PALM-PRINT ON STICKERS AS A REPLACEMENT OF BLOOD-DRAWING FOR DNA TESTS

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Note: Figures appear at end of file

We anticipate that there will be an explosion of demand for genetic testing in the 21st century because of the increasing demand for the DNA profiling and the genetic diagnosis for various diseases. The DNA profile databases in the United States and in Britain already boast hundreds of thousands of records and a national DNA profile database does not appear to be very distant (1).

As automated high-throughput instruments are rapidly developed, sampling can be a bottleneck for the whole process of DNA testing. The traditional source of DNA, blood, has never been satisfactory. Blood drawing is painful and potentially infectious. Furthermore blood requires special care in shipping and storage.

Buccal sampling became a viable alternative for blood drawing because many tests require only a minute quantity of DNA due to PCR (2,3). Buccal sampling appears to be widely used in paternity tests, forensic identification tests and even for population genetic surveys. However, buccal sampling is still intrusive and Introduction may give discomfort to those tested. With regard to paternity and forensic tests, photos and witnesses are necessary to generate notarized documents.

We thought that the skin could be a superior alternative to buccal swabs and blood since there appears to be enough DNA that can be amplified by PCR which, in theory, can generate DNA profiles from a single copy of genomic DNA (3,4). We came up with an idea that sticky tapes (“stickers” from now on) could be helpful in obtaining excess amount of skin material, which should have proportionally more DNA. Indeed, we were able to get DNA profiles out of the skin extracts.

Results and discussion

After an umber of trial and errors, the stickers for DNA sampling from skin were developed from a sheet of a paper coated on both sides with water-resistant film after a number of trial and errors. The adhesive was applied to one side of the sticker and the sticky surface was protected by another non-sticking coated paper.

The sticky surface allowed clear imprint of hands. The palm-prints can be visualized by staining with 1% crystal violet briefly (Figure 1). The fingertip area could be enlarged either with a magnifying glass or electronically after storing the image digitally in the computer. The three fingerprint patterns in Figure 2 demonstrate the clarity of the enlarged images. The fingerprint directly relates the sample donor to the DNA profile.

DNA is extracted easily following a fairly standardized procedure. A 1.5cm x 0.5 cm slice of a sticker is incubated with 500 ul Extraction buffer composed of 5% Chelex-100 ion-exchange resin (Bio-Rad) and 100 ug/ml Protease K (Boehringer Mannheim) at 56°C for 30 min., heated to 100°C for 5 min. and then subjected to ethanol-precipitation. Because the recovered DNA is a miniscule amount, we did not attempt to characterize the extracted DNA directly. We simply assayed the final DNA samples for usability for DNA profiling experiments using the commercially available Profiler-Plus kit (Perkin-Elmer) which allows typing of 9 STR (short tandem repeat) regions together with a part of amelogenin gene.

The construction of the sticker is important for successful DNA extraction from the sticker. The adhesive is essential. Water-impermeable coating of the supporting paper is another critical factor because leakage of various chemicals impregnated within the paper deteriorated subsequent amplification reactions severely (Figure 3). It is possible to construct a sticker with a plastic backing which would release no soluble inhibitors to the subsequent biochemical reactions. We tried diverse kinds of tapes or stickers including Scotch tape and surgery tapes. It appeared that the kinds of adhesives mattered little while the coated papers and plastic media gave the best results.

Boiling is an essential step out of the whole extraction procedure (Figure 4). Without boiling, virtually no peaks were detected. Chelex-100 and Protease K were also obligatory ingredients although the peak heights were moderately affected. Without incubation with Chelex-100 and Protease K, we encountered many non-specific peaks which lowered the confidence of allele reading.

From time to time for unknown reasons, the DNA obtained by the above procedure was not clean enough to give reliable DNA profiles. We found that spin-column chromatography using Sephadex G-50 (Sigma) or comparable measures like Microcon (Amicon) ultrafiltration seemed to be helpful (Figure 5).

The DNA, which can be obtained from a 1.5 x 0.5 cm slice of a sticker, is of good quantity for PCR analysis in most cases. However, depending on the conditions of the hand, which are still not clear to us, some palm-prints of the same person taken on different days gave poor results. This problem is often overcome by collecting another palm-print.

In Figure 6, the mass of the genomic DNA obtained from a 1.5cm x 0.5 cm slice of a sticker was estimated by comparison with a series of dilutions of DNA from blood which could be measured accurately either by UV absorption or by fluorescence after mixing with Hoechst 33258 dye. The DNA was amplified using the Profiler-Plus kit but only for 29 cycles. For simplicity, the peaks were shown only for a single STR site, FGA, out of the 9 simultaneously examined loci. The height of the peaks was reduced gradually below 0.5 ng of blood DNA while little change was observed above 0.5 ng. The height of the peaks obtained with the “sticker” DNA was comparable to the largest peaks indicating that the amount of the DNA extracted from a sticker slice should be greater than 0.5 ng. It is much less than suggested by van Oorschot and Jones (1977) who estimated that more than ten nanograms of DNA were obtained even from the handle of a suitcase (5). It is not clear whether our result is simply an underestimation due to the inhibition by adhesives and leaked chemicals from the paper or if the difference simply reflects the efficiency of the two sample collection methods.

The sticker system has many advantages over the traditional blood or more recent buccal DNA sampling methods. The fingerprint left on the sticker intrinsically identifies the sample donor eliminating the requirement for photographs and witnesses. It can greatly simplify the DNA tests particularly for paternity tests and for forensic identification. This technique is non-invasive. Thus, even soldiers in the army can leave their DNA without conflict and it will be easier to get the cooperation of the imprisoned persons for a DNA database. The DNA on the sticker appears to be very stable without any treatment. Stickers left on the bench for several months still gave the same profiles. The stickers can be filed and delivered like any paper documents at room temperature without any special caution. The weakness of the sticker system is the limited quantity of DNA obtainable from the sticker. However, it is good enough for DNA profiling using PCR and the limited amount could rather be a protection against the abuse of the stored DNA.

References

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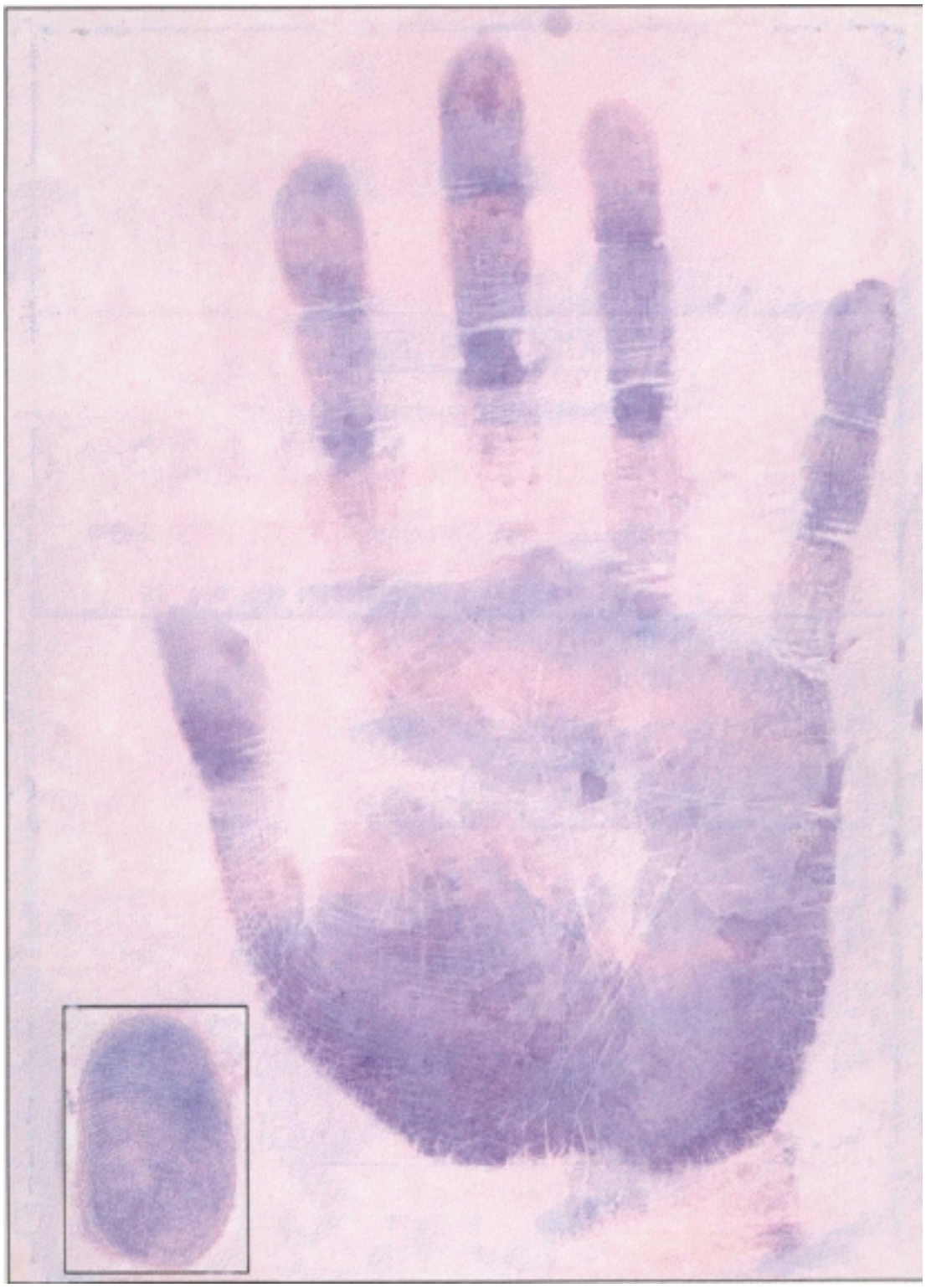

Figure 1. A palm-print stained with 1% crystal violet.

Figure 2. Enlarged fingertip area showing fingerprint patterns.

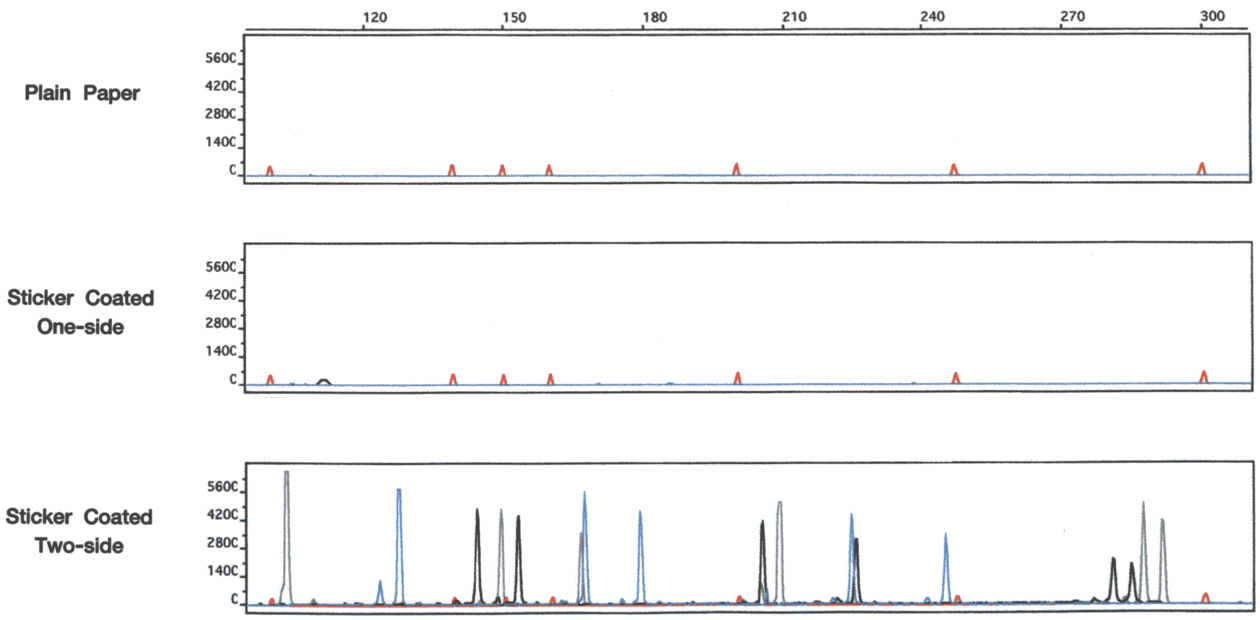

Figure 3. Multiplex PCR assay for the “sticker” DNA. The procedure to obtain DNA from a slice of a sticker is described in the text. The palm-prints were made on plain copy paper, on a common sticker one side of which is coated and on a sticker with both side coated. The electropherograms are results of multiplex STR amplification using Profiler-Plus kit (Perkin-Elmer). The direction of electrophoresis is from right to left with the leftmost peaks being the smallest. The 9 STR co-amplified by Profiler-Plus were labeled in 3 different fluorescent dyes which are reflected by 3 different colors in the electropherogram. The small red peaks are size-markers.

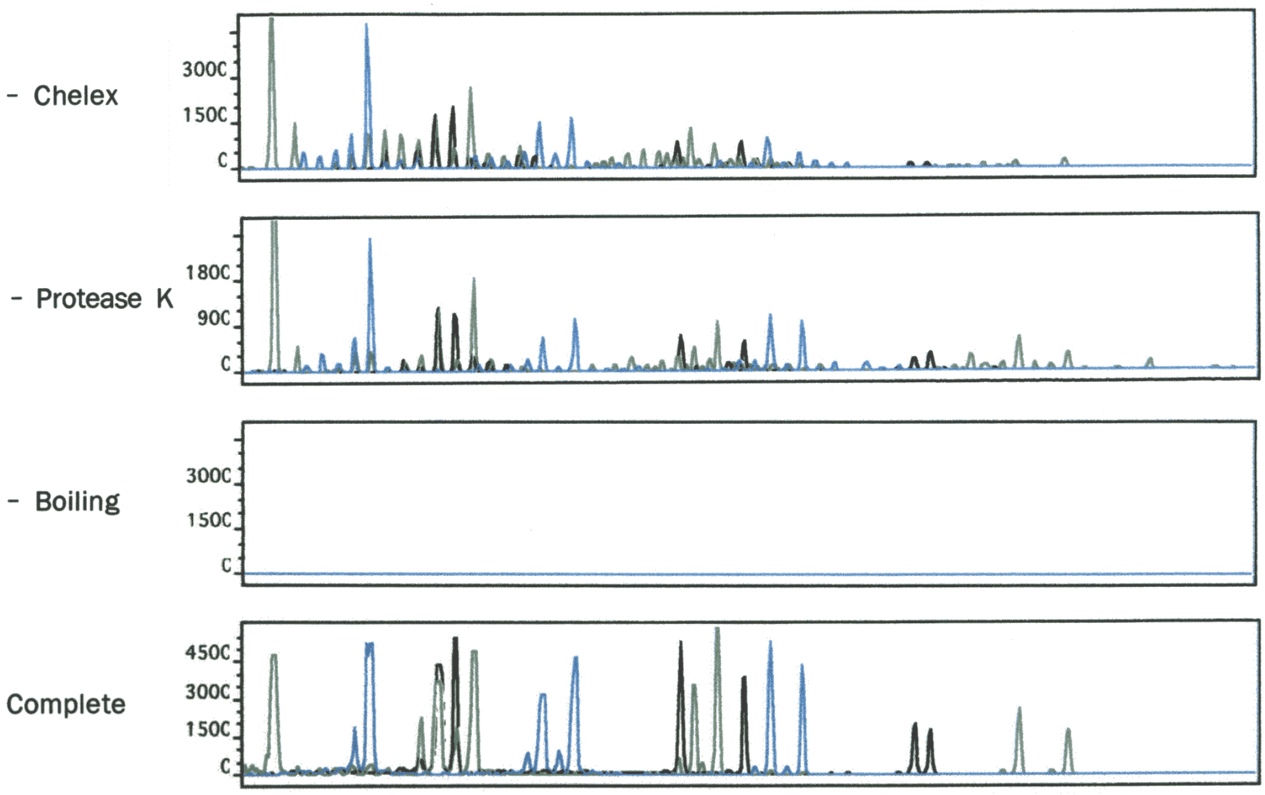

Figure 4. Multiplex PCR assay for the evaluation of the DNA extraction procedure from the sticker. The experiments were essentially the same as in the legend to Figure 3. Note the differences in scale.

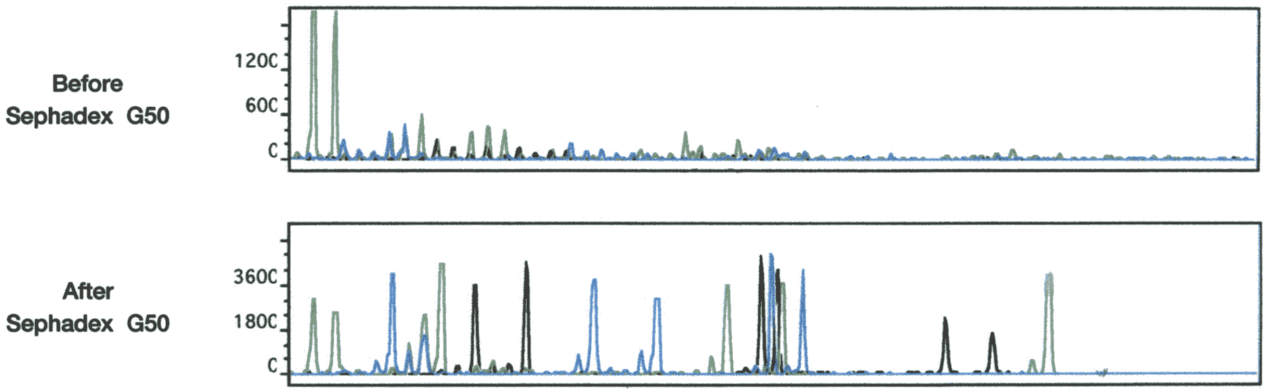

Figure 5. The effect of gel-filtration column. The same multiplex PCR assays were performed under the identical protocol described in the legend to Figure 3.

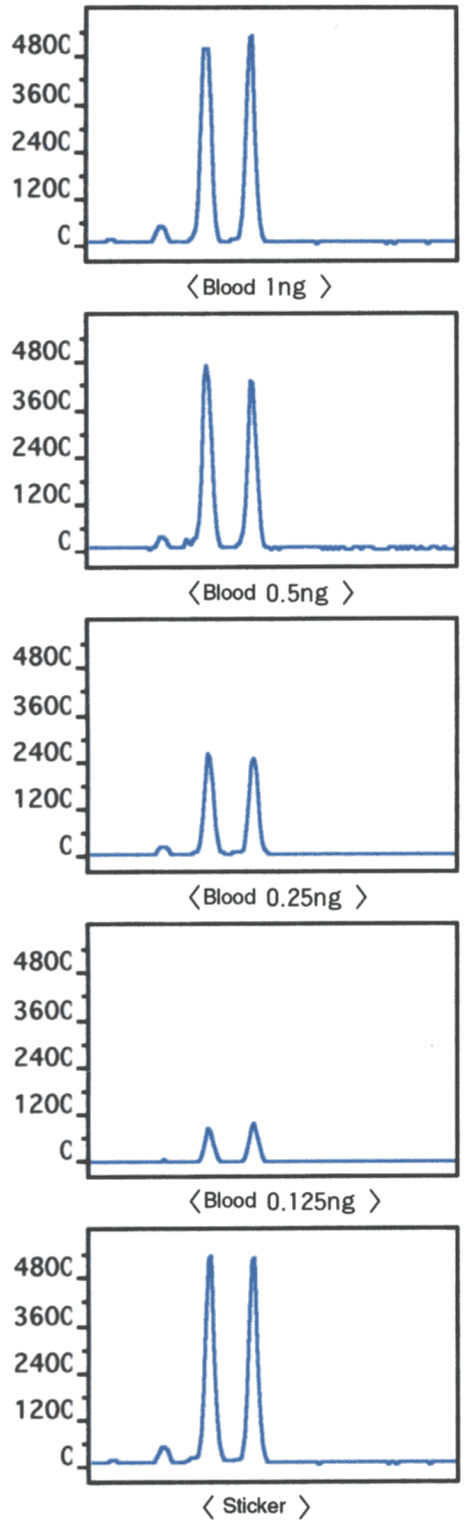

Figure 6. Indirect estimation of the genomic DNA on a sticker. The blood DNA was serially diluted to 0.5 ng/ml and 1 ul of each diluted DNA was spotted on a sticker slice. PCR of blood and “sticker” DNA was performed with Profiler-Plus kit as described in the legend to Figure 3 and for 29 cycles.