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Review Article

Single-cell gene expression profiling using reverse transcription quantitative real-time PCR

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ABSTRACT

Even in an apparently homogeneous population of cells there are considerable differences between individual cells. A response to a stimulus of a cell population or tissue may be consistent and gradual while the single-cell response might be binary and apparently irregular. The origin of this variability may be preprogrammed or stochastic and a study of this phenomenon will require quantitative measurements of individual cells. Here, we describe a method to collect dispersed single cells either by glass capillaries or flow cytometry, followed by quantitative mRNA profiling using reverse transcription and real-time PCR. We present a single cell lysis protocol and optimized priming conditions for reverse transcription. The large cell-to-cell variability in single-cell gene expression measurements excludes it from standard data analysis. Correlation studies can be used to find common regulatory elements that are indistinguishable at the population level. Single-cell gene expression profiling has the potential to become common practice in many laboratories and a powerful research tool for deeper understanding of molecular mechanisms.

1. Introduction

Cells have a remarkable ability to cooperate and jointly construct complex structures such as tissues, organs and whole organisms. These constructions are normally accurately tuned and respond to stimuli with high precision. During development, cells differentiate to specialized cell types, each with particular functions in the environment they reside. In many aspects, individual cells exhibit a high degree of variability and responses to identical stimuli may be very different even in a seemingly homogeneous population [1–6].

Gene expression profiling is a pivotal research tool in molecular biology. By default, measurements are made on large pools of cells as this will increase reliability of the recordings. For tissues, different cell types are mixed uncontrollably and the measured gene expression profile has unknown contribution from different cell types. In addition, cell population measurement will not reveal how a particular transcript is distributed among the cells (Fig. 1A–B). Bulk measurements easily miss potentially important gene correlations (Fig. 1C–D) where single cell analysis would indicate coupled transcriptional regulations, which might be controlled by the same molecular mechanism (Fig. 1C–D) [7]. Observed heterogeneity may indicate the presence of specialized cell types or originate in the random nature of the transcription machinery [1–6].

A typical single cell contains ~1 pg mRNA, which is equivalent to a few hundred thousand molecules transcribed from about ten thousand genes [8]. The high sensitivity of reverse transcription quantitative real-time PCR (RT-qPCR) makes it possible to detect even a single molecule. RT-qPCR is also characterized by high reproducibility and wide dynamic range [9–11]. These properties make RT-qPCR suitable for single-cell gene expression profiling. Even if single-cell gene expression profiling using RT-qPCR has been successfully applied to several different applications [6,12–14], it has still not become common practice for laboratories. In this paper, we describe the workflow of single cell RT-qPCR including: cell collection, cell lysis, RT, qPCR and data analysis.

2. Description of method

Single cell RT-qPCR constitutes several sequential steps, outlined in Fig. 2. Our intention with this paper is to present the most common experimental approaches with suitable references and in

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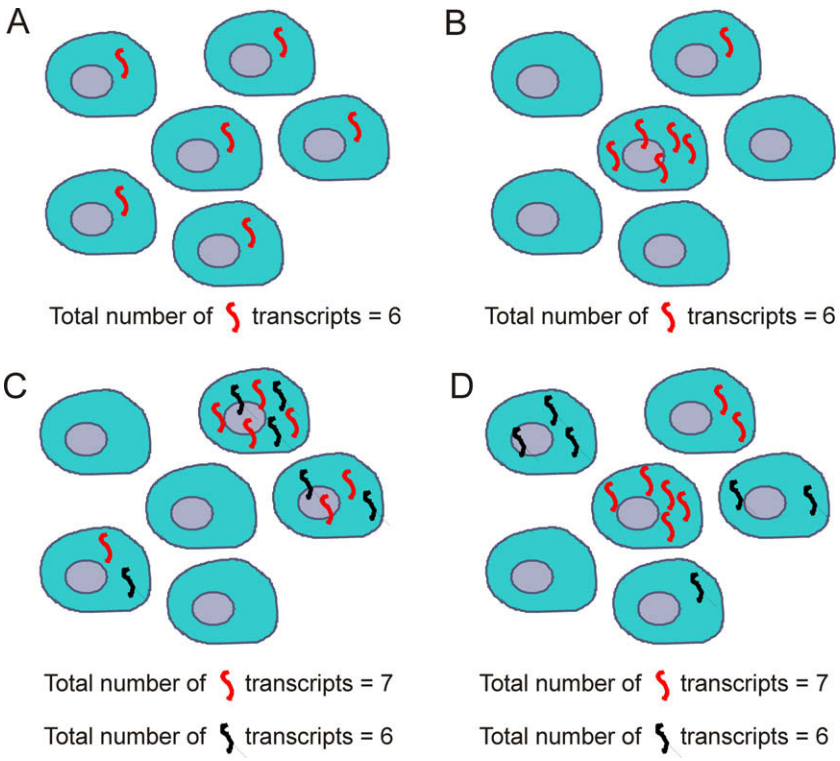

Fig. 1. Cell heterogeneity and correlated transcript levels. Single cell measurements can distinguish the cases in (A) and (C) from (B) and (D), respectively, while cell population measurements cannot.

detail describe the most appropriate experimental setup for analysis of cells in suspension. For successful single-cell gene expression profiling good laboratory practice is essential. All RNA work requires completely RNase free conditions. Furthermore, PCR contamination must be avoided, since even negative (zero values) are used in data analysis. Physical separation, i.e. different rooms, of pre-PCR, PCR and post-PCR is recommended.

3. Single cell collection and lysis

A majority of single-cell studies published deploy one of three methods to collect cells: Flow cytometry, glass capillaries, and laser capture. This review will describe the two former while laser capture and laser microdissection are described in detail elsewhere [15–17]. For some applications, the origin and surroundings of a cell is vital information and this excludes flow cytometry as collection method, leaving laser capture as best option. Where high numbers of cells are needed, and where collections of intact cells are of importance, flow cytometry is recommended. Glass capillaries, as we use them in this article, will also collect intact cells.

Single cell suspensions are prepared from tissue using mechanical separation, enzymatic treatment, and/or non-physiological buffers. The yield of functionally viable, dissociated cells will be dependent on several parameters such as concentrations, incubation times and temperatures. An informative website for different cell dissociation approaches is: www.tissuedissociation.com. As the expression of some genes may be altered by the cell treatment it is recommended that the expression of the genes of interest is quantified also in an untreated sample. For example, one part of the biological sample is saved for total RNA isolation and the remaining is used for cell dissociation and collection.

3.1. Single cell sorting using flow cytometry

Several flow cytometry instruments, such as FACSDiva and FACSVantage (both BD Biosciences, San Jose, CA, USA) can be used to sort out individual cells [18]. PCR plates (96-well) with lysis buffer should be prepared in advance. In addition to standard flow cytometry calibration, the instrument needs to be carefully calibrated to deposit single cells in the center of each collection tube. This can easily be tested by sorting ~50 beads/cells on the plastic

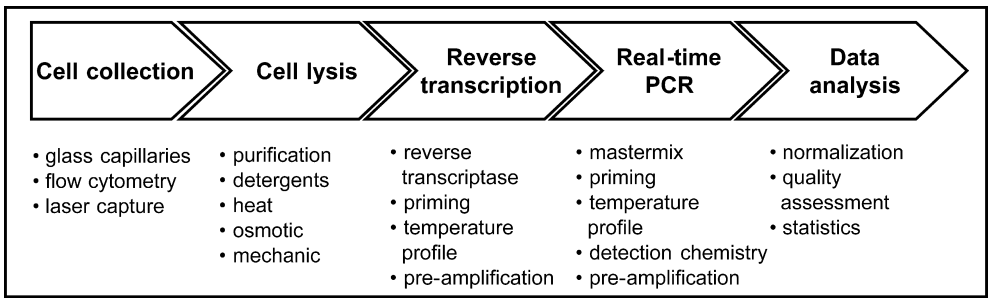

Fig. 2. Overview of single-cell gene expression profiling using RT-qPCR.