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Application of less primer method to commercial kits

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Abstract

A modified PCR method is described. We examined the conditions of commercial kits including concentration of primer, amplification cycle number and annealing and extension time to obtain even PCR products as well accurate genotype analysis. In addition, we tried to arrange the ratio of primer composition in PowerPlex® 16 system to improve the loci of high molecular and add two times of Taq Gold polymerase to decrease the extension time.

The advantages of less primer method are good balance among loci, reproducibility and sensitivity.

Keywords: Less primer method; Multiplex PCR

1. Introduction

In the case of an imbalance from locus to locus, the manufacturer recommends that reducing the number of PCR cycles and amplification using less templates can improve the balance among loci. We reported that the even PCR products and accurate genotype analysis from putrefactive sample and bone sample were obtained by the application of less primer method to Profiler® system [1]. The difference between less primer method and conventional PCR is due to an upper limit and specific amplification at 40 cycles. This method takes 9 h and we try to shorten it and apply it to commercial kits that have high degree of discrimination.

2. Methods

The primer concentration of both Identifiler® system (Applied Biosystems, USA) and PowerPlex® 16 system (Promega, USA) was set at minimum.

The comparison of protocol and less primer method in point of PCR conditions including component of mixture, amplification cycle number, annealing and extension time are described in Table 1. The primer composition in PowerPlex® 16 system was improved by adding Monoplex® primer.

The treatments of PCR product and electrophoresis conditions (ABI 3130XL) are same in both methods.

3. Result and discussion

When Identifiler® system using both methods was compared, the peak of D21S11, D3S1358, D13S317, D16S539 and FGA typing in less primer method were not detected because of lower primer concentration (data not shown). The ratio of primer concentration is suitable for protocol, but not for less primer method.

In the case of PowerPlex® 16 system, the high peak with pull-up in FGA typing and the extreme low peak of D3S1358, TH01, D18S51, D5S818, D13S317, D16S539 and CSF1PO typing in less primer method were detected (data not shown).

We arranged the primer composition by adding MonoPlex® 16 system (D3S1358, TH01, D18S51, PentaE, D5S818, D13S317, D7S820, D16S539 and CSF1PO systems) to improve the balance among loci and added two times of Taq Gold polymerase to decrease the extension time.

The peaks of loci that were added into PowerPlex® 16 system in Fig. 1b were improved and good balance for high molecular locus was obtained.

As molecular weight become higher, the template of high molecular locus reduces, especially in degraded sample. In

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Table 1
Comparison of PCR condition in both methods

	Protocol	Less primer method
Identifiler® system		
Concentration of primer (%)	100	2.5
Annealing time (min)	1	5
Extension time (min)	1	5
Cycle number	28	40
PowerPlex® 16 system		
Concentration of primer (%)	100	2.5–5
Annealing time (min)	0.5	1
Taq Gold polymerase (μl)	0.8	1.6
Cycle number	28–32	40

The other PCR conditions are same in both methods.

protocol, enough PCR products of low molecular loci were detected, however, those of high molecular loci were apt to decrease (Fig. 1a). Because the excess primer in protocol combines with the template immediately, PCR product is depended on the amount of template rather than the concentration of primer. In contrast to protocol, the primer concentration of less primer method is set at minimum. The addition of primer increases the opportunities to encounter between template and less primer and affects directly PCR product. Therefore, the arrangement of primer composition in less primer method promotes the good balance among loci for degraded sample Fig. 1b.

We think that the annealing and extension time is very important because of few opportunities to encounter between template and less primer. It takes longer to anneal between less primer and the template, likewise, compose of less primer–template and polymerase. The larger yield of low molecular locus is produced at 3 min of the annealing and extension time and 5 min promote the amount of PCR product of high molecular locus. PCR condition under 5 min of the annealing and extension time takes about 9 h. Although Taq Gold polymerase is active after 11 min at 95 °C, the active polymerase is half of all and each denature step activates it. In this study, we try to add two times of Taq Gold polymerase in PowerPlex® 16 system to increase the chance to encounter between compose of less primer–template and active polymerase. The extension time is dramatically reduced from 5 min to 45 s.

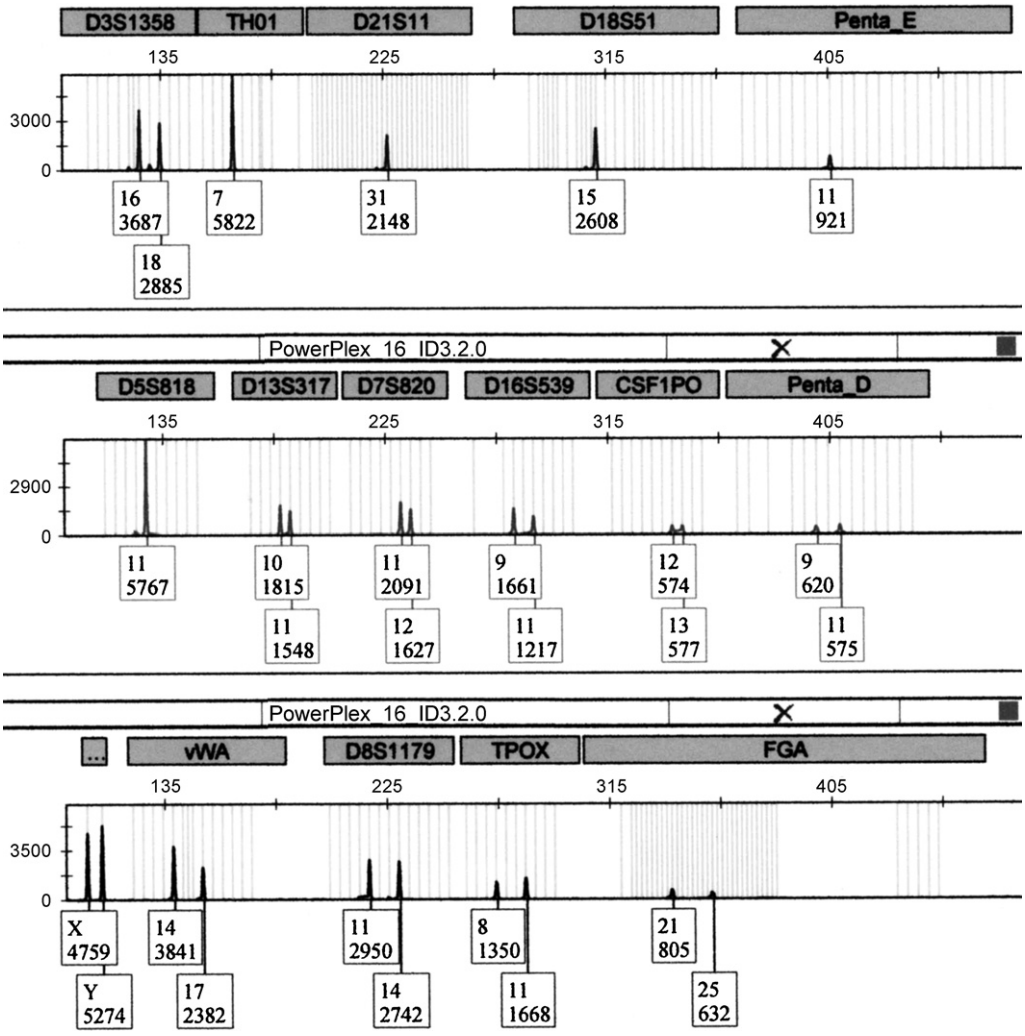

Fig. 1a. Electropherogragrams from putrefactive sample in protocol.

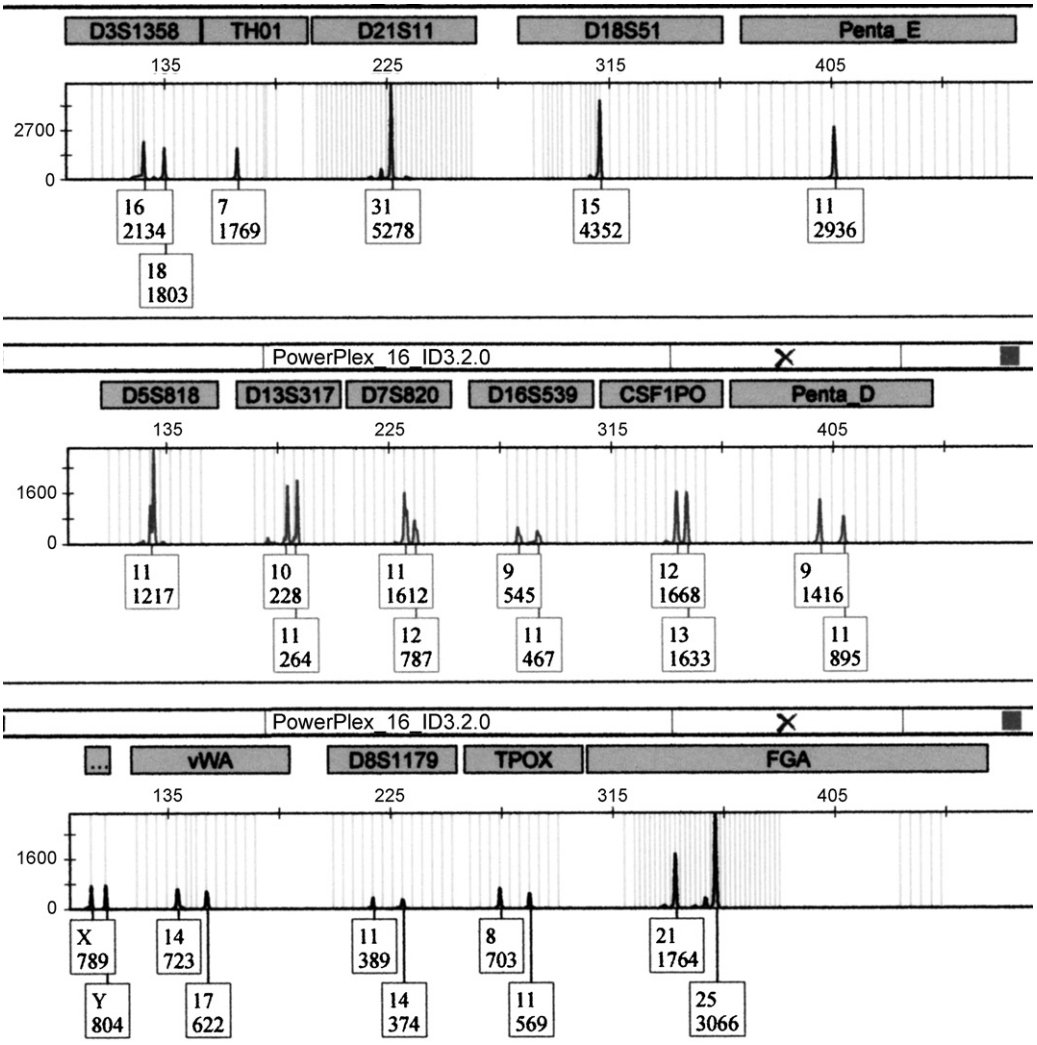

Fig. 1b. Electropherogragrams from putrefactive sample in less primer method.

Conflict of interest

I have a potential conflicting interest for patent applications/registrations.

Reference

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