Stable tranfection of unicellular relative of animals, Corallochytrium limacisporum, using Lonza Nucleofector

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Abstract

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Guidelines

Keeps cells always on ice & all reagents must be ice-cold

Protocol

Start a culture 2 days before transfection (cells at the exponential growth phase)
Step 1.

100ul of culture + 9ml of Marine Broth

Count the cells to reach 1E6 cells / condition
Step 2.

If there are more conditions, it is recommended to pull the cells together to get more visible pellet

Spin cells down at 1500g for 5'
Step 3.

Discard the supernatant

Wash cells with chilled 1xPBS
Step 4.

100ul / condition

Discard the supernatant

Add 20ul of P3 buffer (Lonza) / condition

Step 5.

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

It is recommended not to keep the cells in P3 buffer for too long

Add 10ug of a circular plasmid / condition

Step 6.

Our plasmid contains sequence coding mCherry fluorescent protein & resistance to puromycin under strong tubulin promoter

It is recommended to have a highly concentrated plasmid (2-5ug/ul)

Transfer cells + DNA into a well

Step 7.

Carefully & without creating bubbles

In total 20-22ul

Insert into a Lonza machine and apply code: EN-138

Step 8.

Immediatelly add 80ul of Marine Broth medium (MB) to a well

Step 9.

Mix up and down

Transfer to 1ml of growth medium in 12-well plate (NUNC) and incubate overnight

Step 10.

Check for positive cells 24h later using fluorescent microscopy

Step 11.

Expected efficiency is 50< cells / well.

Add 300ug/ml of puromycin

Step 12.

Increase of positive cells can be observed over time.