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Notes & Tips

Whole-genome amplification of DNA from residual cells left by incidental contact

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Advances that enable investigators to perform analyses on the DNA in cells left behind in latent prints have been made recently in molecular biology. Researchers have been able to lift cells from a variety of different surfaces [1] and have demonstrated successful analysis on processed and archived prints [2,3]. Despite the potential, there are still many problems associated with the use of cells contained in latent prints for forensic analysis. Most prints yield a limited quantity of DNA: up to 50 ng in the case of whole-hand or multiple-contact prints [1] or less than 1 ng for single and archived fingerprints [2,4]. Prints that are successfully lifted can be used for only a limited number of analyses and are often not successfully analyzed [2].

In 2002, Dean et al. [5] published a paper demonstrating whole-genome amplification. The technique involved a new method of multiple displacement amplification (MDA)[1] utilizing Φ29 polymerase, known for its high strand displacement capabilities, low error rate (1 in 10^6–10^7 [6]), and random hexamer primers. The technique has been shown to provide complete, nonbiased amplification of the starting material, producing as much as 20–30 μg of DNA from as few as 10 cells. Here, we demonstrate the application of MDA in processing fingerprints before analysis to alleviate the problems associated with the limited amount of DNA in a print.

We tested the utility of MDA for forensic analysis by comparing the frequency of successful typing reactions from amplified buccal swabs and fingerprints to nonamplified material. IRB-approved buccal swabs were collected from 10 individuals and processed according to the manufacturer's recommendations in the Repli-G kit. Typing was done using the GammaSTR multiplex kit available from Promega. In a second series of reactions, eight fingerprints applied onto cleaned glass slides were lifted using Epicentre Collection Swabs and lysed using Epicentre QuickExtract Solution. Extracted DNA was analyzed by alu-PCR (ACE primers described in [7]). In both cases, parallel reactions were run in which no MDA step was performed.

Both sources of material showed a much greater frequency of successful amplification following MDA; 6 of 10 buccal swabs showed complete STR amplification profiles (all four expected alleles were present) and three of eight fingerprints were successfully analyzed by PCR when samples were preamplified by MDA. Of the 10 MDA-processed buccal swabs, an additional 2 showed partial profiles. Only 3 of 10 buccal samples and no fingerprint samples were successfully analyzed when the preamplification step was omitted. Similar banding patterns were found in the MDA processed samples and those that had not been preamplified.

To demonstrate the sensitivity of MDA, 1, 2, 4, 6, 8, or 10 log-phase MOLT-4 human lyrnphoblastoid cells were placed by micromanipulation into microcentrifuge tubes. Multiple displacement amplification was performed using both the Repli-G kit from Molecular Staging, and the Templiphi kit from Amersham. Postamplification samples were run on a 2% agarose gel in TBE and tested by inter-alu-PCR [8] to determine the quality of amplification. It can be seen in Fig. 1 that MDA was successful in amplifying as little as 8 microdissected cells. This sensitivity was confirmed using serially diluted log-phase MOLT-4 cells. MDA reactions were performed containing 1, 10, 100, and 1000 cells. Fig. 2 shows that reactions containing 10 or more cells were also successfully whole-genome amplified. This is

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[1] *Abbreviation used:* MDA, multiple displacement amplification.

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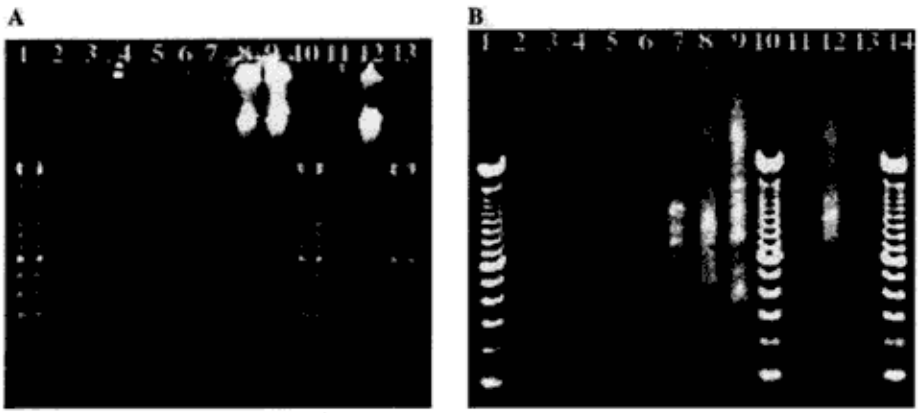

Fig. 1. (A) 2% Agarose gels of varying numbers of human MOLT-4 cells that had been microdissected and then whole-genome amplified. Lanes 1, 10, and 13 contain 100-bp DNA ladders; lanes 2–4 were made from a single cell each; lane 5 was made from 2 cells; lane 6 was made from 4 cells; lane 7 was made from 6 cells; lane 8 was made from 8 cells; lane 9 was made from 10 cells; lanes 11 and 12 contain negative and positive controls, respectively. (B) Lanes 1–10 show the same samples following inter-alu-PCR, loaded in the same order; lanes 11–13 show negative, positive, and negative controls, respectively.

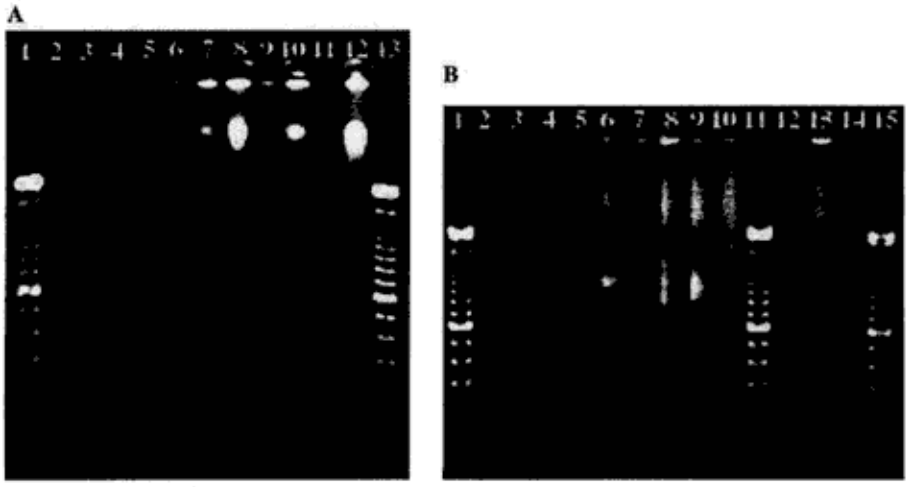

Fig. 2. (A) 2% Agarose gel of serially diluted human MOLT-4 cells following whole-genome amplification. Dilutions estimated to contain 1 cell as starting material in lanes 2–4, 10 cells as starting material in lanes 5 and 6, 100 cells as starting material in lanes 7 and 8, and 1000 cells as starting material in lanes 9 and 10 were prepared. Lanes 11 and 12 contain negative and positive controls, respectively. (B) Same samples following inter-alu-PCR, loaded in the same order. In this case, however, a ladder separates the test samples from the controls: negative, positive, and negative in lanes 12–14. All ladders used were 100-bp DNA ladders.

consistent with the manufacturer's recommendation of a minimum number of 10 cell and with results published by Dean et al. [5].

The quantity of material generated during the amplification reaction was found to be sufficient for a virtually unlimited number of subsequent typing reactions. Dilutions of 1:10, 1:100, and 1:1000 of the amplified product in water were made; 1 μL of each of the diluted materials was used as template material for an alu-PCR (APO primers were used, as published in Watkins et al. [7]). PCR was also performed on the undiluted material. Samples were run on a 2% agarose gel to determine the success of the reaction. It can be seen in Fig. 3 that successful PCR was performed on amplified samples that had been diluted by as much as 1:1000. As expected, the quantity of product was dependent upon the initial dilution. At 1:1000 the product band appears to be very faint but is still a definite band. Perhaps the best results were obtained when the sample was diluted 1:100. Using this experiment it can be estimated that the 50 μL of product produced by whole-genome amplification can be diluted 100 times, producing 5000 μL of usable product for genetic typing analyses. This offers a significant improvement over current technology, as Schulz and Reichert [2] published that they could type only five different STR loci from a single sample.

Care must be taken to avoid contamination when collecting samples for processing using this

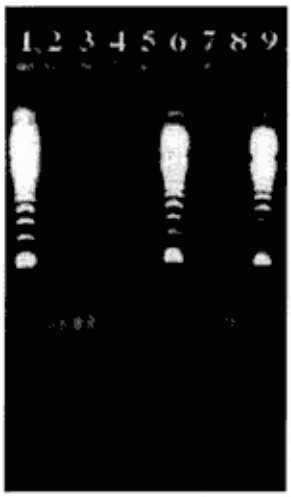

Fig. 3. 2% Agarose gel showing PCR products from serially diluted, whole-genome amplified templates. In lanes 1, 6, and 9 are 100-bp DNA ladders. In lanes 2 through 5 are alu-PCR products. APO primers [5] were used on amplified templates that had been diluted 1:10, 1:100, and 1:1000 (lanes 3–5) or not diluted (lane 2). Lanes 7 and 8 show negative and positive controls, respectively. Note that product can clearly be seen at 1:100 dilution and faintly seen at 1:1000 dilution.

methodology. Because the assay is so sensitive, even trace amounts of DNA will become a significant problem. Contamination can come from several sources, including the technicians handling and collecting the samples. Samples can also become contaminated before collection. Studies by van Oorschot and Jones [1] demonstrated that DNA could be transferred between individuals by a handshake and objects handled by many individuals showed profiles from several people. We are currently investigating methods to either avoid contamination, if possible, or determine whether it has occurred.

This method may allow many previously unusable samples to be analyzed. In cases where only smudged or partial prints can be located traditional fingerprint analysis would be impossible. Using this method, material adequate to perform genetic analyses can be generated. Work in our lab performing multiple displacement whole amplification on bacteria shows results using a single cell, suggesting that the assay may become even more sensitive following optimization. We anticipate that this method will prove useful even in cases where no prints are visible, but skin contact is suspected.

Acknowledgments

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References

[1] R.A. van Oorschot, M.K. Jones, DNA fingerprints from fingerprints, Nature 387 (1997) 767.
[2] M.M. Schulz, W. Reichert, Archived or directly swabbed latent fingerprints as a DNA source for STR typing, Forensic Sci. Int. 127 (2002) 128–130.
[3] A. Zamir, E. Springer, B. Glattstein, Fingerprints and DNA: STR typing of DNA extracted from adhesive tape after processing for fingerprints, J. Forensic Sci. 45 (3) (2000) 687–688.
[4] F. Alessandrini, M. Cecati, M. Pesaresi, C. Turchi, F. Carle, A. Tagliabracci, Fingerprints as evidence for a genetic profile: morphological study on fingerprints and analysis of exogenous and individual factors affecting DNA typing, J. Forensic Sci. 48 (2003) 586–592.
[5] F.B. Dean, S. Hosono, L. Fang, X. Wu, A.F. Faruqi, P. Bray-Ward, Z. Sun, Q. Zong, D. Du, J. Du, M. Driscoll, W. Song, S.F. Kingsmore, M. Egholm, R.S. Lasken, Comprehensive human genome amplification using multiple displacement amplification, Proc. Natl. Acad. Sci. USA 99 (2002) 5261–5266.
[6] J.A. Esteban, M. Salas, L. Blanco, Fidelity of Φ29 DNA polymerase, J. Biol. Chem. 268 (1993) 2719–2726.
[7] W.S. Watkins, C.E. Ricker, M.J. Bamshad, M.L. Carrol, S.V. Nguyen, M.A. Batzer, H.C. Harpending, A.R. Rogers, L.B. Jorde, Patterns of ancestral human diversity: an analysis of alu-insertion and restriction-site polymorphisms, Am. J. Hum. Genet. 68 (2001) 738–752.
[8] D.L. Nelson, S.A. Ledbetter, L. Corbo, M.F. Victoria, R. Ramirez-Soliz, T.D. Webster, D.H. Ledbetter, C.T. Caskey, *Alu* polymerase chain reaction: a method for rapid isolation of human-specific sequences from complex DNA sources, Proc. Natl. Acad. Sci. USA 86 (1989) 6686–6690.