Transformation of DinoIII GFP into O. marina with Use of Lonza 4D-Nucleofector X Electroporation Unit System in 16 -well Nucleocuvette Strips

* University of Connecticut (UConn) fed the O. marina D. tertiolecta 3 days prior to transformation. **Deviation**: The O. marina culture was fed 2 days prior. To add, UConn used a flashlight to concentrate the O. marina within the light. This seems weird as from my experience, the O. marina tend to swim away when under the microscope light.

1. For each transformation well, 16.4 uL of solution SG, 3.6 uL of Supplemental 1 solution, and 2 uL of linear DNA product were used as a transformation solution.
2. O. marina cells were collected in a 50 mL falcon tube. The cells were centrifuged a 2500 g for 3 minutes.
3. Removed remaining medium supernatant except for 2-3 mL.
4. Transferred cells into 1.5 mL Eppendorf tubes and centrifuged at 900 g for 2 minutes. Discarded all remaining supernatant.
5. Cells were resuspended in transformation solution (reference step 1.) and 22 uL were added into each well.
    a. Added 2.5 x 10^5 of O. marina cells into each well. **Deviation**: They calculated the O. marina cell density using a Sedgwick-Rafter counting chamber. However, we do not have an estimated cell density.
6. After an initial optimization test, the following electroporation settings were used for further experiments: DS-137, DS-130, DS-138, DS-134, DS-150, ED-150, DS-120, and no pulse controls (NPC).
7. Immediately after electroporation, 80 uL of the same medium that O. marina was cultured in along with an AKS antibiotic cocktail (100 ug/uL ampicillin, 50 ug/uL kanamycin, and 50 ug/uL streptomycin) was added into each well. **Deviation**: UConn grew their O. marina cultures in autoclaved, filtered seawater. We grew ours in F2 medium + autoclaved, filtered seawater.
8. All of the volume was gently transferred into 24-well plates where each well already contained 1.4 mL of the same F2 SW+AKS medium.
9. The transformed cells were left to recover for 3 days.
10. For the DinoIII-gfp transformations, cells were examined microscopically under blue light for gfp expression.
11. New antibiotic solution was added every 3 weeks but the concentration was dropped down to 200 ug/uL to allow for greater cell growth, and D. tertiolecta in 225 ug/uL rifampin medium was supplied whenever they were no longer detected in the medium.
    a. The D. tertiolecta in 225 ug/uL rifampin medium was added to UConn's DinoIII-arrO cells, so we added the D. tertiolecta within the F2 medium.

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