# Transfection of Capsaspora owczarzaki using calcium-phosphate precipitation

**Helena Parra Acero,Núria Ros,Alberto Perez-Posada,Aleksandra Kozyczkowska,Sebastián R. Najle**

## Abstract

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

**Citation:** Helena Parra Acero,Núria Ros,Alberto Perez-Posada,Aleksandra Kozyczkowska,Sebastián R. Najle Transfection of Capsaspora owczarzaki using calcium-phosphate precipitation. **protocols.io**
dx.doi.org/10.17504/protocols.io.p4adqse
**Published:** 13 May 2018

## 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

## 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 121ºC. 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 4ºC.

***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 121ºC. Store at 4ºC protected from the light.

****Buffer solution (for 1 L)**: 18.1 g KH 2 PO 4 (Sigma, #P5655), 25 g Na 2 HPO 4 (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 4ºC.
**Transfection medium (for 1 L)**: 10 g Peptone, 15 mg Folic Acid in 990 mL distilled water. Autoclave

for 20 min at 121ºC. 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 4ºC.
**2X HBS (for 250 mL)**: Dissolve 4 g NaCl (Sigma, #S3014), 0.18 g KCl (Sigma, #P9541), 0.05 g Na 2 HPO 4 (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 -80ºC.
**1.25M CaCl 2 (for 10 mL)**: 1.84 g CaCl 2 (Sigma, #C1016) in 10 mL autoclaved distilled water. Filter-
sterilise through 0.22 µm, flash-freeze with liquid Nitrogen and store at -80ºC.
**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 -80ºC.

## Protocol

Preparation of cells
**Step 1.**

Seed $10^7$ cells in a flask with 5mL of growth medium, let grow for 24h at 23ºC to have a conflluent culture.

Preparation of cells
**Step 2.**

Take the cells from the confluent culture, seed $2*10^6$ cells per well in 600 µL of growth medium.

Calcium-phosphate precipitation
**Step 3.**

Replace growth medium with 600 µL of transfection medium, incubate 30 min at 18ºC (room temperature).

Calcium-phosphate precipitation
**Step 4.**

While incubating with transfection medium, prepare DNA mix (300 µLper experiment):

1.271 pmol of plasmid in distilled water (add water to reach a total of 120 µL)

150 ul of 2X HBS (to obtain final concentration 1X HBS)

30ul of 1.25 M CaCl2 (add dropwise and while flickering the tube, to obtain a final concentration of 125mM, then invert twice to ensure mixing)

Incubate 10 min at 37ºC.

Calcium-phosphate precipitation

**Step 5.**

Remove medium and add 300 µL of DNA mix dropwise in the center of the wells, incubate 30min at 18ºC (room temperature).

Calcium-phosphate precipitation

**Step 6.**

Add 500 µL of transfection medium, incubate at least 4h at 23ºC.

Calcium-phosphate precipitation

**Step 7.**

Remove medium and perform an osmotic shock with 110 µL of 10% glycerol shock for 1 min.

Calcium-phosphate precipitation

**Step 8.**

Remove glycerol and add 600 µL of growth medium.

Put cells back at 23ºC until screening.

Screening of positive cells

**Step 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.

## 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 (<5x$10^7$ 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