

# Efficient and Reliable Transfection of Mouse Erythroleukemia Cells Using Cationic Lipids

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**Abstract:** Preliminary studies of *cis*-regulatory elements are frequently performed in transiently transfected cells before further analysis in stably transfected cell lines and transgenic mice. However, not all cells are readily transfectable by routine means. For instance, mouse erythroleukemia (MEL) cells have been a valuable model system for studies of their endogenous globin genes, but introduction of DNA using common transfection methods such as electroporation has been very inefficient. This has allowed studies of stably transfected cells, after selection for the rare transfection events, but transient transfection analysis has been problematic. This report describes an efficient and reliable method for transient transfection of MEL cells using commercially available cationic lipids.

**Keywords:** MEL cells, transfection, cationic lipids

## INTRODUCTION

DNA segments involved in gene regulation, such as promoters and enhancers, are commonly identified by adding appropriate DNA fragments to reporter genes in plasmids and then introducing these test plasmids into appropriate cell lines. Such *cis*-regulatory elements cause the level of activity of the reporter gene product to increase in the transfected cell lines. In transient transfection, the reporter gene is expressed prior to integration into a chromosome, and only a fraction of the templates are assembled into nucleosomal chromatin (5, 15). Stably transfected cells carry the test plasmids integrated into a chromosome and hence the template DNA is in a normal chromatin structure. Different kinds of questions can be addressed with the two types of transfections; e.g., transient transfections can provide a rapid means for identifying promoters and enhancers (2) whereas stably transfected cells can reveal position effects (21, 22) or integration-dependent enhancement (14, 16).

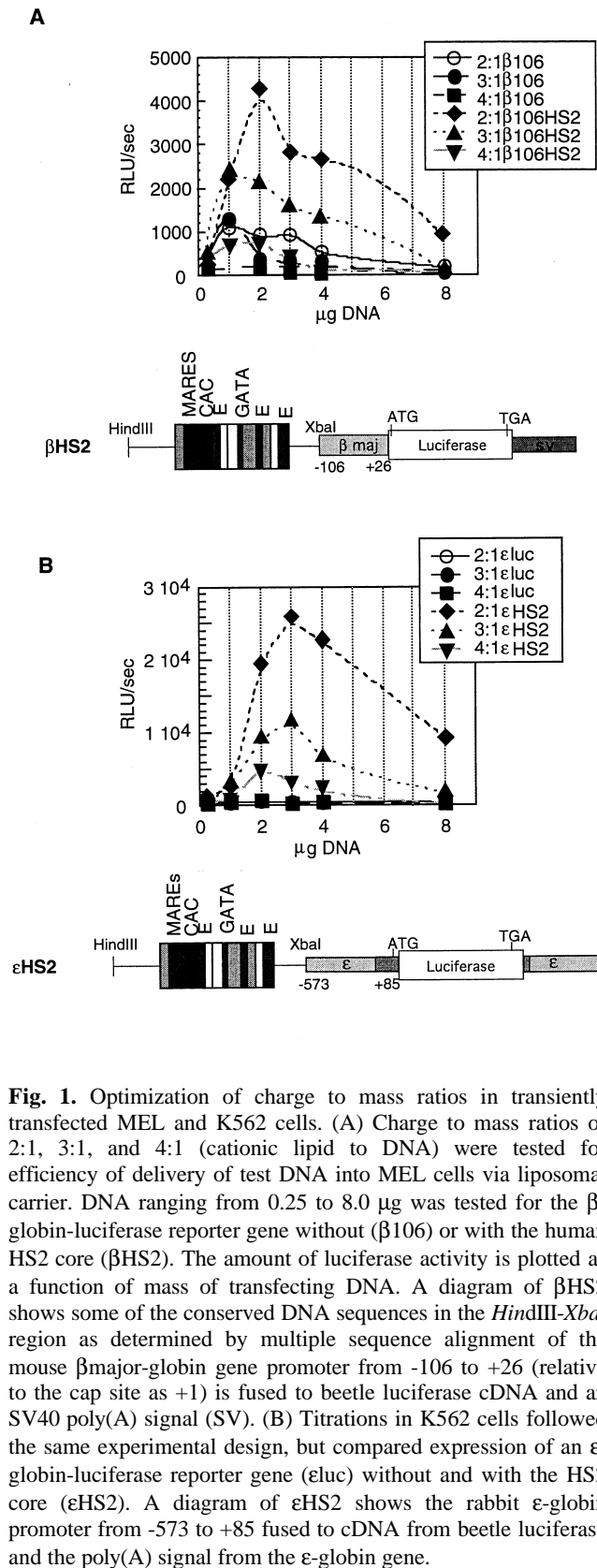
Some of the methods developed for effective transfection of attached cells, such as Ca phosphate precipitation, have not been applied easily to suspension cells. Methods such as

electroporation work well for some but not all suspension cells. Consider the example of mouse erythroleukemia (MEL) cells, which are virally transformed cells maintained at the proerythroblast stage of maturation. Normally they produce a small amount of adult hemoglobin, but they can be induced for further maturation and substantially increased hemoglobin synthesis (8). These cells have provided much information about regulation of the endogenous mouse globin genes. Additionally, many laboratories have examined the role of *cis*-regulatory elements in other mammalian globin genes in pools of stably transfected MEL cells (e.g., 6, 10, 13, 18).

In contrast, few data have been published on transient expression in this cell line, suggesting that introduction of DNA using common transfection methods such as electroporation is inefficient, and only reliable when the rare transfection events are selected for, as in stable transfection. In particular, our attempts to use electroporation for transient transfection of MEL cells (line 745A) gave highly variable results and very little expression, consistent with a previous report that expression is maximal for only 3–6 hr after electroporation of MEL cells, i.e., it is very short-lived (4). Although MEL cells are naturally

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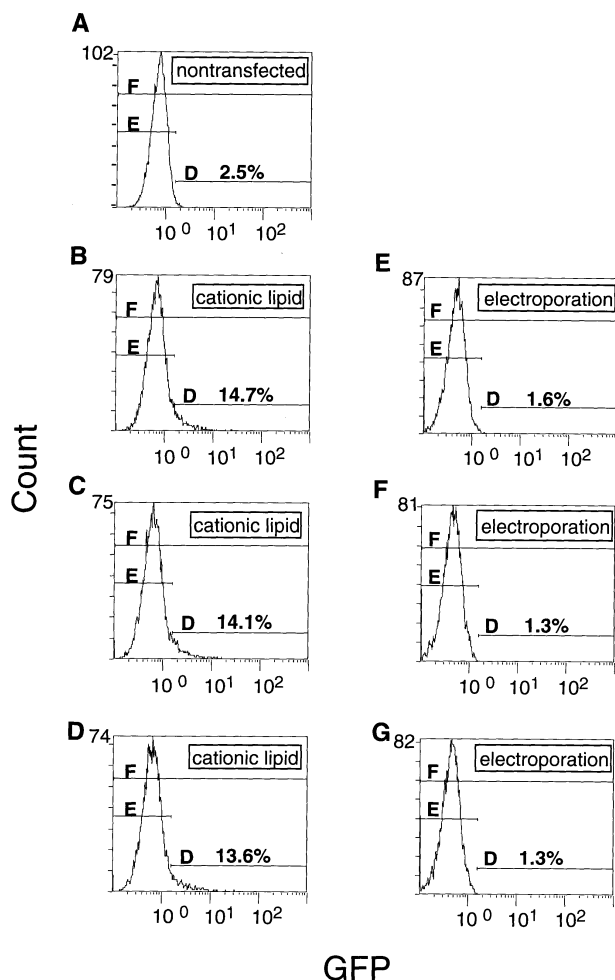
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nonadherent, most previous reports used selected adherent MEL cell lines (1, 11, 17, 20). Transient transfection of nonadherent MEL cells can be accomplished via a transferrin-based transfection procedure (19). We sought a more convenient procedure, utilizing commercially available reagents, that could be applied under identical conditions to both nonadherent MEL cells and other hematopoietic cells. We report here that use of a cationic lipid transfection reagent with MEL cells gives reproducible results and readily detectable expression of a globin-luciferase reporter gene.

## METHODS

The cationic lipid reagent Tfx50 (from Promega) was used to transiently transfect MEL and K562 cells.  $1 \times 10^6$  cells were transfected according to the manufacturer's protocol for suspension cells; the reagent and DNA remained in the cell culture for 48 hr, at which point the cells are harvested. The manufacturer's protocol indicates the amount of cationic lipid necessary to confer an overall positive charge when associated with the DNA (at charge to mass ratios of 2:1, 3:1 or 4:1). For example, 3.0 µL of the Tfx50 solution plus 1.0 µg of DNA gives a charge to mass ratio of 2:1. The optimal amount of reagent for use with MEL cells was determined by titrating increasing amounts of DNA at each ratio. Transfection conditions were determined to be optimal at a 2:1 charge to mass ratio of cationic lipid to DNA. Initially, each plasmid containing a reporter gene was assayed as a titration from 0.25 µg of DNA up to a maximum of 8.0 µg. Then plasmids were tested in triplicate at the single DNA mass most frequently seen to be optimal for enhancement in each cell line, which was 2.0 µg for MEL cells and 3.0 µg for K562 cells. In some assays, plasmids containing either *RSV-lacZ* or *Renilla* luciferase gene driven by the HSV *tk* promoter were included as co-transfection controls. Student's *t*-test was used to determine the probability that paired samples did not belong to distinct, nonoverlapping data sets.



**Fig. 2.** Flow-cytometry to measure the percentage of cells expressing GFP. (A) Nontransfected MEL cells were used to set the gate for GFP negative cells (gate E). MEL cells were transfected by cationic lipid (panels B-D) or electroporation (panels E-G) as described in the methods section. Following 2 days incubation, cells were resuspended in 1.0 mL of culture media and analyzed by flow-cytometry for GFP fluorescence (gate D). The percentage of GFP positive cells in each sample (out of a total of 10,000 cells, gate F) is shown above gate D.

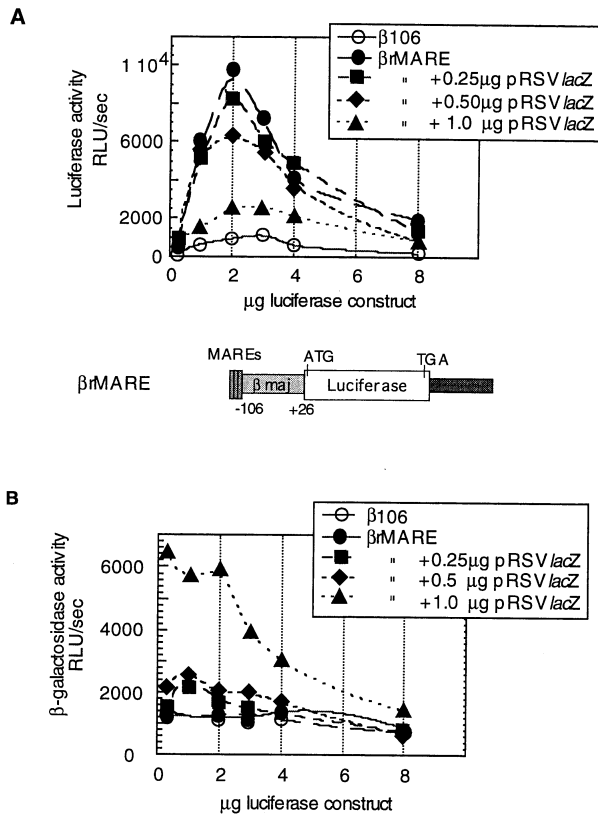
Reporter plasmids for MEL cells contain the promoter of the mouse  $\beta$ -major globin gene (*Hbb-b1*) fused to the coding region from the beetle (firefly) luciferase gene from Promega's pGL2 Basic vector ( $\beta$ 106). The  $\beta$ 106HS2 plasmid contains an enhancer from hypersensitive site 2 (HS2, in particular a *HindIII-XbaI* fragment, 18) from the  $\beta$ -globin locus control region inserted into the  $\beta$ 106 parental plasmid. The  $\beta$ rMARE plasmid contains a tandem repeat of 3 *maf*-

response elements (MAREs, 12) from rabbit HS2 inserted into the  $\beta$ 106 parental plasmid. The reporter gene for K562 cells, called  $\epsilon$ -luc, consisted of the luciferase coding region fused between the first and second exons of the rabbit  $\epsilon$ -globin gene. Thus it contains the promoter, considerable internal sequences and the 3' flanking region of the globin gene (9). The  $\epsilon$ -luc plasmid was also tested after insertion of the *HindIII-XbaI* DNA fragment containing HS2.

In order to measure the fraction of cells expressing a reporter plasmid, MEL cells were transiently transfected using the Tfx50 reagent to introduce 2.0  $\mu$ g of pHS234 $\beta$ GFP, an expression vector for green fluorescent protein containing the human  $\beta$ -globin gene promoter and enhancer elements from hypersensitive sites 2, 3, and 4 from the human  $\beta$ -globin locus control region (modified from (3)). For comparison,  $5 \times 10^6$  MEL cells were transfected by electroporation to introduce 10  $\mu$ g of pHS234 $\beta$ GFP and 40  $\mu$ g of pBluescript as carrier DNA. The conditions for electroporation were the same as those we have used reliably in K562 cells: 500  $\mu$ farads, 500 mseconds, and 450 V/cm. Two days after transfection, cells were counted by flow cytometry to determine the efficiency of DNA transfer for each method.

## RESULTS

Conditions for transfection of uninduced MEL cells by the cationic lipid reagent Tfx50 (from Promega) were optimized by treatment with increasing amounts of DNA at three different ratios of the charge of the lipid to the mass of DNA. MEL cells express the endogenous adult  $\beta$ -major globin gene (*Hbb-b1*), and the reporter was a fusion between the mouse *Hbb-b1* promoter and the luciferase coding region. Expression from the enhancerless plasmid,  $\beta$ 106, was compared to that of a plasmid carrying the human HS2 core upstream of the reporter gene,  $\beta$ HS2. A charge to mass ratio of 2:1 gave the greatest level of expression from both constructs and showed the largest window of enhancement from HS2 (4.5-



**Fig. 3.** Reduced expression of luciferase test plasmids in the presence of pRSVlacZ as a cotransfection control. (A) Enhancement of the β106 reporter gene linked to the rabbit HS2 MARE trimer (βrMARE) was tested in the presence and absence of a potential cotransfection control plasmid, pRSVlacZ while the charge to mass ratio remained constant at 2:1. Luciferase activity (from the βrMARE plasmid) is plotted as a function of mass of transfecting DNA. (B) β-galactosidase activity (from the pRSVlacZ plasmid) was measured for each transfected sample in panel A and plotted as a function of the mass of transfecting luciferase plasmid. The β-galactosidase activity in the β106 and βrMARE samples, where no pRSVlacZ was added, represents the background activity in this cell line.

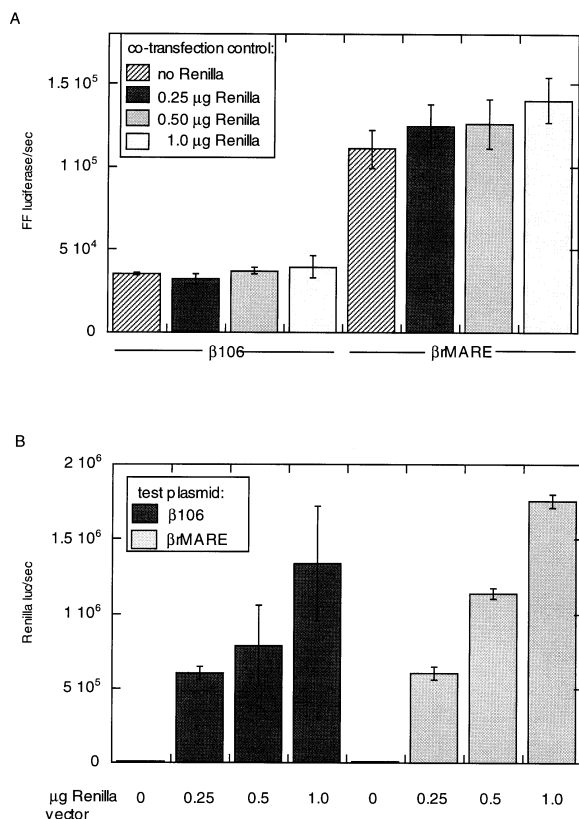
fold), with a peak in the curve for βHS2 at 2.0 µg of transfected DNA (Fig. 1A). Use of a smaller charge to mass ratio (1.5:1) provided no detectable βHS2 activity (data not shown); thus the 2:1 ratio was used in subsequent experiments. The level of expression consistently declined above 4.0 µg of DNA, presumably due to cell death at higher concentrations of the lipid reagent. The peak level of enhancement shown in the DNA titration curves differed for the constructs with and without HS2, so all constructs were tested

initially in a DNA titration experiment to ensure capturing the maximal window of enhancement.

Although K562 cells are readily transfected by electroporation, conditions for transfection with the cationic lipid reagent were also optimized for these cells in order to compare results with MEL cells. K562 cells are human bipotential cells that express the endogenous embryonic ε-globin gene (*HBE*), and the reporter plasmid contained a hybrid *HBE*-luciferase gene. Expression was optimal at a charge to mass ratio of 2:1, progressively declining as the ratio increased to 3:1 and 4:1 (Fig. 1B). In these experiments the reporter plasmid containing HS2 gave 25-fold enhancement over the expression from the enhancerless ε-luciferase gene, peaking at 3.0 µg of DNA. The amount of DNA used for transfection of K562 cells by cationic lipid (0.25 to 4.0 µg) is substantially less than that used in electroporation (e.g., 50 µg comprising 10 µg test plasmid plus 40 µg carrier DNA).

Once the optimal conditions for transfection were determined, the efficiency of transfection by electroporation and cationic lipid were compared in MEL cells. By using an EGFP (Clontech) reporter gene driven by a human β-globin gene (*HBB*) promoter, we could determine the fraction of cells expressing "enhanced" green fluorescent protein by flow cytometry. Obviously cells have to take up the plasmid DNA in order to express it, so this assay provides a minimum estimate of the transfection efficiency. In three independent transfections of MEL cells using cationic lipids, an average of 1,400 out of 10,000 cells produced GFP (Figs. 2 B-D). The GFP-positive cells are in gate D, which is a shoulder to the much larger peak of GFP-negative cells in gate E. The cells were refractory to transfection by electroporation, yielding no detectable GFP (the fraction of cells in the "GFP-positive" window was no greater than that of the nontransfected MEL population; see Fig 2, gate D in panels A and E-G).

The requirement for a specific ratio between lipid transfection reagent and mass of DNA, coupled with the toxicity of high levels of the lipid reagent, precluded the use of pRSVlacZ



**Fig. 4.** Co-transfection of MEL cells with beetle luciferase and *Renilla* luciferase vectors. A titration of the mass of *Renilla* luciferase vector from 0.25 to 1.0 µg of DNA was conducted in the presence of a constant mass of beetle luciferase test vector (β106 or βMARE), at 1.0 µg. (A) Luciferase activity is plotted as beetle luciferase units per second for each titration point. Shading is used to depict the amount of *Renilla* DNA present in each sample. All transfections were done in triplicate, the means are plotted and the standard deviation shown as error bars. (B) A plot of *Renilla* luciferase activity measured at each titration point. The sample described above were sequentially measured for beetle luciferase, quenched, and measured for *Renilla* luciferase which has a completely different substrate. The mass of *Renilla* vector used in each transfection is shown below each bar in the graph and shading depicts which beetle luciferase construct was present.

DNA as a co-transfection control. Even using a reporter plasmid that produced a relatively high level of enhancement in MEL cells (10-fold enhancement by βrMARE, Fig. 3A), the pRSVlacZ DNA interfered with the expression assay. When increasing amounts of the pRSVlacZ control were co-transfected with βrMARE, raw luciferase values decreased proportionately, resulting in only a 3-fold enhancement when

assayed in the presence of 1.0 µg of pRSVlacZ (Fig. 3A). This amount of pRSVlacZ was the only one to produce β-galactosidase activity above endogenous levels (Fig. 3B). Thus the amount of co-transfecting control plasmid required to see a signal in this assay was also inhibitory to the enhancement under study, suggesting competition between the two reporter gene promoters.

An alternative co-transfection control, utilizing *Renilla* luciferase (from sea pansy; pRL-TK, Promega), was effective in MEL cell transfections. Because of their distinct evolutionary origins, beetle and *Renilla* luciferase differ in their enzyme structures and substrate requirements, allowing them to be assayed sequentially in the same reaction tube. Transfection conditions were as described above, adding increasing masses of the *Renilla* luciferase test plasmid (0.25 to 1.0 µg DNA) in the presence of a constant amount (1.0 µg) of firefly luciferase test plasmid. Enhancement of β106 expression levels by the MARE elements was unaffected by increasing masses of *Renilla* DNA (Fig 4A). *Renilla* luciferase activity increased proportionally with increasing mass of transfected pRL-TK plasmid (Fig. 4B).

## CONCLUSION

We have shown that cationic lipid carriers provide a reliable, effective, and reproducible method of transfecting MEL cells. This method uses only small amounts of DNA and is compatible with the use of a highly sensitive co-transfection control plasmid such as pRL-TK. Additionally, the *Renilla* luciferase encoded by this plasmid is conveniently assayed in the same reaction tube as the beetle (firefly) luciferase. Thus one can now use transient transfection of MEL cells as means to define and dissect *cis*-regulatory elements of gene encoding globins and other erythroid-specific proteins. The ability to do these assays in MEL cells is significant, since it is one of the very few fully erythroid cell lines available. Many others, such as K562 or HEL cells, are multipotential and incapable of a full response in inducers of erythroid maturation, e.g.,

forming substantially less hemoglobin. Our own studies utilizing this method have revealed a previously unrecognized negative element in the HS2 enhancer and have defined a subregion of HS2 with potent enhancement activity (7). Application of this transient transfection methodology to MEL and other hematopoietic cell lines growing in suspension may reveal additional regulatory elements.

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