

Effectene[™] Reagent yields high transfection efficiencies with *Drosophila melanogaster* S2 cells

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Drosophila melanogaster Schneider line 2 (S2) cells are useful for in vitro analysis of mammalian and non-mammalian genes or proteins. Despite their multiple uses and the recent advancements in transfection technologies, S2 cells are still typically transfected using the relatively inefficient calcium phosphate method. We report the ability to greatly increase transfection efficiency with S2 cells by using Effectene[™] Transfection Reagent, a non-liposomal lipid reagent from QIAGEN.

The ability to efficiently and reproducibly transfect cells is critical to studying the activity of genes and gene products in a controlled environment. One cell line commonly used for such analysis is the *Drosophila melanogaster* Schneider line 2 (S2). This cell line is derived from a primary culture of 20 to 24-hour-old embryos (1), and is easy to use since the cells can grow at room temperature without controlled CO₂ levels, and do not need trypsinization prior to passaging. S2 cells also have a wide variety of uses including functional analysis of transcription factors and cis-regulatory elements (2), the study of cell-adhesion molecules (3), and as expression systems for mammalian proteins (4). Despite their multiple uses, S2 cells are still routinely transfected using the calcium phosphate method although this method yields relatively low transfection efficiencies (5, 6) and requires large amounts of DNA. The development of new transfection technologies has increased the ease and efficiency of transfection, even with S2 cells (6). Here, we report the success of Effectene Reagent, an easy-to-use, non-liposomal lipid transfection reagent, in significantly increasing transfection efficiency with S2 cells.

Materials and methods

Transfections

Transfections were performed using *Drosophila melanogaster* S2 cells cultured in Shields and Sang M3 Insect Medium (M3; SIGMA) supplemented with 12.5% heat-inactivated (60°C for

30 min) fetal bovine serum (FBS; Life Technologies), and 100 U/ml penicillin (pen) and 100 µg/ml streptomycin (strep). Cells were grown in 75 cm² vented flasks (FALCON®; Becton Dickinson) on a benchtop and routinely passaged every 3 days when the cell density reached approximately 5 × 10⁶ cells/ml. 27 h prior to transfection, the cells were seeded into a 25 cm² flask (Costar Corning) at a density of 1 × 10⁶ cells/ml in 5 ml medium. Transfection of a luciferase reporter constitutively driven by the promoter element of the long terminal repeat (LTR) of the copia transposable element (copia-*luc*; pGL3-Basic; Promega) was used to compare the transfection efficiencies with calcium phosphate, the non-liposomal lipid Effectene Reagent (QIAGEN), and the activated-dendrimer SuperFect[™] Reagent (QIAGEN). Additional transfections using either calcium phosphate or Effectene Reagent were carried out using a reporter that is not activated by factors endogenous to S2 cells. The CME-*lacZ* reporter plasmid consisted of a multimerized (6x) central nervous system midline enhancer element (CME) fused to a *lacZ* reporter. The expression plasmids pAct-*sim* and pAct-*tgo*, expressing *single-minded* (*sim*) and *tango* (*tgo*), respectively from the *actin5C* promoter (2), were cotransfected as a source of CME-activating proteins. Plasmid DNA was purified using the QIAGEN[®] Plasmid Maxi Kit and transfected cells were harvested 48 h after transfection and lysed using Passive Lysis Buffer (PLB; Promega). ▶

References

1. Schneider, I. (1972) Cell lines derived from late embryonic stages of *Drosophila melanogaster*. *Embyol. Exp. Morph.* **27**, 353.
2. Sonnenfeld, M. et al. (1997) The *Drosophila* tango gene encodes a bHLH-PAS protein that is orthologous to mammalian Arnt and controls CNS midline and tracheal development. *Development* **124**, 4571.
3. Nose, A., Mahajan, V.B., and Goodman, C.S. (1992) Connectin: a homophilic cell adhesion molecule expressed on a subset of muscles and the motoneurons that innervate them in *Drosophila*. *Cell* **70**, 553.
4. Johansen, H. et al. (1989) Regulated expression at high copy number allows production of a growth-inhibitory oncogene product in *Drosophila* Schneider cells. *Genes Dev.* **3**, 882.
5. Han, K., Levine, M.S., and Manley, J.L. (1989) Synergistic activation and repression of transcription by *Drosophila* homeobox proteins. *Cell* **56**, 573.
6. Han, K. (1996) An efficient DDAB-mediated transfection of *Drosophila* S2 cells. *Nucleic Acids Res.* **24**, 4362.
7. Fehon, R.G. et al. (1990) Molecular interactions between the protein products of the neurogenic loci notch and delta, two EGF-homologous genes in *Drosophila*. *Cell* **61**, 523.

Transfection with calcium phosphate was performed in a manner similar to that of Fehon et al. (7). A 1-ml mixture of DNA with 2 M calcium chloride and BES buffer (N,N-bis[2-Hydroxyethyl]-2-aminoethanesulfonic acid; Sigma) was incubated for 25 min and then added to the cells. After 17 h, the transfection complexes were removed, the cells were washed with 2 ml complete medium (M3+FBS+pen/strep), and 5 ml fresh complete medium was added to the cells. Transfection using Effectene Reagent was optimized according to the manufacturer's specifications for adherent cells in a 60-mm dish (roughly the same surface area as a 25 cm² flask). The DNA, buffer, and Enhancer were incubated for 5 min before adding the Effectene Reagent and incubated for 15 min to allow transfection complex formation. During this time, the medium was removed from the cells and they were washed with 2 ml complete medium, and then resuspended in 4 ml complete medium. 1 ml complete medium was mixed with the Effectene-DNA complexes, added to the cells, and left on the cells until evaluation (48 h). Transfections with SuperFect Reagent were performed as recommended by the manufacturer.

Evaluation of transfection efficiencies

Luciferase expression of the copia-*luc* reporter was assayed using the Luciferase Assay System (Promega). 2 ml of cells were removed from the culture, pelleted by

centrifugation, resuspended in PBS, and recentrifuged. The pelleted cells were resuspended in 200 µl PLB and placed on a rocker for 15 min, after which the lysate was cleared by centrifugation. 5 µl of a 1:100 dilution of lysate in PLB was added to 100 µl luciferase substrate and quantified using a monolith 2010 luminometer (Analytical Luminescence Laboratory). Reporter activity was normalized to protein concentration using a Bradford assay (Bio-Rad Protein Assay) with protein levels quantified using an EC 340 Bio Kinetics Reader (Bio-Tek Instruments).

β-galactosidase (β-gal) expression of the CME reporter plasmid was assayed using the lacZ/Galactosidase Quantitation Kit (Molecular Probes). Cells were lysed and diluted as above with the exception that 20 µl cell extract was added to 50 µl 8 mM 3-carboxyumbelliferyl β-D-galactopyranoside (CUG, C-1488), and quantified according to the manufacturer's recommended procedure using a luminescence spectrometer (Perkin Elmer). Reporter activity was normalized to luciferase activity of a cotransfected copia-*luc* reporter.

Results and discussion

Under the conditions tested, cells transfected using Effectene Reagent showed significantly higher transfection efficiencies than cells transfected using either calcium phosphate or SuperFect Reagent. With the same quantity of transfected DNA (2 µg), Effectene Reagent

Comparison of Transfection Efficiencies

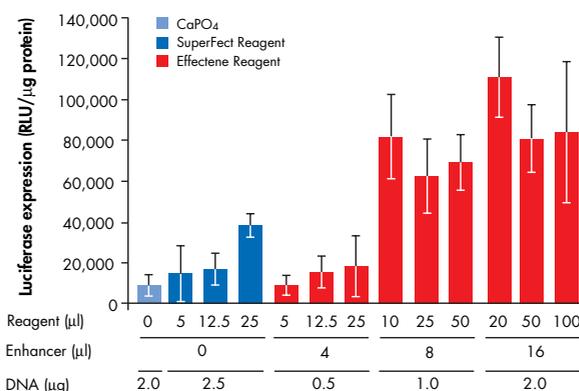


Figure 1 Effectene Reagent increases the transfection efficiency with *Drosophila* S2 cells. S2 cells in 25 cm² flasks were transfected with 0.5–2.5 µg of a constitutively driven copia-*luc* reporter using calcium phosphate, SuperFect Reagent, or Effectene Reagent. 48 h after transfection, the cells were lysed and the luciferase levels were quantitated using a luminometer. The results were normalized to protein concentration using the Bradford assay. Luciferase activity was expressed in relative light units (RLU) per µg of protein ± SEM (n = 3–6).

yielded luciferase levels 7–10-fold higher than calcium phosphate (Figure 1). Furthermore, with only half the quantity of DNA used for the calcium phosphate method (i.e., 1 µg versus 2 µg), Effectene Reagent still gave 6–7-fold higher luciferase activities (Figure 1). With an increase to 10 µg DNA, the calcium phosphate-transfected cells still had only half the luciferase activity of cells transfected using Effectene Reagent and 2 µg DNA (data not shown). 1–2 µg DNA was optimal for transfection with Effectene Reagent. The optimal DNA:Effectene ratio was 1:10, although the results were not significantly different from those obtained using DNA:Effectene ratios of 1:25 or 1:50.

When compared to SuperFect Reagent, the cells transfected using Effectene Reagent produced up to a 2.5-fold higher luciferase activity with a comparable amount of DNA (2 µg versus 2.5 µg), and as much as 2-fold higher with half as much DNA as with SuperFect Reagent (Figure 1). Transfection with SuperFect Reagent yielded 2-fold higher transfection efficiencies than calcium phosphate when using comparable amounts of DNA (2.5 µg versus 2.0 µg).

To verify that Effectene Reagent did not induce some endogenous factor that activated the copia-LTR promoter in a manner unrelated to transfection efficiency, we tested the activity of a reporter that was not dependent on endogenous factors (Figure 2). Cotransfection experiments were carried out using an exogenous expression/reporter system, previously shown to work in S2 cells (2). The expression plasmids, pAct-sim and pAct-tgo, generate Sim and Tgo proteins, and the reporter plasmid (CME-lacZ) contains 6 Sim:Tgo binding sites cloned into the promoter-lacZ vector. 5 µg and 0.5 µg per plasmid were used for calcium phosphate- and Effectene-mediated transfection, respectively. For transfection with Effectene Reagent, a 1:10 DNA:Effectene ratio was used. Transfection with Effectene Reagent produced >2-fold higher β-gal activity despite using 10-fold less DNA. This effect was seen only when all three components (pAct-sim, pAct-tgo,

Activation of CME-lacZ by Sim and Tgo

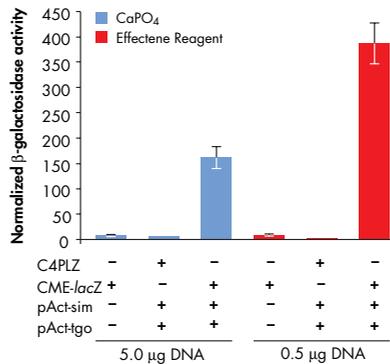


Figure 2 Activity of a CME-lacZ reporter in *Drosophila* S2 cells transfected with Effectene Reagent or calcium phosphate. S2 cells in 25 cm² flasks were transfected with combinations of: (1) a CME-lacZ reporter in the C4PLZ vector, or the C4PLZ vector alone, and (2) a source of CME-activating proteins, pAct-sim and pAct-tgo. 5 µg per plasmid was used for calcium phosphate transfected cells and 0.5 µg per plasmid was used for cells transfected with Effectene Reagent. A 1:10 DNA:Effectene ratio was used for Effectene-transfected cells. Cells were lysed 48 h after transfection and β-gal expression assayed using the lacZ/Galactosidase Quantitation Kit (Molecular Probes). The results were normalized using luciferase activity and expressed in arbitrary β-gal units as a mean of two independent experiments ± SEM (n = 2).

and CME-lacZ) were cotransfected, demonstrating that the increased transfection efficiency was not due to Effectene-mediated activation of factors endogenous to S2 cells. Leaving the Effectene–DNA complexes on the cells for 12 h instead of 48 h did not influence the β-gal activities obtained (data not shown).

Conclusions

Compared to both calcium phosphate and SuperFect Reagent, Effectene Reagent gave significantly enhanced transfection efficiencies. Its ease of use, the ability to use small amounts of DNA, and its low toxicity make it an excellent choice for transfection of *Drosophila melanogaster* S2 cells. ■

Reader Inquiry No. 99404

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For ordering information, see page 21.

Ordering Information

Product	Contents	Cat. No.
QIAamp Viral RNA Mini Kit (50)	For 50 nucleic acid preps: 50 QIAamp Spin Columns, Carrier RNA, Collection Tubes (2-ml), RNase-free Buffers	52904
QIAamp Viral RNA Mini Kit (250)	For 250 nucleic acid preps: 250 QIAamp Spin Columns, Carrier RNA, Collection Tubes (2-ml), RNase-free Buffers	52906
QIAamp Viral RNA Mini Kit (1000)	For 1000 nucleic acid preps: 1000 QIAamp Spin Columns, Carrier RNA, Collection Tubes (2-ml), RNase-free Buffers	52908

Ordering Information

Product	Contents	Cat. No.
For protein purification and detection (see pages 3–6)		
RGS-His Antibody, BSA-free (100 µg)	100 µg mouse anti-RGS(H) ₄ (lyophilized, BSA-free, for 1000 ml working solution)	34650
Penta-His Antibody, BSA-free (100 µg)	100 µg mouse anti-(H) ₅ (lyophilized, BSA-free, for 1000 ml working solution)	34660
Tetra-His Antibody, BSA-free (100 µg)	100 µg mouse anti-(H) ₄ (lyophilized, BSA-free, for 1000 ml working solution)	34670
6xHis Protein Ladder	6xHis-tagged marker proteins (lyophilized, for 50–100 lanes on western blots)	34705
Ni-NTA Magnetic Agarose Beads (2 x 1 ml)	2 x 1 ml nickel-charged magnetic agarose beads (5% suspension)	36111
Ni-NTA Magnetic Agarose Beads (6 x 1 ml)	6 x 1 ml nickel-charged magnetic agarose beads (5% suspension)	36113
12-Tube Magnet	Magnet for separating magnetic beads in 1.5-ml or 2-ml tubes	36912
For QIAGEN Transfection Reagents and QIAGEN Plasmid Kits (see pages 7–9)		
Effectene Transfection Reagent (1 ml)*	For 40 transfections in 60-mm dishes or 160 transfections in 12-well plates	301425
Effectene Transfection Reagent (4 x 1 ml)*	For 160 transfections in 60-mm dishes or 640 transfections in 12-well plates	301427
SuperFect Transfection Reagent (1.2 ml)*	For 40 transfections in 60-mm dishes or 160 transfections in 12-well plates	301305
SuperFect Transfection Reagent (4 x 1.2 ml)*	For 160 transfections in 60-mm dishes or 640 transfections in 12-well plates	301307
Transfection Reagent Selector Kit	0.3 ml SuperFect Transfection Reagent, 0.3 ml Effectene Transfection Reagent	301399
QIAGEN Plasmid Maxi Kit (10) [†]	10 QIAGEN-tip 500, Reagents, Buffers	12162

* Bulk quantities available; please inquire.

† Other kit sizes and options available; please inquire.