Improving heterologous protein expression in transfected *Drosophila* S2 cells as assessed by EGFP expression

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Abstract *Drosophila melanogaster* S2 cells were co-transfected with plasmid vectors containing the enhanced green fluorescent protein gene (EGFP), under the control of metallothionein promoter (pMt), and the hygromycin selection gene, in view of establishing parameters for optimized gene expression. A protocol of transfection was worked out, leading after hygromycin selection, to ~90% of S2MtEGFP fluorescent cells at day 5 after copper sulfate (CuSO_4) induction. As analyzed by confocal microscopy, S2MtEGFP cell cultures were shown to be quite heterogeneous regarding the intensity and cell localization of fluorescence among the EGFP expressing cells. Spectrofluorimetry kinetic studies of CuSO_4 induced S2MtEGFP cells showed the EGFP expression at 510 nm as soon as 5 h after induction, the fluorescence increasing progressively from this time to attain values of 4.6×10^5 counts/s after 72 h of induction. Induction with 700 μM of CuSO_4 performed at the

exponential phase of the S2MtEGFP culture (10^6 cells/mL) led to a better performance in terms of cell growth, percent of fluorescent cells and culture intensity of fluorescence. Sodium butyrate (NaBu) treatment of CuSO_4 induced S2MtEGFP cell cultures, although leading to a loss of cell culture viability, increased the percent of EGFP expressing cells and sharply enhanced the cell culture fluorescence intensity. The present study established parameters for improving heterologous protein expression in stably transfected *Drosophila* S2 cells, as assessed by the EGFP expression.

Keywords Schneider *Drosophila* cells · EGFP · Cell transfection · Gene expression · Sodium butyrate

Abbreviations

EGFP	Enhanced green fluorescent protein gene
pMt	Metallothionein promoter
S2	Schneider 2 <i>Drosophila melanogaster</i> cells
S2MtEGFP	S2 cells transfected with Mt inducible plasmid containing the EGFP gene
NaBu	Sodium butyrate
CuSO_4	Copper sulfate
DES	<i>Drosophila</i> expression system
Hygro	Hygromycin

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Introduction

Drosophila melanogaster Schneider 2 (S2) cells have become increasingly utilized over the past few years for the expression of heterologous proteins. High levels of protein expression with pharmacological and biotechnological interest can be achieved using the *Drosophila* expression system (DES) procedure whereby a plasmid encoding a gene of interest is co-transfected with a plasmid encoding resistance to hygromycin B. After three weeks of selection this system, using *Drosophila* promoters, is able to generate a stable polyclonal cell line processing up to 1,000 gene copies per cell. Proteins of prokaryotic, eukaryotic and viral origin have been expressed in this system showing to be appropriately processed and biologically active (Angelichio et al. 1991; Culp et al. 1991; Han 1996; Li et al. 1996; Delm et al. 1999; Nilsen and Castellino 1999; Lee et al. 2000; Hill et al. 2001; Shin et al. 2003; Perret et al. 2003; Chang et al. 2005). Based on these evidences DES has an important potential as a gene expression system.

Nevertheless, in view of higher expression, some bioprocess parameters have to be optimized. In this context, the transfection procedure, aiming the introduction of foreign DNA into cells, is an important step. The calcium phosphate method has been recommended for gene transfection of S2 cells (Van der Straten et al. 1989) but procedures based on liposome reagent have been reported to be more efficient (Han 1996; Park et al. 1999; Shin and Cha 2002).

Besides the metallothionein promoter induction by copper sulfate (CuSO_4) (Huang et al. 2004) it has been studied the enhancement of gene expression by agents, such as the sodium butyrate (NaBu), a histone deacetylase inhibitor that lead to chromatin exposure and allow the transcriptional factors binding and increased gene expression (Dorner et al. 1989; Li and Li 2006; Zhao et al. 2006; Ping et al. 2006; Song et al. 2006).

The heterologous gene encoding the enhanced green fluorescent protein (EGFP), a mutant from jellyfish *Aequorea Victoria* (Ward 2006; Phillips 2006), has been proved to be very helpful for the estimation of gene expression in several systems,

becoming very attractive as a reporter gene expression (Shin and Cha 2002; Shin et al. 2003; Ward and Lippincott-Schwartz 2006; Hazelrigg and Mansfield 2006). This protein is a 238-amino acid polypeptide that undergoes an intramolecular cyclization to give a brightly fluorescent molecule. It remains remarkably stable in the presence of many denaturants and proteases, as well as over a broad range of pH and temperature (Ward 2006).

We report here in this study an optimization of EGFP stable transfection and inducible conditions for expression using the S2 cells. A protocol for EGFP stable transfection was established by using a lipotransfection method taking into account the cell density, the ratio of DNA concentration and the amount of cellfectin reagent. Conditions for inducible EGFP expression were established by using the metallothionein promoter and CuSO_4 and NaBu as expression inducers. We have evaluated the kinetics cell growth and EGFP expression in a stable transfected, hygromycin selected, polyclonal S2 cell line by confocal microscopy, flow cytometry, and spectrofluorimetry. Our data point to optimized experimental conditions for heterologous protein expression by *Drosophila melanogaster* S2 cells.

Materials and methods

Cell line, plasmids and enzymes

Drosophila melanogaster Schneider 2 cells (Invitrogen) were grown at 28 °C under normal atmosphere in T-25 flasks in SF900-SFMII medium (Invitrogen). For the EGFP expression, we used the pMT/BiP/V5-His (3.6 KB) expression vector (Invitrogen) which has a metallothionein promoter (pMt) for heavy metals inducible expression. It contains also a sequence of C-terminal peptide containing V5 epitope and a polyhistidine (6× His) tag, besides the elements (pUC ori and ampicillin) for replication in bacteria. For selection of co-transfected cells, we used the pCoHygro (Invitrogen) selection vector containing the hygromycin resistance gene. This gene is under control of the constitutive *Drosophila* copia 5'LTR promoter. *E. coli* DH5 α was

used as a primary host for constructing and propagating plasmids. *E. coli* were grown and maintained in LB medium containing 50 µg/mL of ampicillin. DNA restriction enzymes and ligases were used according to manufacturer's instructions (Promega and Fermentas).

Recombinant plasmid construction

cDNA encoding the EGFP was originally isolated from pGie1EGFP plasmid (kindly provided by Dr. Bergmann Moraes from the Brasilia University, Brasília, Brazil). The fragment was isolated from electrophoresis gel of pGeie EGFP digested with *Apa* I and *Not* I restrictions enzymes (Fig. 1). The fragment was extracted from the gel and purified using a commercial kit (Promega). Cloning was done according to standard techniques (Sambrook and Russel 2001) and the construct (pMtEGFP) was sequenced to check for in-frame insertion.

Cell culture and transfection

Drosophila S2 cells were grown at 28 °C in SF900-SFMII medium and co-transfected with pMtEGFP and pCoHygro using cellfectin reagent (Invitrogen) for stable expression. Both plasmids (pMtEGFP and pCoHygro) at a ratio of 20:1 and cellfectin reagent (20 µL) were diluted separately in 500 µL of SF900-SFMII medium and then mixed for 15 min at room temperature, constituting the transfection solution (Table 1).

5×10^6 cells were seeded in T-25 flasks and 24 h after the medium was discarded and 1 mL of transfection solution added. After 5 h of incubation with slowly agitation at room temperature,

4 mL of SF900-SFMII medium was added. The cells were incubated for 2 days and the medium replaced with 600 µg/mL of hygromycin B for selection. Hygromycin B resistant cells were then selected as stable polyclonal populations.

Kinetics experiments of EGFP expression by S2MTEGFP cells were performed upon CuSO₄ induction (500 or 700 µM) and/or NaBu (5 mM) treatment of cell cultures performed in 100 mL shake flasks with 20 mL of SF900-SFMII medium incubated at 100 rpm and 28 °C. The cell seeding was the same for all experiments (5×10^5 cells per mL). Cell concentration was determined by viable cells (trypan blue exclusion) counting in hemacytometer.

Confocal microscopy

For confocal EGFP detection, CuSO₄ induced S2MTEGFP cells were scanned using the 488 nm laser line and light emitted between 500 and 600 nm was collected. Images were collected on a META LSM 510 laser scanning confocal microscope equipped with a 63.0 × 1.2 W objective (Carl Zeiss Ltda). The same settings for image acquisition and processing have been applied for all samples to allow comparison of the fluorescence intensities among different samples

Flow cytometry

After induction with the indicated amounts of CuSO₄, 400 µL of S2MTEGFP cells (10^6 cells) growing in suspension were analyzed by flow cytometry using a FACS-CALIBUR (Becton Dickinson). EGFP fluorescence was excited at 488 nm and emission was measured with a

Fig. 1 Schematic construction of pMtEGFP vector

Table 1 Optimized protocol for transfection of *Drosophila* S2 cells

Parameters	Established transfection protocol
S2 cell concentration	5×10^6 cells/ T-25 flasks
Ratio expression/selection vectors	20:1
Total DNA	2 μ g
Cellfectin [®] reagent	20 μ g
SF900 SFMII medium (dilution)	500 μ l
Incubation time (complex formation)	15 min RT
Incubation time (transfection)	5 h

530/30 nm band pass filter. S2 cells were used as a control for auto fluorescence and to set the gate for EGFP positive cells. 10.000 events/cells were gated from the 400 μ L samples. The geometric mean values were used to compare the intensity of fluorescence in different samples as well as the percentage of fluorescent cells.

Spectrofluorimetry

Cell suspensions were analyzed in spectrofluorimeter PTI for fluorescence measurements with a 4 nm band pass. Cell cultures prepared in shake flasks were induced with 700 μ M of CuSO_4 and samples (10^7 cells) were harvested at different times post induction and washed with PBS (1 $\times$). The cell suspension (1 mL) was evaluated for fluorescence emission at 490–750 nm when excited with blue light (470 nm).

Statistical analysis

Data were analyzed by the variance *F* test and statistical differences among values were established by the *Tukey* test with a significance level of 5%.

Results

EGFP expression vector construction and transfection in S2 cells

We have generated an expression vector encoding the EGFP cDNA by cloning the EGFP fragment

into a pMt expression vector in *Apa* I/*Not* I cloning sites (Fig. 1). The EGFP gene, under control of the inducible *Drosophila* metallothionein gene promoter, was inserted in a *Drosophila* expression vector. This vector was co-transfected with a *Drosophila* selection vector (carrying hygromycin B resistance gene) into *Drosophila* cells and a population was selected for their resistance to hygromycin B.

For the co-transfection of pMtEGFP and pCOHygro into S2 cells, we have developed a transfection protocol (Table 1) which allowed us to select a stable polyclonal cell population (S2MtEGFP) with hygromycin B after 2 weeks of culture.

EGFP expression upon CuSO_4 induction

Figure 2 shows the EGFP expression and cell growth of S2MtEGFP cells induced with 500 or 700 μ M of CuSO_4 at day 3 of culture. We observed that after 2 days of induction, 700 μ M of CuSO_4 were able to induce the EGFP expression in 80% of the cells, in contrast to 500 μ M of CuSO_4 that induced the EGFP expression in only 60% of cells. Nevertheless, after 5 days of induction both CuSO_4 concentrations led to EGFP expression in about 85% of cells. The fluorescence intensity also increased with the CuSO_4 concentration and at day 5 of induction we had an intensity of fluorescence of 100 units for cultures induced with 500 μ M of CuSO_4 and 142 units for those induced with 700 μ M of CuSO_4 . The cell growth, at the exponential phase, was shown to be comparable for control or CuSO_4 induced cultures, indicating that the CuSO_4 , at these concentrations, had no influence on the cell growth (1.5 to 2×10^7 cells/mL). When the CuSO_4 induction was performed together with cell seeding at the beginning of the culture we have observed a significant decrease in the cell multiplication as well as lower expression levels of EGFP as evaluated by the number of fluorescent cell or the intensity of fluorescence of the cell population (data not shown).

Confocal analysis of CuSO_4 induced S2MtEGFP cells showed an heterogeneous EGFP expression among the cell population (Fig. 3A). Kinetics of EGFP expression as

Fig. 2 Kinetics of cell growth (A), % of fluorescent cells (B) and fluorescence intensity (C) of S2 and S2MtEGFP cells induced with 500 or 700 μM of CuSO_4 at day 3 of culture. ●, S2MtEGFP cells + 700 μM of CuSO_4 ; ■, S2MtEGFP cells + 500 μM of CuSO_4 ; ◆, S2 cells; ○, S2MtEGFP cells. Arrows indicate the time of induction at day 3. Results are the average of at least three independent experiments. *: statistically different

assessed by flow cytometry analysis of S2MtEGFP cells (Fig. 3B) showed in a non-induced S2MtEGFP cell population a low percent of fluorescent cells (8%) which sharply increased upon CuSO_4 induction attaining high levels (88%) at day 5 of induction. By spectrofluorimetry we could better examine the time response of S2MtEGFP cells upon CuSO_4 induction, by analyzing the fluorescence intensity expressed by the cell population in early times after induction. Figure 4 shows that specific fluorescence at 510 nm could be already detected as early as 5 h after induction, showed significant levels at

24 h and increased progressively to attain high levels at 72 h.

Improving heterologous protein expression by using sodium butyrate (NaBu).

The effect of NaBu on the EGFP expression by CuSO_4 induced S2MtEGFP cells was analyzed. Figure 5 shows that the NaBu (5 mM) treatment led to a decreased cell growth. At day 4 of induction, cell cultures treated with NaBu attained 0.8×10^7 cells/mL in contrast to control cells that attained 1.6×10^7 cells/mL (Fig. 5A). Following NaBu treatment an increased number of cells expressing the EGFP was observed at 24 h of induction (90%) in comparison to control cell cultures (75.6%) (Fig. 5B). Moreover, the intensity of fluorescence showed by the treated culture sharply increased from 54 units in control cultures to 257 units in the treated ones (Fig. 5C), indicating a remarkable effect of NaBu in promoting the EGFP expression in induced cells. When a higher NaBu concentration (7 mM) was used, the enhancement of EGFP expression was of lower magnitude, possibly due to a higher detrimental effect on the cell metabolism (data not shown).

Discussion

The control and improvement of transfected gene expression and recombinant protein synthesis is a central point of study for several purposes in biology, engineering and medicine when one consider fundamental research or immunobiological production.

In the present study we have investigated the influence of various parameters for the optimization of high gene expression in stably transfected *Drosophila melanogaster* S2 cells. The enhanced green fluorescent protein (EGFP) reporter gene, under the control of the inducible metallothionein (Mt) promoter and co-transfected with the hygromycin selection gene was employed in the study. Protocols for vector transfection and gene induction in cell cultures were established.

Several parameters have to be considered for improving gene expression optimization. First of all, together with the gene vector construction,

Fig. 3 S2MtEGFP cell culture as evaluated by confocal microscopy (**A**) and flow cytometry (**B**). S2MtEGFP cultures were induced with 700 μ M of CuSO_4 . At day 5 of induction they were observed in a confocal microscopy and at different times after induction they were evaluated by flow cytometry. For confocal microscopy: (I), optical microscopy; (II), fluorescence microscopy; (III) merged image. For flow cytometry they were observed at days 0, 2, 5, 8, 9 and 12 of induction and the % of fluorescent cells is indicated as M1. Data are representative of independent experiments

an efficient and reliable transfection procedure is required to provide the recombinant cell population with a high percent of properly transfected cells. For this purpose standardizations in transfection or co-transfection protocols must take into account parameters such as the ratio of vectors (expression and selection), the cell concentration, the metabolic state of the cell population, the concentration of transfection and selection reagents and time and temperature of incubation.

In the present study we have established an optimized protocol for gene co-transfection (Table 1) of S2 cells using 20 μ g of cellfectin: 5×10^6 cells seeded and cultured for 24 h in T-25 flasks (5 mL), ration of expression: selection vectors of 20:1 (2 μ g DNA) and a transfection incubation period of 5 h at 28 °C in a volume of 500 μ L. Hygromycin selection was performed with 600 μ g/mL of hygromycin for 2 weeks. The above described transfection protocol led to an

enriched S2MtEGFP polyclonal cell population (88% fluorescent cells).

Following the transfection step, it was of importance to establish parameters of optimized gene expression based on the promoter induction. Experiments were carried out to optimize the concentration of CuSO_4 to induce the Mt promoter. Kinetic parameters of cell growth (cell/mL), cell expressing the gene (% of fluorescent cells) and the degree of gene expression intensity (fluorescence intensity) were analyzed by confocal microscopy, flow cytometry and spectrofluorimetry, in suspension cell cultures, mimicking large scale conditions. S2MtEGFP cells were shown to be not influenced, at their exponential growth phase, by the CuSO_4 induction, even though some detrimental effects could be observed after the stationary phase, leading to a higher loss of viability when compared to non-induced cultures. S2MtEGFP cells could, in these conditions, attain a cell concentration of 1.5 to

Fig. 4 Kinetics of EGFP intensity expression by S2MtEGFP cells as evaluated by spectrofluorimetry. S2MtEGFP cell culture was induced with 700 μ M of CuSO_4 and at different times the EGFP fluorescence emission was detected at 510 nm (**A**) and the kinetics curve was recorded (**B**). Data are representative of independent experiments

2×10^7 cells/mL and expressed EGFP as soon as 5 h after CuSO_4 induction. 700 μ M of CuSO_4 were able to induce EGFP expression in about 85% of cells with heterogeneous fluorescence intensity, which reached an average of 142 units, after 5 days of induction (Fig. 2–4). Nevertheless, the overall data of cell growth and EGFP expression were substantially affected when CuSO_4 induction was performed at the beginning of cell culture together with cell seeding, indicating a detrimental effect of CuSO_4 on the cell metabolism during the lag phase (data not shown) and the importance of CuSO_4 induction of the EGFP gene during the exponential cell growth phase.

We found a high heterogeneity of EGFP expression among the cells in the population. This could be due to cells harboring plasmids with rearrangements, since plasmid DNA transfection into a cell culture leads to a high frequency of genetic instability (Calos et al. 1983; Razzaque et al. 1983). Besides, since the expression of an heterologous transfected gene, in the cell context, depends on its properly insertion into the cell genome, it is conceivable that a variable number of gene copies, upon cell transfection and gene integration, became inserted in not transcribed regions of the genome. So, even efficient procedures of gene transfection may lead to gene integrated copies that are not expressed.

Based on published studies (Dorner et al. 1989; Chen et al. 2002; Li and Li 2006; Zhao et al. 2006; Ping et al. 2006; Song et al. 2006), sodium butyrate (NaBu), is a histone deacetylase inhibitor, affecting the chromatin structure and leading to the destabilization of nucleosomal structure. As a consequence, the NaBU influence on chromatin exposure may facilitate binding of transcription factors to DNA resulting in an increased and deregulated cell protein synthesis.

We have investigated the enhancement of EGFP expression by S2MtEGFP cell treatment with NaBu. As expected, the cell treatment with NaBu led to loss of cell multiplication and viability, since the NaBu is a potent inducer of G1 cell cycle arrest and provokes apoptosis by activating an intrinsic apoptotic pathway (Li and Li 2006). On the other hand NaBu treatment may expose cell genome regions bearing an heterologous transfected gene and so foster the synthesis of their products (Dorner et al. 1989; Ping et al. 2006; Song et al. 2006). Indeed, our data clearly show that S2MtEGFP cell treatment with NaBu led to a decreased cell multiplication (from 1.6×10^7 in controls with only addition of CuSO_4 , to 0.8×10^7 cells/mL) and decreased viability, which was accompanied by not only a clear cut enhancement of fluorescence intensity of the cell population (~ 5 fold) but also an early increase in the percent of fluorescent cells among the induced cell population (from 75.6% in controls to 90%) at one day post-induction (Fig. 5).

Drosophila S2 cells is a promising cell system for heterologous gene expression due to its

Fig. 5 Kinetics of cell growth (**A**), % of fluorescent cells (**B**) and fluorescence intensity (**C**) of S2MtEGFP cells induced with 700 μM of CuSO_4 and treated with 5 mM of NaBu. At day 3, cell cultures were induced with CuSO_4 and/or treated with NaBu. Cell samples were periodically taken for evaluation of viable cell concentration by hemacytometer

or fluorescence by flow cytometry. ○, S2MtEGFP cells; ●, S2MtEGFP cells + 700 μM of CuSO_4 ; △, S2MtEGFP cells + 5 mM NaBu; ▲, S2MtEGFP cells + 700 μM of CuSO_4 + 5 mM NaBu. Arrows indicate the time of induction at day 3. Results are the average of at least three independent experiments. *: statistically different

characteristics of easy handle cell culture procedures associated to the possibilities of stable and induced gene expression. EGFP, as a reporter gene, is a useful tool to establish suitable param-

eters for improving heterologous protein expression. We show here in this study an optimized vector transfection protocol that enabled us to establish a primary stable transfected S2MtEGFP

cell population that once induced by CuSO_4 , expressed the recombinant EGFP in a high percentage of the cells. Its expression, by the CuSO_4 induced cell population, could be further sharply enhanced by sodium butyrate (NaBu). The procedure shown here was developed in view of a general application for gene expression in the stable transfected *Drosophila* S2 cell system.

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References

- Angelichio ML, Beck JA, Johansen HE, Ivey-Hole M (1991) Comparison of several promoters and polyadenylation signals for use in heterologous gene expression in cultured *Drosophila* cells. *Nucleic Acids Res* 19:5037–5043
- Calos MP, Lebkowski JS, Botchan MR (1983) High mutation frequency in DNA transfected into mammalian cells. *Proc Natl Acad Sci USA* 80:3015–3019
- Chang KH, Yang JM, Chun HOK, Chung IS (2005) Enhance activity of recombinant β -secretase from *Drosophila melanogaster* S2 cells transformed with cDNAs encoding human β 1,4-galactosyltransferase and Gal β 1,4-GlcNAc α 2,6-sialyltransferase. *J Biotechnol* 116:359–367
- Chen T, Sun H, Lu J, Zhao Y, Tao D, Li X, Huang B (2002) Histone acetylation is involved in *hsp70* gene transcription regulation in *Drosophila melanogaster*. *Archi. Biochem Biophys* 408:171–176
- Culp JS, Johansen H, Hellmig B, Beck J, Matthews TJ, Delers A, Rosenberg M (1991) Regulated expression allows high level production and secretion of HIV-1 gp 120 envelope glycoprotein in *Drosophila* Schneider cells. *Bio/technology* 9:173–178
- Delm L, Wolf H, Wagner R (1999) High level expression of hepatitis B virus surface antigen in stably transfected in *Drosophila* Schneider-2 cells. *J Virol Methods* 79:191–203
- Dorner AJ, Wasley LC, Kaufman RJ (1989) Increased synthesis of secreted proteins induces expression of glucose-regulated proteins in butyrate-treated Chinese hamster ovary cells. *J Biol Chem* 264:20602–20607
- Han K (1996) An efficient DDAB-mediated transfection of *Drosophila* S2 cells. *Nucl Acids Res* 24:4362–4363
- Hazlerigg T, Mansfield JH (2006) Green fluorescent protein applications in *Drosophila*. *Methods Biochem Anal* 47:227–257
- Hill RM, Brennan SO, Birch NP (2001) Expression, purification and functional characterization of the serine protease inhibitor neuroserpin expressed in *Drosophila* S2 cells. *Protein Expr Purif* 22:406–413
- Huang EP, Marquis CP, Gray PP (2004) Process development for recombinant chinese hamster ovary (CHO) cell line utilizing a metal induced and amplified metallotionein expression system. *Biotechnol Bioeng* 88:438–450
- Lee JM, Park JH, Park JO, Chang KH, Chung IS (2000) Expression of recombinant erythropoietin in stably transformed *Drosophila melanogaster* S2 cells. *In Vitro Cell Dev Biol Anim* 36:348–350
- Li B, Tising S, Kosaka AH, Nguyen B, Osen EG, Bach C, Chan H, Barnett J (1996) Expression of Human Dopamine β Hydroxylase in *Drosophila* Schneider 2 Cells. *Biochem J* 313:57–64
- Li RW, Li CJ (2006) Butyrate induces profound changes in gene expression related to multiple signal pathways in bovine kidney epithelial cells. *BMC genomics* 7:234
- Nilsen SL, Castellino FJ (1999) Expression of human plasminogen in *Drosophila* Schneider S2 cells. *Protein Expr Purif* 16:136–143
- Park HJ, Kim HY, Han KH, Chung SI (1999) Optimization of transfection conditions for expression of green fluorescent protein in *Drosophila melanogaster* S2 cells. *Enzyme Microb Technol* 25:558–563
- Perret BG, Wagner R, Lecat S, Brillet K, Rabut G, Bucher B, Pattus F (2003) Expression of EGFP-amino-tagged human mu opioid receptor in *Drosophila* Schneider 2 cells: a potential expression system for large-scale production of G-protein coupled receptors. *Protein Expr Purif* 31:123–132
- Phillips GN Jr (2006) The three-dimensional structure of green fluorescent protein and its implications for function and design. *Methods Biochem Anal* 47:67–82
- Ping W, Ge J, Li S, Zhou H, Wang K, Feng Y, Lou Z (2006) Baculovirus-mediated gene expression in chicken primary cells. *Avian Dis* 50:59–63
- Razzaque A, Mizusawa H, Seidman MM (1983) Rearrangement and mutagenesis of a shuttle vector plasmid after passage in mammalian cells. *Proc Natl Acad Sci USA* 80:3010–3014
- Sambrook J. and Russel D.W (2001) *Molecular cloning: a laboratory manual*. New York Cold Spring Harbor Laboratory Press
- Shin HS, Cha HJ (2002) Facile and statistical optimization of transfection conditions for secretion of foreign proteins from insect *Drosophila* S2 cells using green fluorescent protein reporter. *Biotechnol Prog* 18:1187–1194
- Shin HS, Lim HJ, Cha HJ (2003) Quantitative monitoring for secreted production of human interleukin-2 in stable insect *Drosophila* S2 cells using a green fluorescent protein fusion partner. *Biotechnol Prog* 19:152–157

- Song J, Liang C, Chen X (2006) Transduction of avian cells with recombinant baculovirus. *J Virol Methods* 135:157–162
- Van der Straten A, Johansen H, Rosenberg M, Sweet R (1989) Introduction and constitutive expression of gene products in cultured *Drosophila* cells using hygromycin B selection. *Curr Methods Mol Cell Biol* 1:1–8
- Ward WW (2006) Biochemical and physical properties of green fluorescent protein. *Methods Biochem Anal* 47:39–65
- Ward TH, Lippincott-Schwartz J (2006) The use of green fluorescent protein in mammalian cells. *Methods Biochem Anal* 47:305–337
- Zhao YM, Chen X, Sun H, Yuan ZG, Ren GL, Li XX, Lu J, Huang BQ (2006) Effects of histone deacetylase inhibitors on transcriptional regulation of the hsp70 gene in *Drosophila*. *Cell Res* 16:566–576