Application Note

Using the QIAscout® for efficient and targeted isolation of single cells selected based on their morphology and fluorescence

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The QIAscout provides an easy, fast and cost-effective method to isolate single cells from any sample. Other solutions are highly complex, expensive, and accessible only to large laboratories. In addition, they may stress the cells creating additional experimental variables. Powered by a novel approach to single-cell isolation, QIAscout can accurately select single cells within minutes through visual control using an inverted microscope. This application note shows how easily you can use QIAscout with a variety of selection parameters.

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 manipulating the cellular status.

The need to select a specific single cell of interest from a heterogeneous cell population is fundamental to many aspects of biological research. Such selections form the basis of genetic screens or generation of stable transformants and cell lines. In many instances, cells need to be selected on the basis of subtle morphological features or complex parameters such as cell response or fluorescent labeling. These types of selections become difficult when following existing conventional approaches like pipetting and serial dilution. In contrast, the fast and efficient QIAscout technology allows you to first screen the cells from a given population before a cell of interest is picked.

In this application note, we have compared the QIAscout technology with the two common methods for cell isolation: pipetting and serial dilution. The QIAscout instrument (Figure 1) is ideal for various cell types like adherent or suspension cells, primary cells or cell lines, and fluorescent cells. Additionally, the core technology, the 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, without any risk of cross-contamination at any given stage. ▷

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: Isolation of cells selected based on fluorescence

A mixture consisting of three different cell lines (in the ratio of 1:1:1) was prepared containing one specific mutation per cell line:

1) HT-29 (specific mutation: BRAF c.1799T>A)
2) LoVo (specific mutation: APC c.3340C>T)
3) MDA-MB-231 (red fluorescent cells, specific mutation: BRAF c.1391G>T)

From this cell mixture, single fluorescent cells (MDA-MB-231) were targeted for isolation using three different methods: QIAscout, pipetting and serial dilution.

a) One QIAscout array was prepared by loading 2 ml of cell culture media containing 6000 cells from the cell mixture (2000 cells per cell line). Cells in the array were left overnight in a standard cell culture incubator.

   Next day, cells were screened for fluorescence and 47 cells were selected. All 47 microrafts each containing one selected fluorescent single cell were dislodged and transferred to a secondary vessel containing 4 µl PBS (=starting volume for whole genome amplification).

b) Following a second method, cells from the cell mixture were counted using a cell counter. Cell mixture was diluted to 1 cell/4 µl PBS and to 0.4 cells/4 µl PBS. As cell distribution in the tubes follows Poisson distribution, 0.4 cells/4 µl PBS maximizes the chance of having only a single cell in each reaction tube while reducing the number of tubes containing more than one cell. In total, 47 (1 cell/4 µl PBS) and 45 (0.4 cell/4 µl PBS) tubes of diluted cells were prepared.

c) In a third method, a suspension of cell mixture was transferred to a cell culture dish and 48 single cells were isolated by capturing only one cell at a time with the pipet tip and transferring each cell into separate reaction tubes containing 2 µl PBS.

All samples collected were further processed for amplification of genomic DNA from the single cells 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). The PyroMark® PCR Kit was used to amplify the DNA and this was then analyzed by Pyrosequencing® using the PyroMark Q48 Autoprep. Three Pyrosequencing reactions were performed per sample in order to identify each single cell for the presence of one cell line-specific mutation:

1) BRAF 600 assay (c.1799T>A; HT-29-specific)
2) APC 1114 assay (c.3340C>T; LoVo-specific)
3) BRAF 464 assay (c.1391G>T; MDA-MB-231-specific)

For the PCR and Pyrosequencing reactions, refer to Protocols: PyroMark PCR Kit and *PyroMark Q48 Autoprep User Manual*. The BRAF Pyro Kit* was used for the detection of HT-29- and MDA-MB-231-specific mutations, and a Pyrosequencing assay was designed using the PyroMark Assay Design Software for the detection of LoVo-specific mutation. Genomic bulk DNA of each cell line was analyzed as positive control.

Experiment 2: Isolation of cells selected based on morphology

A mixture consisting of three different cell lines (in the ratio of 1:1:1) was prepared containing one specific mutation per cell line:

1) HT-29 (round cells, specific mutation: BRAF c.1799T>A)
2) LoVo (elongated cells, specific mutation: APC c.3340C>T)
3) SW48 (round cells, specific mutation: EGFR c.2155G>A)

HT-29 LoVo SW48

* Availability depends on the country.

From this cell mixture, single elongated cells (LoVo) were targeted for isolation using three different methods: QIAscout, pipetting and serial dilution.

a) A QIAscout array was prepared as described in Experiment 1a.

   Next day, cells were screened using brightfield light and 37 elongated cells were selected. All 37 microrafts each containing one selected elongated single cell were dislodged and transferred to a secondary vessel containing 4 µl PBS (=starting volume for whole genome amplification).

b) A serial dilution was performed as described in Experiment 1b. In total, 44 (1 cell/4 µl PBS) and 48 (0.4 cell/4 µl PBS) tubes of diluted cells were prepared.

c) Using the pipetting method similar to that described in Experiment 1c, 37 single cells were collected in separate reaction tubes containing 2 µl PBS each.

All samples collected were further processed for amplification of genomic DNA from the single cells using the REPLI-g Single Cell Kit (see protocol in the corresponding handbook mentioned earlier). The PyroMark PCR Kit was again used to amplify the DNA, followed by Pyrosequencing analysis using the PyroMark Q48 Autoprep. Three Pyrosequencing reactions were performed per sample in order to identify each single cell for the presence of one cell line-specific mutation:

1) BRAF 600 assay (c.1799T>A; HT-29-specific)
2) APC 1114 assay (c.3340C>T; LoVo-specific)
3) EGFR Exon 18 assay (c.2155G>A; SW48-specific)

For the PCR and Pyrosequencing reactions, refer to the protocol and user manual mentioned earlier. The BRAF and EGFR Pyro Kits* were used for the detection of HT-29- and SW48-specific mutations, and a Pyrosequencing assay was designed using the PyroMark Assay Design Software for the detection of LoVo-specific mutation. Genomic bulk DNA of each cell line was analyzed as positive control.

Results and Discussion

Experiment 1: Highly efficient isolation of single fluorescent cells using the QIAscout method

The aim of this experiment was to selectively isolate fluorescent cells from a mixture of two non-fluorescent (HT-29 and LoVo) and one fluorescent (MDA-MB-231) cell lines using three different isolation methods. Isolated cells were identified by three different Pyrosequencing assays covering one specific mutation per cell line. The PyroMark Q48 Autoprep allows a cost-efficient and highly reproducible Pyrosequencing analysis of 48 samples in parallel, enabling fast and reliable identification of the isolated single cells. By using the PyroMark Q48 Autoprep standard protocol, both the template preparation and subsequent sequencing were performed automatically with only minimal hands-on time in the beginning of the Pyrosequencing run. As a positive control, genomic bulk DNA of each cell line was analyzed in parallel with the same Pyrosequencing assays (Figure 2A-C).

* Availability depends on the country.

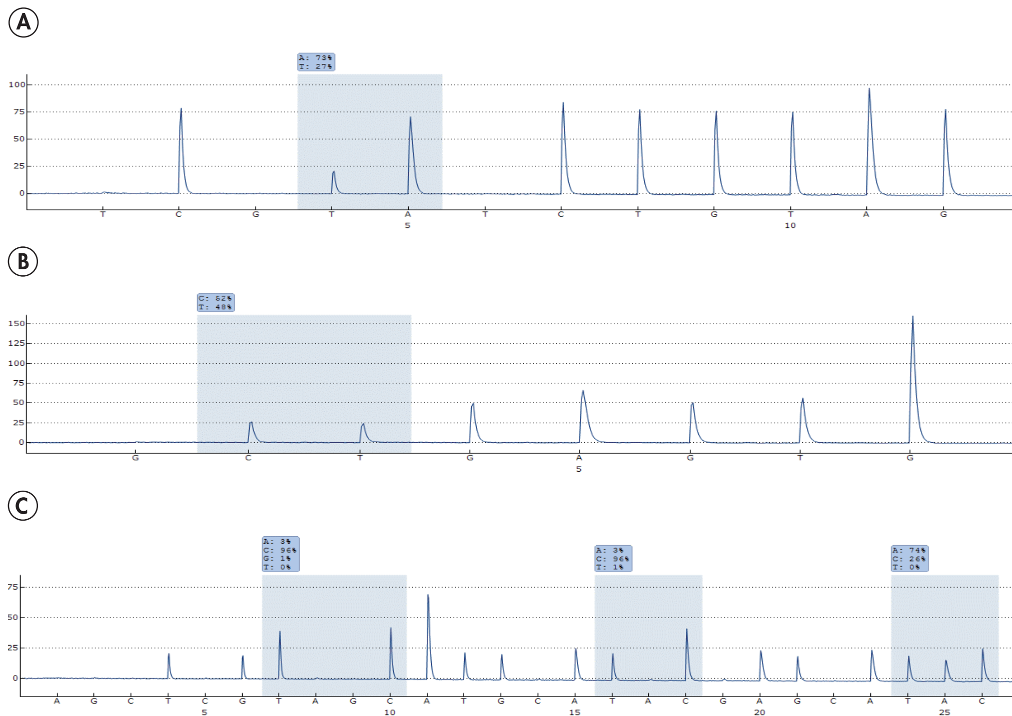

Figure 2. Representative pyrogram of bulk samples of the three different cell lines. A. Representative pyrogram showing the c.1799T>A point mutation (here shown as A>T mutation as reverse strand was sequenced) in the human *BRAF* gene in bulk DNA of HT-29 cells. **B**. Representative pyrogram showing the c.3340C>T point mutation in the human *APC* gene in bulk DNA of LoVo cells. **C**. Representative pyrogram showing the c.1391G>T point mutation (here shown as C>A mutation as reverse strand was sequenced) in the human *BRAF* gene in bulk DNA of fluorescent MDA-MB-231 cells.

Using the QIAscout, 98% of the isolated microrafts contained single fluorescent MDA-MB-231 cells (Figure 3). This result clearly demonstrates how efficiently and reliably targeted single-cell isolation works with the QIAscout method. In contrast, selective isolation of fluorescent cells was not possible using pipetting or serial dilution method.

Manual cell picking for conducting single-cell assays is a skill-dependent technique that may or may not have a good level of success. While performing pipetting, some fluorescent cells could be isolated but 50% of the tubes contained one of the two other cell lines, or two cells, or even no cell. Selectively picking fluorescent single cells is difficult as cells are less fluorescent when they are in suspension and it is difficult to pick one fluorescent cell without picking a non-fluorescent cell (rapid shift between bright field and fluorescent light is necessary). Surprisingly, in our experiment, the percentage of fluorescent cells is high considering the equal distribution of the three cell lines. However, it was later shown that HT-29 and LoVo cells tend to sink much faster compared to MDA-MB-231 cells, thus explaining the observed discrepancy. In summary, pipetting cannot be considered as an ideal method of choice for targeted isolation of single cells.

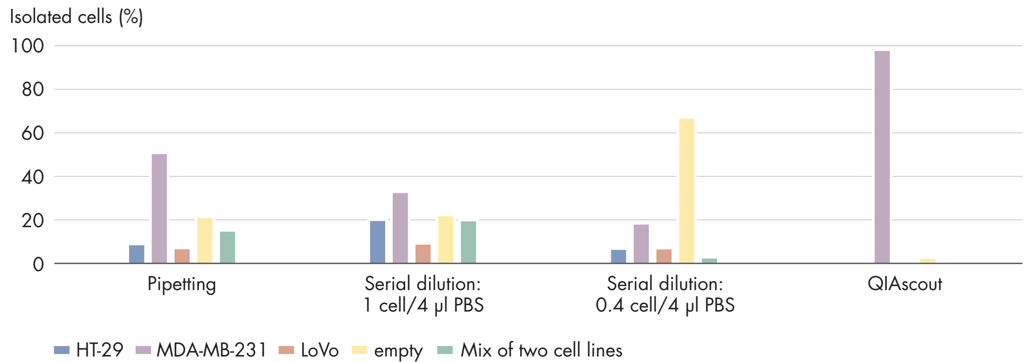

Figure 3. Comparison of three different methods used for selective isolation of single fluorescent cells. Aim of this experiment was to isolate single fluorescent cells using pipetting, serial dilution (using two different concentrations of cells per 4 µl PBS) and the QIAscout method.

Experiment 2: Highly efficient isolation of single elongated cells using the QIAscout method

The aim of this experiment was to selectively isolate elongated cells from a mixture of two round (HT-29 and SW48) and one elongated (LoVo) cell lines using three different isolation methods. Isolated cells were identified by three different Pyrosequencing assays covering one specific mutation per cell line. As a positive control, genomic bulk DNA of each cell line was analyzed with the same Pyrosequencing assays (Figure 4A-C).

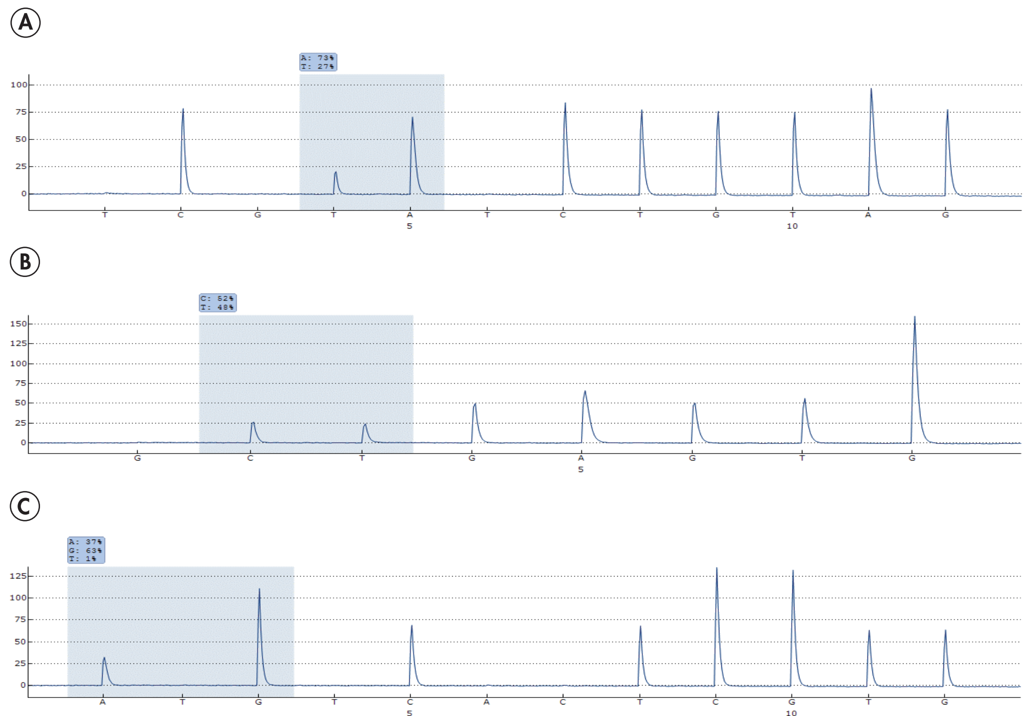

Figure 4. Representative pyrogram of bulk samples of the three different cell lines. A Representative pyrogram showing the c.1799T>A point mutation (here shown as A>T mutation as reverse strand was sequenced) in the human *BRAF* gene in bulk DNA of HT-29 cells.
B Representative pyrogram showing the c.3340C>T point mutation in the human *APC* gene in bulk DNA of LoVo cells.
C Representative pyrogram showing the c.2155G>A point mutation in the human *EGFR* gene in bulk DNA of SW48 cells.

When using the QIAscout, 97% of isolated microrafts contained single elongated LoVo cells (Figure 5). The observed success rate of the QIAscout is remarkable and only one pierced cell did not appear to be the cell type of interest. In contrast, selective isolation of elongated cells was not possible using pipetting or serial dilution. In addition, our results demonstrate the varying success rates of two serial dilutions (1 cell/4 µl PBS and 0.4 cells/4 µl PBS) with respect to the tubes containing not more than one single cell. The lower the concentration of cells, the higher the number of empty tubes and also the lower the number of tubes with more than one single cell.

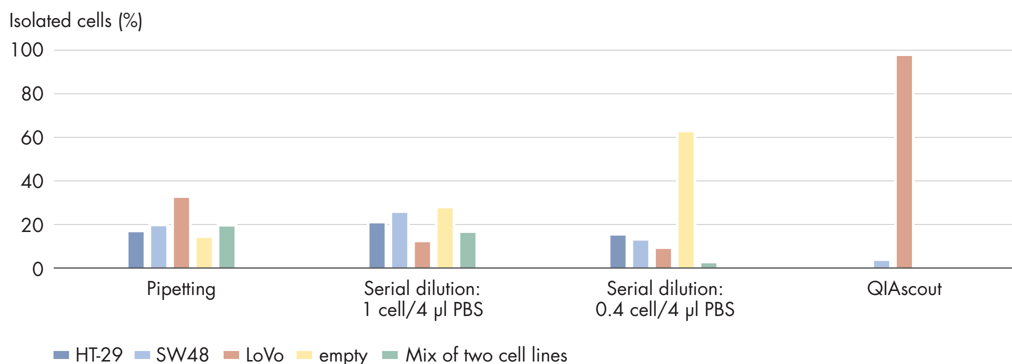

Figure 5. Comparison of three different methods used for selective isolation of single elongated cells. Aim of this experiment was to isolate single elongated cells using pipetting, serial dilution (using two different concentrations of cells per 4 µl PBS) and the QIAscout method.

Conclusions

- The QIAscout method allows efficient and reliable isolation of single targeted cells. Selection of cells can be based on different cellular parameters like morphology or fluorescence.
- Conventional methods like pipetting and serial dilution are less successful in selective isolation of single cells when compared to the novel QIAscout method.

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 Autoprep System	PyroMark Q48 Instrument, multistep pipet, software, documentation and installation	9002470
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
EGFR Pyro Kit (24)†	For 24 reactions: Sequencing Primers, PCR Primers, Unmethylated Control DNA, PyroMark PCR Master Mix, CoralLoad Concentrate, Buffers and Reagents	970480
BRAF Pyro Kit (24)†	For 24 reactions: Sequencing Primers, PCR Primers, Unmethylated Control DNA, PyroMark PCR Master Mix, CoralLoad Concentrate, Buffers and Reagents	970470

* Larger kit sizes available.
† Availability depends on the country. Please contact QIAGEN for more information.

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.

The PyroMark Q48 Autoprep System, BRAF and EGFR Pyro Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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