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Nucleofector Protocol for Dinoflagellates using Lonza's 4D-Nucleofector X Unit V.1

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Senjie Lin

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Protocol status: In development
We are still developing and optimizing this protocol

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Attachments

DOCX

Nucleofector Protoco...

14KB

1 Harvest dinoflagellate cells using centrifuge method. Determine the lowest centrifuge speed and shortest time at which you can achieve both a good pellet of your species with minimal damage to your species

2 Add 1400ul of normal culture medium (e.g, F/2, L1) into a 24 wells plate

3 Prepare Lonza's nucleofector solutions: Per reaction use 16.4ul solution SF, SG, or SE and 3.6ul Supplement 1 and your DNA/Vector of choice (*Note do not add more than 10% the volume of the 20ul reaction)

4 Count your cells and harvest 200,000 cells/reaction using the lowest centrifuge speed stated above

5 Once the cells have been pelleted, remove all the seawater, resuspend the cells VERY GENTLY with one of the three solutions provided by Lonza (SF, SG, SE). Use 20ul of the solution mix per reaction.

6 Place the 20ul cell solution into Lonza's strip kit wells.

7 Perform the optimization procedure that is preprogrammed.

8 After the electroporation is completed add 100ul of normal culture liquid and resuspend the cells VERY GENTLY and transfer them to the 24 well plate

9 Return the plate to normal culture conditions and look for expression of your foreign gene.

10 Add selectable agent 24-72 hours after transfection and determine the best setting for your species. Once narrowed down, use Lonza's chart to help target the optimal setting for your species.