Patch-based Colony PCR Protocol for Screening Phaeodactylum tricornutum

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Abstract

This protocol describes a method for screening transgenic lines of *Phaeodactylum*, specifically for those obtained via conjugation with episomal plasmids. The assay can also be used for screening genomic integrants obtained through biolistics. The assay uses patched colonies of diatom grown on agar plates for biomass and does not require the use of kits or any pre-processing (boiling, etc) of the samples prior to analysis. This allows for relatively high-throughput screening of transgenic lines in a single day.

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Materials

- OneTaq 2X Master Mix with Standard Buffer - 500 rxns M0482L by New England Biolabs
- Gel Loading Dye, Purple (6X), no SDS - 4.0 ml B7025S by New England Biolabs
- 1 kB DNA ladder #N3232 by New England Biolabs

Protocol

Prepare primers working stock

Step 1.

Prepare 5'/3' primer working stock by diluting each primer 1:10 into a single clean Eppendorf tube from an intial 100 uM stock (ex: 16 uL ddH_20 + 2 uL 5' primer + 2 uL 3' primer in 20 uL total). Each PCR reaction will require 1 uL of primer mix, but due to pipetting error, it is good practice to generate at least 5-10 extra samples. Final concentration of each primer in the mix in 500 nM.

Setting up PCR master mix

Step 2.

To set up the PCR master mix, use the table below:

#colonies to screen/#rxns to prep	Single reaction	12/16	48/56	96/112
5'/3' mix	1	16	56	112

2x OneTaq Master Mix	10	160	560	1120
ddH_2O	9	144	504	1008
Final volume (uL)	**20**	**320**	**1120**	**2240**

REAGENTS

OneTaq 2X Master Mix with Standard Buffer - 500 rxns M0482L by New England Biolabs

Prepare PCR reaction tubes

Step 3.

Pipette 20 mL of PCR master mix into appropriate PCR tubes (keep tubes/plate on ice).

Picking Phaeodactylum biomass from agar plates

Step 4.

Using a 10 uL pipette tip, pick a small amount of *Phaeodactylum* biomass from the patch on an agar plate. This is roughly equivalent to the amount of biomass one would select for screening *E coli* colonies and is just enough to see the diatom biomass on the end of the tip. If you overload the reaction with biomass, a nonspecific DNA band can be visualized on an agarose gel at roughly 800bp-1kb. Place the tip with biomass into the appropriate PCR tube on ice and leave it there. Continue picking samples from agar plates, placing pipet tips in the PCR mixes on ice.

Setting up positive/negative control reactions

Step 5.

If a positive control plasmid is available, add 1 uL of 0.5-1.0 ng/uL purified plasmid to a tube containing 19 uL of PCR master mix or 1 uL of ddH20 for negative/water control.

Dispacing diatom biomass into PCR reaction tubes

Step 6.

Once picking is finished, use a P20 pipet set at 10 uL to pipet each reaction up and down multiple times, releasing the cells from the pipet tip. The color of the reaction will be a light brown if the appropriate amount of biomass was collected. We also suggest using a multichannel pipet for clearing out the tips when a larger number of colonies are being screened (24 and up). If desired, briefly spin the PCR tubes to remove bubbles from pipetting. We have not seen any difference in PCR efficiency when tubes are spun versus leaving in the bubbles.

PCR Thermocycler Programming

Step 7.

Set up the PCR program on thermocycler. Our standard protocol for PCR using OneTaq is:

Step 1: 95°C for 2 min

Step 2: 95°C for 10 sec

Step 3: Annealing temp for 20 sec (we often aim for 60°C at this step)

Step 4: 72°C for appropriate extension time (1kb/min with OneTaq)

Step 5: Go to Step 2 29 additional times (30 total cycles)

Step 6: 72°C for 5 min

Step 7: Hold at 10°C or end program

Note: Once the cycles are finished, the samples can be stored at 4°C overnight (-20°C for longer).

Agarose gel analysis of PCR reactions

Step 8.

To analyze the reaction on a gel, add 4 uL of 6x DNA loading dye to each tube. Briefly spin the tubes to mix buffer with sample and flick tubes to mix. On a 1% agarose gel, load 16 uL of samples + dye mix. If 1ng of positive control plasmid was used, load 8 uL of final reaction so to not blow out the gel when imaged. Run gel and image with appropriate DNA stain/imaging system. This protocol was developed using gels stained with EtBr and imaged on a BioRad GelDoc XR System. Other systems and stain combinations may require loading more or less of the final PCR reaction.