

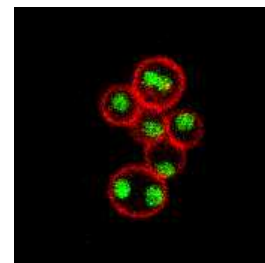
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Version 2

Stable transfection of unicellular relative of animals, *Corallochytrium limacisporum*, using Lonza Nucleofector V.2

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Abstract

This protocol describes the transfection of *Corallochytrium limacisporum*, one of the unicellular relatives of animals. The transfection is performed using Lonza Nucleofector System X and reporter cassettes that contain endogenous regulatory elements of *C. limacisporum*.

Guidelines

Keeps cells on ice during the whole procedure & all reagents must be ice-cold

Materials

Main materials:

P3 PrimaryCell 4D-Nucleofector Kit S (32 rxn) Kit Catalog# H3V4XP-5032

pUC19 carrier plasmid

highly concentrated reporter construct

1XPBS

Marine Broth



Cell Culture preparation

- 1 Inoculate 100 μ l of *C. limacisporum* culture in 5ml of Marine Broth in 25cm² flask. Keep at 23°C air incubator for two days.

Cell count

- 2 Scrape gently and count cells to reach 1.5E6 cells / condition using hemocytometer
If there are more conditions, it is recommended to pull the cells together to get more visible pellet

Medium removal

- 3 Spin down the cells at 1500xg for 5'
Discard the supernatant.

Washing step

5m

- 4 Wash the pellet with an ice-cold 1XPBS (100 μ l / condition)
Spin down and discard the supernatant.

Prepare DNA mix

- 5 Mix DNA plasmids: 10 μ g of an reporter plasmid + 20 μ g carrier DNA (pUC19)
It is recommended that plasmid is highly concentrated (2-5 μ g/ μ l) to avoid final buffer dilution with the DNA solution
Our plasmid contains mCherry coding sequence & resistance to puromycin gene (*pac*) under constitutive promoter: actin (Addgene: #104446) or tubulin (Addgene#129560)

Note: 10 μ g of reporter plasmids guarantees the highest efficiency, however a successful transfection can be also obtained with 5 and 1 μ g of the plasmid

- 5.1 Transfer DNA mix to 20 μ l of ice-cold P3 buffer
It is recommended not to keep the cells in P3 buffer for too long

- 6 Add DNA mix to the cell pellet



- 7 Transfer the mix (cells + DNA plasmid) into a strip (from the manufacture kit)
Do it carefully & without creating bubbles
Final volume: 20-25 μ l

Transfection of plasmid DNA into *C. limacisporum*

- 8 Insert into Lonza nucleofection system and apply code: EN - 138

Recovery step

- 9 After transfection, immediately add 80 μ l of Marine Broth medium (MB) to a well
Mix up and down
- 9.1 Transfer to 12-well plate (NUNC) that contains 1 ml of growth medium (MB) and incubate overnight

Check-out for results

- 10 Check for positive cells 24 - 48h post-transfection using fluorescent microscopy

Enrichment & selection of transfected cells

- 11 Replace 500 μ l of medium with fresh MB that contains 300 μ g/ml of puromycin
Increase of mCherry-positive cells can be observed over time.