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Montastraea faveolata host genotyping
Last updated 01/22/09 by Shini

*Status: needs to be updated

Overview

This protocol describes the genotyping of *M. faveolata* host colonies by using microsatellite markers described in Severance et al. (2004, Molecular Ecology Notes, Vol. 4: 74-76). The general methodology is based on the publication by Schuelke (2000, Nature Biotechnology, Vol. 18: 233-234). In principle, this method can be used to genotype any organism, if microsatellite markers are available. The first step includes the amplification of the target sequence using a primer mix that contains an M13-tagged forward primer, a reverse primer and a fluorescently labeled M13 primer. The PCR products are subsequently analyzed on an ABI3700 sequencer.

A) PCR-protocol

The method takes advantage of amplifying the target sequence using an M13-tagged forward primer, which is limited in amounts, so that once it is used up, the dye-tagged M13-primer will amplify the M13-tagged target sequence.

Note: Before you start the amplification of microsatellites with the primer mixes described below, you need to make sure that the microsatellite primers (without the M13-dye tagged primer) produce a single band at the expected size range!

1. Set up primer mixes

Each primer mix will contain the M13-tagged forward primer (in limited amounts), a reverse primer, and a dye-tagged M13 forward primer.

For each microsatellite, set up:

	Primer stock conc.	1x (2 uL /rxn)*
M13-dye (e.g. FAM,VIC,NED) primer	5 uM	0.6 uL
M13-tagged forward primer	5 uM	0.133333 uL
Reverse primer	5 uM	0.6 uL
Total:		2 uL

* scale up all primers according to the number of individuals to be genotyped

2. Set up PCR reactions

For each reaction, set up:

	1x (20 uL rxn)*
PCR-grade H_2O	14.4 uL
10 x PCR buffer	2 uL
dNTPs (10 mM)	0.4 uL
Primer Mix (see above)	2 uL
Taq polymerase (5U/uL)	0.2 uL
Template DNA (~20 ng/uL)	1 uL

* scale up all primers according to the number of individuals to be genotyped and number of microsatellites to be used.

3. Run the following program:

Hot-start: 94°C – 2’

35 cycles: 94°C – 30’

51°C – 30”

72°C – 1’

1 cycle: 72°C – 2’

For ever: 4°C

4. Check PCR amplicons:

Run out 5 uL of the PCR reaction on a 1% agarose gel.

5. Sizing PCR products on ABI 3700

To be updated

Materials

M. faveolata microsatellite primer sequences

maMS8_M13-fw
CACGACGTTGTAAAACGACTCTTGCCTATCAGCAGAGGAG

maMS8_rv TCTGCAAACCAATGTACCATCT

maMS11_M13-fw
CACGACGTTGTAAAACGACCAGACGGATTAAATAGTCTCCCA

maMS11_rv GACGAATTTTGCCGAGTCAC

maMS12_M13-fw
CACGACGTTGTAAAACGACGGACCTAAACGGGAACACAA

maMS12_rv GAAAGGCTATTCAAAGCTGGG

maMS2-4_M13-fw
CACGACGTTGTAAAACGACTGCTTTGACAGCTACGCAAT

maMS2-4_rv CCGGGAATTTAGCTATTTGG

maMS2-5_M13-fw
CACGACGTTGTAAAACGACTTGAAGTAAACAGTACGGAAAGG

maMS2-5_rv TTCATGTAAACCTGTCGCTGTC

maMS2-8_M13-fw
CACGACGTTGTAAAACGACCCCCTTTGTCACACATCTTTC

maMS2-8_rv ATGAAGGATAGGCCGCACT

maMS2-17_M13-fw
CACGACGTTGTAAAACGACTACCCAGGGCCTCTCTTTTT

maMS2-17_rv CACGTAATGGCAACGTATGG

M13-Tag:

CACGACGTTGTAAAACGAC