Flow cytometric analysis of human primary cells using the Agilent 2100 bioanalyzer and on-chip staining

A fast and accurate method to detect green fluorescent protein expression in samples of low cell numbers

Application

Samuel Chan
Gerd Luedke
Tobias Preckel

Introduction

In previous Application Notes we demonstrated how the Agilent 2100 bioanalyzer can be used to monitor transfection efficiency in mammalian cell lines[1,2,3]. We described how the 2100 bioanalyzer produces high quality data which compares well with data obtained by conventional flow cytometry. This Application Note addresses the major problems that many researchers working with primary cells have, for example, limited availability, quantity and lifespan. Using the Agilent 2100 bioanalyzer and Cell Fluorescence LabChip kit the transfection efficiency of primary cells can be determined using green fluorescent protein (GFP) as a reporter molecule. This study expands on the applications described in previous Application Notes [1,2]. Two types of cells were transfected and analyzed: human umbilical vein endothelial cells (HUVEC) and normal human dermal fibroblasts (NHDF). The advantages of using the compact 2100 bioanalyzer for monitoring transfection efficiency in primary cells include low cell consumption, high reproducibility of results, fast on-chip staining procedure and easy-of-use.

Materials and Methods

Primary cell culture
HUVEC, NHDF, culture media, and trypsin/EDTA solution were obtained from Clonetics. HUVEC cells were maintained in EGM-2 medium and NHDF cells were cultured in FGM-2 medium.

Transfection
Cells were trypsinized and seeded into 12-well culture plates at a density of 3×10^5 cells/mL in 1 mL of culture medium and incubated for 20 hours under cell culture conditions. Prior to transfection the growth medium was replaced by 1 mL of OPTI-MEM I (Gibco). DNA-Lipofectamine 2000 (Invitrogen) complex was prepared and added to the cells according to the supplier's protocol. After six hours of incubation, the transfection medium was removed and replaced with 1 mL of culture medium. The cells were harvested 18 hours later.

On-chip CBNF staining and steps for handling few cells
On-chip CBNF staining and steps for handling few cells are described in reference 2, using procedure B.

Experiments and Results

We have developed tools for measuring the transfection efficiency using a GFP reporter plasmid in primary cells on the 2100 bioanalyzer. Great emphasis was placed on being able to perform the analysis with a minimum of cells and to eliminate steps in the protocol where cells may be lost. These protocols facilitate working with very few cells. A concentrated cell buffer ("2x CB") was added to the Cell Fluorescence Lab Chip kit. This buffer allows the exact adjustment of cell concentration and density of samples with few cells. Cells were transfected and stained on-chip with the live cell stain CBNF as described in reference 2. Only 30,000 cells were required for on-chip staining of primary cells and subsequent measurement on the 2100 bioanalyzer. Figure 1 shows an optimization study conducted to reach best transfection efficiency in HUVEC cells. HUVEC cells were transfected with decreasing DNA:Lipofectamine (LP) ratios (1:1 to 1:10). Figure 1A shows the transfected samples as viewed through a fluorescence microscope 18 hours after transfection and figure 1B shows the corre-

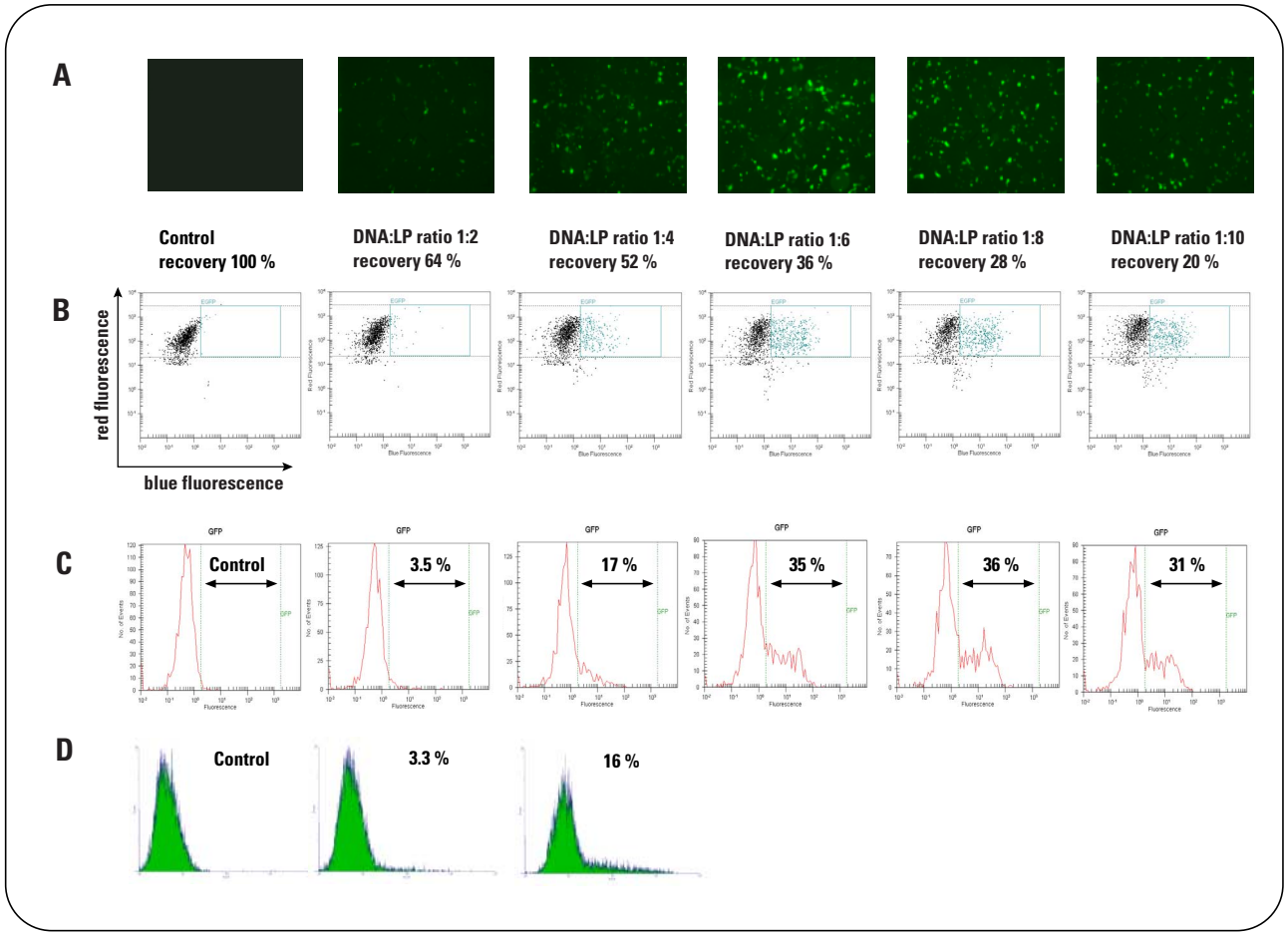

Figure 1
Optimization of transfection efficiency in human umbilical vein endothelial cells (HUVEC). HUVEC cells were transfected with pEGFP-C2 at varying Lipid ratios (DNA:LP ratio). Cells were then stained on chip with the live cell dye CBNF and analyzed on the 2100 bioanalyzer and on a conventional flow cytometer. A) Fluorescence micrographs of control and transfected cell samples obtained with a fluorescence microscope. B) Dot plots of control- and EGFP-transfected cells. C) GFP histograms of control- and GFP-transfected cells obtained with the 2100 bioanalyzer. D) GFP histograms obtained by measuring the same samples on a flow cytometer. 10,000 events were acquired per sample on the flow cytometer whereas 750 events were analyzed on the 2100 bioanalyzer.

sponding dot plots generated on the 2100 bioanalyzer. The selected region shows the positively transfected live cells. Figure 1C shows the GFP-histograms of the samples with the percentages of double positive cells. The recovery of live cells, as determined by manual counting under microscope after trypan blue straining, decreased as the amount of Lipofectamine was increased (compare listed percentages of recovery in figure 1A) due to the toxic effect of Lipofectamine on the cells. However, the transfection efficiency steadily increased up to a DNA:Lipofectamine ratio of 1:8. When more Lipofectamine was used transfection efficiency decreased again. This data shows that to obtain a maximum number of live transfected cells it is important to take the toxicity of the transfection reagent into account. For this experiment, a ratio of 1:6 was found to be optimal. In those cases where enough cells were obtained, samples were measured in parallel on a flow cytometer (figure 1D - only GFP histograms are shown). We also determined the optimal transfection condition for NHDF using a similar protocol. Figure 2 shows the results obtained when the cells were

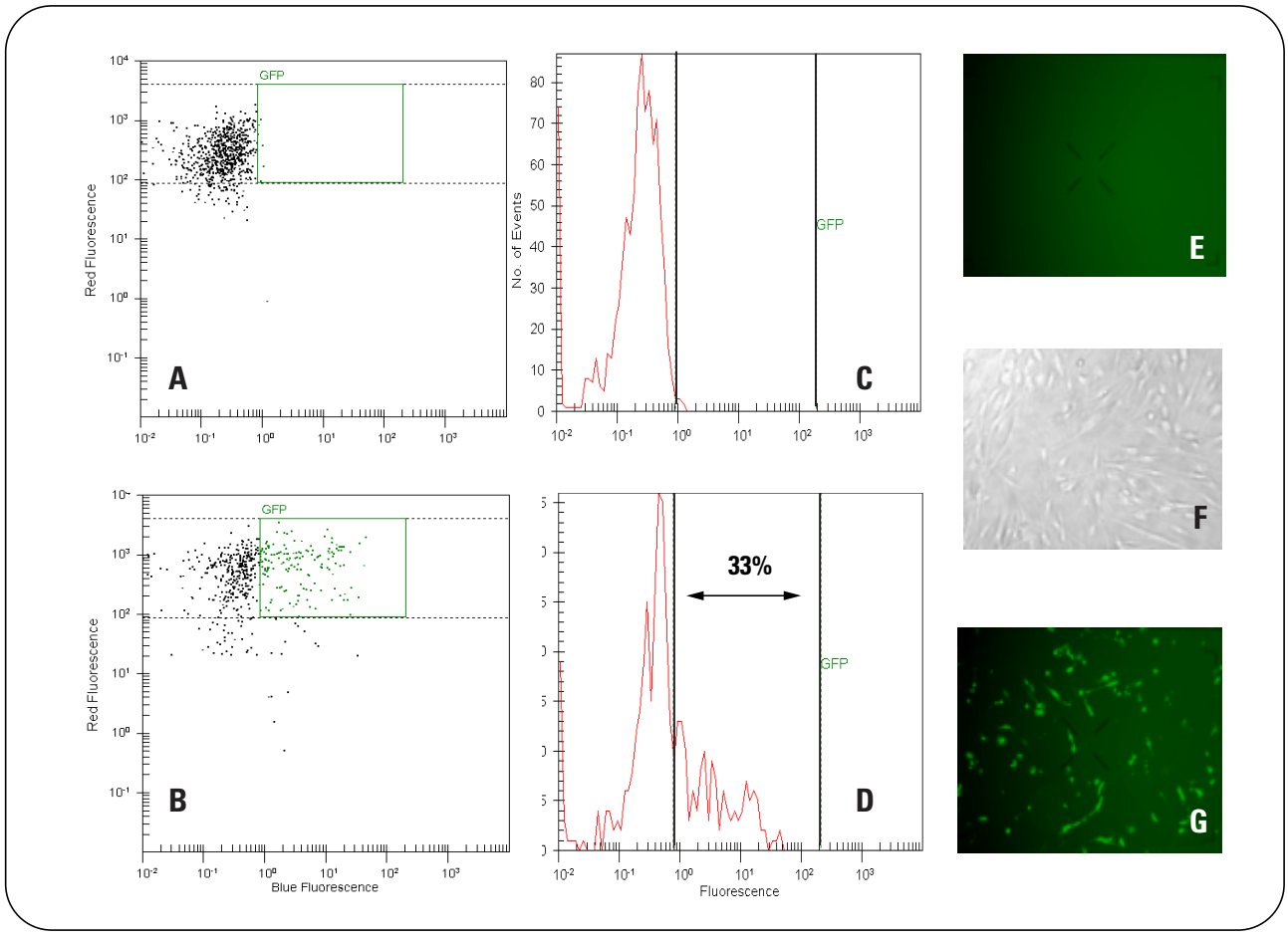

Figure 2
Analysis of transfection efficiency in normal human dermal fibroblasts (NHDF). NHDF were transfected with pEGFP-C2 and then stained on chip with the live cell dye CBNF and analyzed on the 2100 bioanalyzer. Dot plots of control- (A) and EGFP- (B) transfected cells. GFP histograms of control (C) and GFP (D) transfected cells. Fluorescence micrographs of control (E) and transfected (G) cell samples obtained with a fluorescence microscope. F) Phase contrast photo of untransfected NHDF.

transfected with the optimal DNA:Lipofectamine ratio of 1:8. Figure 2 represents the dot plots of the control (A), the transfected sample (B) and the GFP histograms - control (C) and transfected (D). Although on average only 650 events were counted per sample data quality was very good for the histograms and dot plots. Figure 2E shows the untransfected and figure 2G the transfected samples viewed using a fluorescence microscope 18 hours after transfection. Figure 2F is a phase contrast photograph of the control cells.

Conclusion

Primary cells are valuable samples and are frequently limited in availability and lifespan. It can therefore be difficult to conduct experiments with these cells. The Agilent 2100 bioanalyzer with its low sample consumption together with an on-chip staining protocol proves to be a versatile tool for analysis of primary cells. The complete staining procedure takes place on chip and the analysis requires minimal amounts of cells and reagents, saving both time and reducing sample consumption.

References

1.
Hahnenberger, K. and Chan, S. "Monitoring transfection efficiency by green fluorescent protein (GFP) detection with the Agilent 2100 bioanalyzer." *Agilent Application Note* **(2001)**, publication number 5988-4320EN.

2.
Preckel, T.; Luedke, G. and Chan, S. "Monitoring transfection efficiency in cells using an on-chip staining protocol. A rapid and accurate method to detect green fluorescent protein expression." *Agilent Application Note* **(2002),** publication number 5988-7296EN.

3.
Preckel, T.; Luedke, G.; Chan, S.D.H.; Wang, B.N.; Dubrow, R. and Buhlmann, C. "Detection of Cellular Parameters Using a Microfluidic Chip-Based System", **(2002)** *JALA, 7, pages 85-89.*

Samuel Chan is a Staff Scientist at Caliper Technologies, Mountain View, CA, USA.
Gerd Lüdke and Tobias Preckel are Research Scientists at Agilent Technologies Waldbronn, Germany.

www.agilent.com/chem/labonachip

Published November 1, 2002
Publication Number 5988-8154EN

Agilent Technologies