



Transfection of *Lymantria dispar* insect cell lines

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Abstract. Lepidopteran cell lines derived from the gypsy moth, *Lymantria dispar*, have not been widely used in protein expression studies or systems because they are weakly adherent, have specific growth requirements and characteristics, and are generally difficult to transfect. Using lipid-mediated transfection of a reporter plasmid, we modify the standard method for transfection of *L. dispar*-derived embryonic cell lines IPLB-LdEp and -LdEIta, obtain-

ing transfection efficiencies of 34% and 30%, respectively, as determined by image analysis assays. Using the standard lipid-mediated method, we obtain transfection efficiencies for *L. dispar*-derived cell line IPLB-Ld652Y of at least 40% with high mean expression levels, indicating the IPLB-Ld652Y cell line may be a superior choice for expression studies or systems requiring *L. dispar*-derived cells.

Key words: *In vitro*, Liposome, *Lymantria dispar*, Transfection

Introduction

Optimized systems for transfection of insect cells have become increasingly important for generation of stably transformed cell lines and for improving heterologous gene expression using *in vitro* systems. Indeed, insect cell-based systems for large-scale production of recombinant proteins have become indispensable for biotechnological, biopharmaceutical, and industrial applications. Cultured cell lines have been established from a large number of insect species, predominantly from the orders Lepidoptera and Diptera [10]. Lepidopteran-derived cultured cell lines have often been weakly adherent and inherently difficult to transfect, yet these insect cell lines have offered several advantages over competing bacterial and mammalian cell culture systems in the production of biopharmaceuticals and other expression products. Cell lines derived from *Spodoptera frugiperda* (Sf-9 and Sf-21) [18] and *Trichoplusia ni* (High-Five™) have been the most commonly used commercially and in the laboratory for insect *in vitro* expression systems. Currently, there are no commercially available cultured insect cell lines derived from *Lymantria dispar* (gypsy moth), and consequently *L. dispar* cells have not been widely exploited.

Often research objectives may predicate use of a particular insect cell line. Our current research requirements have obligated use of cultured *L. dispar* cells. We have observed the *L. dispar* embryonic cell-derived lines known as IPLB-LdEp [10] and IPLB-LdEIta [10] to be the most permissive to *in*

vitro transformation by the polydnavirus of the braconid *Glyptapanteles indiensis*, a natural parasitoid of the gypsy moth [7, 11]. In addition, the IPLB-LdEp line grows faster than the other available gypsy moth lines, making it more useful in certain situations. The embryonic gypsy moth lines also have the advantage of a wider susceptibility to several insect viruses as compared to the ovarian lines (IPLB-Ld652Y and Sf-21) used here and by other researchers. For example, IPLB-LdEp and -LdEIta lines are susceptible to infection by both *Autographa californica* and *L. dispar* nucleopolyhedroviruses while the ovarian lines can only replicate one of these each [10]. Cell lines such as IPLB-LdEIta may be useful for production of glycoproteins, as this line was recently shown to be capable of the highest percentage fucosylation as compared with other lepidopteran cell lines [9]. Each of the *L. dispar*-derived cell lines tested in this paper grow well in serum-free media and any could be useful in a protein-expression system.

The necessity to use these *L. dispar* cell lines for virus infection, transformation, and transfection experiments in our laboratory, in combination with the difficulty associated with transfection of *L. dispar*-derived cell lines in general, prompted us to examine and optimize the transfection of *L. dispar* cells *in vitro*. Here, we optimize methods for transfection of three different cultured *L. dispar* cell lines, IPLB-LdEp, IPLB-LdEIta, and IPLB-Ld652Y, using cationic lipid-mediated transfection of a reporter plasmid vector expressing the green fluorescent protein (GFP) under the control of a baculovirus

promoter [3] and indirectly assaying protein expression efficiencies in these living cells.

Materials

A. Equipment:

1. Incubator, Model No. 3158.¹
2. Laminar flow hood class II biosafety cabinet, Cat. No. 36209-02Y.²
3. Microscope, inverted, fluorescent, Model fluovert.³
4. Benchtop centrifuge (Eppendorf), Model No. 5804 R.
5. Gilson pipetman, Models P-20, P-200, and P-1000.⁵
6. Pipet-aid, Model No. DRM4-000-110.⁶
7. Rocker platform, Model No. RP-50.⁷
8. Endow GFP filter cube, Cat. No. 41017.⁸
9. Cooled charge-coupled device (CCD) camera system, Model LAS1000.⁹
10. Photoshop 5.5 software.¹¹
11. Colony Count-sort software, component of 1D Advanced Program for Analysis.¹⁰
12. Image Quant Image Guage V 3.3 software.⁹

B. Cell culture medium and reagents:

1. Ex-Cell 400, Cat. No. 14400-78P.¹²
2. Grace's insect cell culture medium, Cat. No. 11590-056 (Important: this is UNSUPPLEMENTED, does not contain lactalbumin hydrolysate or yeastolate).¹³
3. Fetal bovine serum, Cat. No. 16140-063.¹³
4. CELLFECTIN lipid reagent, 1 mg/ml, Cat. No. 10362-101.¹³

C. Plastic and glassware:

1. Culture flasks, 25 cm² and 75 cm², Cat. No. 690160 and 658170.¹⁴
2. Glass pipets, 5 ml, Cat. No. 401746 7740.¹⁵
3. Screw capped polypropylene tubes, 50 ml, No. 2098.¹⁶
4. Polystyrene tubes, 12 × 75-min, 7 ml, No. 2058.¹⁶
5. Aerosol barrier pipet tips, Cat. Nos. RT-20F, RT-200F, and RT-1000F.⁵
6. Cell culture polystyrene cluster plate, 24-well, No. 3524.¹⁷
7. Filter, 0.45 μm (Nalgene), Cat. No. 290-4545.

D. Cell lines:

Established insect cell lines IPLB-LdEp, IPLB-LdEIta, and IPLB-Ld652Y were maintained by routine weekly subculture in the laboratory. The first two lines were established from gypsy moth (*L. dispar*) embryos [10], and the third from pupal ovaries of the same species [6]. Each cell line was maintained at 26 °C in serum free Ex-Cell 400 medium and was at high passage level (over 500).

E. Reporter plasmid:

The reporter plasmid p-166EGFP, expressing *Aequorea victoria* enhanced green fluorescent

protein (EGFP) under control of the *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis virus (OpMNPV) gp64 early/late promoter (gift from Gary W. Blissard, Boyce Thompson Institute at Cornell University, NY), has been described in detail previously by Chang et al. [3].

Procedures

A. Preparation of plasmid DNA for transfection:

Plasmid DNA was prepared via single banding over cesium chloride density gradients as described previously in Sambrook et al. [14]. To purify the plasmid DNA, ethidium bromide was extracted from the DNA band by adding an equal volume of 20X-SSC-saturated isopropanol and shaking. The layers were allowed to briefly separate, then the upper layer containing ethidium bromide was removed. This process was repeated until both layers were colorless. The volume of the plasmid DNA band (lower layer) was raised to 5 ml using distilled deionized H₂O. The plasmid DNA was precipitated in two volumes 100% ethanol (10 ml). The pellet was washed with 70% ethanol, allowed to air dry, then dissolved in 1XTE buffer, pH8, to give a final concentration of 500 ng/μl.

B. Cell preparation and transfection protocols, comparison of two methods:

1. Method of Life Technologies Incorporated (Rockville, MD) for transient transfection of adherent cells using CELLFECTIN lipid reagent, protocol from package insert with several modifications. These include optimized cell seeding numbers, solution volumes, and incubation temperature, necessary for transfecting insect cells. The DNA:lipid ratio was experimentally optimized for IPLB-LdEp cells as described in section C:

- a) In a 24-well tissue culture plate, seed $\sim 3.5 \times 10^5$ *L. dispar* cells per well in 1 ml of the appropriate growth medium (Ex-Cell 400).
- b) Incubate the cells at 26 °C until the cells are 60–80% confluent. (This will usually take 18–24 h, but the time will vary among cell types. Since transfection efficiency may be sensitive to culture confluency, it is important to maintain a standard seeding protocol from experiment to experiment).
- c) Prepare the following solutions in 12 × 75-mm sterile tubes:
 - Solution A: For each transfection, dilute 3 μg (6 μl) of DNA into 150 μl serum-free culture medium.
 - Solution B: For each transfection, dilute

- 10 μ l of CELLFECTIN Reagent (mix well before using to ensure a homogenous sample is taken) into 150 μ l serum-free culture medium.
- d) Combine the two solutions, mix gently, and incubate at room temperature for 15 min. The solution may appear cloudy; however, this will not impede the transfection.
 - e) Wash cells once with 1 ml of serum-free medium. (Note: Cells may be transfected in the presence of serum, if necessary.)
 - f) For each transfection, add 0.35 ml serum-free medium to each well containing cells. (Note: Do not add antibacterial agents to medium during transfection.)
 - g) For each transfection, add the 300 μ l DNA:lipid complexes by dropping slowly and evenly onto the cells in a circular pattern to distribute the complexes. After adding the complexes, rock the plate evenly on a rocking platform for 20 min at a rate of 20 oscillations per minute, to mix thoroughly and completely cover the cells.
 - h) Incubate the cells stationary at 26 °C for a total 5–6 hours incubation in the presence of lipid complexes.
 - i) Replace the DNA-containing medium with 1 ml of normal culture medium containing serum and incubate cells at 26 °C.
 - j) Assay cell extracts for gene activity at 36–40 h post transfection, using digital image analysis as described in section D.
2. Modified method for transfection of certain *L. dispar*-derived cells (IPLB-LdEp and -LdEIta):
- a) Suspend cultured insect cells in their preferred media (Ex-Cell 400).
 - b) Pellet cells for 2 min at room temperature in a bench top centrifuge at 1000 \times g in 50 ml screw-cap polypropylene tube.
 - c) Pour off the media and gently resuspend cells in 10% FBS-Grace's insect cell culture medium at a concentration of 1.0×10^6 cells per ml for IPLB-LdEp, -LdEIta, and -Ld652Y cells. Count cells each time to ensure uniform density of cells from well to well and from experiment to experiment. 10% FBS-Grace's cell culture medium is made by adding 10% v/v FBS to Grace's medium and passing through a 0.45 μ m filter. Grace's cell culture medium alone, without the 10% FBS, may also be used to resuspend cells. In our experience, this results in 2–5% lower percentage of transfectants in *L. dispar* cells.
 - d) For each transfection, aliquot 350 μ l of the 10% FBS-Grace's cell culture medium-suspended cells into each well such that each contains approximately 3.5×10^5 cells per well. This volume is sufficient to just cover the surface of the well. The cells should be approximately 60–70% confluent. Allow 45 to 60 min for cells to attach to the well. The plate should be covered (with kimwipes or paper towels that have been lightly saturated with distilled water) or attachment should take place in a humid environment to prevent cells from drying as a result of the reduced media volume.
 - e) After the cells appear to have adhered to the wells, 30 to 40 min, prepare the DNA:lipid complexes for transfection of the cells in polystyrene 12 \times 75-mm sterile tubes:
 - Solution A: For each transfection, dilute 3 μ g (6 μ l) of DNA into 150 μ l serum-free culture medium.
 - Solution B: For each transfection, dilute 10 μ l of CELLFECTIN Reagent (Mix well before using to ensure a homogenous sample is taken) into 150 μ l serum-free culture medium.
 - f) Combine the contents of both tubes, mix gently, and allow DNA:lipid complexes to form for 15 minutes at room temperature. The solution may appear cloudy; however, this will not impede the transfection.
 - g) After the complexes form, for each transfection, add the 300 μ l DNA:lipid complexes by dropping slowly and evenly onto the attached cells in 10% FBS-Grace's cell culture medium in a circular pattern to distribute the complexes. After adding the complexes, rock the plate evenly on a rocking platform for 20 min at a rate of 20 oscillations per minute, to mix thoroughly and completely cover the cells. The primary focus here should be even and complete distribution of the DNA:lipid complexes without disturbing the newly seeded and attached cells.
 - h) Incubate the cells stationary at 26 °C for a total 5–6 hours incubation in the presence of lipid complexes.
 - i) After this time, aspirate the DNA/lipid/cell media/10%FBS-Grace's medium and replace with 1 ml Ex-Cell 400 or appropriate insect cell culture media. Incubate the cells at 26 °C or as they are normally cultured.
 - j) Assay cells for reporter gene expression 36 to 40 hours post-transfection, using digital image analysis as described in section D.
- C. Optimization of DNA:lipid ratio:
IPLB-LdEp cells were transfected using CELLFECTIN with various DNA:lipid ratios to determine the lipid dose to use for greatest efficiency

of transfection with the least number of detached, clumped, or apoptotic cells. The modified method 2 described above was used, with a constant plasmid DNA dose of 3 μg and varying lipid quantities of 0; 2; 5; 8; 10; 12; and 15 μl , corresponding to DNA:lipid ratios 3:0; 3:2; 3:8; 1:3.33; 1:4; and 1:5 respectively.

D. Digital image capture and analysis:

Digital image analysis was utilized to examine the efficiencies of Ld cell transfections. In this method, fluorescent cell images are captured using sensitive camera equipment and manipulated to reduce background [reviewed in 15]. Successfully transfected cells are then counted from the digital images using computer software. For this study, visible light and UV/fluorescent images for each transfection were captured at 10 \times magnification on a Leitz Fluovert inverted microscope using an Endow GFP filter cube and Fuji LAS1000 cooled CCD camera system. At least three images were captured for each transfection and at least 2×10^3 cells were counted for each. Fluorescent digital images were normalized and levels adjusted using Adobe Photoshop 5.5 software so that all fluorescent images were evaluated with common background. Fluorescent cells were counted on inverted images and analyzed using the Colony Count-sort component of 1D Advanced Program for Analysis (Advanced American Biotechnology). Total cell numbers were counted for each on the visible light image so that percent transfected cells could be evaluated. The expression levels of transfected cells, measured as pixel densities or units/pixel², were determined on the same images using Fuji-quant software Image Gauge V3.3 program (Fuji Medical Systems) to estimate the mean cellular expression level for each transfection.

Results and discussion

A wide variety of methods have been used for transfection of viral or plasmid DNA into mammalian and insect cells, including calcium phosphate (CaHPO_4) coprecipitation [5, 20], carrier methods such as DEAE-Dextran and Poly-Lysine [12], cationic liposome-mediated transfer [2, 21], and electroporation [12, 16] [methods reviewed in 17]. No method has emerged as ideal, especially in transfection of lepidopteran cell lines, which are generally weakly adherent and difficult to transfect, with possible exception of Sf-9 and -21 cells [8]. In our experiences with transfection of lepidopteran cell lines, liposome-based transfection methods, in which DNA is transported to the nucleus through fusion with the transport across the plasma membrane or through endocytosis, have been highly superior to CaHPO_4 coprecipitation-based methods, which can

be toxic to the cells or inefficient. Certain cell lines, such as Sf-9 and -21, grow well in culture and are comparatively more conducive to transfection than other less-utilized cell lines. However, experimental requirements, such as need for a cell line derived from a particular host or a specific tissue, may necessitate use of cell lines of less than ideal adherence, growth, or membrane permeability characteristics.

The IPLB-LdEp cell line, derived from gypsy moth embryos [10], has been an essential cell line in our laboratory because it is permissive to transformation by a braconid polydnvirus. It was our focus for optimizing transfection conditions. We used the cationic lipid reagent CELLFECTIN (Life Technologies Inc.), which was reported by the manufacturer to be superior for the transfection of lepidopteran Sf-9 cells. Despite successful use of LIPOFECTIN (Life Technologies Inc.) lipid reagent by other laboratories for transfection of a variety of lepidopteran cell lines [8], our early trial experiments comparing both lipid reagents indicated that CELLFECTIN was superior for transfection of IPLB-LdEp cells (data not shown). Transfections with DNA in absence of lipid reagent resulted in no fluorescent cells. Digital image analysis of these cells indicated there was no interfering natural autofluorescence of IPLB-LdEp cells. Dose-response results for IPLB-LdEp cell line using our modified method (Figure 1) and gradually increasing lipid indicated that a ratio of 1:3.33 (3 μg plasmid DNA:10 μl CELLFECTIN) was optimal under our experimental conditions (Figure 1). A higher percentage of successfully transfected cells was obtained using a DNA:lipid ratio of 1:4 (3 μg plasmid DNA:12 μl CELLFECTIN), but the higher lipid dose was somewhat toxic, causing some cells to become clumped and detached, or even, rarely, apoptotic. To maintain the integrity of the cell membranes and cellular functions [1], the DNA:lipid dose was modulated for the highest efficiency transfection with the least number of impaired cells.

Our lipid-based transfection method was modified from the published Life Technologies Inc. standard method for transfection using CELLFECTIN reagent. Normally cells are seeded onto tissue culture plates using supplemented Grace's medium (TNM-FH, containing lactalbumin hydrosylate and yeastolate) 12 to 24 hours prior to transfection so that they acquire stronger adhesion. This introduces and additional 12 to 24 hours time to a transfection protocol. In our modified method, lepidopteran cells were resuspended in minimal Grace's insect cell culture medium containing 10% serum and plated immediately. Under these conditions, cell adhesion was strong after 30 to 40 minutes and cells could be immediately transfected. Media volumes were also reduced to facilitate attachment [19]. The presence of serum during transfection, but not during forma-

tion of liposomes, did not inhibit transfection efficiency [4]. In fact, the presence of 10% serum with Grace's cell culture medium during preparation and attachment of the cells gave slightly higher (2–5%) transfection efficiency over Grace's cell culture medium alone (data not shown). We culture the lepidopteran cells used in this experiment in the absence of serum and they are quite sensitive to media composition. However, we suspect that the presence of serum and the absence of additives in the Grace's medium may aid in the adherence of cells to the culture plate, needed because attachment time for the cells is greatly reduced over the traditional protocol. Interestingly, cells prepared and attached in Ex-Cell 400 culture medium alone (instead of 10%FBS-Grace's cell culture medium or Grace's cell

culture medium alone) by our modified method showed greatly reduced transfection efficiencies (data not shown). This suggested the Grace's medium may allow for greater cell membrane permeabilization than culture medium, given the greatly reduced cell preparation and attachment time. Or, additives contained in Ex-Cell 400 culture medium might interfere with cell adhesion. Our modified transfection method, which resulted in time savings of 18–24 hours over the standard protocol, proved to be somewhat more or equally as efficient for transfection of IPLB-LdEp (Figure 2A, Table 1) and IPLB-LdEIta (Figure 2C, Table 1) cell lines compared with the standard method (Figure 2B and D, Table 1) with similar mean expression levels (Table 1). The standard transfection method was

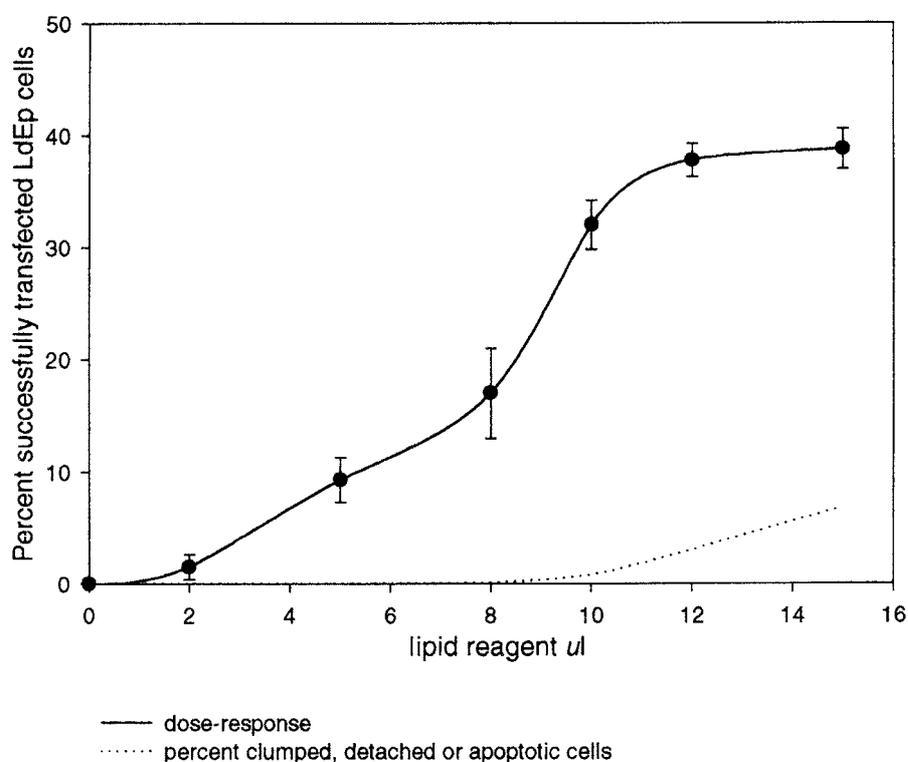


Figure 1. Optimization of DNA:lipid ratio with respect to transfection of IPLB-LdEp lepidopteran insect cells using modified method. 3.5×10^5 LdEp cells were transfected in each experiment, varying lipid dose while holding DNA and all other parameters in the transfection protocol constant. Cells were transfected with a reporter plasmid expressing EGFP. Thirty six hours after transfection, cells were analyzed by image analysis to determine the transfection efficiency.

Table 1. Transfection efficiencies of *L. dispar*-derived cells as estimated by Image Analysis using Image Gauge V3.3 software.

Cell line	Transfection efficiency (percent)		Expression range (intensity units/pixel ²)		Mean expression levels (intensity units/pixel ²)	
	Modified method	LTI method	Modified method	LTI method	Modified method	LTI method
IPLB-LdEp	34%	31%	92–255 ^a	96–255	207.8	211.8
IPLB-LdEIta	30%	29%	107–255	112–255	215.1	214.7
IPLB-Ld652Y	41%	44%	111–255	109–255	215.0	229.6

^a The value 255 represents full saturation detected by this method.

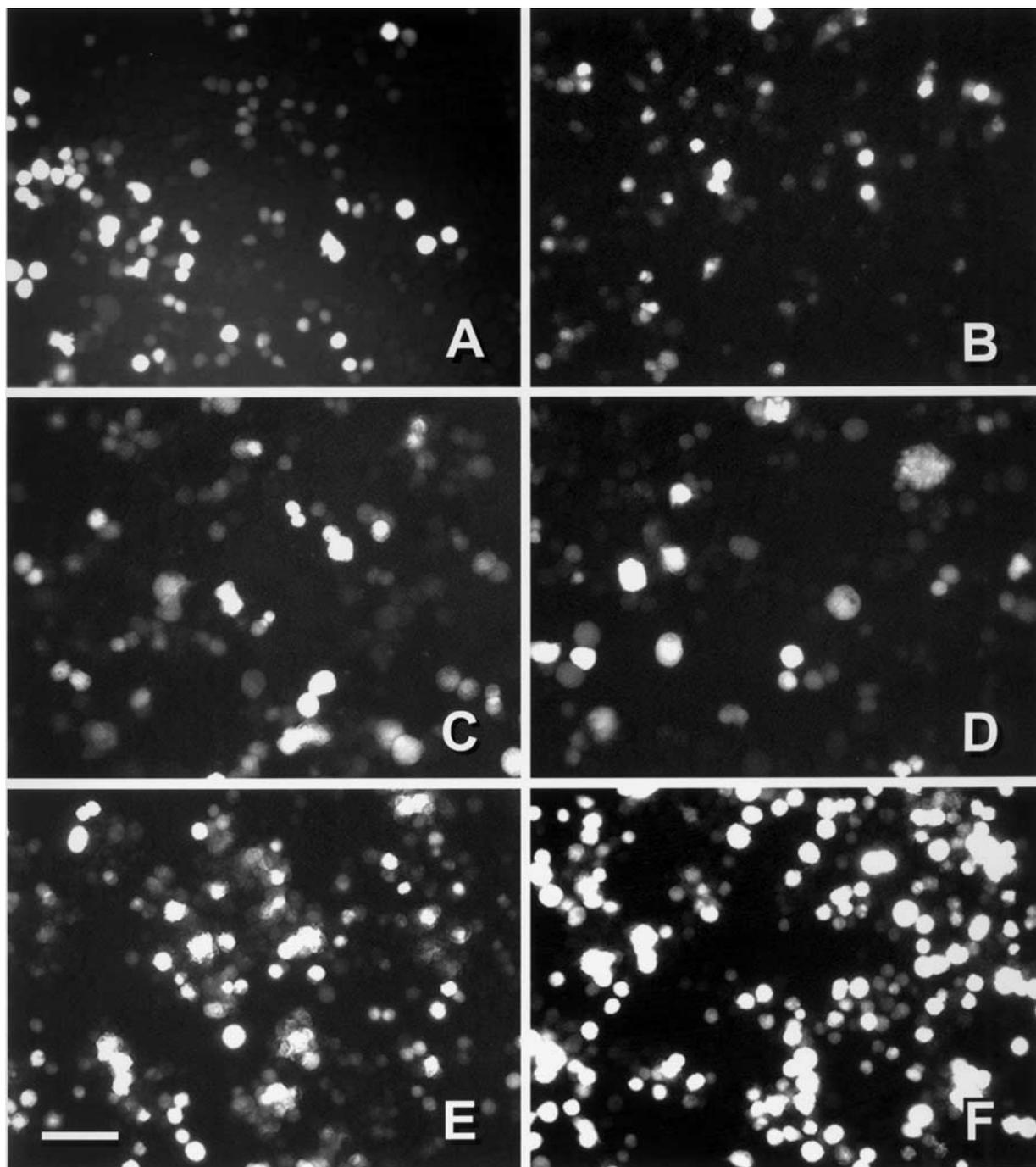


Figure 2. Comparison of transfection of *L. dispar*-derived lepidopteran cell lines IPLB-LdEp, IPLB-LdEIta, and IPLB-Ld652Y by two methods. Constant numbers of IPLB-LdEp (A and B), IPLB-LdEIta (C and D), and IPLB-Ld652Y (E and F) were transfected by our modified method (A, C, and E) or by Life Technologies Inc. method (B, D, and F) with a reporter plasmid expressing EGFP, using CELLFECTIN reagent. At 36–40 hours post transfection, cell images were captured using a cooled CCD camera system (Fuji LAS1000) on a Leitz Fluovert inverted microscope at 10× magnification. Images were analyzed for transfection efficiency by counting fluorescent cells using Colony Count-sort software (Advanced American Biotechnology, Valencia CA) and expression efficiency estimated using Image-quant Image Guage V3.3 software (Fuji Medical Systems, Stamford Connecticut). The bar represents 100 microns.

superior to our method for the transfection of IPLB-Ld652Y cell line (Figure 2E and F, Table 1) in both efficiency of transfection and in mean expression levels (Table 1).

It has generally been difficult to obtain transfection efficiencies equal to or greater than 40% in

lepidopteran cells, with the exception of Sf cells. Clearly, with respect to *L. dispar*-derived cell lines, IPLB-LdEp cells are not the best choice for obtaining the highest numbers of successfully transfected cells with highest expression levels. It appears that IPLB-Ld652Y cells, which were successfully trans-

fectured with efficiencies of at least 40%, regardless of method, may represent the best choice of *L. dispar*-derived cell lines for protein expression.

Notes

1. Forma Scientifics, Marietta, OH 45750, USA
2. Labconco Corporation, Kansas City, MO 64132, USA
3. Leitz Wetzlar, GMBH D-6330, West Germany
4. Brinkmann Instruments, Westbury, NY 11590, USA
5. Rainin, Woburn, MA 01888-4026, USA
6. Drummond Scientifics, Broomall, PA 19008, USA
7. Elmeco, Rockville, MD 20850, USA
8. Chroma Technology Corporation, Brattleboro, VT 05301, USA
9. Fuji Medical Systems, USA, Inc, Stamford, CT 06912, USA
10. Advanced American Biotechnology, Fullerton, CA 92631, USA
11. Adobe Systems Incorporated, San Jose, CA 95110, USA
12. JRH Biosciences, Lenexa, KS 66215, USA
13. Life Technologies, Inc, Rockville, MD 20849-6482, USA
14. Greiner, GmbH D-72636 Frickenhausen, West Germany
15. Corning Inc, Corning, NY 14831, USA
16. Falcon brand, Becton Dickenson and Co, Lincoln Park, NJ 07035, USA
17. Costar brand, Corning Inc, Corning, NY 14831, USA

References

1. Baker EA, Vaughn MW, Haviland DL (2000). Choices in transfection methodologies: Transfection efficiency should not be the sole criterion. *FOCUS* 22(3): 31–33.
2. Bangham AD (1992). Liposomes: realizing their promise. *Hosp Pract* 27: 51–62.
3. Chang M-J, Kuzio J, Blissard GW (1999). Modulation of translational efficiency by contextual nucleotides flanking a baculovirus initiator AUG codon. *Virology* 259: 369–383.
4. Ciccarone V, Hawley-Nelson P, Jesse J (1993). Cationic liposome-mediated transfection: Effect of serum on expression and efficiency. *FOCUS* 15(3): 80–83.
5. Graham FL, van der Eb AJ (1973). A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52: 456–461.
6. Goodwin RH, Tompkins GJ, McCawley P (1978). Gypsy moth cell lines divergent in viral susceptibility I. Culture and Identification. *In Vitro* 14: 485–494.
7. Gundersen-Rindal D, Lynn DE, Dougherty EM (1999). Transformation of lepidopteran and coleopteran insect cell lines by *Glyptapanteles indiensis* polydnavirus DNA. *In Vitro Cellular and Developmental Biology* 35: 111–114.
8. Keith MBA, Farrell PJ, Iatrou K, Behie LA. 2000. Use

of flow cytometry to rapidly optimize the transfection of animal cells. *BioTechniques* 28: 148–154.

9. Kulakosky PC, Hughes PR, Wood HA (1998). N-linked glycosylation of a baculovirus-expressed recombinant glycoprotein in insect larvae and tissue culture cells. *Glycobiology* 8: 741–745.
10. Lynn DE, Dougherty EM, McClintock JT, Loeb M (1988). Development of cell lines from various tissues of lepidoptera. In: Kuroda Y, Kurstak E, Maramorosch K (eds) *Invertebrate and Fish Tissue Culture* (pp 239–242). Tokyo: Japan Scientific Societies Press.
11. McKelvey TA, Lynn DE, Gundersen-Rindal D, Guzo D, Stoltz DA, Guthrie KP, Taylor PB, Dougherty EM (1996). Transformation of gypsy moth (*Lymantria dispar*) cell lines by infection with *Glyptapanteles indiensis* polydnavirus. *Biochem and Biophys Res Comm* 225: 764–770.
12. Neumann E, Schaefer-Ridder M, Wang Y, Hofschneider PH (1982). Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO J* 1: 841–845.
13. Pagano JS, McCutchan JH (1969). Enhancement of viral infectivity with DEAE-dextran: Application to development of vaccines. *Prog Immunobiol Stand* 3: 152–158.
14. Sambrook J, Fritsch EF, Maniatis T (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1.21.
15. Stewart NT, Byrne KM, Hosick HL, Vierck JL, Dodson MV (2000). *Methods in Cell Science* 22: 67–78.
16. Toneguzzo F, Hayday AC, Keating A (1986). Electric field-mediated DNA transfer: Transient and stable gene expression in human and mouse lymphoid cells. *Mol Cell Biol* 6: 703–706.
17. Trotter KM, Wood HA (1995). Transfection techniques for producing recombinant baculoviruses. *Methods Molec Biol* 39: 97–105.
18. Vaughn JL, Goodwin RH, Tompkins GJ, McCawley P (1977). The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera; Noctuidae). *In Vitro* 13: 213–217.
19. Verma RS, Giannola D, Schlomchik W, Emerson SG (1998). Increased efficiency of liposome-mediated transfection by volume reduction and centrifugation. *BioTechniques* 25: 46–49.
20. Wigler M, Pellicer A, Silverstein S, Axel R (1978). Biochemical transfer of single-copy eucaryotic genes using total cellular DNA as donor. *Cell* 14: 725–731.
21. Zhu N, Huang L (1994). DNA transfection mediated by cationic liposomes containing lipopolylysine: Characterization and mechanism of action. *Biochem Biophys Acta* 1189: 195–203.

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