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Stable Transfection of Plasmid DNA into Adherent Rodent Cell Lines Using Calcium Phosphate

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We use this protocol and it's working

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Abstract

A traditional strategy for stably transfecting DNA into rodent fibroblast cell lines features calcium phosphate precipitates. Here we describe our laboratory protocol for this strategy. We have assumed that the transfected DNA contains an expression vector for an antibiotic resistance gene, such as the neomycin resistance gene, the hygromycin resistance gene, the puromycin resistance gene, and the like.

Troubleshooting

Introduction

1 A traditional strategy for stably transfecting DNA into rodent fibroblast cell lines features calcium phosphate precipitates. Here we describe our laboratory protocol for this strategy. We have assumed that the transfected DNA contains an expression vector for an antibiotic resistance gene, such as the neomycin resistance gene, the hygromycin resistance gene, the puromycin resistance gene, and the like.

Methods

2 ***Cell Lines and Cell Culture***

2.1 NIH/3T3 [1] and C127 [2] adherent rodent cell lines are a generous gift from Daniel DiMaio. PA317 [3], Psi-2 [4], and Psi-Cre [5] recombinant retrovirus packaging cell lines are derived from NIH/3T3 cells and are a generous gift from Daniel DiMaio [6].

2.2 The cell line to be transfected is typically maintained in T-75 (nominally 75 cm^2) cell culture flasks according to published or vendor recommendations [1-5, 7]. The cell line is transfected no less than two passages and no greater than five passages after being established from a frozen vial of archived cells.

2.3 Cells to be transfected should be seeded at three densities to account for experiment-to-experiment variations in cell growth rate and plating efficiency. Thus, for each transfection, 1×10^5, 2×10^5, and 3×10^5 cells are seeded into a 60 mm (diameter) cell culture dish. In other words, three dishes of cells, each at a different density, are prepared for each transfection. Prepare enough plates of cells to allow for mock and control transfections. Cells are incubated under standard conditions for 24-48 hours to allow cell recovery. Cells should be at 50% confluence at the time of transfection.

3 ***Preparation for DNA Transfection***

3.1 Two hours prior to transfection, the set of plates that are closest to 50% confluence are selected, while the other sets of plates are discarded. The medium should be changed on the selected set of plates using 5 mL of standard (pre-warmed to 37^oC) culture medium and the cells should be returned to the 37^oC incubator.

3.2 During this two-hour incubation, a sterile 5 mL snap-cap tube (Falcon 352063; VWR 60819-728 [8]) should be labeled for each transfection, including mock and control transfections.

3.3 In a biosafety cabinet, 200 uL of 2x HEPES-buffered saline (2x HEBS – see recipe below) should be added to each 5 mL snap-cap tube.

3.4 In a biosafety cabinet, add 10 ug of uncut plasmid DNA to the appropriate 5 mL snap-cap tube(s).

3.5 Using sterile technique, make a fresh solution of 250 mM $CaCl_2$ by diluting sterile 2 M $CaCl_2$ with deionized water. Each transfection requires 200 uL of 250 mM $CaCl_2$. The 250 mM $CaCl_2$ solution should be formulated in a sterile 1.5 mL microcentrifuge tube or a sterile 50 mL centrifuge tube to facilitate sterile pipetting.

3.6 Prepare the calcium phosphate precipitates of DNA in a biosafety cabinet. Approximately 90 minutes after changing the medium on the cells to be transfected, use a micropipetter to add 200 uL of 250 mM $CaCl_2$ to each HEBS/DNA solution while simultaneously blowing air bubbles into the HEBS/DNA solution using a pipetter and a 1 mL sterile serological pipette. This facilitates formation of calcium phosphate precipitates of DNA.

3.7 Vortex to mix the precipitates and incubate for 20 minutes at room temperature in the biosafety cabinet.

4 *DNA Transfection and Shock*

4.1 Drip each precipitate onto a 60 mm dish of cells with swirling. Confirm the presence of precipitates by microscopic inspection. Incubate 4-6 hours at 37^{o}C.

4.2 Shock cells as follows:

- Prepare the glycerol/HEBS shock solution using a 1:1 mixture of 30% glycerol and 2x HEBS
- Prepare complete (pre-warmed to 37^{o}C) culture medium containing 5 mM NaButyrate.
- Aspirate medium from each plate.
- Add 0.7 mL glycerol/HEBS solution and incubate 45 seconds.
- Aspirate glycerol/HEBS solution and wash twice with PBS.
- Add 4 mL complete culture medium supplemented with 5 mM NaButyrate.
- Incubate 12-16 hours at 37^{o}C.

5 *Passage Cells and Select for Transfected Cells*

5.1 Use standard cell culture techniques to subculture each 60 mm dish of transfected cells into three 100 mm (diameter) cell culture dishes.

5.2 Incubate 16-24 hours at 37^{o}C.

5.3 Select for transfected cells using the appropriate antibiotic.

5.4 Depending on the antibiotic, colonies of stably transfected (antibiotic-resistant) cells typically become apparent following 7-12 days of selection.

Buffer Recipes

6 ***2x HEBS (250 mL recipe)***

6.1 Add the following to a 500 mL Erlenmeyer flask on the benchtop
118 mg Na_2HP0_4-$7H_20$ (62.5 mg anhydrous)
0.5 g Dextrose (D-glucose)
4 g NaCl
185 mg KCl
2.5 g HEPES
Add 230 mL deionized water; stir until the solids are dissolved
Adjust pH to 7.05 w/10 N NaOH
Repeat pH next day (use concentrated HCl if pH is too high)
Adjust volume to 250 mL using deionized water
Sterile filter using a 0.2 um filter and aliquot 10 mL into twenty-five 15 mL screw top tubes
Store at -20^{o} C until use

7 ***1 M NaButyrate ($NaC_4H_7O_2$ – 250 mL recipe)***

7.1 Add the following to a 500 mL Erlenmeyer flask in a fume hood
200 mL deionized water
27.5 g NaButyrate
Stir until the solid is dissolved
Adjust volume to 250 mL using deionized water
Sterile filter using a 0.2 um filter into a sterile 250 mL media bottle
Store at 4^{o} C until use

8 ***30% Glycerol (250 mL recipe)***

8.1 Add the following to a 500 mL Erlenmeyer flask in a fume hood
150 mL deionized water
75 mL Glycerol
Stir until the two liquids are thoroughly mixed
Adjust the volume to 250 mL using deionized water
Sterile filter using a 0.2 um filter into a sterile 250 mL media bottle
Store at 4^{o} C until use

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