Optimization of transient gene expression in mammalian cells and potential for scale-up using flow electroporation

Janet H. Parham*, Marie A. Iannone, Laurie K. Overton & Jeff T. Hutchins

Glaxo Wellcome, 5 Moore Drive, Research Triangle Park, NC 27709, U.S.A.

E-mail: parham-jh@glaxo.com

Received 25 August 1998; accepted 25 August 1998

Key words: cell cycle, electroporation, Epstein Barr nuclear antigen, flow electroporation, mammalian transfection, T-antigen

Abstract

The goals of this study were to identify mammalian cell lines which could be efficiently transiently-transfected and scaled-up for protein production. The transfection efficiencies of eight cell lines (NSO, NSO-TAg, CV-1, COS-7, CHO, CHO-TAg, HEK 293, and 293-EBNA) were measured using electroporation for DNA delivery and green fluorescent protein (Evans, 1996) as the reporter gene. In addition, we have evaluated the effects of stable expression of viral proteins, cell cycle manipulation, and butyrate post-treatment in small scale experiments. The cell lines varied widely in their GFP transfection efficiencies. Stable expression of simian virus 40 large T-antigen or Epstein Barr nuclear antigen failed to significantly increase transfection efficiency above that seen in the parental lines. Aphidicolin (a DNA polymerase inhibitor), which blocked cells from S or G2/M, brought about an increase in transfection efficiency in two cell lines. The primary effect of butyrate (a histone deacetylase inhibitor) post-treatment was an increased intensity of the fluorescent signal of green fluorescent protein, as measured by flow cytometry (1.0 to 4.2-fold, depending on the cell line). The combined use of aphidicolin pretreatment followed by butyrate treatment post-electroporation yielded increases in fluorescence intensities ranging from 0.9 to 6.8-fold. Based on their high transfection efficiencies in small scale experiments, rapid growth, and ability to grow in suspension culture, CHO, CHO-TAg, and 293-EBNA were selected to assess the feasibility of using flow electroporation for large-scale transfections. Using secreted placental alkaline phosphatase as a reporter, 293-EBNA cells produced the highest protein levels in both the presence and absence of butyrate. These data indicate that flow electroporation provides an efficient method of DNA delivery into large numbers of cells for mammalian protein production.

Abbreviations: APH – aphidicolin; BT – butyrate; EBNA – Epstein Barr nuclear antigen; GFP – green fluorescent protein; MESF – molecules of equivalent soluble fluorochrome; SPAP – secreted placental alkaline phosphatase; SV40 – Simian virus 40; TAg – T-antigen; TE – transfection efficiency.

Introduction

The advent of genetics strategies, mechanism-based drug design and high through-put screening in the pharmaceutical industry has generated an increased need for the rapid, large-scale production of mammalian recombinant proteins. In this context, modest levels of protein are needed for antibody pro-

duction, target compound screening, and *in vitro/in vivo* validation studies. Achieving high levels of protein production is dependent on efficient methods of DNA delivery and gene expression, RNA stability, translation efficiency, and proper post-translational modification. DEAE-dextran and calcium phosphate precipitation were two of the early methods developed for DNA delivery (Pari, 1997). A number of commercial cationic lipid products that facilitate the

* Author for all correspondence.

Figure 1. Large-scale SPAP electroporations were performed as described in Section Materials and Methods. Medium was sampled and filtered daily for seven days following electroporation. Samples were refrigerated and assayed at one time. Results are the average of two experiments.

uptake of DNA into mammalian cells have been developed (Hawley-Nelson, 1996; Schenborn, 1998). Other compounds currently being used to deliver DNA into cells include polyethylenimine (Boletta, 1997), polybrene/DMSO (Aubin, 1997), and viral vectors such as Semliki Forest (Berglund, 1998), adenovirus (Konesdi, 1997), and baculovirus (Possee, 1997). Alternatively, electroporation offers a non-chemical method of DNA delivery for mammalian cell transfection (Lurquin, 1997). Several of these methods, while very efficient on a small scale, are not practical for use in large scale transfections.

Mammalian cell lines vary widely in their ability to be transfected with recombinant DNA. While fi-

broblasts and myeloid lines are known to be difficult to transfect, cell lines such as CHO, COS-7, 293-EBNA, and BHK are in common use in transfection experiments because they take up DNA and express recombinant proteins very efficiently. A number of other factors may influence gene expression following transfection; for example, cell cycle manipulation has been shown to enhance the transfectability of cells (Nicolau, 1982; Strain 1985). In addition, SV40 large T-antigen (TAg) and Epstein Barr nuclear antigen (EBNA), multi-functional proteins which immortalize mammalian cells and increase growth potential, can also increase transcription, thereby enhancing gene expression (Harvey, 1997; Manfredi, 1994; Parker,

Table 1. Cell line comparison of transfection efficiency (TE) and fluorescence intensity (MESF) using GFP as a reporter gene

Cell line	Doubling time (hr)	Max. express.	TE (%)	Fold-increase	Fluor. intensity (MESF $\times 10^6$)	Fold-increase
NSO	20.8	Day 1	8.43		0.22	
NSO-TAg	25.4	Day 1	9.82	1.2	0.22	1.0
CV-1	44.8	Day 2	61.03		12.67	
COS-7	41.1	Day 3	71.23	1.2	8.00	0.6
CHO	19.1	Day 2	62.42		7.08	
CHO-TAg	19.2	Day 2	79.22	1.3	8.29	1.2
293	46.7	Day 3	68.74		12.75	
293-EBNA	14.2	Day 2	82.99	1.2	5.71	0.4

Three pairs of cell lines (+ and – stably expressed T-antigen) and one pair of cell lines (+ and – stably expressed EBNA-1 of Epstein Barr virus) were compared for their transfection efficiency using GFP as a reporter gene. Doubling times were calculated over a 48 hr period. Electroporation data are expressed as the percentage of total cells expressing GFP (TE). Fold-increase represents the increase in transfection efficiency or fluorescence intensity over the parental cell line, presumably due to the presence of T-antigen or EBNA. Transfection efficiency and MESF were measured 17 hr (Day 1), 41 hr (Day 2), and 65 hr (Day 3) following electroporation. The transfection efficiency presented for each cell line was the maximum measured for that cell line over the three day post-transfection period. Results are the average of two experiments.

1996; Rice, 1993). Furthermore, protein production following transfection can be enhanced by histone deacetylase inhibitors such as butyrate and trichostatin (Kruh, 1982; Tang, 1994; Yoshida, 1990; Chen, 1997).

In these experiments we have evaluated the potential of several commercially available cell lines, as well as two cell lines developed internally, for potential use in large-scale electroporation experiments. In addition, we examined the effects of viral proteins, cell cycle manipulation, and the post-electroporation effects of butyrate on transfection efficiency and protein expression. The conditions for optimal protein production, using SPAP as a reporter gene and flow electroporation as the method of gene delivery, are described for the CHO, CHO-TAg and 293-EBNA cell lines. The selection criteria for large-scale protein production by these cell lines were rapid growth (doubling time <24 hr), high transfection efficiency by electroporation (>60%) and ability to grow in serum-free suspension culture.

Materials and methods

Cell lines

Chinese Hamster Ovary (CHO, ATCC) and CHO-TAg cells (J. Northrop, E. Tate; Affymax, Palo Alto,

CA) were routinely maintained in DMEM/F12 (Life Technologies; Gaithersburg MD) + 5% Fetal Clone II (HyClone; Logan UT). African Green Monkey kidney cells (CV-1, ATCC), SV 40 transformed African Green Monkey kidney cells (COS-7, ATCC), human embryonic kidney (HEK 293, ATCC) and 293-EBNA (InVitrogen; Carlsbad CA) were maintained in DMEM/F12 + 10% Fetal Clone II. Mouse myeloma (NSO – CellTech; Berkshire UK) and NSO-TAg cells (isolated in our laboratory following transfection with the T antigen construct from J. Northrop) were grown in a defined growth medium (RD002) supplemented with 2X glutamine, lipids, peptone, and B cyclodextrin.

Chemicals

Aphidicolin (Sigma; St. Louis MO), prepared as a 1000X stock in DMSO was added to logarithmically growing cells for 24 hr. Following a 6 hr rest period, cells were harvested for cell cycle analysis or electroporation. Butyrate (Sigma), prepared as a 100X stock in distilled water and used at a final concentration of 2 mM, was added to cultures immediately following electroporation.

Table 2. Effects of aphidicolin (APH) pretreatment on cell cycle progression

Cell line	Treatment	Cell cycle phase		
		G0/G1	S	G2/M
		—————(%)—————		
NSO	Control	57.1	27.6	15.3
	APH	26.5	55.5	18.1
NSO-TAg	Control	62.4	26.9	10.7
	APH	29.3	54.8	15.8
CV-1	Control	64.2	18.4	17.4
	APH	21.2	69.4	9.4
COS-7 ^a	Control	66.2	23.0	10.7
	APH	44.1	55.4	0.47
CHO	Control	54.6	28.1	17.3
	APH	0.75	81.0	13.2
CHO-TAg	Control	27.3	56.1	16.6
	APH	3.8	82.5	13.7
HEK 293	Control	56.1	19.4	24.5
	APH	55.1	34.4	10.5
293-EBNA	Control	44.5	45.1	10.4
	APH	14.6	82.3	3.1

Log-phase cultures were pre-treated with aphidicolin for 24 hr. Aphidicolin was removed and the cells were allowed to recover for 6 hr before being trypsinized for electroporation. Cells were harvested for cell cycle analysis immediately prior to electroporation. Results are average of two experiments.

^a Cell cycle analysis demonstrated that the COS-7 cells were aneuploid, with two separate cycling populations. The COS-7 results presented in the table represent the percentages of the cell population with higher DNA content.

Electroporations

Small-scale electroporation experiments were performed using a Bio-Rad Gene Pulser (BioRad; Hercules CA). Twenty four μg of GFP (pGreen Lantern – Life Technologies; Gaithersburg MD) DNA was added to 6×10^6 cells in 0.4 mL of cold RPMI (Life Technologies) with 20% Fetal Clone II and 10 mM HEPES (Life Technologies) in 0.4 cm BioRad cuvettes. Optimal electroporation conditions for each cell line had been previously determined and were as follows: COS-7 and CV-1 (250 V, 960 μF); CHO and CHO-TAg (290 V, 960 μF); 292 and 293-EBNA

(300 V, 500 μF); and NSO and NSO-TAg (1500 V, 3 μF , 2 pulses).

Large-scale electroporations were performed using an Electro Flow Porator T9000 (BTX Inc., a Division of Genetronics; San Diego CA). The pump was calibrated to pump 18.5 mL min^{-1} and the flow electroporation chamber holds 1.54 mL of medium. The instrument can be programmed to deliver an infinite number of sequential pulses. The voltage was set at 400 V and the capacitance at 2100 μF for CHO and CHO-TAg (time constant of ~ 11 mseconds) and at 500 V, 800 μF for 293-EBNA (~ 4.5 mseconds). 10^8 cells were suspended with 200 μg of plasmid DNA in 1.54 mL of electroporation medium for each pulse. The electroporation medium consisted of cold RPMI with 20% Fetal Clone II and 10 mM HEPES. Following electroporation the cells were placed in suspension culture in Techne stir flasks in 50 mL of either CHO-S-SFM II (Life Technologies) or IS 293 (Irvine Scientific; Irvine CA) at a density of 10^6 cells mL^{-1} .

Flow cytometric analysis

Cultures were harvested for analysis at various times following electroporation and cells were counted in a coulter counter. 1×10^6 cells were resuspended in 0.5% paraformaldehyde for flow cytometric analysis using a FACStar^{PLUS} (Becton Dickinson; San Jose CA) equipped with an INNOVA 90 laser (Coherent; Palo Alto CA) operated at 200 mW and tuned to an excitation wavelength of 488 nm. GFP fluorescence was determined in FL1 using a 510/20 bandpass filter. Cells were evaluated for transfection efficiency (percentage of total cells expressing GFP), as well as the fluorescence intensity of GFP. Quantitative flow cytometric analysis of GFP was conducted using Quantum Fluorescence for MESF Units of FITC (Sigma). When GFP fluorescence intensity was brighter than the standards, a neutral density filter (0.1, 1.0 or 2.0; Oriol Instruments, Straford, CT) was placed before the FL1 bandpass filter such that GFP light signal was on the same scale as the bead standards (same instrument settings). The GFP fluorescence values were converted to MESF values using a calibration plot. The filtered GFP MESF values were corrected mathematically after measuring the actual percent light of the brightest bead standard in the presence or absence of the neutral density filter.

Cells were immunofluorescently-stained for TAg using a modification of Su et al. (1997). A monoclonal antibody to TAg (isolated in house)

Table 3. Effects of aphidicolin (APH) pretreatment on electroporation efficiency and GFP fluorescence intensity

Cell line	Treatment	TE (%)	Fold-increase	Fluor. intensity	Fold-increase
NSO	Control	8.43		0.22	
	APH	16.46	2.0	0.25	1.1
NSO-TAg	Control	9.82		0.22	
	APH	23.73	2.4	0.33	1.5
CV-1	Control	61.03		12.67	
	APH	49.53	0.8	12.84	1.0
COS-7	Control	71.23		8.00	
	APH	59.22	0.8	10.55	1.3
CHO	Control	63.64		7.08	
	APH	73.12	1.2	10.87	1.5
CHO-TAg	Control	79.22		8.29	
	APH	88.59	1.1	19.79	2.4
HEK 293	Control	68.74		12.75	
	APH	61.00	0.9	15.13	1.2
293-EBNA	Control	82.99		5.71	
	APH	65.34	0.8	9.83	1.7

Log-phase cells were treated for 24 hr with $2 \mu\text{g mL}^{-1}$ aphidicolin and then allowed to recover for six hours prior to electroporation. The GFP percentages shown represent the maximal value for each cell line over the three day expression period. Fold-increase represents the increase in the total cell GFP expression or fluorescence intensity after aphidicolin pretreatment compared to the untreated control cells. Fluorescence intensity was measured as indicated in Materials and Methods and is expressed as $\text{MESF} \times 10^6$.

was used as the primary antibody and a goat-antimouse phycoerythrin-conjugated antibody (Southern Biotechnology Associates, Inc; Birmingham AL) was used for detection and quantitation as described above. Cultures to be used for cell cycle analysis and electroporation were in log phase (50–70% confluent) at the beginning of the experiment. Cells were harvested immediately prior to electroporation, fixed in 70% ethanol, and stained later with propidium iodide ($50 \mu\text{g mL}^{-1}$) containing DNase-free RNase (1.0 mg mL^{-1}) in PBS. The various cell cycle phases were measured and separated according to DNA content using a FACScalibur analytical cytometer (Becton Dickinson; San Jose CA) using Modfit LT software (Verity Software House Inc.; Topsham ME).

SPAP assays

SPAP was assayed (hydrolysis of the p-nitro-phenyl phosphate substrate) using a modified version of the assay described by Berger et al. (1988). Unknown samples were read against an SPAP standard curve containing known amounts of SPAP in a SpectraMax 250 spectrophotometer and data was analyzed using SpectraMax Pro software.

Results and discussion

Cell line comparison of transfection efficiency

Eight mammalian cell lines were evaluated for their growth characteristics and transfection potential using GFP as a reporter gene. NSO, CHO, and CV-1 were

Table 4. Effects of butyrate (BT) post-treatment and combined APH pretreatment and BT post-treatment on transfection efficiency (TE) and fluorescence intensity of GFP

Cell line	Treatment	TE (%)	Fold-increase	Fluor. intensity	Fold-increase
NSO	Control	8.43		0.22	
	BT	29.60	3.5	0.29	1.3
	APH+BT	25.23	3.0	0.33	1.8
NSO-TAg	Control	9.82		0.22	
	BT	36.36	3.7	0.38	1.7
	APH+BT	35.30	3.6	0.63	2.9
CV-1	Control	61.03		12.67	
	BT	53.90	0.9	13.03	1.0
	APH+BT	42.89	0.7	10.37	0.8
COS-7	Control	71.23		8.00	
	BT	57.44	0.8	15.45	1.9
	APH+BT	35.04	0.5	13.77	1.7
CHO	Control	62.74		7.08	
	BT	64.79	1.0	12.58	1.8
	APH+BT	66.55	1.1	33.18	4.7
CHO-TAg	Control	79.22		8.29	
	BT	77.66	1.0	34.08	4.1
	APH+BT	87.17	1.1	56.77	6.8
HEK 293	Control	68.74		12.75	
	BT	63.50	0.9	31.58	2.5
	APH+BT	53.53	0.8	35.86	2.8
293-EBNA	Control	82.99		5.71	
	BT	82.97	1.0	24.09	4.2
	APH+BT	60.15	0.7	10.97	1.9

Cells were post-treated with butyrate or pretreated with aphidicolin and post-treated with butyrate as described in Materials and Methods. Transfection efficiencies (TE) shown were the maximum obtained for each cell line over the three day expression period. Fold-increase represents the increase in transfection efficiency or GFP fluorescence intensity with butyrate treatment or combined aphidicolin/butyrate treatment compared to untreated control cells. Results are the average of two experiments.

the immortalized, parental lines for NSO-TAg, CHO-TAg, and COS-7 respectively, all of which stably expressed SV40 large TAg. 293 was the immortalized, parental line for 293-EBNA which had been stably transfected with EBNA-1. GFP expression was measured by flow cytometry for three days following electroporation to determine the time of maximal expression for each cell line. The maximal transfection efficiency for each cell line was found to vary widely (Table 1). The highest transfection efficiencies were obtained with 293-EBNA and CHO-TAg (82.99 and 79.22%, respectively), followed by COS-7 (71.23%), 293 (68.74%), CHO (62.42%), and CV-1 (61.03%). NSO and NSO-TAg had very low transfection efficiencies (8.43 and 9.82%, respectively).

SV40 TAg and EBNA are origin-binding proteins that have been used to transform and immortalize cells (Manfredi and Prives, 1994; Grossman and Laimins, 1996). These processes usually involve an enhancement of growth potential, loss of dependence on growth factors, expanded life span in culture, and alterations in cell cycle – all of which may affect gene expression. CHO and NSO had doubling times of 20 hr or less and expression of TAg had no effect on doubling times (Table 1). The expression of TAg in COS cells caused only a slight growth enhancement (i.e. a small decrease in doubling time). However, expression of EBNA in 293 cells reduced the doubling time from ~47 to 14.2 hr (Table 1). The abnormally low doubling time of 293-EBNA was averaged over

a 48 hr period in which there was a very rapid burst of proliferation in the first 24 hr, followed by approximately one doubling in the second 24 hr period (data not shown).

In addition, the stable expression of TAg has been reported to increase the transient expression of genes from transfected vectors (Northrop et al., 1993; Alwine, 1985; Harvey et al., 1997) and has been implicated as being a universal activator of many different transcriptional promoters (Alwine, 1985; Rice, 1993). TAg expression also amplifies the copy number of the expression plasmids boosting the protein expression level of the transfected plasmid (Harvey, 1997). In contrast, EBNA has not been shown to be associated with universal transactivation, having more in common with papillomavirus proteins than with TAg (Parker, 1996; Grossman, 1996). EBNA has been reported to enhance the replication of plasmids which include EBNA-1 and oriP (Yates, 1985). In these experiments, the stable expression of TAg in three cell lines failed to significantly enhance transfection efficiency or the fluorescence intensity of GFP (Table 1). Similarly, the expression of EBNA had no significant effect on 293 cells (Table 1). However, only one EBNA-containing cell line was tested. It should also be noted that the GFP plasmid used for the transient transfections did not contain the Epstein Barr virus origin of replication, but rather an SV40 ori and the cmv promoter region.

The fact that all four parental cell lines were already transformed and immortalized may explain why TAg and EBNA failed to affect transfection efficiencies. The expression of these viral proteins in non-transformed cells may have generated more striking differences. However, for our purposes, nontransformed cell lines were not deemed practical for use in large scale transfections.

Quantitation of T-antigen expression

To determine whether there was a correlation between the level of stable TAg expression and transfection efficiency, TAg expression was quantitated by flow-cytometric analysis concurrently with the initial transfection experiments. COS-7 showed the highest level of TAg expression (582,136 MESF), followed by CHO-TAg (181,863 MESF), and NSO-TAg (8,061 MESF). We hypothesized that cell lines expressing higher levels of T antigen would have higher transfection efficiencies than the parental lines; how-

ever (as was shown in the previous section), T antigen had no effect on transfection efficiencies.

Effects of viral proteins and aphidicolin on cell cycle and GFP expression

In murine melanocytes (Zepter, 1995), stable expression of TAg increased the percentage of cells in G2/M when compared to the parental line, and has also been reported to induce S-phase in 3T3 cells (Ogris, 1993). As shown in Table 2, TAg had no effect on the cell cycle progression of NSO cells which may be a function of the low level of expression of TAg in NSO cells since varying amounts of TAg are required to induce different cellular functions (Ogris, 1993; Price, 1994). COS-7, CHO-TAg and 293-EBNA cells showed a 2-fold increase in the percentage of cells in S phase compared to the parental lines. This increase in the percentage of S-phase cells was not accompanied by a similar increase in transfection efficiency (Table 1). It should be noted that the numbers for COS cells reflect the percentages in aneuploid cells that made up the majority of the population.

Studies have shown that cells in G2/M demonstrated enhanced transfectability (Nicolau, 1982; Strain, 1985). By blocking cell cycle progression with aphidicolin (a DNA polymerase inhibitor), we sought to determine whether increasing the percentage of cells in S or G2/M would translate into increased transfection efficiency or fluorescence intensity in our cell lines. Table 2 represents a 24 hr pretreatment with aphidicolin followed by a 6 hr rest, demonstrating the movement of the majority of the cells out of G0/G1 in the 8 cell lines tested (Table 2). Aphidicolin pretreatment enhanced transfection efficiency in only two of the eight cell lines tested – by 2.0- and 2.4-fold in NSO and NSO-TAg cells, respectively (Table 4), correlating with the 2-fold increase in S-phase cells (Table 2). Although COS, CHO and CHO-TAg cells had 2.4, 2.9-fold and 1.5-fold respective increases in the percentage of S phase cells (Table 2), their transfection efficiencies were statistically unchanged (Table 3). The fluorescence intensity, as a measure of GFP expression levels, did increase in the NSO-TAg, COS-7, CHO, CHO-TAg, HEK-293, and 293-EBNA cell lines (Table 3).

Effects of butyrate and combined aphidicolin and butyrate treatment on GFP expression

Sodium butyrate has been reported to produce many morphological and biochemical changes in cells, in-

cluding increased stable and transient expression of recombinant proteins (Gorman, 1983), which may be linked to butyrate's inhibition of histone deacetylase (Kruh, 1982). In this context, we tested the effects of post-electroporation treatment with butyrate alone and in combination with aphidicolin on GFP expression levels in our eight cell lines. When compared to untreated controls, butyrate treatment alone increased transfection efficiency in two of eight cell lines (NSO – 3.5-fold and NSO-TAg – 3.7-fold), as well as GFP fluorescence intensity, indicating increased production of the GFP protein (Table 4). Five other cell lines showed increases in the fluorescent intensity of GFP (MESF) ranging from 1.3-fold in NSO to 4.1 and 4.2-fold in CHO-TAg and 293-EBNA (Table 4). Combined aphidicolin pre-treatment and butyrate post-treatment further increased the GFP fluorescence intensity in NSO-TAg, CHO, and CHO-TAg by 3.0, 4.7, and 6.8-fold respectively (Table 4). Despite the additive effect of aphidicolin and butyrate in some cell lines, aphidicolin pre-treatment was not considered to be practical for use in large-scale electroporation experiments because of the extra time and manipulation involved. In subsequent large-scale experiments, only butyrate post-treatment was used.

Large-scale electroporation of CHO, CHO-TAg and 293-EBNA cells

Based on their high transfection efficiencies and rapid growth, CHO, CHO-TAg and 293-EBNA were selected for evaluation as large-scale protein expression vehicles. As prerequisites for analysis, the cell lines had doubling times of 24 hr or less and the ability to grow in suspension culture in Techne stir flasks under serum-free conditions. Large-scale electroporation experiments were carried out using a BTX 9000 Electro Flow Porator. After optimizing electroporation conditions for each cell line (see Section Materials and Methods), levels of SPAP expression were measured in mock-transfected (no DNA) control cells and SPAP-transfected cells, with and without post-electroporation butyrate treatment (Figure 1). Cell counts taken on Day 1 following electroporation with SPAP showed 73, 86, and 71% surviving cells in CHO, CHO-TAg and 293-EBNA respectively. SPAP-transfected CHO cells secreted very little SPAP, with butyrate treatment increasing the SPAP concentration 1.7-fold on Day 7 (Figure 1). SPAP production reached a plateau in CHO-TAg on Day 4 following electroporation (250 U/10⁶ cells), while butyrate treat-

ment increased the SPAP levels 2.5-fold (Figure 1). In 293-EBNA, the expression of SPAP in the media continued to rise throughout the seven day assay period, reaching a level of 854 U/10⁶ cells on Day 7; butyrate increased this concentration by 2.2-fold to 1865 U/10⁶ cells (Figure 1). These results correlate with our small-scale observations measuring the transfection efficiency and fluorescence intensity of GFP. As a follow-up to the SPAP expression studies, several excreted proteins have been expressed in the mg/liter (10⁹ cells) range in 293-EBNA cells using this system (data not shown).

Conclusions

In summary, electroporation utilizing a flow chamber is a efficient and practical method for performing large-scale transfections. Although several cell lines were evaluated for growth rate, transfection efficiency, and GFP fluorescence intensity, 293-EBNA was found to produce the highest levels of SPAP protein in large-scale experiments. Butyrate post-treatment was found to enhance protein production, particularly in CHO-TAg and 293-EBNA, and is now routinely included in our large-scale transfection experiments.

Acknowledgements

We would like to acknowledge Jeff Northrop and Emily Tate of Affymax for kindly providing us with the CHO-TAg cells and the TAg plasmid. Also thanks to Jeff Robbins and Martin Rink for the large-scale preparation of high quality DNA, Eric Dixon for assistance with the SPAP assays, and Tom Kost for critical review of the manuscript.

References

- Alwine J (1985) Transient gene expression control: effects of transfected DNA stability and trans-activation by viral early proteins. *Mol Cell Biol* 5: 1034–1042.
- Aubin RA, Weinfeld M, Taghavi M, Mirzayans R and Paterson MC (1997) Highly effective delivery of foreign DNA to adherent cells via polybrene/DMSO-assisted gene transfer. *Methods Mol Biol* 62: 319–342.
- Berger J, Hauber J, Hauber R, Geiger R and Cullen BR (1988) Secreted placental alkaline phosphatase: a powerful new quantitative indicator of gene expression in eukaryotic cells. *Gene* 66: 1–10.

- Berglund P, Sjöberg M, Garoff H, Atkins GJ, Sheahan BJ and Liljestrom P (1993) Semliki Forest virus expression system: production of conditionally infectious recombinant particles. *Biotechnol* 11: 916–920.
- Boletta A, Benigni A, Lutz J, Remuzzi G, Soria MR and Monaco L (1997) Nonviral gene delivery to the rat kidney with polyethyleneimine. *Human Gene Therapy* 8: 1243–1251.
- Chen WY, Bailey EC, McCune SL, Dong J-Y and Townes TM (1997) Reactivation of silenced, virally transduced genes by inhibitors of histone deacetylase. *PNAS* 94: 5798–5803.
- Evans K, Hawley-Nelson P, Schifferli K and Jessee J (1996) pGreen LanternTM-1, a superior green fluorescent protein mammalian cell transfection reporter. *Focus* 18: 40–42.
- Gorman CM and Howard BH (1983) Expression of recombinant plasmids in mammalian cells is enhanced by sodium butyrate. *Nucleic Acids Research* 11: 7631–7648.
- Grossman SR and Laimins LA (1996) EBNA1 and E2: a new paradigm for origin-binding proteins? *Trends in Microbiology* 87: 87–89.
- Harvey TJ, Macnaughton TB and Gowans EJ (1997) The development of a SV40 T-antigen positive cell line of hepatic origin. *Journal of Virological Methods* 65: 67–74.
- Hawley-Nelson P and Ciccarone V (1996) Transient transfection efficiency of human keratinocytes in two serum-free media. *Focus* 18: 43–44.
- Konesdi I, Brough DE, Bruder JT and Wickham TJ (1997) Adenoviral vectors for gene transfer. *Current Opinions in Biotechnology* 8: 583–588.
- Kruh J (1982) Effects of sodium butyrate, a new pharmacological agent, on cells in culture. *Molecular and Cellular Biochemistry* 42: 65–82.
- Lurquin PF (1997) Gene transfer by electroporation. *Mol Biotechnol* 7: 5–35.
- Manfredi JJ and Prives C (1994) The transforming activity of simian virus 40 large tumor antigen. *Biochimica et Biophysica Acta* 1198: 65–83.
- Nicolau C and Sene C (1982) Liposome-mediated transfer in eukaryotic cells: dependence of the transfer efficiency upon the type of liposome used and the host cell cycle stage. *Biochimica et Biophysica Acta* 721: 185–190.
- Northrop JP, Ullman KS and Crabtree GR (1993) Characterization of the nuclear and cytoplasmic components of the lymphoid-specific nuclear factor of activated T Cells (NF-AT) complex. *Journal of Biological Chemistry* 268: 2917–2923.
- Ogris E, Mudrak I and Wintersberger E (1993) Distinct amounts of polyoma-virus large T antigen are required for different functions of the protein. *Oncogene* 8: 1277–1283.
- Pari GS and Keown WA (1997) Experimental strategies in efficient transfection of mammalian cells: calcium phosphate and DEAE-dextran. *Methods in Molecular Biology* 62: 301–316.
- Parker GA, Crook T, Bain M, Sara EA, Farrell PJ and Allday MJ (1996) Epstein-Barr virus nuclear antigen (EBNA)3C is an immortalizing oncoprotein with similar properties to adenovirus E1A and papillomavirus E7. *Oncogene* 13: 2541–2549.
- Possee RD (1997) Baculoviruses as expression vectors. *Current Opinions in Biotechnology* 8: 569–572.
- Price TNC, Moorwood K, James MR, Burke JF and Mayne LV (1994) Cell cycle progression, morphology and contact inhibition are regulated by the amount of SV40 T antigen in immortal human cells. *Oncogene* 9: 2897–2904.
- Rice PW and Cole CN (1993) Efficient transcriptional activation of many simple modular promoters by SV40 large T antigen. *Journal of Virology* 67: 6689–6697.
- Schenborn E, Goiffon V and Oler J (1998) An efficient new transfection reagent for eukaryotic cells: TransFastTM transfection reagent. *Promega Notes* 65: 2–6.
- Strain AJ, Wallace WAH and Wyllie AH (1985) Enhancement of DNA-mediated gene transfer by high-M_r carrier DNA in synchronized CV-1 cells. *Biochemical Journal* 225: 529–533.
- Su J-L, Kilpatrick KE, Champion BR, Morris DC, Lehmann JM and Kost TA (1997) Fluorescent microtiter screening assay for immunochemically reactive antibodies. *Biotechniques* 22: 320–324.
- Tang DC, Johnston SA and Carbone DP (1994) Butyrate-inducible and tumor-restricted gene expression by adenovirus vectors. *Cancer Gene Therapy* 1: 15–20.
- Yates JL, Warren N and Sugden B (1985) Stable replication of plasmids derived from Epstein-Barr virus in various mammalian cells. *Nature* 313: 812–815.
- Yoshida M, Kijima M, AM and Beppu T (1990) Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by trichostatin A. *Journal of Biological Chemistry* 265: 17174–17179.
- Zepter K, Haffner AC, Trefzer U and Elmetts CA (1995) Reduced growth factor requirements and accelerated cell-cycle kinetics in adult human melanocytes transformed with SV40 large T antigen. *Journal of Investigative Dermatology* 104: 755–762.