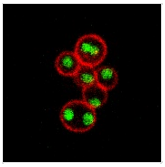

Version 2

Nov 10, 2020

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

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1 *Works for me* This protocol is published without a DOI.

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Aleksandra Kozyczkowska

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.*

PROTOCOL CITATION

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https://protocols.io/view/stable-transfection-of-unicellular-relative-of-ani-bpfgmjjw
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KEYWORDS

transfection, electroporation, unicellular organism, stable

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GUIDELINES

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

MATERIALS TEXT

Main materials:
P3 PrimaryCell 4D-Nucleofector Kit S (32 rxn) Kit Catalog# H3V4XP-5032
pUC19 carrier plasmid
highly concentrated reporter construct
1XPBS
Marine Broth

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.*

Cell Culture preparation

1 Inoculate 100 µl of *C. limacisporum* culture in 5ml of Marine Broth in 25cm^2 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 µl / condition)
Spin down and discard the supernatant.

Prepare DNA mix

5 Mix DNA plasmids: 10 µg of an reporter plasmid + 20 µg carrier DNA (pUC19)
It is recommended that plasmid is highly concentrated (2-5 µg/ul) 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 µg of reporter plasmids guarantees the highest efficiency, however a successful transfection can be also obtained with 5 and 1µg of the plasmid

5.1 Transfer DNA mix to 20 µl 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 µl

Transfection of plasmid DNA into *C. limacisporum*

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

Recovery step

9 After transfection, immediately add 80 µl 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 µl of medium with fresh MB that contains 300 µg/ml of puromycin
Increase of mCherry-positive cells can be observed over time.