Application note

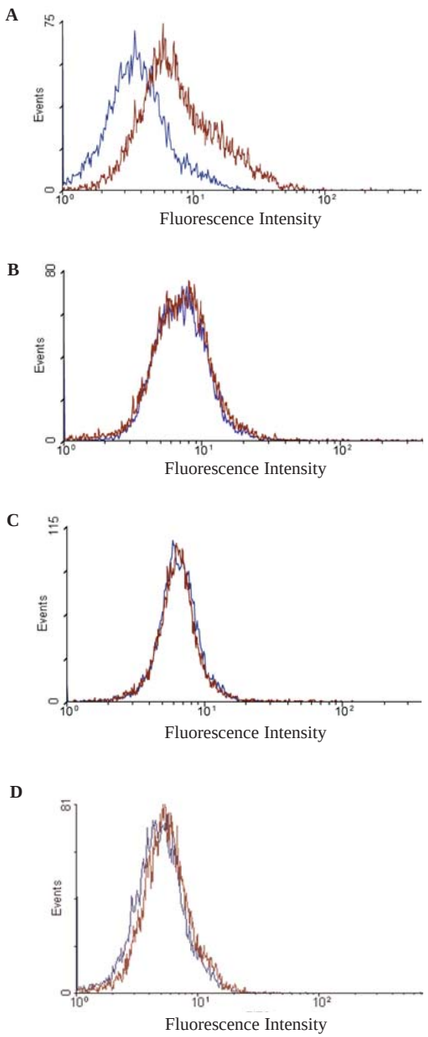
FACS Analysis of Different Transfection Methods for a Primary B Cell line

Kimberly Foreman, Loyola University Medical Center, Maywood, IL 60153 USA

Introduction

BC-3 cells (a primary fusion lymphoma cell line of B-cell lineage latently infected with a herpesvirus, HHV-8) were transfected with pEGFP-N1, a plasmid expressing GFP, using two different transfection reagents (GenePORTER™ 2 and Superfect™) and two different electroporation procedures. Subsequent FACS analysis showed that transfection with GenePORTER 2 transfection reagent gave the best efficiency compared to SuperFect (which was inefficient and not reproducible) and two methods of electroporation (which were reproducible, but not efficient).

Figure 1. FACS analysis of GFP Expression in BC-3 Cells



Transfection methods used:

A. GenePORTER 2 reagent
B. SuperFect reagent
C. Electroporation method I
D. Electroporation method II

Materials & Methods

The pEGFP-N1 plasmid vector (Clontech) was transfected into BC-3 cells using GenePORTER 2 transfection reagent, Superfect transfection reagent (Qiagen), and two methods of electroporation. Evaluation of GFP expression was performed 48 hours post transfection using a Beckman Coulter FACS machine.

Transfection with GenePORTER 2

Cells were resuspended at 2 x 10^6 cells/ml in normal growth media and 1 ml was transferred to each well of a 6-well plate. 4 µg of DNA and 100 µl of DNA Diluent B were mixed and incubated for 1-5 minutes. GenePORTER 2 reagent (20 µl) was mixed with 80 µl of RPMI. The DNA solution and the GenePORTER 2 solution were mixed and incubated at room temperature for 5-10 minutes. The DNA/GenePORTER 2 mixture was added directly to the cells. After 24 hours of incubation, 1 ml of fresh media was added to the cells. Cells were analyzed for GFP expression 48 hours post transfection.

Transfection with SuperFect

Transfection with SuperFect was performed in a 60 mm dish following manufacturer's protocol with 5 µg of DNA and 20 µl of SuperFect reagent.

Electroporation Method I [1]

BC-3 cells (1 x 10^7) were placed in 0.5 ml of electroporation buffer (RPMI 1640 containing 15% fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 1X nonessential amino acids) and were mixed with 10 µg of DNA. Cells were then electroporated at 250 V and 960 µF using a Cell-PORATOR (Life Technologies, Rockville, MD). After pulse application, cells were immediately pelleted by centrifugation and then kept for 20 min at room temperature. Transfected cells were cultured in RPMI 1640 supplemented with 20% heat-inactivated fetal bovine serum.

Electroporation Method II

BC-3 cells (5 x 10^6) were placed in 0.5 ml of RPMI serum-free electroporation buffer and were mixed with 10 µg of DNA. The cells were placed on ice for 5 minutes and were then electroporated at 250 V and 330 µF. Cells were placed on ice for 3 minutes after pulse application and then cultured in serum-containing RPMI 1640 supplemented with 15% heat-inactivated fetal bovine serum.

Results and Discussion

Results in Figure 1 show that 12% of the cells were transfected and GFP expression positive with GenePORTER 2 reagent, although this is likely an underestimate of the percent transfected. The gate was set from the bottom of the control peak all the way to the far right hand side. Clearly, a larger proportion of the cells have shifted to the positive side, but are still overlapping the tail end of the control peak. These cells would not be counted in the above-mentioned 12%, but are presumably expressing at least low levels of GFP. In comparison, the transfection efficiency obtained with SuperFect and electroporation were 1.3% and 1.4 - 1.5% respectively.

In conclusion, the best transfection of B cells was achieved with GenePORTER 2 reagent. Furthermore, transfection of B cells was simple, reliable, and reproducible, unlike results obtained with SuperFect (which was inefficient and not reproducible) and electroporation (which was reproducible, but not efficient).

Reference:

1. Huang, L. *et al.* (2001) *J. Biol. Chem.* **276(16)**: 13427-32

Product	Quantity*	Catalog no.	Price
GenePORTER 2 Transfection Reagent			
	75 transfections (0.75 ml)	T202007	$170
	150 transfections (1.5 ml)	T202015	$325
	750 transfections (5 x 1.5 ml)	T202075	$1,375

*Each transfection is for delivering 2 µg of DNA.