Technical Advance

A New Method for Histological Microdissection Utilizing an Ultrasonically Oscillating Needle

Demonstrated by Differential mRNA Expression in Human Lung Carcinoma Tissue

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Molecular analysis of microdissected tissue samples is used for analyzing tissue heterogeneity of histological specimens. We have developed a rapid one-step microdissection technique, which was applied for the selective procurement of tissue areas down to a minimum of 10 cell profiles. The special features of our microdissection system consist of an ultrasonically oscillating needle and a piezo-driven micropipette. The validity of this technique is demonstrated in human lung large-cell carcinoma by real-time quantitative reverse transcriptase-polymerase chain reaction assays of vimentin, cyclin D1, and carcinoembryonic antigen after linear RNA amplification. mRNA expression values of microdissected samples scattered around those of bulk tumor tissue and showed differential mRNA expression between samples of tumor parenchyma and supportive stromal cells for vimentin and carcinoembryonic antigen as confirmed by immunohistochemistry. In conclusion, this procedure requires simple equipment, is easily performed, and delivers microdissected tissue samples of oligocellular clusters suitable for further molecular analysis. (Am J Pathol 2001, 158:1985–1990)

The study of molecular genetic alterations that occur in tumors requires a precise correlation of molecular genetic characteristics to defined cell populations. In addition to the heterogeneity of the neoplastic cell, tumor tissues are composed of a variable admixture of stromal cells, inflammatory infiltrates, endothelial cells, and pre-

existing tissue. The presence of multiple cell types may dilute out the significant changes that occur in specific cells and therefore most sophisticated methods in molecular pathology are of limited value when applied to bulk tissue.¹ Several mechanical techniques for microdissection have been developed to isolate cells for analysis from histological sections. These include starch-based adhesive fractionation,² the use of scalpel blades,³ fine needles,^{4–6} and pipettes,⁷ either hand-held or connected to micromanipulators. In an alternative method, isolation involves the destruction of unwanted genetic material by UV irradiation after ink protection of selected cells.⁸ Although these methods achieve good precision they are time-consuming and laborious. UV lasers are used to ablate unwanted tissue or to cut around selected cell(s) to avoid contamination from adjacent tissue. Cells thus isolated were then retrieved with needles (laser-assisted cell picking)^{9–11} or catapulted with slightly defocused laser shots directly into caps of PCR tubes to procure cells in a noncontact manner (laser pressure catapulting, LPC).¹² Membrane-mounted tissue facilitates removal of selected cells for either needle transfer or LPC.^{12,13} In laser capture microdissection (LCM)¹⁴ an infrared laser focally melts a transfer film, which fuses to underlying selected cells. Both, LCM and LPC allow an especially rapid dissection and are easy to perform; however, the equipment required for laser-assisted methods is very expensive. To overcome these drawbacks of current microdissection methods, we developed a new mechanical technique, using an ultrasonically oscillating needle and a piezo-driven micropipette for rapid one-step histological microdissection. To validate our technique parenchymal and stromal cells of human lung large-cell carcinomas were quantitatively an-

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alyzed for differential mRNA expression of the intermediate filament vimentin, carcinoembryonic antigen (CEA), and the proliferation-associated antigen cyclin D1 and the results were compared to their immunohistochemical distribution. We used an analytical protocol that allows quantitation of multiple transcripts from a single microdissected sample as small as 10 cells, using linear amplification of RNA followed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR).

Materials and Methods

Tissue Preparation

Parallel blocks of fresh tumor tissues were fixed in 4% buffered formaldehyde and embedded in paraffin or flash-frozen in liquid nitrogen and stored at -80°C until further analysis. Paraffin sections were immunostained for vimentin (monoclonal antibody M7072, 1:400 dilution; DAKO, Hamburg, Germany) and CEA (monoclonal antibody M7020, 1:100 dilution; DAKO) using the DAKO ChemMate Detektionskit (DAKO) according to the manufacturer's protocol.

For microdissection, 10- μm sections were cut from frozen blocks on a standard cryostat and mounted on plain glass slides. Sections were immediately hematoxylin and eosin-stained and dehydrated in graded alcohols and xylene (10 seconds each). All solutions were prepared with diethyl pyrocarbonate-treated H_2O . To improve RNA stability, slides of uncovered cryosections were stored at 4°C on silica gel in an exsiccator.

Microdissection

Microdissection was performed using the prototype of a new commercial microdissection device (MicroDissector; Eppendorf AG, Hamburg, Germany). The system consists of a cutting head, a micropipette, and an electronics box to control them. Both tools (weight <50 g) were mounted on joystick-controlled three-axis motorized micromanipulators (TransferMan, Eppendorf AG) that were attached to an inverted microscope (Axiovert 35M; Carl Zeiss, Oberkochen, Germany) (Figure 1). In the cutting head longitudinal vibrations of an electrolytically sharpened steel needle (tip radius, $<0.5\ \mu\text{m}$) are induced by a monolithic low-voltage piezoelectric actuator ($5 \times 2 \times 5\ \text{mm}^3$; CeramTech, Lauf, Germany). The cutting head is machined from a solid piece of aluminum, where the vibrating beam taking up the needle is linked to the body by two parallel members via flexure hinges. The micropipette works with two normally convex brass membranes (diameter, 35 mm) with glued-on piezo ceramic disks mounted face to face (Piezosignalgeber-Membran EPZ-35; Bürklin, Munich, Germany). With a DC voltage from between -30 and $+250\ \text{V}$ the membranes flatten, thus effecting an arbitrarily variable displacement of up to $30\ \mu\text{m}$.

For microdissection the cryosections were covered with a pool of $15\ \mu\text{l}$ of xylene. Under excellent visualiza-

Figure 1. Microdissection device. **Inset:** Schematic illustration of the one-step preparation process. By use of the oscillating needle the tissue for analysis is fragmented into subcellular particles that are aspirated into the pipette tip.

tion, areas of interest could be easily dissected while moving the ultrasonically oscillating needle in a meander-formed course through the selected tissue area (Figure 2). Along the path of the needle a 10- μm wide gap (Figure 2, inset) was developed where the tissue was detached from the glass slide and fragmented into subcellular particles. The appropriate settings of frequency and amplitude (25 to 55 kHz and 0 to $2\ \mu\text{m}$) were dependent on the resonance of the needle and were adjusted by observing the interaction of the needle with the tissue. The micropipette, equipped with a GELoader Tip (Eppendorf AG), enabled a continuous aspiration of the generated tissue particles with xylene. Following this protocol, preparation and removal of tissue to be microdissected could be done simultaneously in a one-step procedure (Figure 1, inset). The pipette tip was positioned close to the area to be dissected, with a distance between needle and tip of 10 to $20\ \mu\text{m}$ at the beginning of dissection. There is no necessity to move both together during preparation. A distance up to $400\ \mu\text{m}$ is acceptable when

Figure 2. Photomicrographs of a human large-cell lung carcinoma showing tumor parenchyma cell clusters during dissection using the oscillating needle. The generated tissue particles were aspirated into the pipette tip (not shown). **Inset:** Cutting line in tumor stroma developed along the path of the oscillating needle. Xylene-covered 10- μm cryosection. H&E stained; original magnifications: $\times 100$, $\times 200$ (inset).

Table 1. Primer Sequences for PCR

Primer name	Position	Nucleotide	Sequence	PCR product	
				Size (bp)	Melting point (°C)
β -Actin	1687–1704	Sense	5'CTGGGAGTGGGTGGAGGC3'	57	84.0
	1743–1723	Antisense	5'TCAACTGGTCTCAAGTCAGTG3'		
Vimentin	1792–1813	Sense	5'TTTTCCAGCAAGTATCCAACC3'	59	76.5
	1850–1827	Antisense	5'GGAGTTTCCAAAGATTATGAA3'		
CEA	2859–2882	Sense	5'TTTCTCCCTATGTGGTCGCTCCAG3'	98	76.0
	2956–2933	Antisense	5'AGCAGATTTTATTGAACCTGTGC3'		
Cyclin D1	3879–3894	Sense	5'CGCCCCACCCCTCCAG3'	221	91.0
	4099–4081	Antisense	5'CCGCCAGACCCTCAGACT3'		

Sequences were taken from GeneBank, accession X00351 for β -actin, Z19554 for vimentin, M29540 for CEA, and X59798 for cyclin D1.

larger tissue areas were prepared. Tissue damage was avoided by the elasticity of the pipette tip. The inner diameter of the pipette tip, 0.15 mm, resulted in a high velocity of xylene flow dragging tissue particles into the tip. The rate of xylene aspiration was regulated at the control unit and adjusted to the speed of dissection. Normally, a total volume of $\sim 15 \mu\text{l}$ was continuously aspirated in <2 minutes allowing the dissection of up to 1000 cells. The process of dissection was paused and restarted by controlling the ultrasonic actuation of the needle as well as suction with the micropipette by foot pedals. This was particularly useful for collecting multiple disseminated tumor-cell clusters. For subsequent analysis the xylene, containing generated tissue particles, was transferred into a microcentrifuge tube using the micropipette in its ejection mode.

RT-PCR results of samples prepared with the oscillating needle were compared to two tumor parenchyma cell clusters that were microdissected as single fragments according to Going and Lamb⁴ with the modification that the tissue was covered with lysis buffer (PUREscript RNA Isolation Kit; BIOzym Diagnostik, Hess., Oldendorf, Germany) instead of proteinase K buffer solution.

Isolation of Total RNA and RNA Amplification from Microdissected Samples

Total RNA of the cell clusters microdissected as single fragments were extracted with the PUREscript RNA isolation kit (BIOzym Diagnostik) and for tissue particles generated with the oscillating needle with the Micro RNA isolation kit (Stratagene, Heidelberg, Germany) after evaporation of xylene in a vacuum concentrator (concentrator 5301; Eppendorf AG, Germany) according to the manufacturers' protocols. Total RNA was dissolved in $5 \mu\text{l}$ of diethyl pyrocarbonate-treated H_2O and digested 15 minutes with 5 U RNase-free DNase I (Roche Molecular Biochemicals, Mannheim, Germany) and 20 U RNase inhibitor (Life Technologies GmbH, Karlsruhe, Germany) followed by incubation at 95°C for 5 minutes. Control PCRs for genomic contamination were always negative.

The protocol for cDNA synthesis using an oligo-dT primer with an additional T7 promoter sequence and subsequent RNA amplification has been previously described in detail [Barry C, Pat Brown Lab: Modified Eberwine "antisense" RNA amplification protocol (http://cmgm.stanford.edu/pbrown/protocols/ampprotocol_2.txt)].

The only modifications were: 1) after double strand cDNA (ds cDNA) synthesis the alkaline digestion of cellular RNA was omitted; 2) before *in vitro* transcription (ds cDNA) was washed three times with $200 \mu\text{l}$ of diethyl pyrocarbonate-treated H_2O using a Microcon YM 100 centrifugal filter (Millipore GmbH, Eschborn, Germany); and 3) amplified RNA (aRNA) was precipitated after organic extraction with an equal volume of isopropanol in the presence of $20 \mu\text{g}$ of glycogen carrier. The aRNA pellet was washed with 70% ethanol and dissolved in $4 \mu\text{l}$ of diethyl pyrocarbonate-treated H_2O .

RT-PCR from Microdissected Samples

First-strand cDNA was prepared from aRNA using the Expand Reverse transcriptase kit (Roche Molecular Biochemicals) and random hexamers according to the manufacturer's protocol. The cDNA was diluted 1:2 (40- μl final volume), and aliquots of $1 \mu\text{l}$ were analyzed by real-time PCR with a LightCycler instrument¹⁵ using the primer pairs given in Table 1. The reaction was set up with the LightCycler-FastStart DNA Master SYBR Green I (Roche Molecular Biochemicals) according to the manufacturer's protocol containing $1 \mu\text{mol/L}$ of each primer and a Mg^{2+} concentration of 2.25 mmol/L . Amplification conditions were modified to 95°C for 5 minutes, followed by 55 cycles of 95°C for 15 seconds, 60°C for 5 seconds, and 72°C for 12 seconds with a temperature transition rate of 5°C/second from annealing to extension. PCR products were identified by melting curve analysis and by agarose electrophoresis (Table 1).

RT-PCR Analysis from Bulk Tumor Tissue

Isolation of total RNA from frozen tumor blocks (High Pure RNA tissue kit, Roche Molecular Biochemicals) and oligo-dT primed cDNA synthesis (Expand Reverse transcriptase kit, Roche Molecular Biochemicals) were prepared according to the manufacturers' protocols. Real-time PCR analysis was performed as described above.

Data Analysis

Expression data were calculated using the LightCycler software. PCR product accumulation was determined by

measuring the fluorescence once per cycle at the end of the extension phase to generate an amplification curve for each sample. The cycle number at the intersection of the log-linear region of the amplification curve with a threshold of constant fluorescence for each sample was used to calculate the expression data relative to a standard. Individual standard dilution series were analyzed for each transcript where the standard curve is a plot of log dilution factors to corresponding cycle numbers. Expression of target genes was then normalized to β -actin expression to correct for losses during sample preparation and the different cell amount in samples.

Quantitation of low-copy number transcripts is hampered by the appearance of unspecific PCR products, which can be discriminated from specific product by melting curve analysis, because of the nonselective ds DNA binding of the SYBR Green I dye. Unspecific PCR products were only detected in samples found negative for the analyzed transcript.

Statistical analysis was performed by use of SigmaStat for Windows (Jandel). Data that passed the appropriate constraints of equivalent variances and normal distribution were analyzed with unpaired Student's *t*-test, whereas other data were analyzed by the Mann-Whitney rank-sum test. *P* values <0.05 (two-tailed) were considered indicative of a statistically significant difference. Data are presented as means $\pm$ SE.

Results

The use of the oscillating needle allows a micrometrically precise dissection of cells for analysis and their easy detachment from the glass slide. Even in rigid tumor stroma the oscillating needle retains its excellent cutting ability (Figure 2). The time required for sample preparation varied depending on the procured cell number and tissue architecture. Small tumor-cell clusters were dissected in ~20 seconds and areas of 5000 cells were dissected in ~6 minutes. During preparation of larger areas the xylene pool became enriched with the tissue particles. Remaining tissue particles can be removed under visual control by repeated covering and aspiration with xylene and pooled together with the initial sample. Negative controls (xylene, aspirated from the section) before and after microdissection were collected and subsequent RT-PCR after linear amplification revealed a level of β -actin in a ratio of 0.1% compared to a 200-cell sample. Controls for genomic DNA contamination failed to generate a detectable signal for a 110-bp fragment of the β -globin gene (data not shown).

Our method was tested for the selective procurement of 5000 down to a minimum of 10 cell profiles. RT-PCR results are shown in Figure 3. Except for CEA, mRNA expression values of microdissected samples scattered around those of bulk tumor tissue. In samples of parenchyma vimentin mRNA expression was lower in both tumors, CEA mRNA expression was higher in tumor B, and cyclin D1 mRNA expression was higher in tumor A, when compared to stroma samples (statistical analysis is given in legend Figure 3). mRNA expression values of the

Figure 3. Results of RT-PCR analysis. Each symbol represents expression values of microdissected samples (**red circle**, tumor parenchyma A, *n* = 6; **green circle**, tumor stroma A, *n* = 4; **red square**, tumor parenchyma B, *n* = 5; **green square**, tumor stroma B, *n* = 4). The prepared cell number is given in each symbol (*k* = $\times 1000$, *c* = $\times 100$). Samples with indicated cell number of 3k were microdissected as single fragments (see Materials and Methods). **Lines** as indicated represent expression values for bulk tissue of tumors A and B. Expression values are given normalized to β -actin mRNA. Note: expression values cannot be compared between different transcripts (see Data Analysis). 0, PCR product of target gene was not detected. Expression values of microdissected samples were different between tumor parenchyma and stroma for: vimentin 0.15 ± 0.003 versus $0.25 \pm 0.078^{**}$ (tumor A) and 0.04 ± 0.013 versus $0.13 \pm 0.042^{*}$ (tumor B), CEA 5.9 ± 4.2 versus $0.0002 \pm 0.0002^{**}$ (tumor B), and cyclin D1 2.6 ± 0.64 versus $0.2 \pm 0.06^{**}$ (tumor A). *, Student's *t*-test; **, Mann-Whitney rank-sum test.

two samples (3k), which were microdissected as single fragments were in the range of those prepared with the ultrasonically oscillating needle. The threshold cycle of β -actin was 18.79 ± 1.33 for 5000 cells (*n* = 5), 23.35 ± 1.06 for 500 cells (*n* = 8), and $27.82/27.39$ (tumor A/B, respectively) for 50 cells.

Immunohistochemical staining for vimentin showed similar patterns of distribution in tumors A and B, revealing a positive stromal reactivity and detection of a few scattered cells in parenchyma (Figure 4A). In tumor B >70% of parenchyma cells showed immunohistochemical staining for CEA, whereas parenchyma of tumor A and the stroma of both tumors remained negative (Figure 4B).

Discussion

Analysis of pure cell populations is a prerequisite in the study of differential gene expression in complex tissues, especially with regard to advanced molecular techniques. As microdissection comes into widespread use there is an increasing need for an economical method to be applicable in routine work. In the present report we describe a new mechanical technique using an ultrasonically oscillating needle and a piezo-driven micropipette for rapid and precise histological microdissection. The main advantage of our method over current mechanical

Figure 4. Photomicrographs of immunohistochemical staining (brown) of human large-cell lung carcinoma for vimentin, showing the typical detection of tumor stroma and scattered cells in tumor parenchyma (**A**) and CEA-positive parenchyma of tumor B (**B**). Paraffin sections; original magnification, $\times 400$.

approaches is the improved speed of dissection and the easy detachment of tissue from the slide. The method can be applied to either paraffin sections or unfixed cryostat sections that are often recommended for mRNA extraction. This can be an advantage because compromised LCM after short drying periods of frozen sections has been reported.¹⁶ The preparation with the oscillating needle allows a sharp demarcation between the dissected area and unwanted tissue that remain intact for further analysis. An additional example of the efficiency of our method is demonstrated by dissecting individual colonic crypts without collecting any adjacent stroma (Figure 5).

The most sophisticated and certainly the best techniques are laser-assisted methods suitable for microdissection of single cells with minimized risk of contamination.^{12,17} However, our technique competes with laser-assisted methods concerning preparation time and precision for microdissection of oligocellular clusters. For studies that focus on tissue samples in sizes as analyzed in this report, our method offers an alternative at moderate cost (estimated to be approximately \$16,000 US dollars, excluding inverted microscope and manipulators). The system is flexible and can be assembled using a conventional inverted microscope with micromanipulators that are frequently already present in the laboratory.

Figure 5. Photomicrograph of colonic mucosa showing completed microdissection of single colonic crypts. The surrounding connective tissue remained entirely intact. Paraffin section; original magnification, $\times 200$.

A manual micromanipulator has been tested and is adequate for positioning the micropipette. The system is robust and maintenance-free, running costs are limited to disposable pipette tips and needles. In analogy to laser-assisted cell picking⁹⁻¹¹ where selected cells are isolated by photolysis of adjacent tissue, our method should also be useful for the microdissection of single cell profiles. Adjacent tissue can be removed with the oscillating needle and micropipette and cells thus isolated can be retrieved by conventional methods.

It is a general problem of microdissection that dry noncoverslipped slides do not allow fine cytological detail. The preparation under xylene provides excellent optical conditions, which can be particularly helpful for the dissection of premalignant lesions.¹⁸ Furthermore, in contrast to aqueous buffer solutions, the tissue does not suffer variable adherence to the glass slide, which can diminish the precision of dissection^{4,5} and RNA stability is improved in an anhydrous environment.

The validity of this technique is demonstrated by the RT-PCR data (Figure 3). Quantitative analysis of mRNA for vimentin and CEA are in concordance with immunohistochemistry, ie, differential mRNA expression between tumor parenchyma and stroma was shown for these genes. Additionally, differential mRNA expression between parenchyma and stroma was shown for cyclin D1 in one tumor. mRNA for CEA always showed highest expression in CEA immunohistochemically positive areas of tumor B, however none of the expression values reached that of bulk tumor tissue, indicating an inhomogeneous distribution of CEA expression in tumor B. Microdissection of immunophenotypically defined cell populations allows cell-specific mRNA analysis according to antigen expression.^{10,16} Different amplification efficiencies for CEA and β -actin during linear amplification could also be a cause for the lower relative expression of CEA in microdissected samples. Linear amplification of RNA has been shown to be reproducible between individual LCM dissected cells and was successfully used to obtain gene expression profiles with cDNA microarrays.¹⁹ In our protocol, linear amplification of polyA RNA and subsequent hot-start PCR facilitated quantitative analysis of

multiple transcripts from a single sample, especially if only few cells are prepared or at low-copy number transcripts. As assessed from further dilution steps of standard series additional PCR cycles were critical for a precise quantitation, because of the appearance of unspecific products.

In conclusion, we describe a new mechanical method for histological microdissection. The use of an ultrasonically oscillating needle and a piezo-driven micropipette were demonstrated as sufficient tools, allowing a precise and efficient procurement of homogeneous tissue samples. Our data support the utility of this technique for the determination of gene expression in defined cell populations. The moderate cost and low maintenance of the system will provide a technique that could be accessible to a large number of scientists.

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References

1. Going JJ, Gusterson BA: Molecular pathology and future developments. *Eur J Cancer* 1999, 35:1895–1904
2. Turbett GT, Barnett TC, Dillon EK, Sellner LN: Single-tube protocol for the extraction of DNA or RNA from paraffin-embedded tissues using a starch-based adhesive. *Biotechniques* 1996, 20:846–853
3. Youngson BJ, Anelli A, Van Zee KJ, Borgen PI, Norton L, Rosen PP: Microdissection and molecular genetic analysis of Her2/neu in breast carcinoma. *Am J Surg Pathol* 1995, 19:1354–1358
4. Going JJ, Lamb RF: Practical histological microdissection for PCR analysis. *J Pathol* 1996, 179:121–124
5. Moskaluk CA, Kern SE: Microdissection and polymerase chain reaction amplification of genomic DNA from histological tissue sections. *Am J Pathol* 1997, 150:1547–1552
6. Lee JY, Dong SM, Kim SY, Yoo NJ, Lee SH, Park WS: A simple, precise and economical microdissection technique for analysis of genomic DNA from archival tissue sections. *Virchows Arch* 1998, 433:305–309
7. Zhuang Z, Bertheau P, Emmert-Buck MR, Liotta LA, Gnarra J, Linehan WM, Lubensky IA: A microdissection technique for archival DNA analysis of specific cell populations in lesions <1 mm in size. *Am J Pathol* 1995, 146:620–625
8. Shibata D: Selective ultraviolet radiation fractionation and polymerase chain reaction analysis of genetic alterations. *Am J Pathol* 1993, 143:1523–1526
9. Fink L, Seeger W, Ermer L, Hänze J, Stahl U, Grimminger F, Kummer W, Bohle RM: Real-time quantitative RT-PCR after laser-assisted cell picking. *Nat Med* 1998, 4:1329–1333
10. Fink L, Knife T, Seeger W, Ermer L, Kummer W, Bohle RM: Immunostaining for cell picking and real-time mRNA quantitation. *Am J Pathol* 2000, 157:1459–1466
11. Becker I, Becker KF, Röhrl MH, Höfler H: Laser-assisted preparation of single cells from stained histological slides for gene analysis. *Histochem Cell Biol* 1997, 108:447–451
12. Schütze K, Lahr G: Identification of expressed genes by laser-mediated manipulation of single cells. *Nat Biotech* 1998, 16:737–742
13. Böhm M, Wieland I, Schütze K, Rübner H: Microbeam MOMEt: non-contact laser microdissection of membrane-mounted native tissue. *Am J Pathol* 1997, 151:63–67
14. Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, Weiss RA, Liotta LA: Laser capture microdissection. *Science* 1996, 274:998–1001
15. Wittwer CT, Herrmann MG, Moss AA, Rasmussen RP: Continuous fluorescence monitoring of rapid cycle DNA amplification. *Biotechniques* 1997, 22:130–138
16. Fend F, Emmert-Buck MR, Chuaqui R, Cole K, Lee J, Liotta LA, Raffeld M: Immuno-LCM: laser capture microdissection of immunostained frozen sections for mRNA analysis. *Am J Pathol* 1999, 154: 61–66
17. Suarez-Quian CA, Goldstein SR, Pohida T, Smith PD, Peterson JI, Wellner E, Ghany M, Bonner RF: Laser capture microdissection of single cells from complex tissues. *Biotechniques* 1999, 26:328–335
18. Walch A, Komminoth P, Hutzler P, Aubele M, Höfler H, Werner M: Microdissection of tissue sections: application to the molecular genetic characterisation of premalignant lesions. *Pathobiology* 2000, 68:9–17
19. Luo L, Salunga RC, Guo H, Bittner A, Joy KC, Galindo JE, Xiao H, Rogers KF, Wan JS, Jackson MR, Erlander MG: Gene expressions profiles of laser-captured adjacent neuronal subtypes. *Nat Med* 1999, 5:117–122