Application Note

Rapid and Efficient Method of Isolation and Recovery of Highly Viable Single Cells Free of Contamination

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This application note describes a rapid and cost-effective method for the isolation and recovery of single cells using the QIAscout™. The cells obtained are viable and show no traces of contamination. Cells isolated using the QIAscout are suitable for various downstream applications, such as whole genome amplification and whole transcriptome amplification or for clonal expansion.

Introduction

While bulk cell analysis is critical for understanding the biological system as a whole, it also leads to "cellular averages" masking the intrinsic differences across individual cell subpopulations. On the other hand, single-cell analysis is capable of bringing into focus the individual contribution of every cell, without obscuring a biological response that may otherwise occur when cells are assessed in bulk. Although single-cell research is currently gaining momentum, it remains challenged by the lack of affordable methods to precisely isolate a single cell from a heterogeneous cell population without causing high cellular stress.

Cell viability is crucial when isolating single cells for the purpose of clonal expansion or for studying cellular differentiation. QIAscout technology aims to provide a sufficiently gentle mechanism of isolation and recovery of single cells. This allows the live cells to maintain their integrity resulting in high viability, and the possibility for further cultivation.

Purity of the isolated single cell is another critical parameter when analyzing cellular DNA and RNA. Isolating the cell of interest by excluding any other contamination (e.g., cell fragments, free-DNA, etc.) is of prime importance.

In this study, we have addressed some of these issues by using a novel single-cell isolation platform, the QIAscout (Figure 1). The instrument is ideal for various cell types like adherent or suspension cells, primary cells or cell lines and fluorescently-labeled cells. Additionally, the core technology, QIAscout 12,000-Microraft Array, provides all cells with a suitable environment for growth and viability similar to any standard cell culture dish. Microrafts carrying individual cells can be pierced, dislodged and transferred to secondary vessels for further processing using a magnetic wand, with no risk of contamination at any given stage as verified by whole genome amplification (WGA) followed by qPCR and Pyrosequencing®. ▷

Figure 1. The QIAscout system.

Steps in the QIAscout workflow include:

- Seeding and cultivating cells in medium on the QIAscout array
- Placing release device containing release needle on the microscope objective
- Placing array on the microscope stage
- Identifying microrafts containing single cells
- Piercing microraft containing cell of interest
- Transferring microraft to secondary vessel using a magnetic wand
- Processing the collected cell for downstream application or further cultivation

Material and Methods

Experiment 1: Piercing of microraft by release needle

Two independent QIAscout arrays were prepared by loading 2 ml of cell culture media on each. Sheep DNA (42 ng) equivalent to DNA amounting from 6000 cells was then added to the first array. After 3 hours of incubation, the array containing the sheep DNA was pierced 15 times to simulate the usage of the release needle in an experiment. The second array containing no sheep DNA was then pierced for another 15 microrafts using the same release needle without performing a wash step in between.

All 15 microrafts from the second array were further processed for amplification of genomic DNA using the REPLI-g® Single Cell Kit (refer to *REPLI-g Single Cell Handbook* for further details, see Protocol: Amplification of Genomic DNA from Single Cells). A positive DNA control reaction was set up using 10 ng of sheep genomic DNA, and water was used as the negative control. To test for contamination, all samples were further subjected to qPCR analysis using the QuantiNova® Probe PCR Kit in combination with the *mericon*® Sheep Kit designed to specifically detect sheep DNA in real-time.

Experiment 2: Transfer of microraft by magnetic wand

Two independent QIAscout arrays were prepared by loading 2 ml of cell culture media on each, with one array containing 6000 SW48 cells (a cell line containing a G to A point mutation - c.2155G>A in codon 719 in the human EGFR gene (p.G719S)) and another array containing equal number of HT-29 cells without this inherent mutation. Cells in each of the arrays were left overnight in a standard cell culture incubator. Four pierced microrafts with single cells were dislodged and transferred using the magnetic wand from the array containing HT-29 cells, to be treated as a negative control. The magnetic wand was washed with sterile PBS at this step. Four microrafts with single cells were dislodged and collected using the magnetic wand from the array containing the SW48 cells, to be treated as positive control and to simulate the usage of the magnetic wand. Directly thereafter, 24 microrafts containing single HT-29 cells were pierced and transferred using the unwashed magnetic wand.

All the microrafts collected were further processed for amplification of genomic DNA from single cells using the REPLI-g Single Cell Kit (see Protocol: Amplification of Genomic DNA from Single Cells, in the corresponding handbook mentioned earlier). To test for contamination, the PyroMark® PCR Kit was used to amplify the DNA and this was then analyzed by Pyrosequencing using the PyroMark Q48 Autoprep for mutation analysis. For the PCR and Pyrosequencing reactions, the primers for the detection of the G719S mutation from the *therascreen*® EGFR Pyro Kit* were used (see Protocols: PyroMark PCR Kit and *PyroMark Q48 Autoprep User Manual*).

Experiment 3: Cultivation of pierced microraft containing a single cell in a cell culture dish

Two independent QIAscout arrays were prepared by loading 2 ml of cell culture media along with 6000 cells of SW48 and MCF-7 cell lines, respectively. Cells in each of the arrays were left overnight in a standard cell culture incubator. Microrafts containing only single cells were pierced in each case and thereafter transferred to individual cell culture dishes. These dishes were then left undisturbed and cell growth was monitored for several days as a measure of cell viability.

Experiment 4: Cultivation of single cells on the QIAscout array

A QIAscout array was prepared by loading 2 ml of cell culture media along with 6000 HT-29 cells. Cells were seeded such that several microrafts on the array only contained a single cell. To monitor cell viability and growth, the QIAscout array was further cultivated for up to 90 hours (in a standard cell culture incubator) and images from selected microrafts containing a single cell were captured at various time points. Orientation on the QIAscout array is defined by the well number (please refer to *QIAscout User Manual* for more information on cell tracking).

* The *therascreen* EGFR Pyro Kit is not available in the US and Canada.

Results and Discussion

Experiment 1: No DNA contamination upon piercing using release needle

None of the 15 microrafts pierced using the unwashed needle showed any sheep specific signal and behaved just like the water control, indicating that the samples were free of any cross-contamination via the release needle (Figure 2A-B). Here, we show that the needle is not contaminated after piercing and upon reuse; hence, it is not possible to transfer DNA from one array to another. During a standard experiment, we may seed approximately 6000 cells but no free DNA; and even though here we used free DNA equivalent to 6000 cells, we could detect no contamination in our qPCR analysis. Therefore, it is highly unlikely that any contamination among different arrays will be observed when the release needle is reused.

The success of PCR reaction was confirmed by the positive control of 10 ng DNA having a specific signal at a C_T value of 27 (Figure 2C).

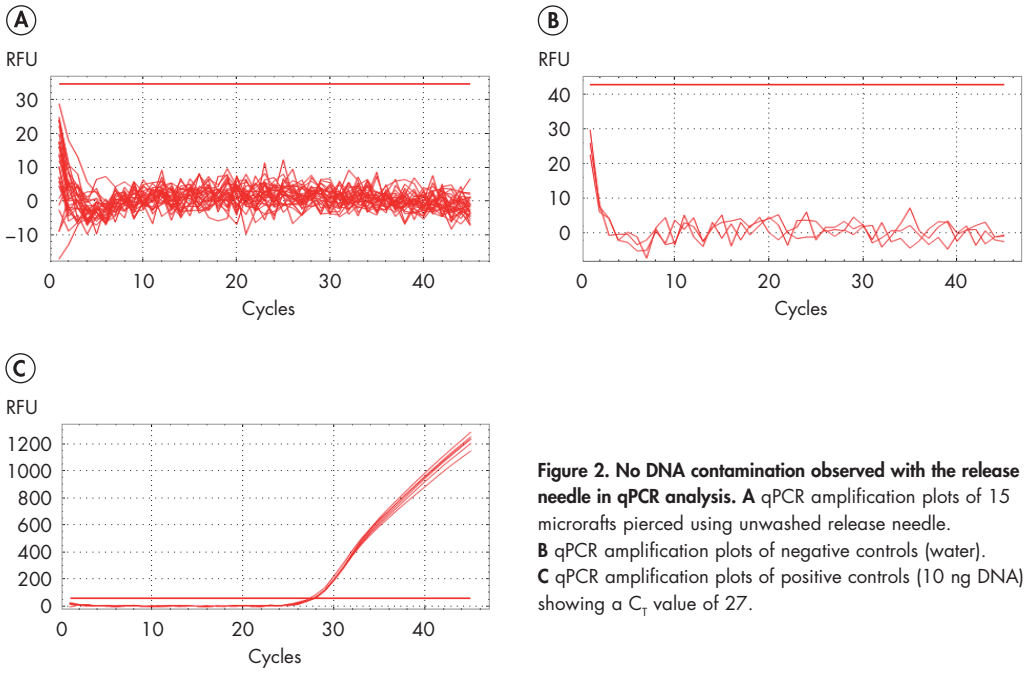

Figure 2. No DNA contamination observed with the release needle in qPCR analysis. A qPCR amplification plots of 15 microrafts pierced using unwashed release needle. **B** qPCR amplification plots of negative controls (water). **C** qPCR amplification plots of positive controls (10 ng DNA) showing a C_T value of 27.

Experiment 2: No cell contamination upon transfer using magnetic wand

To confirm whether the microrafts with single HT-29 cells were contaminated by DNA from SW48 cells, the frequency of A allele corresponding to the SW48 specific G>A point mutation in the human EGFR gene was analyzed by Pyrosequencing. As a positive control, pierced microrafts of the QIAscout array containing SW48 cells were tested and shown to have a significant frequency of A allele (Figure 3A). The negative controls (pierced microrafts of the second QIAscout array containing HT-29 cells that were transferred using a washed magnetic wand) were free of this

mutation. As an additional control, genomic bulk DNA of SW48 and HT-29 cells were analyzed and shown to give the same results as the positive and negative controls.

It is expected that contamination between transferred microrafts of two arrays with two different cell lines would lead to significant detection of the mutation of the first cell line in samples with the second cell line. In this case the G>A mutation of the SW48 cells would be detectable in samples with single HT-29 cells. However, all 24 tested microrafts with single HT-29 cells were free of contamination, indicating no traces of SW48 DNA (Figure 3B-C).

If the magnetic wand is contaminated with DNA from the cells of the SW48 array, it would be possible to detect the mutation of SW48 cells in the samples of the HT-29 array. Since this is not the case, we conclude that the magnetic wand is not contaminated after microraft transfer and hence it is not possible to transfer DNA from one array to the next.

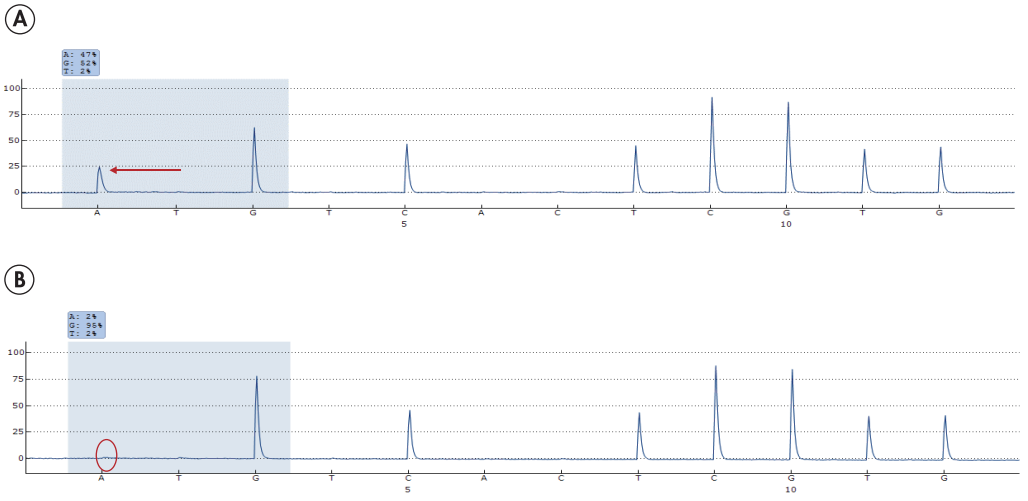

Figure 3. No cell contamination observed with the magnetic wand in Pyrosequencing analysis. A Representative pyrogram showing the G>A point mutation in the human EGFR gene in a sample with single SW48 cells (positive control). B. Representative pyrogram showing absence of the A allele in single HT-29 cells, transferred with the same unwashed magnetic wand as the SW48 cells. **C** Frequency of the A allele in two pierced HT-29 cells (negative control), two pierced SW48 cells (positive control) and 24 pierced HT-29 cells. Single SW48 cells transferred using a clean magnetic wand (positive control) show a mean frequency of A allele (=point mutation) of 50.7%. Pierced single HT-29 cells transferred using an unwashed magnetic wand and analyzed for the presence of SW48 cell contamination showed a similar frequency of A allele when compared to the pierced single HT-29 cells transferred using the clean magnetic wand (negative control), indicating no contamination between the two cell lines, the arrays and the microrafts.

Experiment 3: Pierced cells remain viable after piercing

Single cells can be used for clonal expansion, which is often carried out in standard cell culture plates or dishes. In order to examine whether single cells from pierced microrafts can divide and form colonies if further cultivated on cell culture dishes, we dislodged and transferred single cell containing microrafts from two different arrays each containing a different cell line. If the cell is viable after piercing, it will grow and divide to form a new colony of cells. Figure 4A-B demonstrates that a pierced cell from the SW48 cell line formed a new cluster in a cell culture dish, indicating high viability even after several days. The pierced cells could grow in the presence of cell culture media, just like regular cells plated under similar conditions. Position of the microraft also appeared to be of little concern for further cultivation of single cells (Figure 4C).

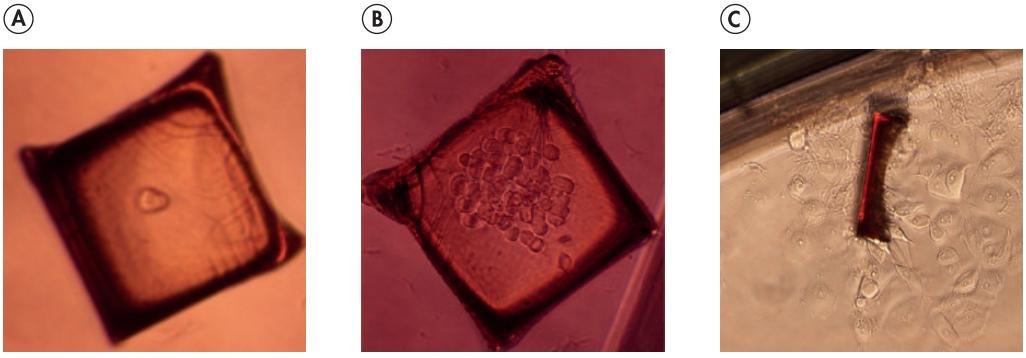

Figure 4. Cells remain viable after piercing. A-B Pierced microraft containing single SW48 cell. The microraft containing a colony of SW48 cells after several days of cultivation in a cell culture dish. **C** Viable colony formed from a single MCF-7 cell. The position of the microraft in the cell culture dish is not important for further cultivation of pierced single cells.

Experiment 4: Cells remain viable on the QIAscout array

To assess whether further cultivation of single cells leading to subpopulations is possible on the QIAscout array, HT-29 cells were seeded on the array in a way that many microrafts only contained a single cell. After cultivating the cells for over 90 hours on the array, it was observed that the cells were still viable and could form subcolonies on the microrafts, just as they would on a standard cell culture dish (Figure 5). The viability test was performed for different cell lines and the outcome remained similar (one cell line per array, data not shown).

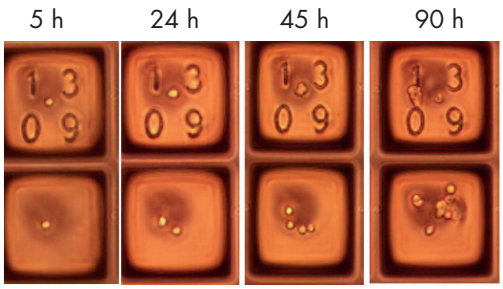

Figure 5. Cells remain viable on the QIAscout array. Successful cultivation of a single HT-29 cell to form subcolonies on the QIAscout array after 90 hours confirms cell viability on the array.

Conclusions

- The release needle and the magnetic wand do not act as sources of contamination while isolating single cells using the QIAscout. This was verified using two approaches, qPCR and Pyrosequencing, after processing cells using the REPLI-g Single Cell Kit.
- Cells remain viable for several days and form subpopulations, irrespective of whether the pierced microrafts containing single cells are transferred to a cell culture dish or single cells present on microrafts are left intact and allowed to grow on the QIAscout array itself.

This novel single cell isolation method using the QIAscout is optimal for viable single cell recovery and clonal expansion and poses no risk of contamination.

Ordering Information

Product	Contents	Cat. no.
QIAscout	Includes instrument platform and starter pack of 5 arrays	9002733
QIAscout 12,000-Microraft Arrays	5 arrays	928031
REPLI-g Single Cell Kit (24)*	REPLI-g sc Polymerase, Buffers, and Reagents for 24 whole genome amplification reactions (yields up to 40 µg/reaction)	150343
PyroMark PCR Kit (200)*	For 200 reactions: 2x PyroMark PCR Master Mix (includes HotStarTaq DNA Polymerase and optimized PyroMark Reaction Buffer containing 3 mM $MgCl_2$ and dNTPs), 10x CoralLoad Concentrate, 5x Q-Solution, 25 mM $MgCl_2$, and RNase-Free Water	978703
PyroMark Q48 Advanced Reagents	Reagents for 4 x 48 PyroMark Q48 Autoprep standard reactions: PyroMark Advanced Enzyme Mix, PyroMark Advanced Substrate Mix, Denaturation Solution, Annealing Buffer, Binding Buffer, Nucleotides	974002
therascreen EGFR Pyro Kit (24)†	For 24 reactions: Sequencing Primers, PCR Primers, Unmethylated Control DNA, PyroMark PCR Master Mix, CoralLoad Concentrate, and therascreen Buffers and Reagents	971480
QuantiNova Probe PCR Kit (100)*	For 100 x 20 µl reactions: 1 ml 2x QuantiNova Probe PCR Master Mix, 250 µl QN ROX Reference Dye, 500 µl QuantiNova Yellow Template Dilution Buffer, 1.9 ml Water	208252
mericon Sheep Kit (24)*	For 24 reactions: PCR Assay Sheep, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292063

* Larger kit sizes available

† The therascreen EGFR Pyro Kit is not available in the US and Canada.

The QIAscout is intended for molecular biology applications. The applications described here are not intended for diagnostic use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.