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Transfection of *Capsaspora owczarzaki* using calcium-phosphate precipitation

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We use this protocol and it's working

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Abstract

This is a protocol to transiently transfect *Capsaspora owczarzaki*, a unicellular eukaryote

Guidelines

- This protocol is set up for transfecting *Capsaspora* in 12 wells/plate. All volumes are defined per well.
- Prepare reagents in advance
- When replicates are to be done, a larger DNA mix can be set up

Safety warnings

! - Work in a laminar flow hood to ensure the experiment is not contaminated

-There are certain critical steps in this protocol that affect efficiency:

CRITICAL STEP 1: Adherent stage cells in confluency. Cultures should be fresh to maximize transfection efficiency. Ideally, they should be maintained weekly, and used for transfection at their exponential growth phase. Do not let cultures reach higher cell densities ($<5 \times 10^7$ cells mL⁻¹).

CRITICAL STEP 2: DNA-Calcium- phosphate precipitates formation. Check the cultures periodically under the microscope to check crystal size. Big cloudy precipitates may compromise transfection efficiency. Instead, verify that small grains of refractant material are spread homogeneously in the plate.

CRITICAL STEP 3: Glycerol shock. Incubation with glycerol at this concentration should not exceed 1 min, counting from the first droplet, to avoid excessive cell death

Before start

Transfection Reagents preparation

Growth medium (for 1 L): 10 g Peptone (BD, #211677), 10 g Yeast Extract (BD, #212750), 1 g Yeast nucleic acid (Ribonucleic Acid, Type VI from Torula Yeast) (Sigma, #R-6625), 15 mg Folic acid (Sigma, #F8758) in 880 mL distilled water. Autoclave for 15 min at 121°C. Cool down and aseptically add 0.4 mL of Hemin stock solution* (Sigma, #H9039), 20 mL Buffer solution** and 100 mL of heat-inactivated Fetal Bovine Serum (Sigma, #F9665-100ml). Filter-sterilise through 0.22 µm and store at 4°C.

***Hemin stock solution (for 200 mL):** 400 mg NaOH in 200 mL dH 2 O. Add 500 mg of Hemin and autoclave 20 min at 121°C. Store at 4°C protected from the light.

****Buffer solution (for 1 L):** 18.1 g KH₂PO₄ (Sigma, #P5655), 25 g Na₂HPO₄ (Sigma, #S5136) in 1 L distilled water. Adjust final pH to 6.5 with HCl 37% and filter-sterilise through 0.22 µm. Store at 4°C.

Transfection medium (for 1 L): 10 g Peptone, 15 mg Folic Acid in 990 mL distilled water. Autoclave for 20 min at 121°C. Aseptically add 10 mL HEPES 1 M (Sigma, #H4034) to a final concentration of 10 mM and 2.1 g Bis-Tris methane (Sigma, #B9754) final concentration 0.21% w/w. adjust pH to 7.1 with NaOH, filter-sterilise through 0.22 µm and store at 4°C.

2X HBS (for 250 mL): Dissolve 4 g NaCl (Sigma, #S3014), 0.18 g KCl (Sigma, #P9541), 0.05 g Na₂HPO₄ (Sigma, #S5136), 2.5 g HEPES and 0.5 g D-glucose (Sigma, #G8270) in autoclaved distilled water. Adjust pH to 7.1 with NaOH. Filter-sterilise through 0.22 µm, flash-freeze with liquid Nitrogen and store at -80°C.

1.25M CaCl₂ (for 10 mL): 1.84 g CaCl₂ (Sigma, #C1016) in 10 mL autoclaved distilled water. Filter-sterilise through 0.22 µm, flash-freeze with liquid Nitrogen and store at -80°C.

10% glycerol (for 4 mL): 0.8 mL of filter-sterilised 50% (v/v) glycerol (Sigma, #G7757) in 1.2 mL autoclaved distilled water and 2 mL 2X HBS. Filter-sterilise through 0.22 µm, flash-freeze with liquid Nitrogen and store at -80°C.

Preparation of cells

- 1 Seed 10^7 cells in a flask with 5mL of growth medium, let grow for 24h at 23°C to have a confluent culture.
- 2 Take the cells from the confluent culture, seed 2×10^6 cells per well in 600 μ L of growth medium.

Calcium-phosphate precipitation

- 3 Replace growth medium with 600 μ L of transfection medium, incubate 30 min at 18°C (room temperature).
- 4 While incubating with transfection medium, prepare DNA mix (300 μ L per experiment):

1.271 pmol of plasmid in distilled water (add water to reach a total of 120 μ L)
150 μ L of 2X HBS (to obtain final concentration 1X HBS)
30 μ L of 1.25 M CaCl_2 (add dropwise and while flickering the tube, to obtain a final concentration of 125mM, then invert twice to ensure mixing)

Incubate 10 min at 37°C.
- 5 Remove medium and add 300 μ L of DNA mix dropwise in the center of the wells, incubate 30min at 18°C (room temperature).
- 6 Add 500 μ L of transfection medium, incubate at least 4h at 23°C.
- 7 Remove medium and perform an osmotic shock with 110 μ L of 10% glycerol shock for 1 min.
- 8 Remove glycerol and add 600 μ L of growth medium.
Put cells back at 23°C until screening.

Screening of positive cells



- 9 If the transfected plasmid contains a fluorescent marker, positive cells can be screened using a fluorescent microscope or by flow cytometry. Screening can be done 18h post transfection.