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Cell Fusion and Transfection of HL3T1 and HeLa-*tat* Cell Lines

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I. CELL FUSION PROTOCOL

This protocol allows for fast and efficient fusion of two or more cell types and the creation of transient heterokaryons (1,2). Cells should be seeded so that they are close to confluency. More than 95% of cells fuse by the PEG treatment.

Reagents

HL3T1 Cells	Repository catalog #1115
Tat-producing Cell Line	HLtat (Catalog #1293) and CB2MX3 (6) cell lines have been used successfully; HeLa-*tat*-III (Repository catalog #502) should also be effective.
PBS	Dulbecco's PBS, calcium and magnesium-free
PEG Solution	Baker-analyzed PEG1000 (Baker catalog #U218-07), in DMEM; see step 1 (below) for preparation. Alternatively, ready-to-use PEG solution can be purchased from GibcoBRL (PEG 4000, catalog #14030-035)
Dowex-Mixed Bed Resin	Ion exchange resin AG501-X8(D) (Bio-Rad catalog #142-6425)
Culture Medium	DMEM supplemented with 10% fetal bovine serum

1. Purchase ready-to-use PEG solution, or prepare PEG solution as follows:

   a. Melt 500 ml of PEG using repeated heat cycles in a microwave oven (temperature should not exceed 45°C).

   b. Add 10 g of Dowex-mixed bed resin to the PEG bottle and incubate the mixture in a 37°C shaker for 4 hours.

   c. Filter the solubilized PEG by applying vacuum through 10 g of fresh Dowex mixed bed resin placed atop a Whatman #1 filter. Maintain 37°C throughout the procedure. Warming the filter apparatus with a hair dryer helps to prevent clogging of the filter due to PEG solidification.

   d. Determine the weight of the filtered PEG and add DMEM to make a 50% (w/w) solution.

   e. Filter-sterilize the PEG solution using a Nalgene 0.2 µ filter and store aliquots at -20°C. The working stock can be stored at 4-8°C for several weeks.

2. Prewarm all solutions used throughout this protocol to 37°C.

3. From an established cell culture, seed a mixture containing 8 x 10^5 cells each of HL3T1 cells and the Tat-producing cell line into a 60 mm tissue culture dish (5 ml total volume). If a 6-well tissue culture plate is used, seed 2.5 x 10^5 cells of each cell line in 3 ml of total medium per well.

4. The following day, wash the cells once with 5 ml of PBS.

5. Gently distribute 2 ml of the PEG solution over the entire dish and incubate at room temperature for exactly 3 minutes.

6. Aspirate off the PEG solution and wash the dish 3 times with 5 ml of PBS.

7. Feed the cells with 5 ml of fresh culture medium.

8 Harvest the cells after 24-48 hours and quantitate the level of CAT activity as described (7).

II. TRANSFECTION PROTOCOL

This simple transfection protocol (3,4) allows for high level expression in HL3T1 cells. These cells contain integrated copies of the HIV-1 promoter linked to the CAT gene. Activation by Tat or other *trans*-activators leads to a dose-dependent expression of CAT enzyme, which can be easily quantitated. After transfection, at least 30% and as many as 50% of cells express high levels of the introduced marker. This protocol also allows for high-level expression of many genes linked to the HIV-1 LTR promoter when used with HLtat cells, which express a truncated, functional form of Tat (5). The quality of DNA is critical for efficient transfections; we use DNA isolated by Triton X-100 lysis followed Qiagen column purification. The efficiency of trans-activation depends on the correct maintenance of the cell line and the quality of the DNA and the solutions used in the protocol.

Reagents

HL3T1 Cells	Repository catalog #1115
Culture Medium	DMEM supplemented with 10% fetal bovine serum
Serum Free Culture Medium	DMEM
2X HBS	274 mM NaCl, 10 mM KCl, 1.5 mM Na_2HPO_4, 12 mM dextrose, 42 mM HEPES, pH 7.1; filter-sterilize and store at 4°C.
Carrier DNA	Any plasmid DNA, purified by Qiagen column
$CaCl_2$ Solution	2 M $CaCl_2$, prepared in sterile aliquots and stored at -80°C

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1. Seed 8 x 10^5 HL3T1 cells into a 60 mm tissue culture dish and feed with 5 ml culture medium.

2. The following day, perform the transfection as described below. Make sure all solutions are prewarmed to room temperature.

3. Adjust the DNA to be examined (usually 0.1 to 5 µg) first to 17 µg using carrier DNA, then adjust to a final volume of 219 µl using sterile, endotoxin-free water, and add 31 µl of sterile 2 M $CaCl_2$.

4. Add the DNA mixture dropwise to 250 µl of 2X HBS. Mix the two solutions gently and thoroughly by applying a sterile stream of filtered air to the surface of the HBS solution (avoid vortexing).

5. After 15-20 minutes, gently distribute the DNA solution onto the cell monolayer of the entire plate using a standard 1 ml blue pipet tip inserted below the surface of the culture medium. Avoid touching the cell monolayer. Incubate the transfected cells at 37°C in a CO_2 incubator for 6 hours.

6. After incubation, a successful precipitate will look like very fine sand on top of the cells. Aspirate the precipitate, then wash the cells twice using 5 ml of serum-free culture medium and refeed with 5 ml of fresh culture medium.

7. Harvest cells for analysis after 16 to 48 hours and assay for CAT activity as described (7).

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