Commercial Plasmid Preparation Methods Yield DNA with Different Transfection Capabilities

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Introduction

Transfection efficiency is dependent upon a number of factors, including method of transfection, cell type, and DNA concentration and purity. Liposome-mediated transfection of mammalian cells has proven to yield higher transfection efficiencies and is more reproducible than other methods (Felgner et al. 1987; Rose et al. 1991). However, we have noted a significant difference in transfection efficiency among cell lines and among different DNA preparations with liposome-mediated transfections. We have sought to determine which method ensures optimal transfection efficiencies. We prepared plasmid DNA by four commonly employed methods and measured its transfection efficiency in three different cell lines. DNA prepared using modified alkaline lysis followed by purification on Bio-Rad's Quantum Prep® DNA binding matrix gave superior transfection efficiencies in all three cell lines.

Method

DNA PREPARATION

DNA from the plasmid pHSVlac (Geschwind et al. 1994) was prepared using four different methods. For each method, pHSVlac DNA was prepared from pellets of overnight cultures (250–500 ml, grown with antibiotic) by a modified alkaline lysis procedure with further purification according to each supplier's recommended protocol and buffer composition. Method A was a noncommercial reference method; we used the protocol of Saporito-Irwin et al. (1997), which purifies plasmid DNA by repeated precipitation with ammonium acetate. Method D was the protocol supplied by Bio-Rad with its Quantum Prep maxiprep kit, which contains a patented DNA-binding matrix. Methods B and C were protocols provided by two other commercial suppliers of DNA-binding matrices.

Following purification, the DNA concentration of each sample was determined by absorbance at 260 nm, and an aliquot was digested with restriction enzymes to establish DNA purity.

TRANSFECTIONS

Three cell lines, RR1, VERO and NIH3T3, were maintained in appropriate growth media and plated to approximately 80% confluency in 24-well plates the day before transfection. All transfections were performed in triplicate. For each experiment, cells were either transfected with 0.2 µg (determined by absorbance) of pHSVlac prepared by method A, B, C or D, or were mock transfected (no DNA). For each well, pHSVlac was added to 1.5 µl LipofectAMINE™ reagent (Gibco/BRL) and 23.5 µl of Opti-MEM® (Gibco/BRL) in a final volume of 50 µl. Following a 45 min incubation, another 200 µl of Opti-MEM was gently mixed with the LipofectAMINE:DNA complex, and added to cells prewashed with Dulbecco's MEM to remove serum and antibiotics. The transfection continued for 5 hr, at which time the LipofectAMINE:DNA complex was replaced with high-glucose Dulbecco's MEM containing 5% fetal bovine serum, penicillin and streptomycin, and the cells were returned to 37 °C at 5% CO_2.

Cells successfully transfected with the pHSVlac plasmid express lacZ. Following overnight incubation, duplicate plates of transfected cells were used for X-gal staining and for chemiluminescent β-galactosidase activity measurements to assess transfection efficiency. The lacZ-expressing cells were detected by direct staining with a solution containing X-gal. Treated cells were washed with PBS buffer and fixed with 1% glutaraldehyde for 5 min, washed again with PBS, and incubated overnight at 37 °C in a solution containing 1 µg/µl X-gal. The average number of lacZ-expressing blue cells was determined for each set of triplicates.

Cells on the duplicate plates were lysed and used in a microplate chemiluminescent reporter assay for the detection of β-galactosidase activity according to the manufacturer's specifications (Galacto-Light Plus™, Tropix, Inc.). Enzyme activity was measured using a LumiCount™ microplate luminometer (Packard Instrument Company).

QUANTITATIVE PCR

Quantitative PCR* (Heid et al. 1996) was used as an alternative technique to measure the concentration of pHSVlac for each plasmid preparation. We used a primer/probe set specific for the lacZ gene and an ABI PRISM® 7700 sequence detector (PE Corporation). Reaction mixtures (50 µl) contained 1 ng of DNA (by absorbance), 1x TaqMan® universal PCR buffer (Roche Molecular Systems, Inc.), 45 pmol of each primer and 5 pmol of probe. Serial dilutions of pHSVlac were used to generate a standard curve. Diluted plasmid was quantified by photoimaging in an ethidium bromide-stained 0.8% agarose gel.

Results and Discussion

EFFECT OF PURIFICATION METHOD ON PLASMID YIELD

Spectrophotometric measurements and restriction enzyme digestion demonstrated that the plasmid preparations prepared by each of the four methods were of high purity and were free of RNA and contaminating genomic DNA. However, methods A–D differed in the amount of DNA recovered: 534 µg (A), 216 µg (B), 1,764 µg (C), and 1,644 µg (D). When pHSVlac from method C was visualized by UV transillumination on gels, less DNA was visible than expected. In an effort to investigate the discrepancy between the absorbance at 260 nm and the amount of DNA visualized, we used quantitative PCR to measure the concentration of the plasmid yielded by each method. The concentrations of pHSVlac as determined by absorbance and by quantitative PCR were similar for methods A, B and D. However, for method C, absorbance apparently overestimates the yield by 2-fold. Presumably a copurified compound accounts for half of the absorbance.

TRANSFECTION EFFICIENCIES

RR1 cells were transfected equally well with pHSVlac prepared by methods A, B and D, but were less efficiently transfected with pHSVlac prepared by method C (see table), whether determining transfection efficiencies by counting blue cells or by measuring β-galactosidase activity. The lower than expected concentration of plasmid from method C may partly account for the low transfection efficiencies. Of the four methods, method A has the advantage of lowest cost, but is particularly time consuming. Since method D yielded 2–8 times the amount of DNA per preparation with comparable cost, it would be the method of choice for cells that are easy to transfect.

For cells more difficult to transfect, we used VERO and 3T3 cells to compare transfection efficiency of pHSVlac purified by each method. The results, shown in the table, demonstrate that transfections with pHSVlac prepared using method D result in higher β-galactosidase activity than using methods A, B or C. When the number of blue cells was counted, methods A, B and D were comparable. Because of the discrepancy between the yield of pHSVlac prepared using method C as measured by absorbance and by quantitative PCR, we repeated transfections on VERO cells with increasing amounts of this DNA. Approximately 5 times more was needed to achieve a similar number of blue cells to that achieved with DNA prepared by method D, although β-galactosidase activity remained lower; still higher amounts of DNA resulted in a decrease in transfection efficiency (data not shown). Thus, plasmid prepared by method D not only gave the highest yield, it also gave optimal transfection efficiency. This was especially noteworthy in cells that are more difficult to transfect.

Transfection of cultured cells with 0.2 µg pHSVlac prepared by 4 different methods. (Control is transfection in the absence of DNA.) Average ± S.D. of 3 transfections.

Method	X-gal-positive cells/10^4	β-galactosidase activity, RLU
RR1 Cells		
Control	0	20 ± 9
A	1.00 ± 0.15	4,382 ± 202
B	0.75 ± 0.10	4,616 ± 98
C	0.40 ± 0.08	613 ± 38
D	0.96 ± 0.17	5,178 ± 280
VERO Cells		
Control	0	48 ± 12
A	1.28 ± 0.38	1,730 ± 101
B	0.70 ± 0.18	1,247 ± 58
C	0.05 ± 0.04	76 ± 2
D	1.53 ± 0.39	2,852 ± 432
3T3 Cells		
Control	0	47 ± 15
A	0.30 ± 0.13	288 ± 7
B	0.23 ± 0.04	194 ± 35
C	0.04 ± 0.04	23 ± 2
D	0.67 ± 0.23	883 ± 301

Preparation of DNA and subsequent transfections can be both time intensive and costly. Our study demonstrates that, while many time-saving methods for DNA purification are commercially available, DNA prepared by these methods varies in total yield and transfection efficiency. Plasmid DNA preparation using the Quantum Prep matrix produces high-quality DNA with optimal transfection efficiencies.

References

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* The polymerase chain reaction (PCR) process is covered by patents owned by Hoffman-LaRoche. Use of the PCR process requires a license.

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