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Technical note

DNA recovery from biological material on mini tapes using a simple extraction buffer and solid phase reversible immobilisation (SPRI) purification

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ABSTRACT

In this study, we compare the performance of a simple PVP extraction method with a commercially available and widely used kit for recovering DNA from adhesive tapes. This novel method shows almost 60% higher DNA recovery from blood deposits on SceneSafe Fast™ minitapes when compared to the PrepFiler™ BTA Forensic DNA Extraction Kit. We also demonstrate how a simple modification of the magnetic bead-based purification step can lead to better recovery and removal of PCR inhibitors.

Introduction

Tape lifting as a method of evidence collection was first introduced in the early 2000s as a way to recover residual cellular and gunshot material from firearms in a single technique [1]. Over the years, tape lifting has become a commonly used technique for the recovery of trace DNA evidence [2], where the main advantage of tapes over other methods is that it is a non-destructive way of evidence collection [3].

Several studies have shown that tape-lifting is a superior DNA recovery method for touch and trace DNA compared to other techniques, such as swabbing [4–7]. Although, there is also some evidence showing that there is not much difference in DNA recovery between cotton swabs and tapes [8]. Tapes with stronger adhesion tend to result in better DNA recovery and are especially recommended over the wet/dry swab technique on fabrics [4]. However, if the item sheds a lot of fibre, both techniques result in similar DNA yields with no clear advantage for either method [4]. Minitapes appear to perform better than wet swabs when recovering DNA from cotton [7].

Over the years, several studies have compared recovery rates between commercial kits and organic, in-house protocols [7,9,10], some showing that the non-commercial extraction techniques can outperform the already established and widely validated methods [7,9]. A recently published novel method for extracting DNA from cotton swabs, which utilises an elution buffer containing a high molecular weight polymer and detergent, demonstrated a substantial increase in DNA recovery efficiency of at least 60% [11]. Considering the common use of tape lifting and swabbing for DNA recovery, we applied this novel method to samples collected using tape lifts and compared its efficiency to the established commercial paramagnetic-based method, the PrepFiler™ BTA Forensic DNA Extraction Kit. Choosing the right type of adhesive tape has an impact on DNA collection and recovery, as not all tapes are suitable for the collection of biological material [2]. SceneSafe Fast™ minitapes have been shown to outperform other tape lift methods due to their stronger adhesion [4].

In this work, we investigated four different sources of DNA deposits, including cell-free trout DNA, mouse cells, bovine blood, and human saliva. The sources of DNA used in these experiments originate from different species and allow the use of species-specific primers, eliminating any concerns over cross-contamination.

Materials and methods

Tapes

The tapes used in this experiment were DNA-free SceneSafe Fast™ minitapes (SceneSafe, Burnham-on-Crouch UK). The adhesive part of the tape was divided into six pieces with a clean scalpel, and each part was used for an individual DNA deposit, five replicates for each DNA type.

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DNA sources

Trout DNA was extracted from frozen rainbow trout liver with a chloroform phenol extraction [12] and then sonicated to create 400–600 bp fragments. The sonicated DNA was then quantified using a Qubit fluorometer (Qiagen, Manchester, UK) and stored at – 20 °C.

Mouse embryonic fibroblasts were removed from culture flasks by trypsinisation and washed in phosphate-buffered saline (PBS) pH 8.0 by centrifugation. The cells were then DAPI (4′,6-diamidino-2-phenyl-indole) stained, and their nuclei were counted in a haemocytometer to determine cell density followed by resuspension at a concentration of 1×10^6/mL in PBS 20% glycerol. The resuspended cells were stored at – 20 °C until required. Prior to use, the cells were centrifuged and resuspended in Tris-buffered saline (TBS) to the required concentration.

Human saliva was collected from a volunteer and used within 30 min of collection.

Bovine blood was sourced from a local abattoir (ABP Perth, Inveralmond Industrial Estate, Ruthvenfield Road, Perth) and treated with 12.5% (v/v) of anticoagulant ACD immediately after sample collection. The blood was divided into aliquots, which were frozen and stored at – 20 °C until required.

Sample deposits

Each DNA source was deposited in a volume of 5 μL directly onto sections of tape cut from a single tape (five sections for each deposit type), constituting five replicate tape pieces, and left until dry. The tapes were then placed in individual 2 mL Eppendorf tubes and stored at – 20 °C until required.

The cell-free DNA and mouse cell DNA were deposited in two different solutions, a 10 mM Tris-Cl, pH 8.5 buffer (EB) (Qiagen, Manchester, UK) and synthetic sebum solution [13]. The reason for choosing those two solutions is that the DNA and cells in sebum solution have been shown to better imitate components of touch DNA samples than the same material resuspended in a buffer [14]. Approximately 50 ng (as estimated by Qubit) of trout DNA was deposited for cell-free DNA samples. Cellular DNA was also deposited at 50 ng, but due to cell suspensions being prone to clumping, the input DNA is inherently variable to some extent.

Human saliva was deposited directly on the adhesive tapes with no prior preparation.

Bovine blood was left to thaw and then deposited directly on the tapes. For the purification test, 5 μL blood aliquots were deposited directly into the buffer and extracted immediately.

DNA extraction

The recovery of DNA from adhesive tapes was carried out with two extraction methods. The first method was the PrepFiler™ BTA Forensic DNA Extraction Kit (Thermo Fisher Scientific Inc, Waltham, Massachusetts, United States) following the manufacturer's instructions for DNA extraction from tape lifts. Briefly, samples were incubated in 230 μL of lysis buffer at 56 °C for 40 min shaking at 900 rpm. The elution of DNA from the SPRI (solid-phase reversible immobilization) beads was carried out at 70 °C for 10 min at 900 rpm. The second method of DNA recovery, to test its efficacy in the extraction of DNA from tapes, was the extraction technique described in detail by Gray et al. [11]. Briefly, the lysis and extraction were carried out in 250 μL of lysis buffer containing (1% polyvinylpyrrolidone (PVP), 1% tween 20 in 20 mM Tris-HCl with 20 μg/mL proteinase K) for one hour at 56 °C with shaking at 1000 rpm. This was followed by a 10-minute incubation at 95 °C with shaking at 300 rpm to inactivate proteinase K.

DNA purification by SPRI beads

DNA purification was based on the method described by DeAngelis et al. [15] with slight modification. Sera-Mag™ Carboxylate-Modified Magnetic Beads (Cytiva, Marlborough UK) supplied at 50 mg/mL were diluted 5-fold and washed three times in 0.5 M EDTA pH 8.0 and resuspended at a final concentration of 10 mg/mL in 0.5 M EDTA. To each extracted sample, an equal volume (250 μL) of 20% PEG 8000 in 2.5 M NaCl was added, followed by the addition of the required volume of 10 mg/mL beads (15 μL). The samples were then vortexed and centrifuged briefly and placed in a shaker at 1000 rpm for 1 hr at room temperature. The samples were then placed on a magnetic stand for 5 min to pellet the beads. After the removal of the supernatant, the beads were washed by the addition of 750 μL of 70% ethanol and vortexing to resuspend the pellet. The beads were centrifuged briefly and placed back on the magnetic stand, and the ethanol wash was removed. A further two 70% ethanol washes were carried out with or without further vortexing. After removing all the residual ethanol, the samples were left on the magnetic stand to air dry for about 2–3 min. DNA was eluted from the beads by the addition of 50 μL of 10 mM TrisHCl pH 8.0 (elution buffer) and a 10-minute incubation at 45 °C with shaking at 700 rpm. The eluted DNA was transferred to a clean Eppendorf tube.

DNA Quantitation

The species-specific primers for trout, mouse and bovine DNA were designed with the NCBI genome browser tools [16]. The primers for each species were as follows: trout forward TCAGCAATCA-GATGGGGAGG, trout reverse TTTCAATGATGGCCTAGTGGGT with a 110 bp product, mouse forward GACGAGGGGGAGCTTTACTTG, mouse reverse ATTGACTGTCTTGTGGACATGGG with a 231 bp product and bovine forward GATCACCCCGTCCCAGTGCC, bovine reverse TTGACGCCCCGCTCCTTTGT with expected product size 208 bp. A set of GAPDH primers were used for human DNA samples with AAAGGGCCCTGACAACTCTTT forward and TCAGTCTGAGGAGAACA-TACCA reverse primers and an expected product size of 400 bp. The primers for trout, mouse and human DNA were obtained from Eurofins Scientific, Lancaster, UK, while the bovine primers were obtained from Sigma-Aldrich (Gillingham, Dorset, UK). The DNA was quantified by qPCR using Luna® Universal qPCR Master Mix (New England Biolabs Hertfordshire UK Ltd) according to the manufacturer's instructions in a total volume of 10 μL consisting of 5 μL of sample and 5 μL of master mix. The analysis was performed on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific Inc, Waltham, Massachusetts, United States) with the following cycling mode: Initial hot start at 95 °C for 2 min, followed by 40 cycles of 30 s at 95 °C denaturing, 30 s annealing at 56 °C and elongation for 30 s at 70 °C.

Data analysis

The statistical analysis was carried out with GraphPad Prism. The p-value was calculated using a paired sample T-Test.

Results and discussion

Comparison of extraction methods

In initial experiments, it was found that when used on tapes, the swab extraction method failed to produce a yield of DNA comparable to that obtained in extracting DNA from cotton swabs. It was suspected that glue components from the tape were interfering with qPCR. Therefore, we included an SPRI purification step in the protocol as per the PrepFiler™ method, which resolved this problem. A comparison of the two extraction methods for cell-free DNA shows a higher yield from the deposits extracted with the PrepFiler kit (Fig. 1), with, on average, over 30% higher recovery for the deposits in EB and 15% higher recovery for the synthetic sebum samples. Despite the higher recovery for the PrepFiler™ method, the difference in results was not statistically significant. Over 20% more DNA was recovered from mouse cell deposits using the

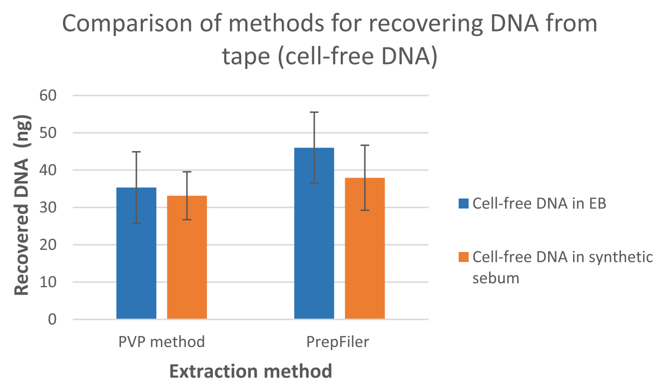

Fig. 1. The average DNA recovery yield from cell-free trout DNA in EB and in synthetic sebum solution deposited on SceneSafe Fast™ minitapes and extracted using the PVP-based extraction method and PrepFiler™ BTA Forensic DNA Extraction Kit.

PVP extraction method for EB and synthetic sebum solution samples (Fig. 2). For both DNA sources, the difference in recovery was statistically significant, with the p-value of 0.018 for cells in EB and $p = 0.015$ for the sebum deposits. The discrepancies between the amounts of DNA recovered from cells in EB and synthetic sebum solution can be explained by the tendency of cells to be preserved better in synthetic sebum than in a buffer during the process of drying the sample onto the surface [14].

The recovery rate of DNA from saliva aliquots on tape was 13% higher for the PrepFiler extracts (Fig. 3). However, despite the better performance of the PrepFiler method, the difference between results was not statistically significant. The opposite outcome was observed for the blood deposits (Fig. 3), where the PVP extraction significantly outperformed the PrepFiler extraction method ($p = 0.003$) and resulted in almost 60% higher DNA yield.

With higher DNA recovery rates observed for half of the tested source materials extracted with the PVP method, the results of this short study clearly show that the PVP extraction method can rival the commercially available extraction kits. A similar outcome was observed in a study by Stoop et al. [7], where the phenol-chloroform extraction method was more effective for DNA recovery from saliva on tapes when compared to commercially used kits, one of which was PrepFiler Express BTA™ Kit. Stoop et al. [7] speculate that the reason for the less efficient performance of the PrepFiler kit was a possible interference of the glue from the adhesive tapes with the beads. However, this explanation cannot be applied in our case as both methods included bead purification as part of the extraction process. We did not compare our method to phenol-chloroform extraction due to concerns with handling the toxic chemicals involved.

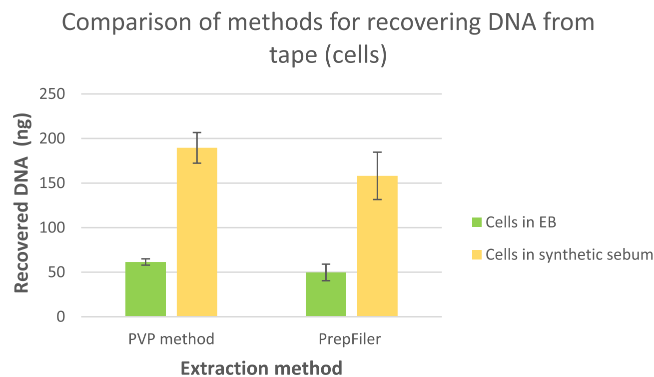

Fig. 2. The average DNA recovery yield from mouse cells in EB and in synthetic sebum deposited on SceneSafe Fast™ minitapes and extracted using the PVP-based extraction method and PrepFiler™ BTA Forensic DNA Extraction Kit.

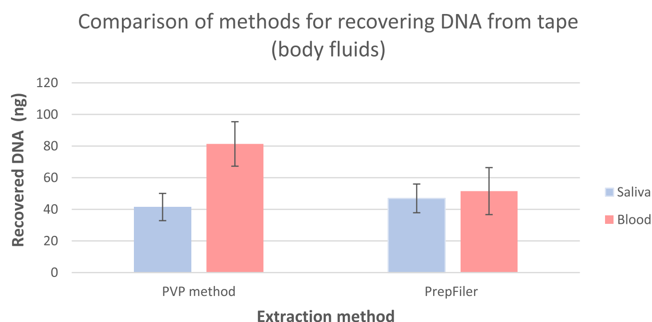

Fig. 3. The average DNA recovery yield from human saliva and bovine blood deposited on SceneSafe Fast™ minitapes and extracted using the PVP-based extraction method and PrepFiler™ BTA Forensic DNA Extraction Kit.

One of the reasons that could explain the loss of DNA in the PrepFiler kit is frequent transfers of the supernatant into a new, clean tube. In the PVP extraction method, the whole extraction process is undertaken in a single tube until the final step of transferring eluted DNA post-bead purification. Minimised approaches to DNA extraction have been shown to increase DNA yield and outperform methods involving multiple purification and sample transfer steps [17]. This simplified approach has also been successfully applied to DNA recovery from tapes [9]. One-tube reaction not only saves time but also limits the chance of sample contamination. The main advantage of the PVP-based extraction is the low cost per sample when compared with the PrepFiler™ method. The estimated cost per sample using the PVP method is less than 12p versus approximately £ 3.50 per sample for the PrepFiler™ kit (not including the cost of multiple Eppendorf tubes used during extraction).

The efficiency of SPRI bead purification

The PrepFiler™ protocol includes incubation of DNA extracts with 15 μL of magnetic beads and pellet resuspension by vortexing for the wash step. It is important to note that this step could not be replicated exactly due to the unknown composition and concentration of the PrepFiler™ magnetic particles. A comprehensive comparison of the effect of bead concentration and buffer composition is given in a detailed study by Liu et al. [18]. It would appear that for the SPRI bead precipitation method to work, certain basic parameters must be followed, such as controlling the salt and peg8000 concentrations as detailed by Liu et al. [18]. Given the high binding capacity of the SPRI beads (>3 μg DNA/μL beads), it is improbable that we are using a bead concentration that is limiting. In order to determine if the slightly different SPRI purification steps had any impact on DNA recovery, we tested different approaches to the SPRI bead-based parts of the methods, comparing the protocols with and without the vortexing step and using either 8 or 15 μL of the SPRI beads. The samples tested were saliva and blood deposits from tape and blood directly to buffer, and the purification protocol for the PVP extraction was carried out for the purification step from the PrepFiler™ manual to obtain an accurate comparison between the two extraction methods.

The results of this comparison are shown in Fig. 4. Samples were quantified directly after extraction, with no purification step, which resulted in no detectable results for any of the blood deposits and just over 3 ng of DNA present in the saliva samples (Fig. 4). Failed detection of DNA in the blood samples is expected due to the presence of PCR inhibitors [19,20] in the DNA extracts. Incubating with 8 μL (approximately half of the volume used in the initial protocol based on the PrepFiler™ method) of Sera-Mag™ Carboxylate-Modified Magnetic Beads (10 mg/mL) and processing as described in materials and methods but leaving the samples on the magnetic stand during the ethanol wash steps, resulted in a lower DNA recovery compared to including the vortex step for all the blood deposits. However, the difference in recovery was only statistically significant for the

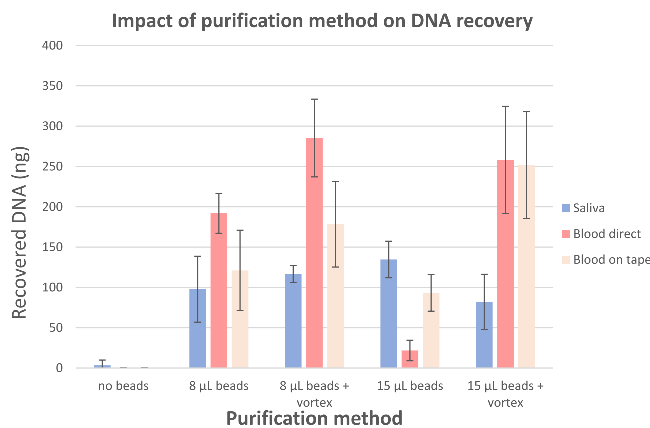

Fig. 4. The comparison of purification methods for DNA recovery from saliva aliquots on tape and blood deposits on tape and directly into the PVP extraction buffer.

direct-to-buffer blood deposits ($p = 0.0278$). The improvement in recovery with the addition of the vortex step was also observed when using 15 μL of SPRI beads (Fig. 4), with significantly higher values for both the direct-to-buffer deposits ($p = 0.002$) and blood on tape samples ($p = 0.003$) This suggests that adding a vortex step may help in the removal of strong PCR inhibitors leading to an apparent increase DNA recovery.

Overall, a clear improvement in DNA recovery could be observed after the addition of the purification step, with almost 100 ng average recovery for saliva tape deposits and close to 200 and 120 ng of DNA recovered from direct and blood-on-tape deposits, respectively (Fig. 4). This is not surprising as magnetic beads have been shown to aid with elimination of PCR inhibitors [21]. The difference in DNA recovery between direct blood deposits and blood aliquots could be explained by the possible interference of the glue from the adhesive tapes with the beads [7].

Increasing the volume of Carboxylate-Modified Magnetic Beads (10 mg/mL) from 8 μL to 15 μL per sample without the vortexing step did not result in any significant increase in DNA recovery, and in the case of the direct purification from blood resulted in a decrease in the recovered DNA. The addition of the vortexing step with the 15 μL of beads resulted in an increase in recovery compared to non-vortexed samples, with perhaps a slight reduction in recovery from saliva. The greatest impact on DNA yield could be observed in the direct-to-buffer blood aliquots, where the average recovered DNA amount was less than 22 ng. This was a significant decrease from the previous two experiments based on the 8 μL bead volume approach. The blood on tape aliquots also showed lower DNA yield when compared to the results from the 8 μL bead-purification samples. This may suggest that in samples with less potent PCR inhibitors, increasing the concentration of magnetic beads may increase DNA recovery. In the presence of strong PCR inhibitors such as those found in blood [22], increased bead concentration seems to have the opposite effect. Adjusting bead concentrations has been previously shown to impact DNA recovery. Liu et al. demonstrated that decreased concentration of beads and increased DNA recovery can be inversely correlated [18].

The addition of the vortex step has had a significant impact on DNA recovered for direct-to-buffer and blood tape aliquots (Fig. 4) when compared with non-vortexed samples during ethanol wash. In the case of blood on tape samples, this purification method resulted in the highest DNA yield out of four tested clean-up protocols. For the saliva samples, the addition of the vortex step decreased the amount of recovered DNA. Additionally, this purification technique resulted in the lowest DNA yield for these samples out of four tested approaches.

For the three types of sources tested, it was not possible to determine the best purification technique suitable for all sample types. These results demonstrate that the efficiency of the purification method is highly dependent on the type of biological material used in DNA extraction. It is also important to consider how even slight modifications of the purification step can impact DNA recovery.

Based on results from blood aliquots, it seems clear that regardless of the concentration of beads in the sample, pellet resuspension during the alcohol wash in a purification step can significantly improve DNA recovery from biological samples with strong PCR inhibitors.

Relying on the same purification method for all kinds of biological samples as recommended by the manufacturer may not yield the best outcome. It is not the first time that a more individual, sample-by-sample approach and diverting from the established protocols resulted in higher DNA recovery [23–26]. Nevertheless, further tests with various types of biological samples are required to maximise the efficiency of the purification step.

Conclusions

The outcome of this short study shows that alternative DNA extraction methods can be as good as, or more efficient, at DNA recovery than commercial forensic kits. Additionally, the PVP method greatly reduces the number of steps and costs involved in the extraction, lessening the chance of contamination while making the process less time-consuming and more cost-efficient. Furthermore, we have shown that modifying the purification step in the extraction method can lead to a much higher DNA yield. Naturally, this method requires more validation but demonstrates that there is potential for investigating and developing more efficient DNA extraction techniques.

Ethical approval

This project has been approved by the University of Dundee ethics committee. The volunteer signed an informed consent prior to donating their saliva sample for this study.

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CRediT authorship contribution statement

Kuffel Agnieszka: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gray Alexander:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Nic Daeid Niamh:** Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsir.2023.100350.

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