Forensic Science International: Genetics Supplement Series 7 (2019) 237–239

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/fsigss

Visualising latent DNA on tapes

Piyamas Kanokwongnuwut*, K. Paul Kirkbride, Adrian Linacre

College of Science & Engineering, Flinders University, Adelaide, 5042, Australia

ARTICLE INFO

Keywords:
Touch DNA
Diamond dye
Tape-lifting
DNA recovery

ABSTRACT

This study reports a simple method for visualising and screening latent DNA on tapes using a Diamond™ dye (DD) staining process followed by visualisation using a portable fluorescence microscope. Ten types of tapes were tested, which include those used currently by forensic laboratories for tape-lifting. All ten types were tested for: 1) their auto-fluorescence, 2) properties when stained with DD using three different DD solutions, and 3) PCR inhibition through a direct STR amplification technique. No background fluorescence was noted viewing four types stained with 20 x DD diluted with 0.01% Triton-X. Clear tape (Sellotape®), DNA-free tape (Lovell Surgical Solutions) and brown packing tape (Packmate™) did not inhibit direct STR amplification, while the other six types showed the inhibition of the PCR. The three tapes were selected to assess their cellular material recovery efficiency by comparing the number of stained cells within an entire fingermark before and after tape-lifting. Tape-lifting was performed either once, twice or ten times. The DNA-free tape (Lovell) used in many forensic laboratories gave poor recovery compared to the clear tape (Sellotape®) and brown packing tape (Packmate™). This simple visualising technique allows the cell location to be recorded, and only the area of tape where cells are present to be removed for DNA typing. The process is a simple and effective triage procedure that reduces the processing of tape-lift samples where there are no cells present.

1. Introduction

Collection of touch DNA, either by a swab or tape-lift, is based on an assumption as to where a person most likely made a contact. Tape lifting is a technique used for recover of DNA on substrates inappropriate for collection using a swab; for example fabrics, documents and matchsticks [1].

Diamond™ dye (DD) has been reported as highly effective at allowing the visualisation of latent DNA in a range of biological samples [2,3]. The visualised cellular material from fingermarks has been confirmed as corneocytes by cell morphology [3,4] and a correlation between percentage of success DNA profiling and the number of cells [4]. This technique has been reported as a simple triage method within latent DNA on swabs [5] and buccal cells on a tape [6].

Here we report a simple method that applied tape-lifts to determine if, and how much, cellular material has been recovered. This study highlights the tapes most suitable for cell collection based on no inhibition of PCR and maximum recovery of touch DNA. The process is easy to incorporate as a routine step in a forensic laboratory.

2. Materials and methods

Approval from the Social and Behavioural Research Ethics Committee (reference 8109) at Flinders University was obtained prior to initiating this project.

2.1. Types of tapes

Ten semi-adhesive tape types were tested: 1) brown packing tape (Scotch®, SA, AUS), 2) brown packing tape (Packmate™, SA, AUS), 3) clear tape (Scotch®), 4) clear tape (Sellotape®, SA, AUS), 5) clear tape (Nitto Denko™, VIC, AUS), 6) clear tape (Lovell Surgical Solutions Int Pty Ltd, VIC, AUS), 7) insulation tape (Click®, SA, AUS), 8) gaffer tape (Bear SGA Pty Ltd, VIC, AUS), 9) paper sticker note (Post-it®, SA, AUS), and 10) plastic sticker note (Post-it®).

2.2. Fluorescent background

Diamond™ dye (Promega, WI, USA) stock solution (10,000 x) was diluted (1:500) to 20 x working concentration. The DD solution was diluted with three types of solvent 1) sterile water, 2) 75% ethanol and 3) 0.01% Triton-X (Sigma, VIC, AUS). Each of the ten tapes were

* Corresponding author.
E-mail address: kano0015@flinders.edu.au (P. Kanokwongnuwut).

https://doi.org/10.1016/j.fsigss.2019.09.091
Received 5 September 2019; Received in revised form 25 September 2019; Accepted 26 September 2019
Available online 26 September 2019
1875-1768/ © 2019 Elsevier B.V. All rights reserved.

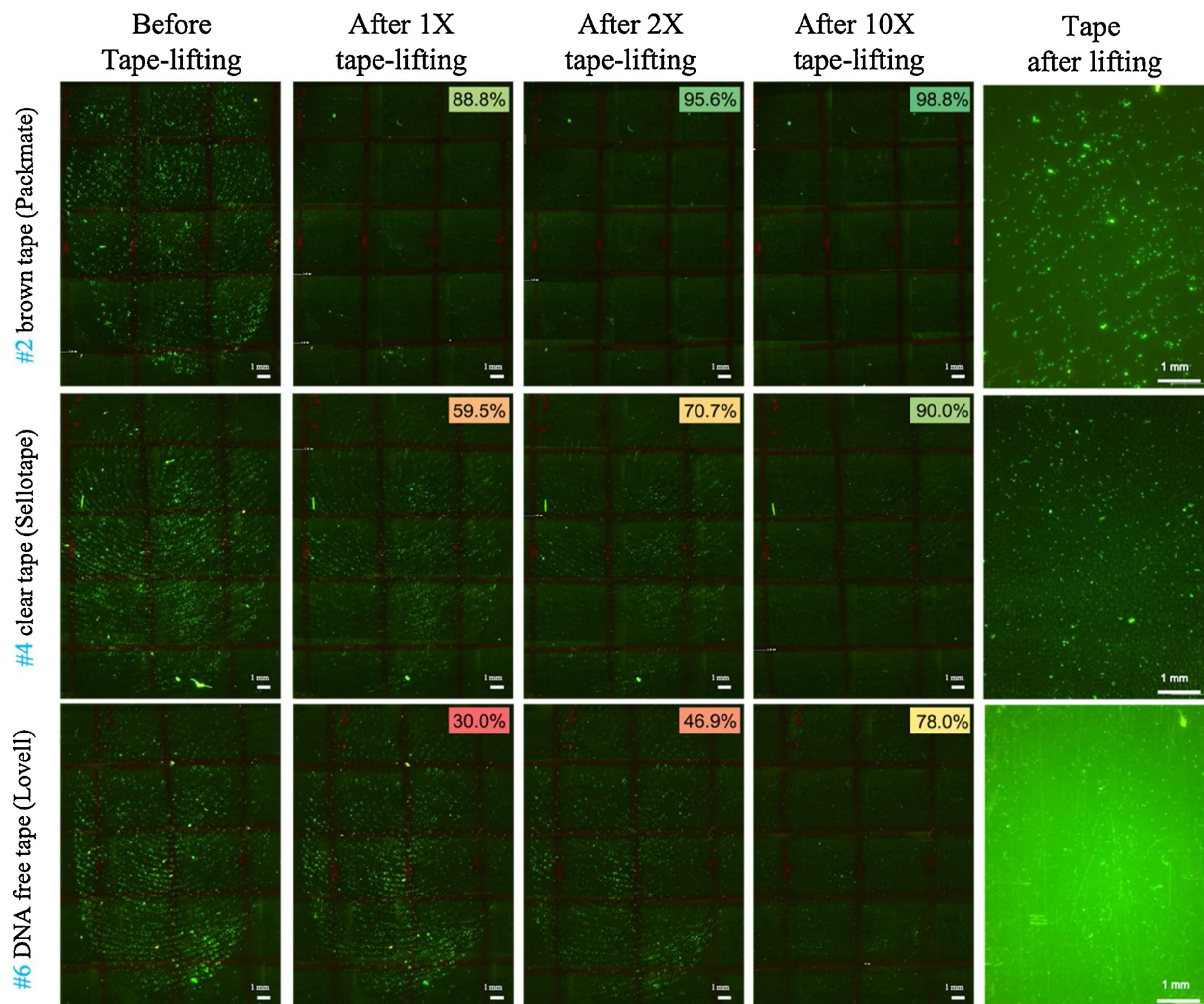

Fig. 1. Shows the remaining cellular material after collection using #2 brown tape (Packmate™), #4 clear tape (Sellotape®), and #6 DNA-free tape (Lovell), and the collected cellular material on each tape. The percentage of recovery each instance is shown at right-top (n = 5).

stained with 5 µL of 20 x DD. The stained tapes were visualised immediately after staining and once the dye was dried, under a Dino-Lite EDGE AM4115T-GFBW digital microscope. Experiments were performed in triplicate.

2.3. Direct PCR inhibition

All tapes were cleaned with 3% solution of sodium hypochlorite and were then irradiated with ultraviolet (UV) light for 15 min. Tapes were cut into approximately 5 mm x 5 mm. Extracted DNA (1 ng) was spiked onto tapes and directly placed into a PCR tube (BioRad, NSW, AUS). Amplifications were performed using the AmpFLSTR® Identifiler® Plus kit (ThermoFisher Scientific, VIC, AUS) following the manufacture's validated protocol of 29 cycles with exception of 2 µL of Prep-n-Go™ (ThermoFisher Scientific) instead of 2 µL of water. PCR product (1 µL) was added to 8.5 µL Hi-Di formamide and 0.5 µL 500 LIZ™, and separated on a 3500 Genetic Analyser. Data were analysed using GeneMapper ID-X (version 1.4). Experiments were performed in triplicates.

2.4. Recovery efficiency

Fingermarks on glass slides were stained with 20 x DD diluted with 75% ethanol. The number of stained cells within a fingermark was recorded based on an average of five frames at 50 x magnification. Tapes were cut into rectangles approximate 2.5 mm x 0.5 mm in size prior to tape-lifting. The number of cells was recorded after tape-lifting once, twice and ten times. Cells within these frames were counted by a cell counting software program. All images were counted using a fix value of five parameters were 1) hSize = 9, 2) Sigma1 = 1, 3) Sigma2 = 3, 4) RBG filter threshold = 10, and 5) Size threshold = 12 which validated for counting the number of cells at 50x magnification. Experiments were performed in five replicates.

3. Results and discussion

3.1. Fluorescent background

Ten types of tapes were visualised before any staining. Tape #8 showed a high fluorescent background and tape #10 showed autofluorescence (green fluorescent spots) over the entire tape. After staining the tapes with 20 x DD diluted in water, background fluorescence was noted on all tapes when the tapes were still wet (except tape #6 and #7), however when the DD solution had dried, no fluorescence was observed. High background fluorescence was observed when tapes were stained with DD diluted with 75% EtOH, both immediately after staining and when the solution was dried (except tape #6 and #7). No background fluorescence was noted from tapes #2, #4, #6, and #7 after staining with 20 x DD diluted with 0.01% Triton-X. DD diluted with 0.01% Triton-X spread easily across tapes unlike when diluted DD

with water, therefore this is the recommended DD solution.

3.2. Effect of tape on direct STR amplification

Four types of tapes (#2, #4, #6 and #10), exhibited no inhibition of direct STR profiling. While the other six types showed inhibition of 38.2% (#1), 9.2% (#3), 25.4% (#5), 22.7% (#7), 53.6% (#8) and 100.0% (#9). This study also indicated that 1 µL of 20 x DD diluted with 0.01% Triton-X spiked directly into the PCR tube does not inhibit direct STR amplification. Only single source STR profiles were obtained from all type of tapes.

3.3. Recovery of cellular material

Fig. 1 shows the recovery efficiency of three tapes. After tape-lifting a fingermark 10 times, tapes #2, #4 and #6 showed 98.8%, 90.0% and 78.0% recovery of cellular material respectively. After only one lift using tape #2 and #4, the recovery was 88.8% and 59.5% respectively. Tape #6 showed the lowest cellular material recovery of 30% after one lift, 47% after two lifts, and 78% after 10 lifts.

The collected cellular material on tape #2 was clearly visualised, as seen in Fig. 1. While, tape #4 showed air-bubbles as background noise and tape #6 showed background auto-fluorescence, which interfered with the detection of the cellular material.

The obtained results showed that brown packing tape (Packmate™) has the highest efficiency for DNA collection, is applicable for the DD staining technique, direct STR amplification and touch DNA recovery.

4. Conclusions

We report a simple method for targeting and triaging latent DNA on tapes after collection using DD staining followed by visualisation using a portable fluorescence microscope. Staining tapes with 20 x DD diluted with 0.01% Triton-X was recommended as there was no indication of background fluorescence, it is easy to spread, and it does not affect direct PCR and STR profiling. Of the 10 tapes tested, three were suitable for further analyses as they did not inhibit PCR or have intrinsic auto-fluorescence. Of the three, the tape used commonly in forensic laboratories performed poorly in comparison with brown packing tape.

The technique described in this study has high potential to apply as an effective triage procedure as a routine step in forensic laboratories to reduce the number of processed samples, which no DNA present on tapes.

Declaration of Competing Interest

None.

Acknowledgements

Funding for this research was provided by the Attorney General's Department through Forensic Science South Australia (FSSA) and the Ross Vining Memorial Research Fund. We would like to thank the Development and Promotion of Science and Technology Talent Project (DPST) Scholarship for supporting Piyamas Kanokwongnuwut and Thai Government scholarship, Thailand. We would also like to thank Joshua Sweaney, Prof. David Powers and Dr. Duncan Taylor for development of the cell counting software program.

References

[1] B. Martin, R. Blackie, A. Linacre, DNA profiles from matchsticks, Aust. J. Forensic Sci. (2019) 1–5.
[2] A.M. Haines, et al., Finding DNA: using fluorescent in situ detection, Forensic Sci. Int. Genet. Suppl. Ser. 5 (2015) e501–e502.
[3] P. Kanokwongnuwut, P. Kirkbride, A. Linacre, Detection of latent DNA, Forensic Sci. Int. Genet. 37 (2018) 95–101.
[4] P. Kanokwongnuwut, P. Kirkbride, A. Linacre, Speed of accumulation of DNA in a fingermark, Aust. J. Forensic Sci. 50 (2018) 1–10.
[5] P. Kanokwongnuwut, P. Kirkbride, A. Linacre, Visualising latent DNA on swabs, Forensic Sci. Int. 291 (2018) 115–123.
[6] A.M. Haines, A. Linacre, Detection of latent DNA on tape-lifts using fluorescent in situ detection, Aust. J. Forensic Sci. (2017) 1–11.