JUL 23, 2018

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.qyjdun

Protocol Citation: Pringle Lab, Grossman Lab 2018. Symbiodinium cp23S RFLP and sequence analysis.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.qyjdun>

Symbiodinium cp23S RFLP and sequence analysis

In 1 collection

Pringle Lab¹, Grossman Lab²

¹Stanford University;

²Carnegie Institution for Science - Department of Plant Biology

Aiptasia Symbiodiniaceae Model System

Christian CR Renicke

Stanford University - Genetics

ABSTRACT

This protocol describes how to perform PCR on extracted *Symbiodinium* DNA to amplify a fragment of the chloroplast 23S rRNA gene. It is based on the method describes in Pochon *et al.*, 2006. The protocol then explains how to perform an RFLP (restriction fragment length polymorphism) analysis on the PCR products as well as either using them directly for sequence analysis by Sanger sequencing or cloning them into a plasmid back bone to separate genes from mixed *Symbiodinium* populations and perform the sequence analysis on several clones per original sample.

The attached file contains reference sequences of the following Symbiodinium strains: Clade A strains SSA01, SSA02, SSA03; SSB01 (clade B), SSB01del6, SSB01del136, SSE01 (clade E) and A001 (Clade D).

ATTACHMENTS

Reference Sequences
 Symbiodinium
 cp23S_final.doc

MANUSCRIPT CITATION:

Pochon, X., Montoya-Burgos, J. I., Stadelmann, B., & Pawlowski, J. (2006). Molecular phylogeny, evolutionary rates, and divergence timing of the symbiotic dinoflagellate genus *Symbiodinium*. *Molecular phylogenetics and evolution*, 38(1), 20-30.

Grawunder, D., Hambleton, E. A., Bucher, M., Wolfowicz, I., Bechtoldt, N., & Guse, A. (2015). Induction of gametogenesis in the cnidarian endosymbiosis model *Aiptasia* sp. *Scientific reports*, 5, 15677.

Xiang, T., Hambleton, E. A., DeNofrio, J. C., Pringle, J. R., & Grossman, A. R. (2013). Isolation of clonal axenic strains of the symbiotic dinoflagellate *Symbiodinium* and their growth and host specificity¹. *Journal of phycology*, 49(3), 447-458.

Pochon, X., Putnam, H. M., Burki, F., & Gates, R. D. (2012). Identifying and characterizing alternative molecular markers for the symbiotic and free-living dinoflagellate genus *Symbiodinium*. *PLoS One*, 7(1), e29816.

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: Jun 13, 2018

Last Modified: Jul 23, 2018

PROTOCOL integer ID:
13035

GUIDELINES**20x Borax running buffer for agarose gels:**

- Dissolve 38.18 g Sodium Tetraborate Decahydrate (Borax) in 900 ml distilled water (you might need to heat up the solution to dissolve everything)
- Bring to 1 L with distilled water
- DO NOT try adjusting the pH, it takes a looooooot of boric acid, even with directly adding pure powder and the gels are running well without doing it.

This results in a 100 mM solution, adjust the amount accordingly, when using other forms of Tetraborate (anhydrous etc.).

Add 50 ml of the 20× buffer to 950 ml distilled water to obtain a 1× solution.

[Sodium boric acid: a Tris-free, cooler conductive medium for DNA electrophoresis](#)

Jonathan R. Brody and Scott E. Kern

BioTechniques 2004 36:2, 214-216

Keywords: Genotyping,
RFLP, Symbiodinium, cp23S

MATERIALS

MATERIALS

HphI - 1,000 units New England Biolabs Catalog
#R0158S

Microcentrifuge Tubes Contributed by users

Orange DNA loading dye Thermo Fisher Scientific Catalog
#R0631

PCR tubes Contributed by users

Sodium Tetraborate Decahydrate Sigma – Aldrich Catalog
#B9876

Agarose Contributed by users

PCR Thermocycler Contributed by users

DNA polymerase and buffers for PCR Contributed by
users

37°C chamber, heat block or water bath Contributed by
users

GeneRuler 50 bp DNA Ladder Thermo Fisher Scientific Catalog
#SM0371

GeneRuler DNA Ladder Mix Thermo Fisher Scientific Catalog
#SM0331

STEP MATERIALS

GoTaq Green Master Mix Promega Catalog
#M7122

GeneRuler DNA Ladder Mix Thermo Fisher Scientific Catalog
#SM0331

GeneRuler 50 bp DNA Ladder Thermo Fisher Scientific Catalog
#SM0371

GoTaq Green Master Mix Promega Catalog
#M7122

GeneRuler DNA Ladder Mix Thermo Fisher Scientific Catalog
#SM0331

GeneRuler 50 bp DNA Ladder Thermo Fisher Scientific Catalog
#SM0371

SAFETY WARNINGS

Depending on the dyes you use for staining DNA gels, follow your institution's guidelines for safety measures (wear gloves/avoid direct contact to the dyes and don't drink them).

If you are using UV-light to detect the bands, take care to not directly expose your eyes or skin to the light.

BEFORE START INSTRUCTIONS

Have/order the primers for the *Symbiodinium* cp23S rDNA locus:

Primer sequences:

23S4F (forward): 5'-GAC GGC TGT AAC TAT AAC GG-3'

23S7R (reverse): 5'-CCA TCG TAT TGA ACC CAG C-3'

PCR amplification of the cp23S rDNA Locus of *Symbiodinium*

- 1 Amplify the cp23S locus by PCR with primers 23S4F and 23S7R.

Primer sequences:

23S4F (forward): 5'-GAC GGC TGT AAC TAT AAC GG-3'

23S7R (reverse): 5'-CCA TCG TAT TGA ACC CAG C-3'

25 µl reactions are sufficient to obtain enough amplicon for RFLP analysis and sequencing. It might be a good idea to include a negative control reaction without template added.

- 2 Example 25 µl PCR setup using Promega GoTaq® 2× master mix:

Component	Volume [µl]
GoTaq® 2× Master Mix	12.5
Forward Primer (10 µM)	1
Reverse Primer (10 µM)	1
<i>Symbiodinium</i> genomic DNA (10-100 ng/µl)	5
Nuclease-free water	5.5
Total volume	25

Thermocycler setup:

Step	Temperature	Time
1	95°C	5 min

	-----	-----	-----
2	95°C		30 s
3	50 C		45 s
4	72°C		1 min
35 cycles of 2-4	-----		-----
6	72°C		5 min

GoTaq Green Master Mix Promega Catalog
#M7122

- 3 Mix 3 µl of the PCR reactions with 2 µl water and 1 µl 6× DNA loading dye and run on a 1% agarose TAE gel in TAE buffer together with a standard DNA ladder.**

This is just to check successful amplification. The amplified fragments should be between roughly 500 and 600 bp.

GeneRuler DNA Ladder Mix Thermo Fisher Scientific Catalog
#SM0331

- 4 Purify the remaining volumes of the PCRs using a PCR purification kit according to the manufacturer's instructions. Elute in 30-50 µl elution buffer (10 mM Tris, pH 8.5) or water.**

Restriction fragment length analysis

- 5 Set up 30 µl restriction enzyme reactions using *HphI* for the RFLP: 10 µl PCR Product; 3 µl CutSmart 10× Buffer (NEB); 16 µl nuclease-free water; 1 µl *HphI* (NEB).**

Using the NEB enzyme and buffer is just an example, enzymes from other manufacturers like Thermo Fisher Scientific (previously Fermentas) will work as well.

- 6 Incubate the reactions for 3 h at 37°C.**

*Alternatively, you can digest your DNA with 0.3 µl *HphI* over night (≥8 h) at 37°C.*

- 7 Mix 10 µl of the digests with 2 µl 6× DNA loading dye and run on a 3% agarose gel, don't forget to load an appropriate (low range) DNA ladder as well (I like the GeneRuler 50bp Ladder).**

You want to achieve a good separation, so run the gel over the whole length of the gel (at least 5 cm).

We are using Borax gels and buffer for gels with these high agarose concentrations (see Guidelines section for recipe). TBE will work probably as well.

8 Expected fragments for different strains:

Strain	DNA fragments
SSA01	343 bp 194 bp 86 bp
SSA02	194 bp 192 bp 152 bp 86 bp
SSA03	194 bp 186 bp 152 bp 86 bp
SSB01	335 bp 296 bp
SSB01 with large deletion	335 bp 160 bp
A001 (clade D1)	356 bp 280 bp 86 bp
SSE01	292 bp 221 bp 131 bp

(Sanger-)Sequencing of the PCR products

9 Follow the instructions of your sequencing service provider to sequence the purified PCR products with primers 23S4F and 23S7F.

You can find a collection of some reference sequences in the 'Reference Sequences Symbiodinium cp23S.doc' file attached to this protocol.

ATTENTION: *If you expect mixes of different strains, you should consider subcloning the PCR fragments into plasmids and sequence a number of clones per original sample. Sanger sequencing of mixed strains will give otherwise hard or impossible to interpret results. For best results use a high-fidelity polymerase to minimize PCR-introduced sequence variations.*