ch</td>
<td>4</td>
<td>3</td>
<td>-</td>
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<tr>
<td>Liquid blood</td>
<td>Ch</td>
<td>12</td>
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<tr>
<td>Skeletal remains</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bone shaft (femur)</td>
<td>[2]</td>
<td>3</td>
<td>3</td>
<td>-</td>
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<tr>
<td>soft tissue</td>
<td>QI</td>
<td>1</td>
<td>1</td>
<td>-</td>
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<tr>
<td>dental pulp</td>
<td>QI</td>
<td>1</td>
<td>1</td>
<td>-</td>
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<tr>
<td>Teaspoon</td>
<td>Ch</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Tin</td>
<td>Ch</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Tissue (post-mortem):muscle, liver, kidney</td>
<td>QI</td>
<td>4</td>
<td>3</td>
<td>1</td>
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<tr>
<td>Tissue embedded in paraffin blocks—liver</td>
<td>[3]</td>
<td>1</td>
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<tr>
<td>Tissue sections—gastric biopsy</td>
<td>QI</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Urine</td>
<td>Ch</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vaginal swab</td>
<td>Ch</td>
<td>3</td>
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</table>

Ch = chelex; Ph = phenol-chloroform; QI = QI Amp-Qiagen.
Table 2
Results of template DNA titration experiments on ABI PRISM 310 genetic analyzer (L = light allele; H = heavy allele)

<table>
<thead>
<tr>
<th>DNA (ng)</th>
<th>D3S1358 (L)</th>
<th>D3S1358 (H)</th>
<th>VWA (L)</th>
<th>VWA (H)</th>
<th>FGA (L)</th>
<th>FGA (H)</th>
<th>X</th>
<th>D8S1179 (L)</th>
<th>D21S11 (H)</th>
<th>D18S51 (L)</th>
<th>D18S51 (H)</th>
<th>D5S818 (L)</th>
<th>D13S317 (H)</th>
<th>D7S820 (L)</th>
<th>D7S820 (H)</th>
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<td>907</td>
<td>701</td>
<td>678</td>
<td>2619</td>
<td>2221</td>
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<td>798</td>
<td>601</td>
<td>1789</td>
<td>1162</td>
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<td>2</td>
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<td>1</td>
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<td>664</td>
<td>435</td>
<td>499</td>
<td>1131</td>
<td>906</td>
<td>1000</td>
<td>597</td>
<td>575</td>
<td>1110</td>
<td>1004</td>
<td>340</td>
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<td>225</td>
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<td>226</td>
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</tr>
</tbody>
</table>

Peak heights (relative fluorescence units)

Genotype of female cell line 9947A: D3S1358 14–15; vWA 17–18; FGA 23–24; D8S1179 13; D21S11 30; D18S51 15–19; D5S818 11; D13S317 11; D7S820 10–11.
profiles resulting in appreciable imbalance between peak heights was observed for 0.25 and 0.5 ng, due to a stochastic effect. Serial dilutions of the GM9947A cell line were also tested to examine the concentration–fluorescence linearity for each locus of the multiplex system (Fig. 1). With decreasing quantities of DNA, a linear trend appeared, except for the longer alleles of systems D7S820 and D18S51, where increased fluorescence was observed in the range 1–2 ng. It was interesting to note that, in the same range, most of the longer fragment loci (from D18S51 to vWa) of the multiplex system showed nearly comparable peak heights, suggesting that within this range, amplification of single loci has the same quality performance as the PCR reaction resulting in a balanced signals, in the range 500–1500 RFU. This indicates that a DNA amount of 1–2 ng is the optimum, thus avoiding differential amplification phenomena. In fact, with greater DNA amounts, an abundance of amplified products was observed for the homozygous loci with fluorescence >1500 RFU.

Unambiguous profiles were obtained for casework analysis when amplification was reached in the range 1–2 ng and quantities of DNA template higher than these did not produce valid results ( locus drop-out phenomenon or negative results). The success rate in typing forensic casework samples was 90%. This percentage included positive results (15%) obtained after the easy additional step of purification by Microcon-30 in DNA extracts that failed to amplify.

References