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Summary

Single cell analysis often generates unsatisfying results because of low gene copy numbers present in the starting sample. Advalytix has introduced AmpliGrid, a superior 1µl amplification platform based on a chemically structured microscope slide. Due to its two-dimensional layout, single cells can easily be deposited to the slide surface, visualised and investigated prior to amplification by common microscopical technique. Thanks to the innovative AmpliGrid system, sensitivity, efficiency and reproducibility are reliable constants for single cell analysis in the AmpliGrid system. In order to offer an easy, sensitive and efficient protocol for single cell qRT-PCR analysis on AmpliGrid, we have developed a RT and PCR pre-amplification step which is run directly on the slide. The pre-amplified amplicons are then transferred onto a standard real-time PCR system for fully quantitative RT-PCR analysis. Together to AmpliGrid, the same protocol can be applied to AmpliCell, an innovative microculture slide chamber where cells are grown and subsequently analyzed by PCR without need of sample preparation. This new platform derived from AmpliGrid has been thought for gene expression studies which can be tightly influenced by sample manipulation. Different examples both on AmpliGrid and AmpliCell will be presented.

Platforms

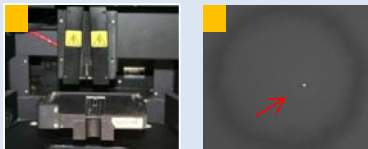
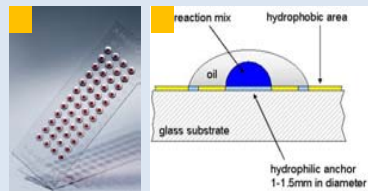


Figure 1: AmpliGrid platform

- AmpliGrid platform with 48 reaction sites, suitable for 1µ PCR reactions
- Hydrophobic and hydrophilic structure of AmpliGrid slide
- High throughput template cell deposition using FACS sorting
- Control of stained cells under a microscope with fluorescence detection

Workflow



Cell deposition on AG/reaction site



Multiplex RT on ASC



Distribution of diluted sample into MTP



qPCR in standard real-time cycler, e.g. using intercalating dye or probe based systems

Results

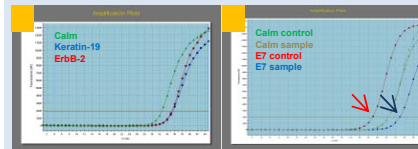


Figure 2: Gene expression analysis by qRT-PCR in single cells

- Expression level of ErbB-2, Calm and Keratin-19 analysed in single SUM149 cells previously deposited on AmpliGrid reaction sites.
- Expression level of E7 in silenced and control single HeLa cells. The blue arrow shows the strong down regulation of E7 in the siRNA treated sample.

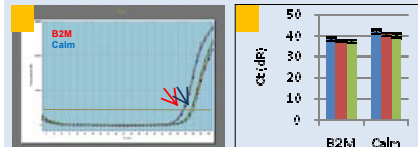


Figure 3: TaqMan® technology for gene expression analysis in single human lymphocytes

- Reproducible Ct (dR) values for B2M or Calm obtained from 6 independent single cells of the same experiment.
- Reproducible Ct (dR) values for B2M or Calm obtained from 3 independent experiments analysing 6 single lymphocytes each.

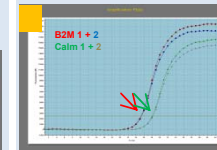


Figure 4: Gene expression analysis including a preamplification step prior qPCR

- In order to increase the amount of cDNA a preamplification step of 5 cycles was added subsequently to the multiplex RT reaction. For low copy genes this step is mandatory to have enough cDNA template before distributing RT sample aliquots into the MTP. A high reproducibility of replicates (B2M or Calm 1+2) can also be shown.

Conclusions:

- The easy, seamless and sensitive workflow makes qRT-PCR analysis on single cells possible
- A high sensitivity and reproducibility is achieved and can even be increased by implementing a preamplification step in the workflow
- All kind of real-time assays adaptable
- The workflow allows multiplexing RT-PCR analyses from one single cell
- Complete control on template deposition thanks to AmpliGrid's 2D format