Calcium Phosphate Transfection of Eukaryotic Cells

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[Abstract] Transfection of DNA into cells is an indispensible protocol in molecular biology. While plenty of lipid-based transfection reagents are commercially available nowadays, a quick, simple, efficient and inexpensive method is to transfect eukaryotic cells via calcium phosphate co-precipitation with DNA (Graham and van der Eb, 1973). The insoluble calcium phosphate precipitate with the attached DNA adheres to the cell surface and is brought into the cells by endocytosis. Calcium phosphate transfection has been optimized and widely used with many adherent and nonadherent cell lines (Jordan *et al*., 1996). Calcium phosphate transfection can result in transient expression of the delivered DNA in the target cell, or establishment of stable cell lines (the latter requires a drug selection process). This protocol is also widely used for co-expression of plasmids for packaging viruses. Efficiency of transfection can be close to 100% depending on the cell lines used. Here, a calcium phosphate transfection protocol is described using a GFP plasmid and the HEK293 cell line.

Materials and Reagents

1. HEK-293 cells (ATCC, catalog number: CRL-1573™)
2. Eagle's minimum essential medium (ATCC, catalog number: 30-2003™)
3. Fetal bovine serum (FBS) (ATCC, catalog number: 30-2020™)
4. Calcium chloride ($CaCl_2$) (Sigma-Aldrich, catalog number: C5670)
5. HEPES (Sigma-Aldrich, catalog number: H4034)
6. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: S5886)
7. Sodium phosphate dibasic (Na_2HPO_4) (Sigma-Aldrich, catalog number: S5136)
8. pEGFP-Actin plasmid (this is an example plasmid; Clontech, catalog number: PT3265-5)
   Note: The pEGFP-Actin Vector expresses the EGFP-Actin fusion protein in mammalian cells; this protein is incorporated into growing actin filaments and allows for visualization of actin-containing subcellular structures in living and fixed cells. http://www.clontech.com/images/pt/dis_vectors/PT3265-5.pdf
9. 2x HBS buffer (see Recipes)
10. Solution-A (see Recipes)
11. Solution-B (see Recipes)

Equipment

1. Tissue culture plates (*e.g.,* 35 mm polystyrene)
2. Cell culture incubator: 37 ºC and 5% CO_2

Procedure

1. Prepare 2 M $CaCl_2$ solution in water, filter sterilize and keep at room temperature.
2. Prepare the 2x HBS buffer (see Recipes).
3. Carry HEK-293 cells in Eagle's Minimum Essential Medium with 10% FBS.
4. 24 h before transfection, trypsinize and reseed log-phase cells into 35 mm tissue culture dishes. For seeding density, cells should reach 60-70% confluence for transfection.
   Note: Best confluence for different cell lines differ. For HEK-293 cells, 60-70% confluence is suggested.
5. 3 h before transfection, replenish cells with fresh medium.
6. For each DNA transfection, prepare mixtures in two separate tubes (Solution-A and Solution-B, see Recipes)
7. Add Solution-B slowly (drop-wise) into Solution-A while mixing Solution-A.
   Note: This is the most important step for forming DNA/calcium phosphate co-precipitate. Mix gently but thoroughly to allow formation of precipitates evenly.
8. After mixing the two solutions, incubate at room temperature for 20-30 min (a shorter incubation time may be used for different cell types; please determined empirically). The solution will become opaque while precipitates being formed.
9. Gently tap the mixture. Add the mixture directly to cells by dripping slowly and evenly into medium (a good way is to let tip touch medium surface).
10. Gently tilt the plate back-and-forth a couple of times to allow even distribution of added precipitate on the cell surface.
11. Incubate the cells at 37 ºC with 5% CO_2 for 24 h and then replenish medium.

Notes

1. GFP expression, if used as your positive, can be detected usually after 24-48 h of cell growth.
2. Transfection efficiency (when using HEK293 cells and the above mentioned sample plasmid) can reach up to 90-100%.

Recipes

1. 2x HBS buffer
   50 mM HEPES
   280 mM NaCl
   1.5 mM Na_2HPO_4
   Adjust pH to 7.0 using HCl. Filter sterilize. The solution can be freezed/thawed once for future use.
2. Solution-A
   100 µl 2x HBS
3. Solution-B
   1-5 µg DNA (*e.g.,* pEGFP-Actin plasmid as suggested above)
   12.2 µl of 2 M $CaCl_2$
   ddH_2O to bring volume up to 100 µl, pipet gently to mix
   Note: Titration of DNA should be carried out to obtain the best efficiency of transfection.

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