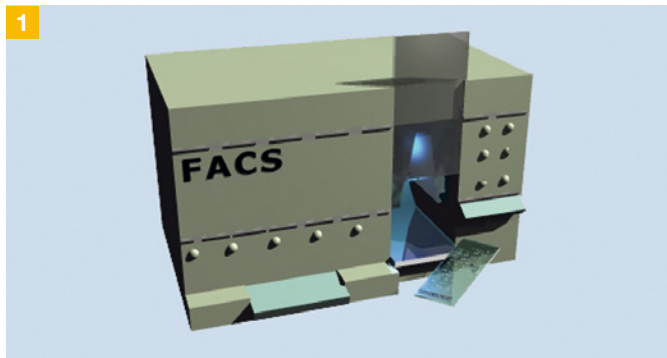


Single Cell RT-PCR

Combining high-speed fluorescence activated cell sorting (FACS) with amplification of mRNA.

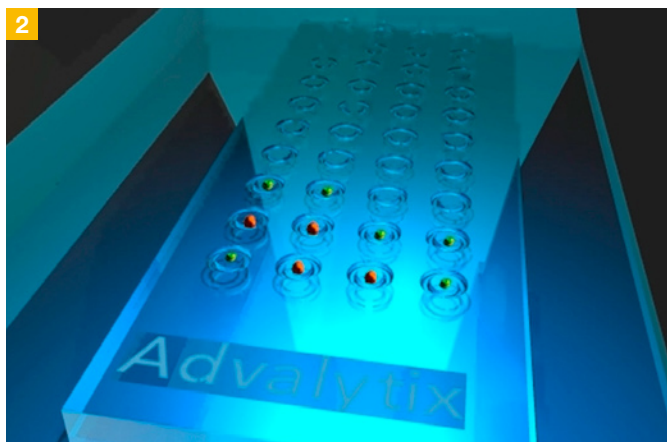
RT-PCR is the most sensitive technique for mRNA detection and quantification currently available. Over the last few years, it has become the preferred method for validating results obtained from microarray analysis as well as other genomic techniques that evaluate gene expression changes on a global scale. Now with the advanced AmpliGrid technology from Advalytix, this technique is sensitive enough to amplify mRNA from single cells without the need of RNA preparation.



Artificial image of cell sorting on an AmpliGrid AG480F slide with FACS

Cell sorting

Different numbers of single cells from the Jurkat cell line were deposited onto each of the 48 hydrophilic AmpliGrid reaction sites by a MoFlo™ High Performance Cell Sorter (Dako Cytomation, fig. 1 and 2). Vital cells were sorted according to their side and forward scatter signals. Slides prepared in this way were stored at room temperature.



Artificial magnification of cell sorting on an AmpliGrid AG480F slide

RT-PCR

Amplification experiments were performed on AmpliGrid AG480F slides using different numbers of single cells (1 to 20) as template at each reaction site. RT-PCR was done

with GeneAmp® EZ r Tth RNA PCR Kit (Applied Biosystems) in the presence of gapdh1 and gapdh2 primers (tab. A). RNasin (Promega) was added to prevent degradation of the RNA by endogeneous RNases. The reaction master mix was prepared as described in table B. 1 µL of the master mix was added to the cells at each AmpliGrid slide reaction site and immediately covered with 5 µL of sealing solution. All these steps were automated on a Hamilton MICROLAB STAR^{LET} (Hamilton Deutschland, fig. 3). Thermal cycling was completed according to the protocol described in table C on the Advalytix AmpliSpeed slide cycler.



Automated high throughput PCR of FACS sorted cells

PAGE analysis

For PAGE analysis, 4 µL of gel loading dye (Fermentas) were added to each reaction site. A volume of 3-5 µL consisting of RT amplification mix and loading dye was transferred to a polyacrylamide gel (8%) and electrophoretically separated.

A Table A: Primer sequences

Primer	Sequence
gapdh1	5'- GACCCCTTCATTGACCTCAAC -3'
gapdh2	5'- CTTCTCCATGGTGGTGAAGAC -3'

B Table B: Components of master mix

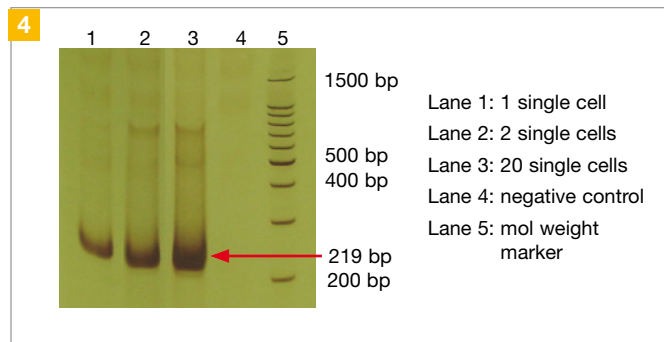
Component	1 µL PCR mix
RNasin 40 U/µL	1 µL
5x EZ-Buffer	8 µL
dNTPs (2.5 µM each)	4.8 µL
Primer gapdh1 15 µM	2.4 µL
Primer gapdh2 15 µM	9.6 µL
25 mM Mn(OAc) ₂	4 µL
r Tth DNA Polymerase	1.6 µL
ddH ₂ O (PCR clean, RNase free)	8.6 µL
Total volume	40 µL

C Table C: Amplification program

Temperature	Duration	
65°C	60 min	
94°C	2 min	
94°C	1 min	
60°C	30 sec	40 cycles
72°C	30 sec	
72°C	10 min	
7°C	∞	

Results

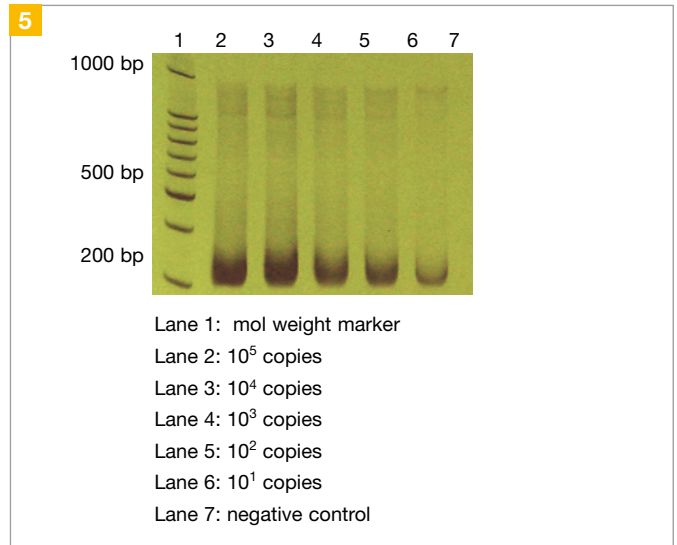
Single cell RT-PCR resulted in a 219 bp amplicon fragment. A scan of the PAGE gel (figure 4) shows all the gapdh fragments detected after FACS sorting and subsequent mRNA amplification of 1, 2 or 20 cells on the AmpliGrid AG480F.



Results of single cell RT-PCR

Discussion

RT-PCR reaction on AmpliGrid has been successfully used for the amplification of RNA template (fig. 5) as well as mRNA from single cells. Furthermore, the system is very efficient even for RT amplification from as little as one single cell, because there is no need of RNA preparation and consequently no loss of template. While conventional methods rely on the isolation of RNA, which is prone to degradation during handling procedures, the AmpliGrid technology provides a simple, efficient and time saving way for successful RT amplification. Moreover, AmpliGrid slides with sorted cells can be stored at room temperature for several weeks, without any loss of the RNA templates. Further analyses of the stability of nucleic acids (DNA/RNA) are on the way.



RNA amplification of sample RNA template with the GeneAmp® EZ r Tth RNA PCR Kit (Applied Biosystems) on the AmpliGrid AG480F

REFERENCES

Polymerase Chain Reaction (PCR) process is covered by patents which are owned by Hoffmann-La-Roche Inc. and F. Hoffmann-La Roche Ltd.