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© Colony PCR for screening transgenic Ostreococcus tauri

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Ostreococcus, colony PCR, PCR, algae, screening

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- 1 Measure cell concentration using a hemocytometer. Ideally, the cell concentration should be around 1-2x10^5 cells/ml
- 2 Mix \Box 50 μ L of cells and \Box 50 μ L sterile water in a PCR tube.
- 3 Boil the PCR tube at § 95 °C for © 00:05:00 in a thermocycler.
- 4 After boiling, briefly centrifuge the PCR tube. Use **1 μL** from the boiled sample as PCR template.
- 5 Mix the PCR components in a sterile laminar hood.

Α	В	
	Volume (uL)	
Component		
	5	
5x green buffer		
dNTPs	0.5	
Forward primer	1.25	
Reverse primer	1.25	
Template	1	
GoTaq	0.125	
MgCl2	1	
H20	14.875	
Total	25	

6 Perform PCR using the following protocol,

Α	В	С
Step	Temperature	Time
	(deg C)	
Initial Denaturation	95	2 min
30 cycles	95	30 s
	Та	60 s
	72	1 min per kb
Final Extension	72	10 min
Hold	4	HOLD

7 Resolve and visualize the PCR product on an agarose gel.