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Application of less primer method to multiplex PCR

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Abstract. A modified PCR method is described. The difference between less primer method and conventional PCR is due to an upper limit and specific amplification even at 40 cycles. The advantages over conventional PCR are good balance among loci, reproducibility and high sensitivity. This new method is very simple and is smoothly applied to PCR in various fields.

Keywords: Less primer method; Multiplex PCR

1. Introduction

Multiplex short tandem repeat (STR) analysis have been indispensable for the forensic genotyping because it can use minute amounts of DNA and has a high degree of discrimination. 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 conditions including concentrations of primer, amplification cycle number and annealing and extension time were examined to obtain even PCR products as well accurate genotype analysis [1].

2. Methods

The primer concentration (Profiler®, Applied Biosystems, USA) was set at minimum required to the plateau below 8000 RFU without pull-up phenomenon. All conditions of PCR and capillary electrophoresis are same except for concentrations of primer (100% in

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protocol to 3% in less primer method), amplification cycle number (28 cycles in protocol to 40 cycles in less primer method) and annealing and extension time (1 min in protocol to 5 min in less primer method). PCR takes about 9 h.

3. Result

When DNA from putrefactive sample is extracted, the template of high molecular locus reduces. The accurate genotyping from degraded samples in this method results from the upper limit and the specific amplification at high number of amplification cycle (Fig. 1b,d). In protocol, peak of high molecular loci are not detected when template is moderate (Fig. 1a). In case of surplus template, the excess PCR products of low molecular locus beyond the polymer capacity shift toward the anode (indicated by horizontal arrow in Fig. 1c). In addition, Amelogenin typing has pull-up phenomenon.

4. Discussion

Contrast to the conventional PCR product that depends on amount of the template, less primer method has the upper limit. If template is enough, the locus of higher efficient

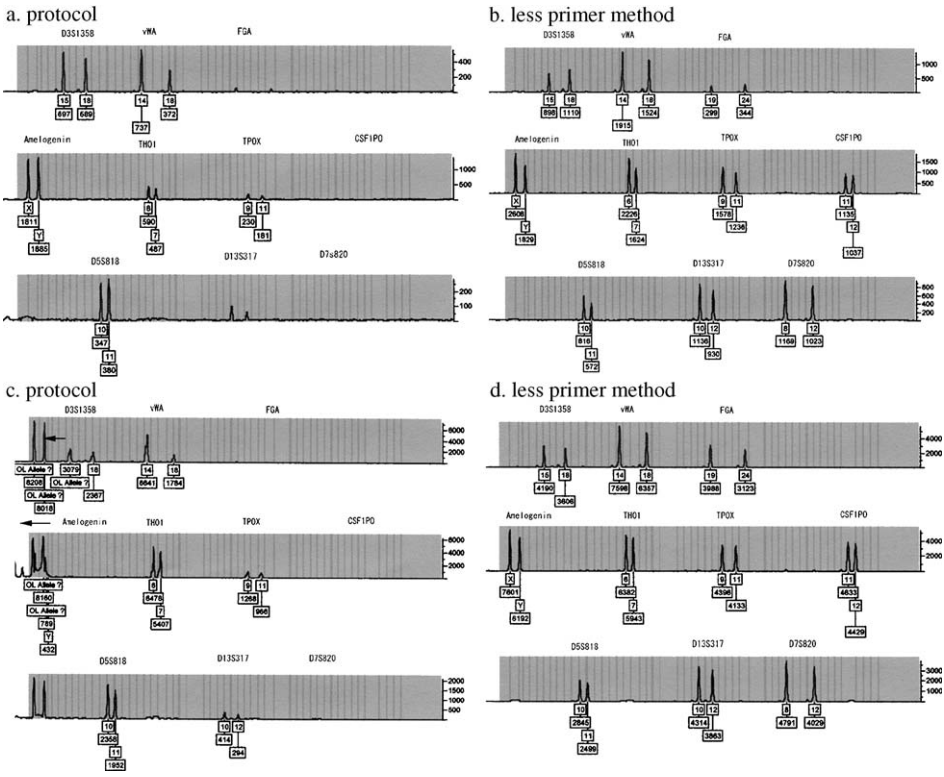

Fig. 1. Electropherograms from putrefactive sample. The amount of templates in both (a) and (b) are same. Each template in both (c) and (d) is 4 times of (a) and (b). The injection time in (a)–(d) is 5 s.

amplification is reached to the plateau during early PCR cycles and the remaining PCR cycles employ to the production of lower efficient locus. Therefore, PCR product in this method is almost constant in every reaction and maintains the reproducibility and good balance among loci.

We think that the annealing and extension time play a key part because of few opportunities to encounter between template and less primer. In conventional PCR, the excess primer combines with the template immediately. 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 dramatically the amount of PCR product of high molecular locus [1].

Less primer with higher number of PCR cycles permits the specific amplification because of the plateau effect. The ordinary primer concentration at 40 cycles results in non-specific PCR because free primer leads to the disordered reaction according to the increase in cycle number. Thus, the cycle number in various kits is limited about 30 cycles. Even if it can not converge on the optimal amounts of PCR products, less primer method at 40 cycles is more sensitive than protocol at 28 cycles (Fig. 1a and b). In the case of a minute template that has not reached to the plateau, 5% primer is more sensitive than 3% primer [1].

As molecular weight becomes higher, the template of high molecular locus reduces, especially in degraded sample. Therefore, high molecular locus needs more primer to encounter less template. The ratio of primer concentration is suitable for protocol, but not for less primer method. The peak of Amelogenin and TH01 typing are considerable high and that of FGA typing is low when amplification is reached to the plateau. Finally, arrangement of the ratio of primer improves less primer method much superior in view of balance among loci, reproducibility and sensitivity.

Reference

[1] M. Kane, S. Masui, K. Nishi, Application of less primer method to PCR, DNA Polymorphism 13 (2004) 34–37 (in Japanese).