Benchmarks

Laser Capture Microdissection of Paraffin-Embedded Tissues

BioTechniques 34:42-46 (January 2003)

Current research on the molecular basis of cancer and other complex diseases involves mapping cellular pathways and identifying molecular alterations associated with disease onset and progression. The ability to charac-

terize gene and protein variation in diseased versus normal tissues requires homogeneous populations of cells free from contamination by other cell types. With advances in genomic and proteomic technologies, nucleic acids and proteins can now be quantified in a small number of target cells obtained from a limited amount of tissue. The increased demand for selective isolation of pure cell populations from small quantities of tissue calls for refined and more efficient methods of tissue microdissection.

Laser-assisted microdissection is an emerging technology with increasing applications in life science research and biotechnology. Laser microdissection permits selective transfer and recovery of cells from histological tissue sections with far greater speed and precision than manual dissection methods. Homogeneous populations of specific cell types can be easily isolated from complex tissues, and the captured material can be verified microscopically. Because the microdissection process maintains the integrity of nucleic acids and proteins, detailed molecular studies of specific cell types can be conducted on heterogeneous primary tissues (2,5,7). For example, laser microdissection has become an important component of cancer research for separating cancer cells from normal cells in heterogeneous carcinomas to better characterize molecular differences between diseased and normal cells. Normal cells have structurally intact genomes and normal gene expression profiles,

Figure 1. Visualization of microdissected cells and cell capture in the target area. Tissue sections from two breast ductal carcinomas in situ, a premalignant form of breast disease in which cells proliferate abnormally within the mammary ducts, mounted on a traditional glass microscope slide (A) and on a foil slide (B). After laser capture of cells mounted on traditional glass slides, it is often difficult to visualize cells that have already been targeted for capture, particularly in dense tissue sections such as in situ tumors (C). In contrast, there is a distinct change of color after laser capture of cells from tissue mounted on reinforced foil slides, making it easy to differentiate captured from uncaptured cells (D). Cells and tissue fragments often remain on glass slides following laser microdissection (E), but foil slides permit more efficient microdissection with virtually no tissue or cell remnants (F). Laser microdissection was conducted on a PixCell II microdissection apparatus, with aperture setting at 30 μ m, laser pulse power at 50 mW, and laser pulse duration at 6 ms.

Figure 2. Electrophoresis of amplified DNA.

Approximately 125 cells were collected from each slide type using 25 laser pulses on each slide (aperture setting, 30 μ m; laser pulse power, 50 mW; and laser pulse duration, 6 ms). DNA was extracted from each sample by overnight digestion in Proteinase K (0.4 mg/mL) at 37°C and purified by centrifugation at 10000 $\times$ *g* through Microcon[®] YM-50 centrifugal filters (Millipore, Bedford, MA, USA). Equal aliquots of purified DNA were then subjected to PCR amplification using the microsatellite marker D2S1045. The ethidium bromide-stained 1% agarose gel demonstrates an improvement in amplification efficiency from DNA extracted from a standard glass slide (lane 2) to DNA extracted from a foil slide (lane 3). Lane 1 contains the EZ Load[™] Precision Molecular Mass Standard (Bio-Rad Laboratories, Hercules, CA, USA) with bands of 1000 bp (100 ng), 700 bp (70 ng), 500 bp (50 ng), 200 bp (20 ng), and 100 bp (10 ng).

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Table 1. Procedure for Mounting and Staining Histological Sections on Foil Slides

Mounting
1. Coat foil slide with gelatin (8 mg gelatin in 40 mL double-distilled water; warm to 40°C for 5 min) to improve tissue adherence.
2. Cut tissue sections at 5 µm and float in 42°C water bath.
3. Adhere tissue section to coated slide and air-dry at room temperature overnight.
Staining
1. Immerse slide sequentially in:
a. Sub X (xylene substitute), two times for 1 min each
b. 100% ethanol, two times for 30 s each
c. 95% ethanol for 30 s
d. 70% ethanol for 30 s
e. double-distilled water for 30 s
f. Mayers Hematoxylin for 3 min
2. Rinse with tap water until clear and then immerse in Bluing Reagent for 30 s.
3. Wash in running tap water for 5 min and then:
a. 70% ethanol for 1 min
b. 95% ethanol for 1 min
c. Eosin Y for 30 s
4. Rinse in double-distilled water until clear.
5. Air-dry at room temperature.

which, if not separated from histologically abnormal cells, may obscure genetic abnormalities such as loss of heterozygosity or aberrant gene expression in developing tumors (3,8,9).

Laser microdissection using the PixCell® II laser-capture microdissection

apparatus (Arcturus Engineering, Mountain View, CA, USA) entails placing a CapSure™ transparent plastic cap (Arcturus Engineering) containing a thermoplastic membrane over a section of tissue, visualizing the tissue microscopically, and selectively adhering

cells of interest to the membrane with a short focused pulse from an IR laser (4). Tissue sections are normally mounted on glass microscope slides to provide support for the CapSure cap during microdissection. However, experience in our laboratory with dense tissue sections characteristic of in situ and invasive breast tumors has shown that capture of cells within the target area is often inefficient because cells and tissue fragments may remain adhered to the glass slide even after repeated attempts at capture. In addition, during the process of laser microdissection on hematoxylin and eosin-stained slides, it is often difficult to discriminate visually cells that have already been captured from cells that have not.

We have found that the efficiency of laser microdissection on paraffin-embedded tissues when using the PixCell II apparatus can be increased substantially by mounting tissue sections on foil slides (Leica Microsystems, Wetzlar, Germany) rather than on traditional glass microscope slides recommended for the PixCell II. The foil slides, consisting of a plastic frame that supports a thin plastic film, are designed for use with an ASLMD Laser Microdissection system (Leica Microsystems). The ASLMD microdissection platform uses laser ablation technology where histological sections are mounted on the plastic film, cells of interest are visualized and outlined on a computer screen using the mouse, a pulsed UV laser cuts the plastic film along the user-drawn outline, and the material excised by the laser falls by the force of gravity into a capture tube located directly beneath the slide.

The protocol for mounting and staining tissue sections on the foil slides (Table 1) is similar to the procedure traditionally used for glass slides. Tissues are processed as permanent sections in formalin, alcohol, xylene, and hot paraffin, and then sectioned at 5 µm using a microtome. Histological staining of mounted tissue sections with hematoxylin and eosin is then used to visualize cellular morphology. When working with foil slides, notable time-saving modifications such as a shorter initial deparaffinization time in Sub X (2 min vs. 15 min) and elimination of the dehydration wash in alcohol

Figure 3. Electropherogram. DNA extracted and purified from cells microdissected from foil slides (panel B) produces greater fluorescent signal intensity (>8000 rfu) in the resulting electropherogram than DNA from cells captured from glass slides (>2000 rfu, panel A). The electrophoretic trace in panel B also contains fewer extraneous peaks, which may be incorrectly scored as alleles, than the trace in panel A. Green bars under each trace represent alleles called automatically by the Genetic Profiler (version 1.1) software. Samples were analyzed on a MegaBACE 1000 capillary electrophoresis DNA analysis system. The allele size range in base pairs for microsatellite marker D22S1045 is indicated along the bottom of each panel and the range of fluorescent signal intensity in relative fluorescence units (rfu) is indicated on the y-axis of each panel.

and Sub X after eosin staining can be used. It is important to note that during laser microdissection, it is necessary to provide support for the CapSure collection cap, which is easily accomplished by mounting (taping) the foil slide to a standard glass slide.

Laser microdissection of tissue sections mounted on foil slides is more efficient than capture of cells mounted on glass slides (Figure 1). On traditional glass slides, it may be difficult to visualize cells that have already been targeted for capture, but tissues mounted on foil slides change color once they adhere to the collection cap, making it easy to discriminate captured from uncaptured cells. In addition, collection of cells from foil slides is more complete than cell capture from glass slides. Using the technique described above ensures capture of virtually all cells in the target area on the first attempt.

We quantified the improved efficiency of laser capture microdissection from foil slides versus traditional glass slides using 25 laser pulses to capture approximately 125 cells from each type of slide. DNA was extracted from each sample and purified by centrifugation. Equal aliquots of purified DNA were then subjected to PCR amplification using a random microsatellite marker D22S1045 (Invitrogen, Carlsbad, CA, USA), and the resulting PCR products were visualized on a 1% agarose gel stained with ethidium bromide (Figure 2). As shown in Figure 2, the PCR amplification from DNA extracted from a foil slide is more efficient (produces more product) than the sample obtained from the standard glass slide. We also examined the electrophoretic traces generated by analyzing the amplified samples on a MegaBACE™ 1000 capillary electrophoresis system (Amersham Biosciences, Piscataway, NJ, USA). The DNA sample extracted and amplified from cells microdissected from foil slides showed (a) greater fluorescent signal intensity (>8000 rfu) in the resulting electropherogram than the DNA from cells captured from glass slides (>2000 rfu, panel A) and (b) fewer extraneous peaks, which could be incorrectly scored as alleles (Figure 3).

Formalin-fixed, paraffin-embedded tissues are an important and widely available source of pathological speci-

mens representing different disease stages and histological tumor types. Numerous studies have successfully isolated DNA of sufficient quality from archival tissues for quantification by real-time PCR (6), cytogenetic heterogeneity analysis by comparative genomic hybridization (1), or loss of heterozygosity assays (10). We have successfully captured cells from formalin-fixed, paraffin-embedded tissues using foil slides for recovery of DNA suitable for PCR amplification and microsatellite genotyping. Our technique for laser capture microdissection yields greater quantities of DNA than amounts obtained from glass slides, improves the robustness of subsequent PCR amplifications, and produces cleaner electropherograms for more reliable fluorescent genotyping.

REFERENCES

1. Aubele, M., A. Mattis, H. Zitzelsberger, A. Walch, M. Kremer, P. Hutzler, H. Hofer, and M. Werner. 1999. Intratumoral heterogeneity in breast carcinoma revealed by laser-microdissection and comparative genomic hybridization. *Cancer Genet. Cytogenet.* 110:94-102.
2. Banks, R.E., M.J. Dunn, M.A. Forbes, A. Stanley, D. Pappin, T. Naven, M. Gough, P. Harnden, and P.J. Selby. 1999. The potential use of laser capture microdissection to selectively obtain distinct populations of cells for proteomic analysis—preliminary findings. *Electrophoresis* 20:689-700.
3. Bertheau, P., L.F. Plassa, F. Lerebours, A. de Roquancourt, E. Turpin, R. Lidereau, H. de Thé, and A. Janin. 2001. Allelic loss detection in inflammatory breast cancer: improvement with laser microdissection. *Lab. Invest.* 81:1397-1402.
4. Emmert-Buck, M.R., R.F. Bonner, P.D. Smith, R.F. Chuaqui, Z. Zhuang, S.R. Goldstein, R.A. Weiss, and L.A. Liotta. 1996. Laser capture microdissection. *Science* 274:998-1001.
5. Fend, F. and M. Raffeld. 2000. Laser capture microdissection in pathology. *J. Clin. Pathol.* 53:666-672.
6. Lehmann, U. and H. Kreipe. 2001. Real-time PCR analysis of DNA and RNA extracted from formalin-fixed and paraffin-embedded biopsies. *Methods* 25:409-418.
7. Maitra, A., I.I. Wistuba, and A.F. Gazdar. 2001. Microdissection and the study of cancer pathways. *Curr. Mol. Med.* 1:153-162.
8. Rubin, M.A. 2001. Use of laser capture microdissection, cDNA microarrays, and tissue microarrays in advancing our understanding of prostate cancer. *J. Pathol.* 195:80-86.
9. Wernert, N., C. Locherbach, A. Wellmann, P. Behrens, and A. Hugel. 2001. Presence of genetic alterations in microdissected stroma of human colon and breast cancers. *Anticancer Res.* 21:2259-2264.
10. Wild, P., R. Knuechel, W. Dietmaier, F. Hofstaedter, and A. Hartmann. 2000. Laser microdissection and microsatellite analyses of breast cancer reveal a high degree of tumor heterogeneity. *Pathobiology* 68:180-190.

Address correspondence to Dr. Darrell L. Ellsworth, Windber Research Institute, 600 Somerset Avenue, Windber, PA 15963, USA. e-mail: d.ellsworth@wriwindber.org

Received 22 July 2002; accepted 2 October 2002.

**D.L. Ellsworth, C.D. Shriver¹,
R.E. Ellsworth, B. Deyarmin,
and R.I. Somiari**

*Windber Research Institute
Windber, PA*

¹*Clinical Breast Care Project
Walter Reed Army Medical
Center*

Washington, D.C., USA

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