Direct isolation of poly-A(+) mRNA from individual laser microdissected cell samples obtained with the PALM® MicroBeam system

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ABSTRACT

Measuring the expression profiles of individual cells is useful in a wide range of research and clinical applications. Laser microdissection and pressure catapulting (LMPC; PALM® MicroBeam System) combined with real-time PCR for expression profiling allows several investigations of individual cells. The PickPen® technology together with QuickPick™ mRNA nano kit is engineered to recover high-quality mRNA consistently from 1-1000 cells very rapidly. Using LMPC and real-time PCR in combination with the PickPen® 1-M tool and QuickPick™ mRNA nano kit, we analyzed expression of murine porphobilinogen deaminase (PBGD) gene from 50, 10 and 1 microdissected cells.

INTRODUCTION

All differences in cell state or type are correlated with changes in mRNA expression levels of genes. Therefore many potential applications in expression profiling exist:

- Identification of complex genetic diseases
- Drug discovery and toxicology studies
- Pathogen analysis
- Differing expression of genes over time, between tissues and disease states

Laser-assisted microdissection allows the analysis of individual cells. Using this technology it is possible to study DNA, RNA and proteins in pure cell populations from specific areas of tissue. An UV-A-laser mediated process dissects selected specimen from various sources and transfers them totally contact free directly into collecting vessels for subsequent analysis, for example mRNA isolation. A primary limitation of any target design for microarray hybridization is the amount of total RNA or poly-A(+) mRNA that can be obtained from limited amounts of cells. The recent developments in mRNA isolation techniques make previously time-consuming mRNA isolations attractive and practical. The conventional methods utilizing column filtration are not very suitable for working with less than 100 cells, as substantial amounts of mRNA may be lost in the resin-bed. The combination of pure cell preparation, QuickPick™ mRNA kits, PickPen® magnetic tools and LMPC is a very potential instrument for rapid isolation of poly-A(+) mRNA from small sample amounts.

PRINCIPLE OF THE QuickPick mRNA KIT

The QuickPick mRNA method is based on the binding of poly-A(+) mRNA to immobilized oligo $(dT)_{30}$ on paramagnetic particles. The sample is first lysed in Lysis/Binding Buffer. Washed magnetic particles are added to the lysed sample, followed by a 4-minute incubation at room temperature during which the poly-A(+) hybridizes to the oligo $(dT)_{30}$ on the particles. The incubation is followed by three quick washes to remove impurities, such as pre-mRNA, rRNA, tRNA and snRNA. Finally the particles are transferred into the Elution Buffer. At this point, it is possible to elute mRNA from the magnetic particles into solution by heating at +70 °C for 5 minutes and removing the magnetic particles. It is also possible to use the mRNA still attached to magnetic particles directly as a solid-phase template in the RT-PCR reaction, which simplifies and speeds up the process.

MATERIALS & METHODS

Tissue preparation and microdissection

Snap-frozen murine liver tissue stored at -80 °C was cut in 7 µm serial sections on a cryotome at -25 °C. Sections were transferred to PALM® MembraneSlides (1 mm glass slides covered with a 1.35 µm thin Polyethylene-naphthalate membrane to facilitate the laser pressure catapulting procedure) and air dried for 10 seconds. After a 5 minute fixation step in 70% ethanol at –20 °C the sections were further processed according to standard histochemical procedures. After staining with cresyl violet acetate (1%) and an increasing ethanol series the sections were air-dried and used immediately. The PALM® MicroBeam System was used to precisely excise the selected cell areas (50 cells /10 replicates, 10 cells/10 replicates, 1 cell/20 replicates, pure

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membrane/20 replicates) and to catapult then the cells of interest into collecting caps filled with 10 µl of mRNA Lysis/Binding Buffer.

mRNA isolation

Sample volume was filled to 100 µl with Lysis/Binding Buffer and mRNA was isolated according to the QuickPick™ mRNA nano kit instructions. Particle-bound mRNA was resuspended in 5 µl elution buffer and used directly as a solid-phase template in RT-PCR.

Reverse Transcription

mRNA samples were reversely transcribed with the First Strand cDNA Synthesis Kit (AMV) (Roche) according to the manufacturers protocol. Briefly, 6 µl of each mRNA solution were transcribed by AMV-RTase with random primers in a total volume of 20 µl for one hour at 42°C.

Real time PCR

For the subsequent PCR analyses 5 µl of each cDNA-solution were used as templates. The PCR amplification of the cDNA was performed in a LightCycler® instrument (Roche) in 20 µl reaction volumes using protocols and components of the LightCycler-FastStart DNA Master^{PLUS} SYBR Green I (Roche). cDNA-specific primers for murine PBGD gene were used as a model system producing a PCR-fragment of 154 bp.

RESULTS

With the PALM® MicroBeam individual cells are easily and reliably dissected (figure 1). Areas corresponding to 50 cells, 10 cells and 1 cell were analyzed. Membrane areas corresponding to the area of one cell were dissected as negative controls to exclude contamination errors.

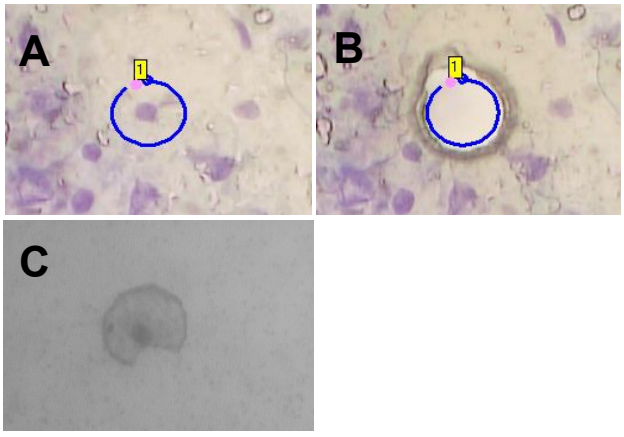

Figure 1: 7 µm section from murine liver tissue, stained with cresyl violet acetate (40 x magnification).
A: selected single cell, B: section after microdissection and catapulting of the single cell, C: isolated single cell in the lid of the collection device

The PickPen® technology recovers high-quality mRNA from cells within 10 minutes. After mRNA isolation samples were reverse transcribed to cDNA and subsequently real-time PCR with the LightCycler was performed. The specificity of the PCR-fragment was analyzed by melting curves

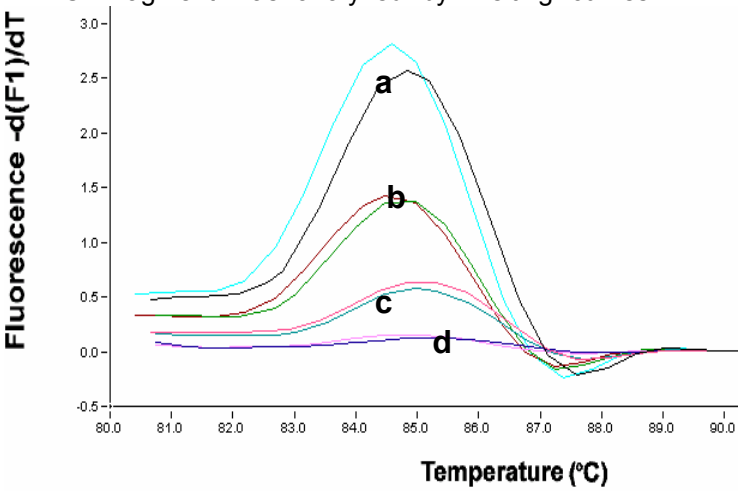

Figure 2: LightCycler specific melting curves.
mRNA was isolated from microdissected mouse liver tissue sections and used for real-time PCR with the LightCycler®. Histological staining was carried out using a cresyl-violet procedure.
a: 50 cells, b: 10 cells, c: 1 cell, d: membrane

As shown in Figure 2 it was possible to reliably study the expression of the murine porphobilinogen deaminase gene from truly one microdissected cell. The melting curves prove the specificity of the products. The fluorescence quotient (d(F)/dT) corresponds with the number of cells.

CONCLUSION

PALM® MicroBeam in combination with the PickPen® magnetic tool and QuickPick™ mRNA kits is a useful tool to study the expression profiles from individual limited cell samples. The PickPen® technology reduces time of mRNA isolation from several hours to 10 minutes without loss of substantial amounts of mRNA. Even single cell

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analysis can be performed in a reliable and reproducible way.

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