Sep 19, 2019

Abeoforma whisleri transient transfection protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.zexf3fn

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ABSTRACT

This is a protocol for transient transfection of the Icthyosporean Abeoforma whisleri. This protocol has an efficiency of 1-2% calculated from fluorescent positive cell from Flow Cytometry data, using the amount of cells and DNA described in the protocol. Increasing DNA from above 1 microgram/microliter does not increase transfection.

MATERIALS

NAME	CATALOG #	VENDOR
Buffer P3	19053	
PBS		
Marine Broth 2216	279110	BD Biosciences
4D-Nucleofector System with X Unit	AAF-1002X	Lonza
A.whisleri cells		

Pre-Transfection

1 Count A.whisleri cells from a culture 1 to 2 weeks old grown in Marine Broth (MB) medium at 13 degrees.

2 Take 2 x 105 cells/per transfection condition, and spin them down at 2000g for 5 min.

3 Discard medium and gently resuspend cells in sterile 1xPBS and spin them down again (same conditions as above)

Transfection

4 Discard PBS and resuspend cells with 20-25 microliters of mixture Buffer P3 Lonza+1 to 5 micrograms of reporter plasmid+ 40 micrograms of carrier DNA (empty vector such as pUC19).

5 Transfer de 20-25 microlters mixture to a 16 well strips from Lonza Nucleofector (X Unit) and apply CODE EN-138.

Post-Transfection

6 Inmediately after transfection add 80 microliters of MB directly to the well. You can transfer directly to a 6 well plate with 1ml of media, or wait for 30 minutes.

7 Screen for transformants after 24 hours of transfection, the number of positive cells will increase untill 48 hours.

IMPORTANT

8 Include always a well of cells with no DNA. A.whisleri shows autofluorescence, so this control is key to compare and decide what is real transfection and what is autofluorescence.
Use high quality DNA and as concentrated as possible. We liofilize DNA prep and resuspend them to have a concentration of at least 1.5 micrograms/microl.
Use ultrapure and concentrated carrier DNA. We use pUC19 at 20micrograms/microliter.