Symbiodiniaceae *ITS2* amplification

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Adapted from Klepac et al. (2013), Eckert 2020, and https://ryaneckert.github.io/Belize_Mcav_Symbiodiniaceae_ITS2/lab_protocol/#symbiodiniaceae_its2_amplification.

We want to try to avoid over-amplification, so we will start with 28 cycles and check on an agarose gel.

Starting with **clean** DNA template that was quantified **fluorescently** will greatly increase amplification success.

ITS2 PCR

Master mix recipe

Reagent	**1 rxn**
RNase free H2O	22.2 µL
10X ExTaq Buffer	3 µL
10 mM dNTP mix	0.6 µL
SYM_VAR 5.8 primer (10µM)	1.5 µL
SYM_VAR REV primer (10µM)	1.5 µL
TaKaRa ExTaq HS	0.2 µL
	+1.0 µL template (20 ng total from 2bRAD dilution plate)
Total :	**30 µL**

PCR profile

95 ºC	5 min	
95 ºC	**30 s**	
56 ºC	**2 min**	**28 cycles**
72 ºC	**1 min**	
72 ºC	10 min	

1. Make master mix and aliquot 29 µL to each well.
2. Spin down DNA (use PCR caps or film) and add 1 µL of 20 ng/µL DNA to each respective well.
3. Cover with PCR film and run PCR machine at above cycles (listed on the PCR machine under “its2 sym var”).
4. Amplify samples with the SYM_VAR forward and reverse primers using cycle checks to obtain a faint but distinct band (Should take ~28 cycles). Avoid over-amplification, don’t run more than 28 cycles. To add cycles, place samples back into the thermocycler and run for the additional number of cycles (no initial heating or final extension steps).
5. Visualize on a large gel using 3 µL of PCR product.

a. Use 3 µL PCR Product and 2 µL loading dye (TriTrack). Use 3 µL Ladder (BR) and 2 µL loading dye for marker wells.
b. Run gel at 150 V for 15–25 min. You should see a distinct band at ~400 bp.
c. If a band is still not visible, redo the reaction using the appropriate number of cycles.
d. ***If making a large gel with the 50CT combs, put ladder at the start, middle, and end of each row. The multichannel pipette will go into every other well so make a gel map ahead of time. Mix PCR product and loading dye in a plate to simplify loading.

6. Clean PCR product with Zymo DNA Clean and Concentrator Kit.
    a. Elute in 12 µL of NFW
7. Qubit samples.
8. Make a dilution plate at 5ng/uL in 20uL of volume.
9. Send to GSAF at UT Austin.
    a. OUTER PCR/ PE250 MiSeq Nano Kit / (full run) 1M reads with 20% PhiX spike-in.
        i. For 81 samples this was ~$3,176.35