EVALUATION OF PRIMER EXTENSION PREAMPLIFICATION (PEP) IN FORENSIC STUDIES

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INTRODUCTION

The content of DNA in hairs is usually limited. Hair root with or without surrounding sheath cells may contain less than 375 ng DNA. The sensitivity of the polymerase chain reaction (PCR) is sufficient to permit the analysis of DNA in a single hair (Higuchi 1988). An efficient method called primer extension preamplification (PEP) has been developed using a random 15-mer oligonucleotide primer (Zhang 1992). This method makes it possible to generate enough template DNA for several PCR-reactions in case the amount of DNA is limited (Huber 1993). The purpose of this study is to evaluate PEP-PCR analysis in forensic studies.

MATERIALS AND METHODS

1. PEP: Template DNA samples were subjected to amplification in a final volume of 60 µl containing 33.33 µM random 15-mer oligonucleotide primer (Operon Technologies, Alameda, CA), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, 1.5 mM $MgCl_2$, 0.1 mM dNTPs and 5 unit AmpliTaq DNA polymerase (Cetus, Emeryville, CA). This was followed by fifty cycles of 1 min at 92 °C, 2 min at 37 °C, 3 min transition at 37-55 °C, and 4 min extension at 55°C.
2. PEP-PCR: Aliquots of the PEP products were further subjected to reamplification with two specific sequences, including VNTR (COL2A1), STR (ACTBP2, TH01) and sex determining primers (Kogan 1987; Witt 1989). Positive and negative controls were always included in each PCR run. The amplified products were analyzed using agarose gel electrophoresis or polyacrylamide gel electrophoresis, then visualized and photographed under ultra-violet light or silver staining.
3. Sequencing of PCR and PEP-PCR products: Subcloning of PCR and PEP-PCR products was performed using the Original TA Cloning Kit (Invitogen, San Diego, CA). PCR and PEP-PCR products were ligated into plasmid pCR™II and transformed into One Shot™ competent cells. The presence of cloned PCR and PEP-PCR products was verified to analyze minipreparations of plasmid DNA. The nucleotide sequence of these PCR cloned products was determined by using a Taq DyeDeoxy™ Terminator Cycle Sequencing Kit

(Applied Biosystems, Inc. CA.) and Taq Dye Primer Cycle Sequencing Kit (Applied Biosystems, Inc. CA.).

RESULTS AND DISCUSSION

1. Both products of PEP-PCR and PCR alone showed identical electrophoretic mobility of the allelic state at the loci of VNTR, and STR and determination of sex. The bands of identical mobility were further used to examine their nucleotide sequences. In our ACTBP2 bands, both products showed identical nucleotide sequences, including the 12-base deletion, compared with the data base (Fig.1). Allelic dropout or mutation caused by the PEP method was not observed.
2. In the COL2A1 locus, 75 pg of template DNA was detected by PEP-PCR, whereas 7.5 ng of template DNA was detected by PCR alone (Fig. 2). The quantities of DNA were calculated before the PEP reaction. This data mean that PEP method enables 10-100 copies of template DNA to be generated for subsequent PCR analyses. These amplified sizes were less than 660 base pairs long. In the ACTBP2, X1 and Y1 loci, the PEP method permits 100 to 1000 copies of template DNA to be generated. These were under 310 base pairs long. The effectiveness of the PEP method was closely related to the primers used for subsequent PCR analyses. The efficiency of PEP-PCR was remarkable in STR primers and sex determining primers.
3. It was possible to analyze PEP products from a single hair using COL2A1, ACTBP2, TH01 and sex determining primers. Thus PEP-PCR was an effective tool for discrimining single hairs. Its use in personal identification from a single hair may be expected.

CONCLUSION

This is the first study to demonstrate identical nucleotide sequences between the products of PEP-PCR and PCR alone. The PEP method permits more copied template DNA to be generated for subsequent locus-specific amplification by PCR. PEP-PCR proved to be an effective tool in forensic science investigation.

REFERENCES

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HSAC04    1' CTACAGTGAGCCGAGGTCATGCCATTGCACTCCAATCTGGGCGACAAGAGTGAAACTCCG
PCR-alone                                     ****************************
PEP-PCR                                       ****************************

HSAC04   61' TCAAAAGAAAGAAAGAAAGAGACAAAGAGAGTTAGAAAGAAAGAAAGAGAGAGAGAGAGA
PCR-alone    ************************************************************
PEP-PCR      ************************************************************

HSAC04  121' AAGGAAGGAAGGAAGAAAAAGAAAGAAAAAGAAAGAAAGAGAAAGAAAGAAAGAGAAAGA
PCR-alone    ************************************************************
PEP-PCR      ************************************************************

HSAC04  181' AAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAAAGAAAGAAAGAAAGAAA
PCR-alone    ************************************************************
PEP-PCR      ************************************************************

HSAC04  241' GAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGGAAGGAAAGAAAGAGCAAG
PCR-alone    ****---------************************************************
PEP-PCR      ****---------************************************************

HSAC04  301' TTACTATAGCGGTAGGGGAGATGTTGTGAGAAATATATATAAACCTCCTTACACCGCGGAG
PCR-alone    ************************
PEP-PCR      ************************
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Fig. 1 Nucleotide sequences of the PCR and PEP-PCR products in the ACTBP2 locus. HSA04: Data base (V00481), Underline shows PCR primers.

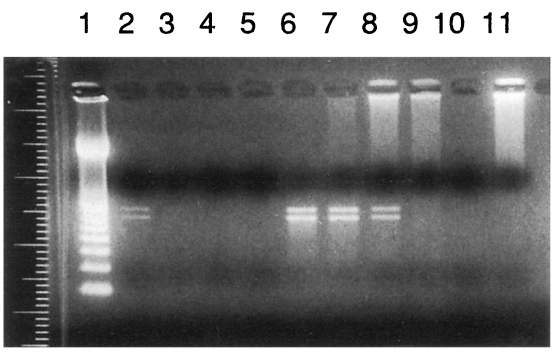

Fig. 2. Electrophoresis of the PCR or PEP-PCR products in the COL2A1 locus. The quantities of DNA were calculated before the PEP reaction. Lane 1: 100 Base-Pair Ladder Marker; lanes 2 and 6: 7.5 ng of DNA; lanes 3 and 7: 750pg of DNA; lanes 4 and 8: 75pg of DNA; lanes 5 and 9: 7.5 pg of DNA; lanes 10 and 11: No DNA was added. Different concentrations of human genomic DNA with or without PEP reaction were analyzed by PEP-PCR (lane 6-9, 11) or PCR alone (lane 2-5, 10).