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ORIGINAL ARTICLE

Optimizing conditions for calcium phosphate mediated transient transfection

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Abstract *Background:* Calcium phosphate mediated transfection has been used for delivering DNA into mammalian cells in excess of 30 years due to its most low cost for introducing recombinant DNA into culture cells. However, multiple factors affecting the transfect efficiency are commonly recognized meanwhile for years, the low transfection efficiency of this approach on higher differentiated and non-tumor cells such as CHO and C2C12 limits its application on research.

Results: In this paper, we systematically evaluated the possible factors affecting the transfection rate of this approach. Two categories, calcium phosphate–DNA co-precipitation and on-cell treatments were set for optimization of plasmid DNA transfection into CHO and C2C12 cell-lines. Throughout experimentation of these categories such as buffer system, transfection media and time, glycerol shocking and so on, we optimized the best procedure to obtain the highest efficiency ultimately.

During calcium phosphate DNA-precipitation, the transfection buffer is critical condition optimized with HBS at pH 7.10 ($P = 0.013$ compared to HEPES in CHO). In the transfection step,

Abbreviations: CHO, Chinese hamster ovary cells; C2C12, mouse myoblast cells; PEG, polyethylene glycol; FBS, fetal bovine serum; Pen-Strep, penicillinstreptomycin; IntDen, integrated density

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FBS is a necessary component in transfection DMEM for high efficiency ($P = 0.0005$ compared to DMEM alone), and high concentration of co-precipitated particles applied to cultured cells in combination with intermittent vortexing is also crucial to preserve the efficiency. For 6-well culture plates, 800 µl of co-precipitated particles (11.25 µg/mL of cDNA) in 1 well is the optimal ($P = 0.007$ compared to 200 µl). For the highest transfection efficiency, the most important condition is glycerol in shock treatment ($P = 0.002$ compared to no shock treatment in CHO, and $P = 0.008$ compared to no shock treatment in C2C12) after a 6 h incubation ($P = 0.004$ compared to 16 h in CHO, and $P = 0.039$ compared to 16 h in C2C12) on cultured cells.

Conclusions: Calcium phosphate mediated transfection is the most low-cost approach to introduce recombinant DNA into culture cells. However, the utility of this procedure is limited in highly-differentiated cells. Here we describe the specific HBS-buffered saline, PH, glycerol shock, vortex strength, transfection medium, and particle concentrations conditions necessary to optimize this transfection method in highly differentiated cells.

1. Introduction

Genetically modified organisms, including transgenic plants, animals and cultured cell lines, have gradually becoming the main experimental models in current food science, agriculture and biology research fields (Liu et al., 2016a,b; Zhou et al., 2016). Delivery of DNA and RNA into cultured cells is a fundamental molecular biology transgenic technique. To explore the function of a gene of interest, transient transfection is normally employed to over-express or knock down gene expression from cells in a controlled setting. To conduct these seminal experiments, recombinant DNA need to be introduced into cell without damaging cell viability. There are currently three main techniques to accomplish this mission. First is lipid-mediated liposome delivery, such as lipofectamine series products manufactured by Invitrogen. This approach displays high efficiency for transient transfection in most cultured cells, but is limited by high cost and is not suitable for large quantity delivery (Junquera and Aicart, 2014; Kaestner et al., 2015; Xiong et al., 2011). The second approach is electroporation, which has the highest efficiency method of the three but also causes the highest death rate of cells (Kalli et al., 2014; Li et al., 2013; Nakamura and Funahashi, 2013). The third method of the transient transfection, and focus of this report, is calcium phosphate mediated transient transfection. In this approach, calcium phosphate forms an insoluble precipitate with DNA, this Ca-DNA complex then attaches to the cell surface where it is transported into cells by endocytosis. Due to its reasonable efficiency and low cost, this technique has been used for delivering DNA into mammalian cells for over 30 years (Dudek et al., 2001; Kwon and Firestein, 2013; Sambrook and Russell, 2001; Sun et al., 2013). However, this approach has largely been neglected in favor of the first two methods due to the myriad of factors affecting transfection efficiency in the hands of experimenters and previous belief that this method does not work well in highly differentiated cells (Inokuchi et al., 2009; Mohammad et al., 2008). In this paper, we systematically documented the optimal conditions for the most effective calcium phosphate mediated transfection in highly-differentiated cell lines, Chinese hamster ovary cells (CHO) and mouse myoblast cells (C2C12).

2. Materials and methods

2.1. Cloning and sub-cloning

Four different plasmids were used in this study, EGFP-N1, EGFP-pIRES, EGFP-pIRES-mCnB, and EGFP-pIRES-mCnA. EGFP-N1 is a commercial product from Clontech. *mCnB* and *mCnA* genes were amplified from total RNA as previously described (Wang et al., 2008; Yoshiga et al., 2002), and cloned into the EGFP-pIRES plasmid with 5′ *Nhe* I and 3′ *Sac* I restriction sites, which were later used to confirm successful insertion.

Recombined plasmid DNAs were transfected into HB101 competent Escherichia coli. Individual colonies were transferred into Kanamycin (20 µg/ml) LB medium and incubated over night at 37 °C with vigorous shaking (250 rpm on a rotary shaker) until the bacteria reached late log phase. Plasmid DNAs were prepared using alkaline lysis with SDS were purified with polyethylene glycol (PEG, 40% PEG6000, 30 mM $MgCl_2$) and were recovered with deionized distilled H_2O or 1 × TE buffer (pH 7.6).

2.2. Cell culture

Cells for transfection were cultured at 37 °C in a humidified incubator with an atmosphere of 5% CO_2, in DMEM high glucose medium (pH 7.4, Gibco) supplemented with 10% inactivated fetal bovine serum (10% FBS, Invitrogen), 100 U/ml penicillin and 100 µg/ml streptomycin (1% Pen-Strep, Invitrogen). Chinese hamster ovary (CHO) cells and mouse myoblast (C2C12) cells were two lines used in the study. Cells were transfected with expression plasmids at 50–50% confluence then sub-cultivated at 80%–90% of cell confluence. The calcium phosphate-DNA co-precipitate transient transfection method was used.

2.3. Calcium phosphate mediated transient transfection of CHO and C2C12 cell-lines

In this study, we optimized the procedure of calcium phosphate mediated transient transfection approach using CHO and C2C12 cell-lines (Supplemental Fig. 1 and detailed in Table 1) in 6-wells plates (NUNC). The traditional steps to