Sep 25, 2019

Nucleofector Protocol for Dinoflagellates using Lonza's 4D-Nucleofector X Unit V.2

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In Development dx.doi.org/10.17504/protocols.io.7n8hmhw

Protist Research to Optimize Tools in Genetics (PROT-G)

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ABSTRACT

This protocol is intended to work for many dinoflagellates but focusses on the method used to successfully transform *Karlodinium veneficum.*

EXTERNAL LINK

http://biorxiv.org/lookup/doi/10.1101/718239

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Faktorova, D. et al. "Genetic tool development in marine protists: Emerging model organisms for experimental cell biology." *bioRxiv* 718239 (2019).

Table 1_DinoIII-neo_sequence.docx

DinoIII-neo-vector.pdf

MATERIALS

NAME	CATALOG #	VENDOR
Lonza Nucleofector 4d	AAF-1002X	Lonza
SF Cell Line 4D-Nucleofector® X Kit S (32 RCT)	V4XC-2032	Lonza

BEFORE STARTING

Make sure your dinoflagellate culture is healthy and narrow down an antibiotic cocktail that can be used to reduce or limit bacteria growth.

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

Karlodinium veneficum CCMP1975 should be cultured as reported (Zhang et al. 2008) and spun at 1500 g for 2 minutes in 1.5mL tubes.

2 Using a 24-well plate, add 1.4 mL of normal culture medium (e.g, F/2, L1) into each well. In order to prevent bacterial growth, add 400ug/mL of ampicillin to the seawater for *K. veneficum* transformation.

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).

For *K. veneficum* only solution SF worked for transformation. Add 2ug of linear DinoIII-*neo* plasmid DNA (Table 1; Figure 1). Digest DinoIII-*neo* with *Eco*RI and concentrate as previously described (Sprecher et al., 2019).

4 Count your cells and harvest 200,000-400,000 cells/reaction using the lowest centrifugation speed stated above
Perform electroporation using Lonza's 16-well Nucleocuvette™Strips.

For *K. veneficum* use 400,000 cells per well. Spin the cells at 1500 g for 2 minutes in1.5mL tubes.

5 Once the cells have been pelleted, remove all the seawater, and resuspend the cells VERY GENTLY with one of the three solutions provided by Lonza (SF, SG, SE). Use 20-22ul of the solution mix per reaction.

Per well, use 22uL of solution 16.4 SF, 3.6 Sol I, and 2 ul linear DinoIII-*neo* plasmid DNA to resuspend *K. veneficum.*

6 Place the 20-22ul cell solution into Lonza's strip kit wells.

7 Perform the optimization procedure that is preprogrammed. The best pulse code for *K. veneficum* is CA-137.

8 After the electroporation is completed add 80ul of normal culture liquid (L1 + 400ug/mL of ampicillin for *K. veneficum*) and resuspend the cells VERY GENTLY, transferring 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.

Add 150ug/mL of kanamycin for *K. veneficum* 72 hours after transformation. After approximately one week, all controls (pulse controls and no pulse controls) should no longer have *K. veneficum* cells and the resistant culture (+DinoIII-*neo*) should be growing. After 2 weeks, new seawater with 150ug/ml kanamycin and 400ug/ml ampicillin should be added to the culture and every 3 to 4 weeks cultures can be reinoculated.

11 After more than a month DNA and RNA can be isolated and PCRs targeted at amplifying the gene of interest can be performed following previously published methods (Zhang et al., 2013; Lin et al., 2006). Often, nested PCRs are needed to detect the gene. Primers neoQF1 (GGTGGAGAGGCTATTCGGCTATG) and neoQR1N (GGAGCAAGGTGAGATGACAGGAG) can be used for the first PCR and primers neoQRF2 (TGGAGAGGCTATTCGGCTATGACT) and neoQR1 (CGGACAGGTCGGTCTTGACAA) can be used in the nested PCR for the introduced *neo* gene.