Electroporation of Pseudo-nitzschia multiseries

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Abstract

Describes novel low conductivity media and multipulse electroporation protocol using a NEPA21 Type II pulse generator with settings optimized to balance transfection efficiency with cell viability for this marine diatom.

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Before start

Pseudo nitzschia multiseries cultures are maintained in sterile seawater enriched with Guillard's Marine Enrichment f/2 medium and transferred during mid-log phase.

Electroporation Buffer

42% w/v 4K PEG
2.5mM Imidazole, pH 7.75

Additives*:

2mM ATP

2mM $MgCl_2$

0.5mM Glutathione

0.5mM Ascorbate

0.2υm filter and store Buffer at 15ºC

*Store additives at -20ºC and add to buffer fresh before each use

Note on additions to buffer:

We found inclusion of these additives to the electroporation buffer to ease the stress of buffer exchange and electroporation.

Additional buffers tried and rejected:

Osmolytes: Mannitol, Sorbitol, Tris for pH--> Reduced Pseudo-nitzschia regrowth as compared to PEG

Additives: Proline --> Increased bacterial growth, did not increase viability.

Recovery media following electroporation:

Sterile seawater ammended with L1 nutrients (Bigelow/ NCMA) at 1:3 NO_3:Si

Additional: Kanamycin (1ug/mL) was added to the recovery culture following electroporationto in order to limit bacterial growth in culture. The microbiome of Pseudo-nitzschia isolates is apparently coupled to cell growth and physiology (Sison-Mangus, 2014). Growth is impeded following stress and culture takeover by exogenous bacteria.

Protocol

Prepare Pseudo-nitzschia cells

Step 1.

Collect cells for electroporation via centrifugation at 3,000 rpm, 20 minutes, remove supernatant by aspiration.

NOTES

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Cells fare best if they are harvested in early-log phase. Recovery following electroporation is greatly reduced in older cultures of Pseudo-nitzschia, even more so than following other manipulations.

ANNOTATIONS

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Cells can also be concentrated by filtration onto 1.2 µm polycarbonate filters (Isopore Membrane Filters RTTP02500) and resuspended in electroporation buffer. However, this requires a pre-

sterilized filtration set-up, otherwise bacterial contamination is greater than observed using the centrifugation method.

Prepare Pseudo-nitzschia cells

Step 2.

Re-suspend pellet in Electroporation Buffer (see before starting for recipe).

Typical cell numbers at begining log phase are in the 10*10^3 cells/mL range. Centrifugal concentration of 100mL of culture yields around 10^6 cells for transformation. Adjust resuspension volume based on how many aliquots you will need for electroporation, (ie 100mLs to final volume of 4mLs in electroporation buffer).

NOTES

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It is preferrable to harvest a greater volume of a younger, less dense culture if needed to achieve 10^6 final cells, rather than harvest an older, higher density culture. Look for healthy chains of Pseudo-nitzschia as opposed to single cells.

Transfer Pseudo-nitzschia cells to cuvette and mix with plasmid

Step 3.

Prechill 0.2 cm gap electroporation cuvettes on ice.

Hold concentrated cell suspension on ice.

Pipette ca. 10 µg DNA into chilled cuvette (≤ 50 µL). Use equivalent volume of TE for preparation of electroporation handling control treatment.

Add 300 µL *Pseudo-nitzschia* cell suspension and gently pipette up and down once to mix with DNA.

Hold prepared cuvettes on ice for 10 min until electroporation.

Electroporation

Step 4.

Electroporation conditions on NepaGene21 using a 0.2 cm gap cuvette:

Poring Pulse: 75V, 5ms Length, 50ms Interval, 7 pulses, 10% D rate, +/- polarity

Transfer Pulse: 9V, 50ms Length, 50ms Interval, 9 pulses, 40% D rate, + polarity

NOTES

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Pilot studies of electroporation with Sytox Green Nucleic Acid Stain (Thermo Fisher Scientifc) demonstrated maximal uptake at 150V, but due to Pseudo-nitzschia's sensitivity to handling and manipulations, keeping a lower voltage will strike a balance between plasmid transformation and cell viability. Voltages between 75-100 will suffice.

Post-electroporation

Step 5.

Remove cuvette from electroporator and hold at room temperature for 5-10 min (until all samples have been electroporated)

Transfer contents from cuvette and resuspend in 30 mLFASW + reduced N-L1 [1:3 N:Si] + Kan

Recovery post-electroporation

Step 6.

Allow to grow at standard conditions + 1 level of screen shade until new growth is observed (by eye).

Post-recovery and Selection

Step 7.

To select: once new growth is observed, spin down entire culture to concentrate. Remove supernatant. Resuspend in 0.5mL sterile seawater. Proceed to LGTA protocol.