

# Laser microdissection and pressure catapulting with PALM<sup>®</sup> to assist typing of target DNA in dirt samples

B.L. Lambie-Anoruo<sup>a,b</sup>, D.V. Prince<sup>a,b</sup>, I. Koukoulas<sup>a</sup>,  
D.W. Howells<sup>c</sup>, R.J. Mitchell<sup>b</sup>, R.A.H. van Oorschot<sup>a,\*</sup>

<sup>a</sup> Victoria Police Forensic Services Department, Victoria 3085, Australia

<sup>b</sup> Department of Genetics and Human Variation, La Trobe University, Australia

<sup>c</sup> Austin Health, Department of Medicine, University of Melbourne, Australia

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**Abstract.** Retrieval of genetic profiles from human biological samples mixed with dirt can be difficult using standard DNA extraction methods. Isolation of target cells from debris, using laser microdissection and pressure catapulting prior to DNA extraction, can improve the recovery of genetic profiles of the target component from such samples. © 2005 Elsevier B.V. All rights reserved.

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## 1. Introduction

Obtaining a DNA profile from small biological samples when they are mixed with debris, such as dirt, can be difficult. Use of the PALM<sup>®</sup> laser microdissection and pressure catapulting process [1,2] may assist in the retrieval of target DNA and subsequent DNA profiling in these situations. Laser isolation has been helpful in generating genetic profiles from sperm cells isolated from a microscope slide [3]. Here we test the capability of PALM<sup>®</sup> to isolate saliva cells from mixtures with dirt.

## 2. Materials and methods

Saliva (sample from a single individual) was mixed with dried dirt (humus rich soil taken from a nearby forest) in a 3:2 volume ratio to produce two mixtures, each of 500 µl.

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\* Corresponding author. Tel.: +61 3 9450 3528; fax: +61 3 9450 3601.

*E-mail address:* roland.vanoorschot@police.vic.gov.au (R.A.H. van Oorschot).

One was used immediately to generate the fresh sample series. The other was dried for 7 days after which time the sample was reconstituted with 300 µl of distilled water to generate the aged sample series. Two 300 µl aliquots of the same saliva were also placed into separate tubes (one used immediately and the other dried for 7 days and reconstituted using 300 µl of distilled water) to be used as controls (fresh and aged, respectively).

Aliquots of 20 µl (~ 12 µl saliva plus 8 µl soil) were placed in separate 1.5 ml centrifuge tubes for DNA extraction or on to a PALM membrane slide (Millennium Science). Samples placed on slides were dried prior to staining with haematoxylin and eosin, cell isolation and DNA extraction.

DNA was extracted using either 5% Chelex® (Bio-Rad) solution [4] followed by Microcon® (Amicon) concentration or organic methodology [5,6]. DNA concentrations and amounts were determined using Quantifiler™ (Applied Biosystems). Samples that did not give quantitative values were purified using QIAquick® (Qiagen) and again quantitated. All samples were typed using AmpFISTR Profiler Plus™, ABI PRISM® 3100 Genetic Analyser and GeneMapper™ software (Applied Biosystems). Cells were isolated from the PALM membrane slides using the roboLPC laser function of the PALM microlaser (Millennium Science) and placed into lids of 0.5 ml microfuge tubes containing 25 µl distilled water.

### 3. Results

Table 1 illustrates that routine DNA extraction methods, such as Chelex and organic, are unable to provide typeable DNA from some biological samples contaminated/mixed with soil. The use of QIAquick clean-up techniques, post Chelex or organic extraction, improved the ability to generate DNA profiles from such samples. The use of PALM to isolate the available target cells from the soiled saliva samples, followed by DNA extraction from these cells for typing, also enhanced the

Table 1

Average amounts (ng) of DNA retrieved, and ability to provide genetic profiles,<sup>a</sup> from soiled saliva samples (based on four replicates each)

Sample	Volume saliva	Chelex	Chelex + QIAquick	Organic	Organic + QIAquick	200 cells PALM Chelex	47 cells <sup>b</sup> PALM Chelex
<i>Fresh</i>							
Control	20 µl	81.3 (4F)		209 (4F)		0.104 (4F)	
Soiled	12 µl	neg <sup>c</sup>	16.5 (2N,2F)	neg <sup>c</sup>	29.7 (4F)	0.457 (4F)	0.168 (P <sup>18</sup> )
<i>Aged</i>							
Control	20 µl	4.8 (4P <sup>14-17</sup> )		34 (4F)		0.130 (4P <sup>13-17</sup> )	
Soiled	12 µl	neg <sup>c</sup>	neg <sup>c</sup>	neg <sup>a</sup>	0.08 (2N,2P <sup>Amel</sup> )		0.013 (P <sup>3</sup> )

<sup>a</sup> The ability to generate profiles is given in parentheses. N=no profile; F=full profile; P=partial profile. The number preceding the capital letter relates to the number of such profiles generated. The number in superscript after the capital letter relates to the number of alleles observed within the partial profiles. Superscript Amel means that only Amelogenin alleles were identified.

<sup>b</sup> It was more difficult to find saliva cells on the membrane of aged soiled samples and only a portion of those isolated had an obvious nucleus. The 47 cells of the fresh soiled sample all had an obvious nucleus. Only one replicate was done for each of these samples.

<sup>c</sup> Negative results were obtained using Quantifiler. No genetic profiles were generated.

ability to provide DNA profiles from such samples. Note, only a very small proportion of the available cells were isolated using PALM for further extraction and typing (i.e. 200 compared to approximately 50,000 and 30,000 estimated to be in the 20 and 12  $\mu$ l saliva samples, respectively) and more could be isolated, if needed, to enhance the ability to create full profiles.

Our results indicate that the use of PALM can improve the chance of generating genetic profiles from biological samples mixed with debris that presently pose difficulties for current DNA extraction methods.

#### **4. Discussion**

The retrieval of DNA from the aged control samples, compared to the fresh samples, was reduced 16- and 6-fold for Chelex and organically extracted samples, respectively. Further, the yield from the aged soiled samples was 360-fold less than the equivalent fresh sample. Whilst there was no reduction in DNA yield from the PALM isolated 200 saliva cells derived from the aged control sample compared to the fresh sample, there was an 8- and 35-fold reduction in DNA yield from the 47 cells isolated from the aged soiled sample compared to that retrieved from the 47 and 200 cells, respectively, isolated from the fresh soiled sample. Aging of the sample appears to have a negative effect on the recovery of DNA when using standard extraction methods but not when applying PALM isolation techniques. The observations also imply that the amount of quality DNA derived from the saliva cells is significantly reduced when left mixed with dirt (at least the type of dirt used here). Early collection and processing of casework samples, in which the biological material is mixed with dirt, may improve the acquisition of genetic profiles from such samples.

Further research is required to improve the isolation, DNA extraction and typing of target cells from various types of casework samples in which the cells are either overwhelmed by inhibiting/degrading substances and/or are in a clear minority within a mixture of cells (of different origin) such that a genetic profile would not normally be identified from them.

#### **Acknowledgments**

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