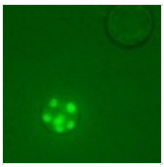

Working

Sep 27, 2018

Transient tranfection of unicellular relative of animals, Creolimax fragrantissima, using Lonza Nucleofector

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Protist Research to Optimize Tools in Genetics (PROT-G) , Multicellgenomelab view 1 more group

Aleksandra Kozyczkowska

Cfra_transient.avi

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

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

Start a culture two days before transfection to get the culture full of amoebas cells

1 300ul of culture + 9ml of Marine Broth in a 25cm2 flask

Count the cells to reach 1.5E6 cells / condition

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

Spin cells down at 2500g for 5'

3 Discard the supernatant

Wash cells with chilled 1xPBS

4 100ul / condition
Discard the supernatant

Add 20ul of P3 buffer (Lonza) / condition

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 1-3 ug of a circular plasmid / condition

6 Our plasmid contains sequence coding H2B-Venus under tubulin promoter
It is recommended to have a highly concentrated plasmid

Transfer cells + DNA into a well

7 Carefully & without creating bubbles
In total 20-22ul

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

8

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

9 Mix up and down

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

10

Check for positive cells 24h later using fluorescent microscopy

11 Expected efficiency is 50< cells / well.