

Bringing Laser Microdissection and On-Chip PCR Together

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We combined laser microdissection and pressure catapulting with low-volume on-chip PCR to obtain full DNA profiles from as few as five cells.

INTRODUCTION

Recent advances in microscopic instrumentation allow direct visualization, dissection and catapulting of defined cells and tissue sections from microscope slides using a focused laser beam. This approach is called laser microdissection and pressure catapulting (LMPC). LMPC is a high-precision laser-manipulation technique that yields pure cellular preparations (1). In the field of forensic medicine, laser microdissection has been reported previously for isolation of sperm and dissection of tissue sections (2–4). Robust amplification methods that generate reliable profiles from very few individual cells are necessary for success.

Downscaling reaction volumes is one possible solution to improve genotyping success. When working with chemically structured chips, complete short tandem repeat (STR) profiles from as little as 32 pg of genomic DNA have been reported (5).

So why not use these techniques together? We have developed a technique that directly links LMPC with low-volume on-chip PCR.

FLYING FROM A SLIDE—LANDING ON A SLIDE: LASER MICRODISSECTION AND SUBSEQUENT CATAPULTING OF CELLS DIRECTLY ON AMPLIGRID® CHIPS

We collected catapulted cells directly onto chemically modified chips that were positioned upside-down on a computer-driven manipulator platform, then performed multiplex PCR amplification of STR loci directly on these chips. We used lymphocytes (dendritic cells) as well as buccal cells of known genotypes. Morphological stains were prepared on polyethylene naphthalate (PEN) membrane slides by a routine Hemacolor® staining procedure. A PALM® MicroBeam (Carl Zeiss MicroImaging) was used for microdissection and capture of cells. We used AmpliGrid® AG480F microliter reaction slides (Advalytix AG) as a collection platform for single-cell deposition and amplification. These slides offer 48 hydrophilic reaction sites, each surrounded by a hydrophobic circle to hold aqueous PCR reagents in place (Figure 1, Panel A).

Different numbers of cells (1, 5, 10 and 20 cells) were catapulted onto reaction sites coated either with water or 1% glycerol. The automated microdissection was performed at 40X magnification.

ON-CHIP PCR

In our study we used the PowerPlex® 16 System^(a-c) (Promega) for multiplex PCR analysis of STR loci. The total reaction volume of 1 µl was covered with 4–5 µl of sealing solution to prevent evaporation. Thermal cycling was performed using an in situ adapter for the Eppendorf Mastercycler® thermal cycler. Standard amplification protocols and rigorous interpretation of STR profiles common to forensic laboratories were used.

LMPC

RESULTS

Interpretable profiles were obtained with as few as one cell when using lymphocytes (Table 1). Full profiles (all alleles detected) were obtained with as few as five lymphocytes (Figure 2).

The staining procedure, which contributed significantly to cell

identification, did not affect the quality of STR profiling, since identical results were obtained with nonstained cells (data not shown). When working with buccal epithelial cells, a significantly lower efficiency of profiling was achieved. Satisfactory profiles were obtained when 20 or more buccal cells were amplified per PCR.

DISCUSSION

The discrepancy between the profiling efficiency for cells of different tissues appears to be due to incomplete cell lysis. For more robust cells such as sperm, the cell lysis procedure needs to be optimized. For this purpose, we are currently developing a protease-based cell lysis protocol. Realistically, two consecutive reaction steps can be performed on chemically structured chips. This is supported by the successful amplification of hypervariable regions of mitochondria followed by subsequent sequencing with chain terminators (6).

Working with on-chip PCR provides several advantages: 1) The procedure described here is more flexible and faster than preparing individual reaction tubes. 2) Due to the glass substrate of the AmpliGrid® AG480F slides, the workflow from cell deposition to the PCR setup stage can be controlled and documented using a standard microscope setup. 3) Robotic pipetting solutions for the AmpliGrid® setup are available from various vendors. 4) Working with small volumes results in lower analysis costs in the long term. The drawback of using chemically structured chips is that replication of the same reaction from cells already catapulted is not possible. The possibility of testing very low amounts of material in parallel compensates for this. Our direct technique lacking further extraction steps might be of major interest when working with single cells to analyze multicopy or abundant targets (mRNAs, mitochondrial DNA).

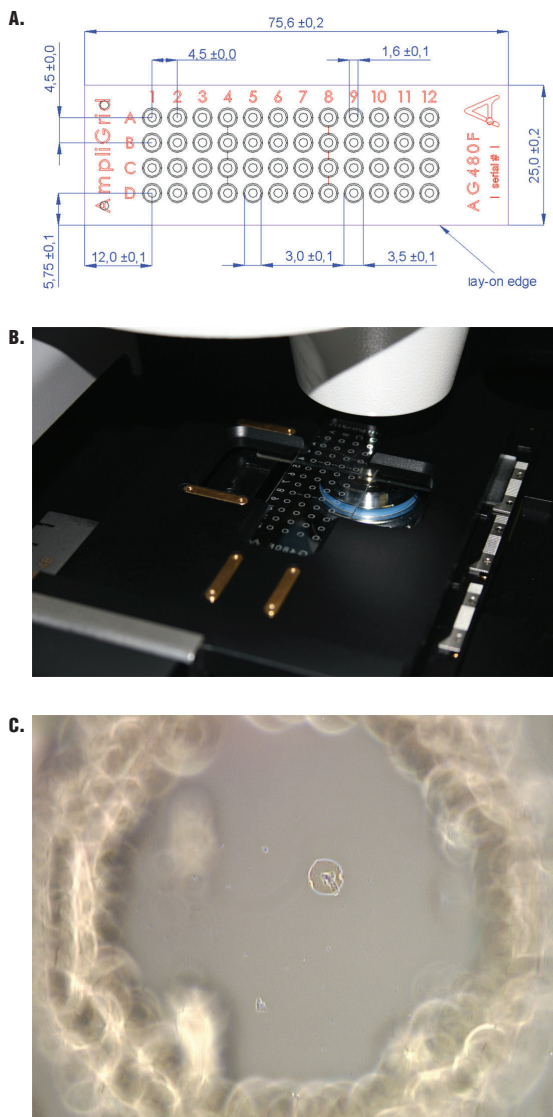


Figure 1. Panel A. AmpliGrid® AG480F microliter reaction slide. Panel B. PALM® Slide Collector 48 with AmpliGrid® slide. Panel C. One cell with a piece of membrane as deposited at one reaction site.

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Table 1. Efficiency of PowerPlex® 16 Profiling of Microdissected Lymphocytes.

Result	Number of Cells per Anchor Spot ¹			
	1 cell	5 cells	10 cells	20 cells
complete allelic profile ²	–	3	3	6
incomplete but satisfactory profile ³	3	2	3	1
unsuccessful amplification ⁴	5	3	2	1

¹Eight replicates (4 stained and 4 unstained) were analyzed for each cell batch.
²All allelic peaks were detected (>100 RFU) and well balanced; no allelic “drop outs”.
³Not all alleles were detected; peak imbalance but still interpretable profiles (more than 50% were typed correctly).
⁴More than 50% of alleles “dropped out”; no alleles were detected.

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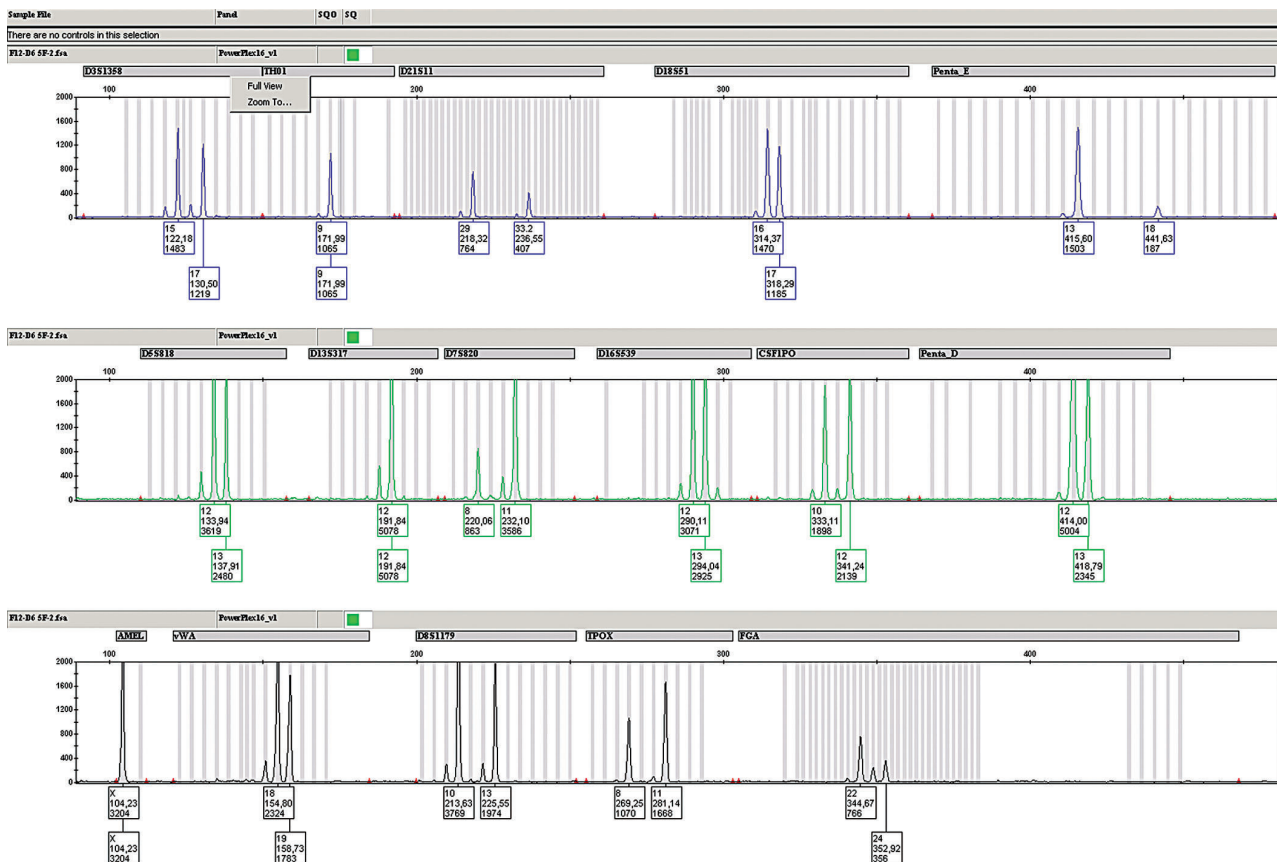


Figure 2. PowerPlex® 16 profile generated from five lymphocytes. A complete profile is reported here, although low-copy number effects (allelic imbalance and higher stutter peaks) remain visible.