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Transfection of *Cryptocodinium cohnii* using labelled DNA

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José A. Fernández Robledo¹

¹Bigelow Laboratory for Ocean Sciences

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José A. Fernández Robledo

Bigelow Laboratory for Ocean Sciences

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Protocol status: In development

We are still developing and optimizing this protocol

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Abstract

José A. Fernández Robledo

Nastasia J. Freyria

Bigelow Laboratory for Ocean Sciences

Reagents

1 **Liquid medium**

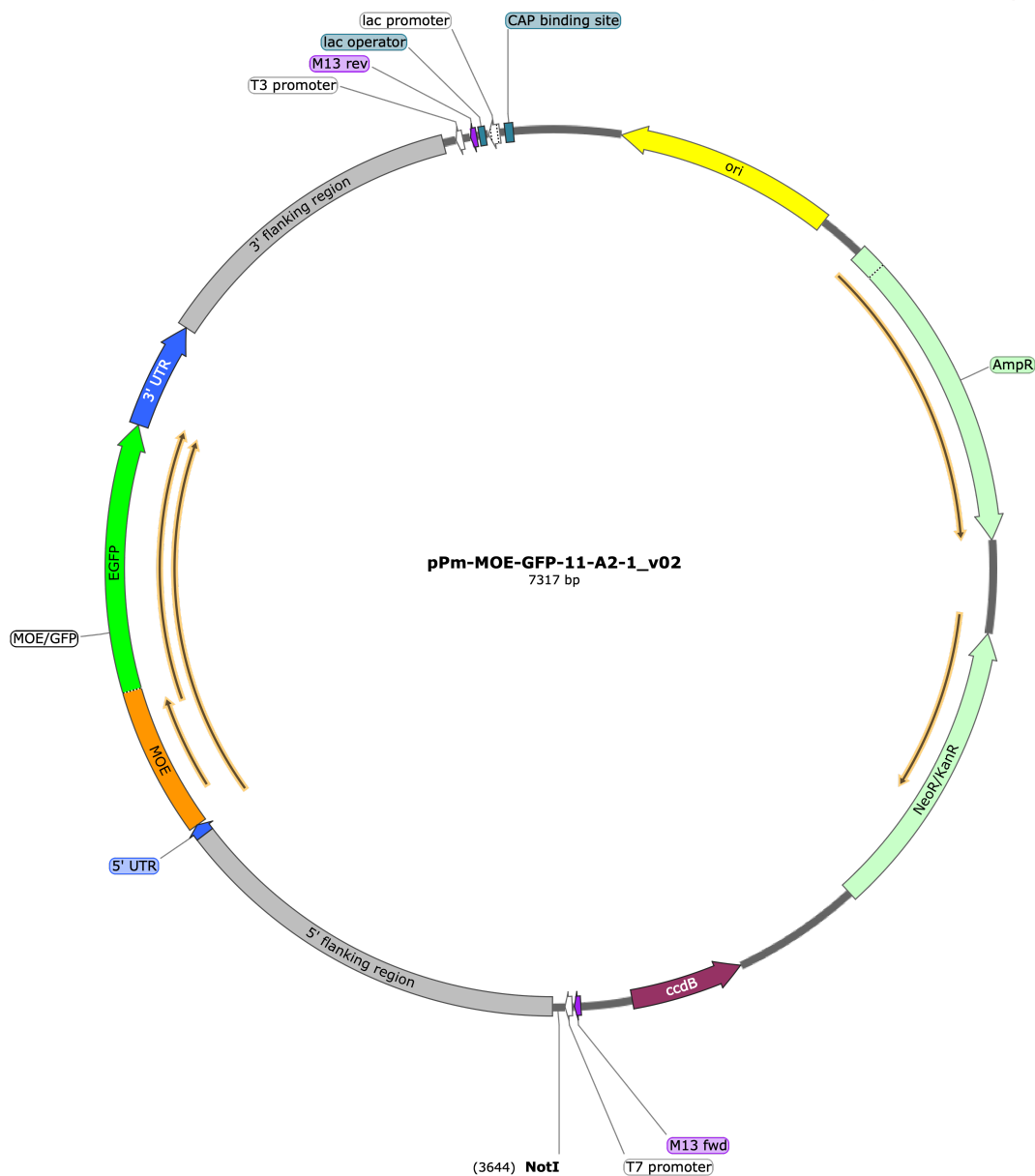
- L1 + 10 gr/L glucose NPN (<https://ncma.bigelow.org/ccmp316#.XLOpDZNKi2w>)
- L1 medium with nitrogen limited (without yeast extract and glucose) to obtain cyst from *C. cohnii* swimming cells

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°C until use

Plasmid vectors

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



pPmMOE[MOE]:GFP-11

- Linearize 20 µg with *NotI* (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 *pPmMOE[MOE]:GFP-11* (Fernández Robledo et al., 2008) to amplify a 739 bp DNA

- Clean the amplicon as above and label 1 µg using Ulysis™ 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

Cryptocodinium 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 *Cryptocodinium cohnii* cells in log phase or spheroplasts (1.0×10^6 cells/mL)
- Electroporated with 5 μ g (2.5 μ g supercoiled, 2.5 μ g *NotI* 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 μ l of Lipofectamine and 1 μ g of labeled DNA in 1.0×10^6 cells
- Monitor cells for green fluorescence using standard FITC excitation/emission filters (488/507nm) under a transmitted-light fluorescence and/or confocal microscope

5

Expected result

Cryptocodinium cohnii viability after electroporation

