ly obtain about 40 µg of DNA from a confluent-plate lysate (ca. 4–5 mL) and 150–300 µg of DNA from 50 mL of liquid lysate. The quality of DNA is suitable for restriction enzyme digestion, as shown in Figure 1, and other molecular biology applications such as cloning, Southern blot hybridization, sequencing and PCR (data not shown). Zinc chloride has been found to precipitate various biological macromolecules including DNA, bovine serum albumin (BSA) and phages other than λ, such as T4 and M13 (3,7). We have isolated single-stranded DNA from M13 by this method as well (data not shown).

We often encountered difficulties with amplifying phage reproducibly and obtaining enough λ DNA for cloning or mapping. By reviewing the present protocols, we have developed a streamlined procedure for phage amplification and DNA isolation. Two days after isolating phage plaques, we routinely obtain enough DNA for cloning and mapping projects.

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Increased Efficiency of Liposome-Mediated Transfection by Volume Reduction and Centrifugation

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Liposome-mediated transfection, or lipofection, has recently been widely and successfully applied to a variety of cell types (4,5,10). Although toxicity sometimes limits the applicability of lipofection, for those cell types that can withstand the treatment, lipofection often proves to be superior to other more classical transfection approaches such as calcium phosphate, DEAE-dextran sulfate and electroporation (8,11–14). Even for cell types susceptible to lipofection, transfection efficiency often varies unpredictably between experiments, probably because of variations in liposome composition and target cell culture conditions.

While studying the efficacy of lipofection for transducing Cos-1 cells, we attempted to improve and regularize the efficiency of lipofection based on biophysical principles. Analogous to recent discussions regarding retroviral infection by Chuck et al. (3), we hypothesized that the relatively large size of the liposomes' results directly limits how fast and how far they can diffuse through the liquid culture medium to reach their target cells. For liposomes ranging in size from 100 to 200 nm, the mean diffusion velocity is approximately 25–50 µm/h. Thus, during our transfections with durations of several hours, each liposome has only a finite chance of coming into contact with a Cos-1 cell. In this way, the size of the liposomes would obligately restrict the number of productive interactions between liposomes and target Cos-1 cells, thereby lowering the transfection efficiency. Such Brownian limitations have been demonstrated by several groups of investigators for retroviral infection (2,3,9), leading to the development of flow sedimentation and centrifugation techniques to improve retroviral infection. We therefore asked whether physical techniques might be used to force an increased interaction between lipo-

somes and Cos-1 cells during the transfection period, either by reducing the liquid volume during transfection or by directly sedimenting the liposomes onto the Cos-1 cells by centrifugation.

pcDNA-CAT plasmid DNA was purchased from Invitrogen (Carlsbad, CA, USA) and purified through QIAGEN Plasmid Maxi Prep Kit (Qiagen, Chatsworth, CA, USA). LIPOFECTIN® reagent (Life Technologies, Gaithersburg, MD, USA) was used as a source of liposomes. Cos-1, a simian virus 40 (SV40)-transformed monkey kidney cell line, was obtained from ATCC (Rockville, MD, USA). Transfection of plasmid DNA as performed using LIPOFECTIN reagent in OPTI-MEM® medium (Life Technologies) without serum as recommended by the manufacturer and previously described, with DNA and LIPOFECTIN reagent mixed and allowed to complex for 15 min prior to introducing the reagent to the cells (1,4). DNA/liposome complexes were incubated overnight with Cos-1 cells that had reached 50%–60% confluence in 12-mm wells (Falcon®; Becton Dickinson Labware, Bedford, MA, USA). After incubation, the transfection medium was replaced with fresh medium, incubated further for 48 h and assayed for chloramphenicol acetyltransferase (CAT) activity.

For centrifugation transfection, LIPOFECTIN reagent (l0 µL) containing plasmid DNA was diluted in 200 µL medium, placed on top of the semiconfluent Cos-1 cells and centrifuged for 10 min at 300× *g* in a tabletop centrifuge (Sorvall, Newtown, CT, USA), using flat-plate centrifugation adaptors. After centrifugation, the plates were incubated overnight in a humidified incubator. After incubation, LIPOFECTIN medium was replaced by the regular growth medium containing 10% fetal bovine serum (FBS). CAT activities were evaluated in these samples to monitor the transfection efficiency after an additional 48 h as previously described (7).

Comparing various transfection methods including calcium phosphate, electroporation and DEAE-dextran sulfate, we initially found that lipofection was the most efficient method for transfecting cDNA into Cos-1 cells (data not shown). Nevertheless, when LIPOFECTIN reagent was co-cultured with the target Cos-1 cells in 1.0-mL cultures, we observed fairly low and inconsistent transfection efficiency (data not shown). To test whether the relatively large size of the liposomes might limit the transfection efficiency by decreasing the frequency and absolute likelihood of productive interactions with the transfection target cells, we directly compared the CAT plasmid transfection between cultures in which the cell volume was either 1.0 or 0.2 mL. In this comparison, we observed significantly higher transfection efficiency in samples in which the transfection was

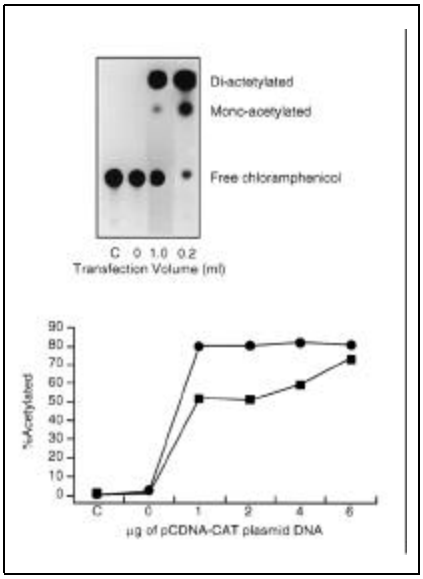

Figure 1. Effect of DNA/liposome complex volume on transfection efficiency. Upper panel: autoradiograms of thin-layer chromatography (TLC) analysis of CAT activity after 72 h of transfection with 2 µg of pcDNA-CAT plasmid. Plasmid suspended into a minimum volume [>2.5 µL distilled (d)H_2O] and incubated with LIPOFECTIN reagent (10 µL) in a total volume of 200 µL or 1 mL using OPTI-MEM medium. After 15 min incubation at room temperature, the whole complex (200 µL or 1 mL) was placed on top of Cos-1 cells and incubated overnight. Lower panel: 1–6 µg of pcDNA-CAT plasmid DNA were combined with 10 µL LIPOFECTIN reagent and 170 µL medium (final vol = 200 µL). After 15 min incubation at room temperature, the DNA/LIPOFECTIN complex was placed on top of Cos-1 cells (**O**—**O**). In another set, the DNA/LIPOFECTIN complex was diluted to 1 mL with OPTI-MEM medium and placed on top of Cos-1 cells (■—■). After 72 h, the cells were lysed and analyzed for CAT enzyme activity. Results are expressed as relative % di-acetylated chloramphenicol as estimated by PhosphorImager® (Molecular Dynamics, Sunnyvale, CA, USA). The data shown are the means of replicates from three experiments, in which the variation between replicates was ±10%.

performed in 0.2 mL per 10-mm well compared to samples in which the transfection volume was 1.0 mL per well as monitored by the increase in the CAT activity (Figure 1, upper panel).

To confirm further, we compared low (0.2 mL) vs. standard (1.0 mL) transfection volumes with a range of plasmid DNA concentrations from 2.0 to 8.0 µg. As seen in Figure 1 (lower panel), we found higher CAT activity in those samples in which low volume (0.2 mL) was used during transfection compared to samples transfected in media with higher volumes at all plasmid concentrations. When the results of four experiments were compared, the CAT activity measured at any given DNA concentration varied between transfections, but transfection efficiency was increased by reducing the culture volume within every experiment.

Recently, Kotani et al. introduced spin infection, or "spinnoculation'' to enhance the retroviral infection into the mammalian cells (9). In this approach, cell cultures containing retroviral supernatant are centrifuged onto a cell monolayer or cell button. To mimic this approach for lipofection, liposome/DNA mixture was centrifuged onto a monolayer of Cos-1 cells for 10 min at 300× *g*, and CAT activities were compared to control liposome infection. While the measured level of CAT activity varied less than ±10% between replicate samples, the absolute transfection efficiency was quite variable between each of three experiments. For each experiment, transfection efficiency was dramatically increased in all samples that were subjected to centrifugation as compared with unspun transfected samples (Figure 2, A and B).

These results document the utility of maximizing the frequency of physical interaction between liposomes and target cells as an approach to increasing transfection efficiency by lipofection. In this study, we have taken two alternative approaches to decrease the distance that the liposomes must travel, by limiting the volume and by directly centrifuging the liposomes onto the target cells. Both approaches had similarly positive effects, with centrifugation somewhat more effective.

Although the approaches taken clearly increase the liposome-cell contacts, the precise mechanisms by which these techniques lead to increased transfection are not known. Because the mechanism of liposome-mediated transfer of foreign DNA into cells is not clearly defined (10–12), fusion, endocytosis or a combination of these two mechanisms could be the mechanism for the transfer of exogenous DNA into the cells. Increasing the frequency of the liposome-target cell interactions could increase membrane fusion, stimulate endocytosis or even have additional amplifying effects. However, whatever the precise steps that occur once the liposomes contact their target cells, maximizing the frequency and efficiency of these interactions is a sensitive step in regulating the overall transfection efficiency. Similar results have been obtained with cell types that are susceptible to lipofection, e.g., T lymphocytes (7) (data not shown). Overall, this approach is immediately generalizable to diverse applications in cell and molecular biology, and can be flexibly adapted to whatever solute particles and target cells are being studied by the investigators.

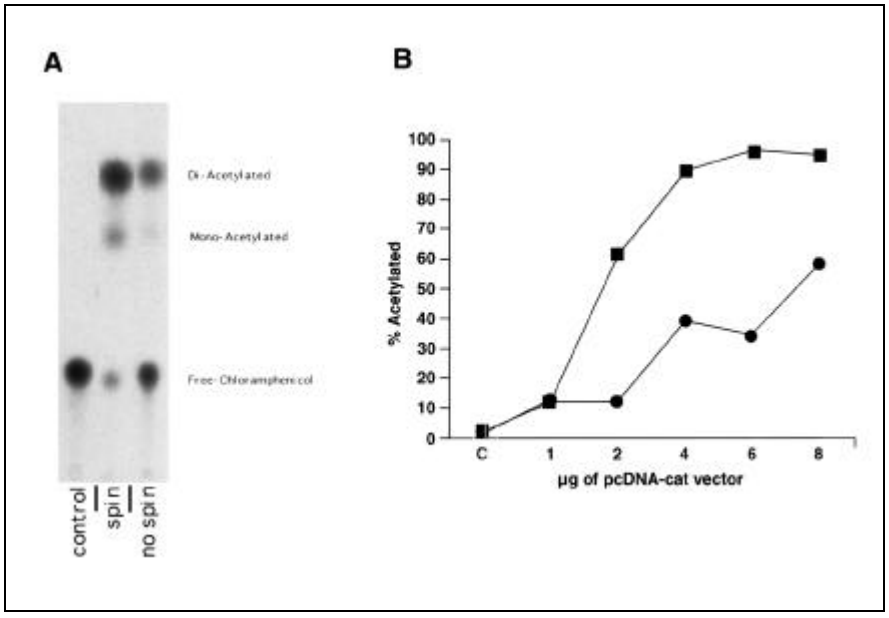

Figure 2. Effect of spinnocculation (9) of transfection efficiency during transfection. (A) Autoradiograms of TLC analysis of CAT activity after transfection using spinnoculation. pcDNA-CAT plasmid DNA (2 µg) was suspended with 10 µL of liposomal transfection reagent in a final volume of 200 µL. One sample was centrifuged for 10 min at 300× *g* and incubated overnight along with the unspun sample. CAT enzyme activity was analyzed after 72 h of transfection. (B) pcDNA-CAT plasmid DNA (1–6 µg) was combined with 10 µL LIPOFECTIN reagent in a final volume of 200 µL. One set of these samples was centrifuged (■—■) and incubated overnight along with unspun samples (O—O). After 72 h of transfection, the cells were lysed and analyzed for CAT enzyme activity. Results are expressed as relative % Diacetylated chloramphenicol as estimated by PhosphorImager. The data shown are the means of replicates from four experiments, in which the variation between replicates was within ±10%.

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Microscope Slide for Enhanced Analysis of DNA Damage Using the Comet Assay

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The comet assay is a powerful tool for evaluating damage to nuclear DNA. It is a relatively quick test and is sufficiently sensitive to detect small differences in DNA strand breaks (1,3). The technique has a wide range of applications and protocols (1–3). Generally, cells are embedded in low-melting-point agarose and layered on microscope slides. The cells are lysed, electrophoresed, stained with propidium iodide and examined using a fluorescence microscope.

Typically, fully frosted microscope slides that are uniformly roughened on one side are used to promote gel adhesion to the slide. These slides are troublesome in that they accumulate stain residue and create a level of background fluorescence that diminishes contrast. Removing the stain from the slides for reuse is labor-intensive, while consistent use of new slides is expensive and does not eliminate background fluorescence.

A simple but effective technique has been developed by our laboratory to maintain the adhesive advantage of the frosted slide while allowing for enhanced sensitivity. Using 19-mm-wide masking tape, standard microscope slides were carefully taped across the center of a metal tray in sets of five. These slides were cautiously sandblasted in a Zero-brand, Blast-in-peen sandblasting cabinet to roughen both ends of the slide without removing too much material. The tape was removed, and the slides were washed to eliminate residues. The resulting slides have rough ends for gel adhesion and a window of smooth glass in the center for analysis with augmented sensitivity.

We performed several comet assays to illustrate the advantage of the custom-frosted slides. Cells were damaged with 0.2 J/cm^2 of UV light to demonstrate the enhanced sensitivity. Along with controls, the damaged cells were assayed on both fully frosted and custom-frosted