Application Note

Profiling miRNA Expression from a Single Cell Isolated Using the QIAscout™ system

Martin Kreutz, Jonathan Shaffer

QIAGEN, Maryland, USA

This application note describes a rapid and cost-effective method for the isolation and recovery of single cells using the QIAscout™. The precise and affordable single-cell isolation technology in combination with miScript® Single Cell qPCR Kit and miScript miRNA PCR Array facilitates the downstream expression analysis of miRNAs from individual cells.

Introduction

MicroRNAs (miRNAs) are a family of ubiquitous, small non-coding RNAs that have a significant impact on cell biology and disease. While bulk transcriptomic analysis of miRNA expression is critical for understanding the biological system as a whole, it also leads to "cellular averages" masking the intrinsic transcriptional variability across individual cell subpopulations. Studying miRNA expression at the single-cell level removes much of the ambiguity and offers greater insight into the differences between cells in the same population. Single-cell expression analysis brings into focus the individual contribution of every cell, highlighting a specific biological response otherwise obscured when assessed in bulk. Moreover, profiling of miRNAs in individual cells becomes a prerequisite in instances where miRNA analysis is often restricted by limited sample availability. 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 losing the cellular message.

This application note explains how a low cost and efficient single-cell isolation platform, the QIAscout (Figure 1), simplifies the isolation and preparation of individual cells to reliably detect miRNA expression signatures. The instrument is ideal for various cell types like adherent or suspension cells, primary cells or cell lines and fluorescent 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 reaction tubes for further processing using a magnetic wand, with no risk of contamination or loss of information 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 release device
- Identifying microrafts containing single cells
- Piercing microraft containing cell of interest
- Transferring microraft to a reaction tube using a magnetic wand
- Processing the collected cell for downstream application

Material and Methods

HCT 116 colorectal cancer cells were left untreated or treated with 5-aza-2′-deoxycytidine (5-aza-2′-dC, 10 µM final concentration in DMEM containing 10% FBS) for 72 hours. Growth media was changed every 24 hours for the full duration of the treatment. After 72 hours, the cells were harvested. Approximately 4500 cells resuspended in 3 ml media were seeded in a 200 micron microraft within the QIAscout array and incubated for 6 hours. After 6 hours, the growth medium was replaced by 1x PBS, and for each condition (untreated and treated), 46 cell rafts containing a single cell each were selected using the QIAscout system. Selected cells were then processed using the miScript Single Cell qPCR Kit. The Human miFinder miScript miRNA PCR Array was then used to profile miRNA expression.

Results and Discussion

Principle Component Analysis (PCA) reveals a heterogeneous response amongst individual cells to 5-aza-2′-deoxycytidine treatment

HCT 116 cells were treated with or without 5-aza-2′-dC demethylation reagent. 5-aza-2′-dC irreversibly inhibits DNA methyltransferase (DNMT) driven methylation reactions by incorporating into DNA and covalently binding to the active site of the DNMT. Individually isolated, treated and untreated cells were processed using the miScript Single Cell qPCR Kit. In real-time PCR, the Human miFinder miScript miRNA PCR Array was used to profile miRNA expression. PCA analysis of individual cells (Figure 2) reveals a differential response amongst the treated cells to 5-aza-2′-dC, thus presenting three distinct populations. Together, the data suggests that miRNA heterogeneity exists within single cells and a bulk cell analysis leads to the consequential "cellular averages" masking the intrinsic transcriptional variability across individual cell subpopulations.

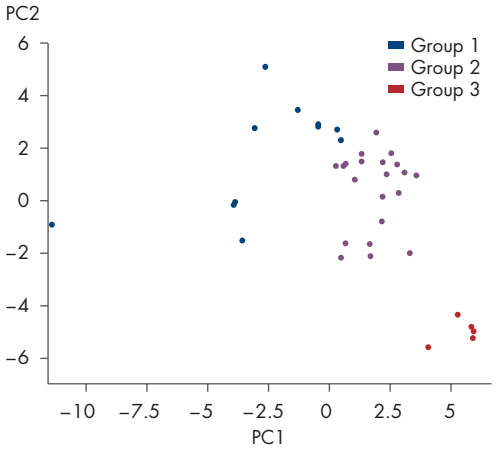

Figure 2. PCA analysis of miRNA expression of single cells. Differential response in miRNA expression amongst treated single cells with 3 distinct populations.

Conclusions

Single-cell analysis for miRNA expression reveals heterogeneity across cell populations. In bulk cell analysis "cellular averages" mask intrinsic transcriptional variability. Distinct cell populations can be effectively revealed by single-cell analysis using the QIAscout for miRNA expression profiling.

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
miScript Single Cell qPCR Kit (24)*	For 24 reactions: Cell lysis, 3' ligation, 5' ligation, reverse-transcription, cDNA cleanup, preamplification reagents, quality control primers	331053
Human miFinder miScript miRNA PCR Array	Array of the 84 most abundantly expressed and best characterized miRNAs in miRBase (www.miRBase.org); available in 96-well, 384-well, or Rotor-Disc 100 format	MIHS-001Z

* Larger kit sizes available

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.