Zooxanthellae Chloroplast Genotyping Protocol

This protocol was originally developed for scoring the size of the PCR products on a Li-Cor automated sequencer. You will need to adjust the protocol (specifically, the information regarding primers) if you are using another type of DNA sequencer.

1) Extract samples according to standard procedures.
2) Establish cladal identity with SS5/SS3Z primers and *Taq* I RFLP (if needed; protocols are available on The Santos Lab website).
3) Using the same DNA template dilution, set up the following PCR reactions in 0.2 mL thin-walled PCR tubes (recipe below is per sample):

		Final Concentration
ddH2O	6.8 µL	
10X PCR Buffer	1.0 µL	1.5 mM $MgCl_2$
10 mM dNTP	0.2 µL	0.2 mM
Taq polymerase	0.2 µL	
1 µM 23SHYPERUP primer	0.2 µL	0.2 pmol
1 µM 23SHYPERDNM13 primer	0.15 µL	0.15 pmol
1 µM IRD800 M13Rev	0.05 µL	0.05 pmol
25 mM $MgCl_2$	0.4 µL	1.0 mM $MgCl_2$
DNA template	1.0 µL	10-25 ng
		2.5 mM $MgCl_2$ final concentration

4) Place samples in MJ96 and run the appropriate PCR program (see "PCR Primers" document on The Santos Lab website; approximate cycling time = 2 hours):

Step #1	94° C	2 minutes
Step #2	94° C	30 seconds
Step #3	50° C	30 seconds
Step #4	72° C	30 seconds
Step #5	Goto #2	35X
Step #6	72° C	5 minutes
Step #7	4° C	HOLD
Step #8	END	

5) Add 5 µL sequencing stop buffer and denature samples for 3-4 minutes. Place immediately on ice.

6) Again, this is specific to a Li-Cor sequencer. If you are using a newer capillary-based sequencer, you can skip this part and treat the samples like any other type of fragment analysis. If you have questions, consult someone who runs microsatellites on that particular sequencer. Otherwise, pour a 25 cm, 0.25 mm 6.5% Long Ranger acrylamide gel:

Urea	12.6 g
50% Long Ranger acrylamide	3.9 mL
10X TBE	3.0 mL
After urea is dissolved:	
Top up to 30 mL with ddH_2O	
Just before pouring gel, add:	
10% APS (0.1 g APS to 1 mL ddH_2O)	200 µL
TEMED	20 µL

Pour gel immediately following addition of APS and TEMED. Allow the gel to polymerize for 2 hours before using.

7) Load 0.5 - 1.0 µL of sample per lane and run gel under the following electrophoresis conditions ("Genotyping"):

Voltage (V)	1500 V
Power (W)	40 W
Current (mA)	40 mA
Temperature (°C)	50°C
Scan Speed	3 - moderate

Alleles should be visible in approximately 90 minutes; gel can be reloaded immediately after first set of alleles are visualized.

Primer sequences:

23SHYPERUP
5′-TCAGTACAAATAATATGCTG-3′

23SHYPERDNM13
5′-GGATAACAATTTCACACAGGTTATCGCCCCAATTAAACAGT-3′