# 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 $CO_2$ 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 x $10^6$ cells/ml. 27 h prior to transfection, the cells were seeded into a 25 cm² flask (Costar Corning) at a density of 1 x $10^6$ 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 *actin*5C 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). ▶

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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-aminoethane-sulfonic 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 monolight 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**

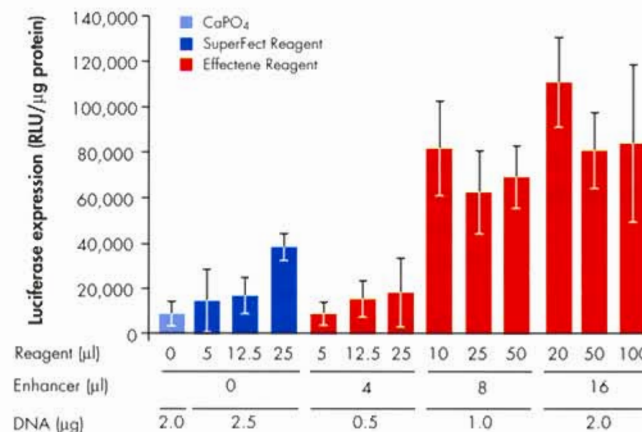


**Figure 1** *Effectene Reagent increases the transfection efficiency with Drosophila S2 cells. S2 cells in 25 cm² flasks were transfected with 0.5–2.5 µg of a constitutively driven copia-luc reporter using calcium phosphate, SuperFect Reagent, or Effectene Reagent. 48 h after transfection, the cells 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).*

**References**

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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,

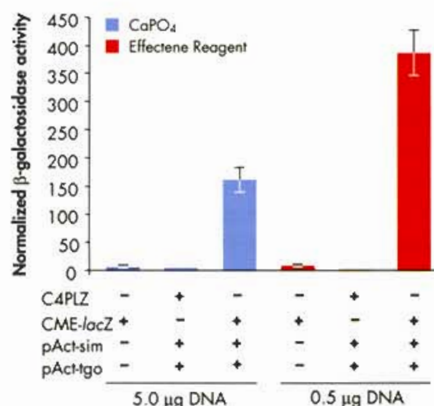


**Figure 2** *Activity of a CME-lacZ reporter in Drosophila S2 cells transfected with Effectene Reagent or calcium phosphate. S2 cells in 25 cm² flasks were transfected with combinations of: (1) a CME-lacZ reporter in the C4PLZ vector, or the C4PLZ vector alone, and (2) a source of CME-activating proteins, 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. ■

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***Acknowledgments***

*The authors would like to thank Pat Estes for providing the copia-luc plasmid, Ferdinand Dabu for the QIAGEN Transfection Reagents, Tom Darlington for helpful discussion, and Sarah Short and Alan Howe for helpful advice. This work was supported by NIH grant RD25251 to S.T.C.*

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