Apr 23, 2019

In devel.

Transfection of Crypthecodinium cohnii using labelled DNA

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dx.doi.org/10.17504/protocols.io.z26f8he

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

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ABSTRACT

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

In progress, unpublished

Reagents

1 **Liquid medium**

- L1 + 10 gr/L glucose NPN (https://ncma.bigelow.org/ccmp316#.XLOpDZNKi2w)
- L1 medium with nitrogen limited (without yeast extract and glucose) to obtain cyst from *C. cohnii* swimming cells

Plates

- Prepare Petri dishes containing L1 medium bacteriological agar (1.5%)
- Prepare PEG 8000 solution (0.4%) with L1 medium, sterilized by using a filter (0.22µm)
- Pour 1 ml of L1-PEG8000 solution onto dry agar plates; alternatively, pour 12.5 ml of L1-PEG8000 solution onto dry agar plates
- Let the PEG800 solution infuse for 24 h
- Store at 4°Cuntil use

Plasmid vectors

- Propage *p*PmMOE[MOE]:GFP-11 (Cold et al., 2016; Fernández Robledo et al., 2008) in JM109 for miniprep

*p*PmMOE[MOE]:GFP-11

- Linearize 20 µg with *Not*I (e.g., New England Biolabs® inc., Ipswich, MA, USA), clean the restriction digestion (e.g., GenElute® PCR Clean-up kit)
- Store at -20°C until use

DNA probe construction and DNA labeling

- *Use forward (5' -CCGCACATGTATGGTGAGCAAGGGCGAGGAGC- 3') the reverse (5' –CGTAGGACATGTCTTGTACAGCTCGTCCATGCCG- 3' primers targeting *p*PmMOE[MOE]:GFP-11 (Fernández Robledo et al., 2008) to amplify a 739 bp DNA
- Clean the amplicon as above and label 1 µg using UlysisTM Alexa Fluor® 488 Nucleic Acid Labeling Kit (ThermoFisher Scientific) following the manufacturer's instructions
- Store labelled DNA were stored at -20°C until use

*Alternatively use any other set of primers/target DNA to generate the amplicon for labeling

References

Cold, E.R., Freyria, N.J., Martínez Martínez, J., Fernández Robledo, J.A., 2016. An agar-based method for plating marine protozoan parasites of the genus *Perkinsus*. PLoS One 11, e0155015.

Fernández Robledo, J.A., Lin, Z., Vasta, G.R., 2008. Transfection of the protozoan parasite *Perkinsus marinus*. Mol Biochem Parasitol 157, 44-53.

Cell Preparation

2 ***Liquid culture***

- Incubate at 24°C in the dark with 125 rpm in a rotatory shaking incubator; the doubling time of *C. cohnii* cells under replete nitrogen

conditions is 24-48 hours
- Harvest cells at log phase
- Centrifuge cells at 2,500 g for 10 min at room temperature
- Discard supernatant and maintain cells on ice until use

Spheroplasts induction

Crypthecodinium cohnii spheroplasts are derived from both swimming cells and cyst cells (Kwok et al., 2007; Pozdnyakov et al., 2014).
- Prepare PEG8000 (20%) (wt/vol) in L1 medium
- Resuspend *C. cohnii* swimming cells with 1 ml of 20% L1-PEG8000 solution and vortex for 10 min
- Spread 1 ml of *C. cohnii* swimming cells or cysts on Petri dishes containing L1 medium bacteriological agar and L1-PEG8000 solution (0.4%)
- Incubate for 2 days at 28°C
- Cover the colonies on the plate with L1 medium, pour off the medium
- Pour new L1 medium and retain the second elutant containing the spheroplasts
- Count spheroplasts using a haemocytometer (x3)

References

Kwok, A.C., Mak, C.C., Wong, F.T., Wong, J.T., 2007. Novel method for preparing spheroplasts from cells with an internal cellulosic cell wall. Eukaryot Cell 6, 563-567.

Pozdnyakov, I., Matantseva, O., Negulyaev, Y., Skarlato, S., 2014. Obtaining spheroplasts of armored dinoflagellates and first single-channel recordings of their ion channels using patch-clamping. Marine drugs 12, 4743-4755.

Electroporation

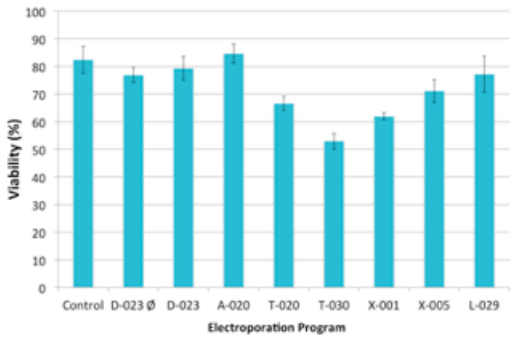
3
- Collect *Crypthecodinium cohnii* cells in log phase or spheroplasts (1.0 x10^6 cells/mL)
- Electroporated with 5 µg (2.5 µg supercoiled, 2.5µg *Not*I linearized) of pPmMOE[MOE]GFP-11 using Amaxa®Cell Line Optimization Nucleofector™ solution V and program X-001 in a Nucleofector™ (Lonza)
- Recover cells from the cuvette and incubate in L1 medium
- As control for plasmid and electroporation, electroporate *P. marinus* PRA240 with pPmMOE[MOE]:GFP-11 as reported elsewhere (Fernández Robledo et al., 2008)
- Monitor cells for green fluorescence using standard FITC excitation/emission filters (488/507nm) under a transmitted-light fluorescence and/or confocal microscope

Lipofection

4
- Lipofectamine® 3000 Reagent kit (ThermoFisher Scientific) in tissue culture plate 24-wells and 6-wells plates containing respectively 2-3 mL of L1 medium
- Use 15 µl of Lipofectamine and 1 µg of labeled DNA in 1.0 x 10^6 cells
- Monitor cells for green fluorescence using standard FITC excitation/emission filters (488/507nm) under a transmitted-light fluorescence and/or confocal microscope

5

Crypthecodinium cohnii viability after electroporation



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