Symbiodinium ITS MiSeq Library Prep

Materials:

Milli-Q Water

AccuStart II PCR ToughMix (2x)

Fwd Primer (ITS-Dino-forward_MAf, 10µM)

Rev Primer (ITS2-rev2-reverse_MAr, 10 µM)

Sample DNA

Notes & Protocol:

1. Combine primers with ToughMix in the following ratios, with enough for triplicate reactions per sample, one negative control per master mix, and pipetting error:

	Single Reaction (µL)	4.5 Reactions	Final Conc.
AccuStart II ToughMix (2X)	6.25	28.125	1X
ITS-Dino-forward_MAf	0.25	1.125	0.2 µM
ITS2-rev2-reverse_MAr	0.25	1.125	0.2 µM
MilliQ Water	5.25	23.625	-

2. Aliquot master mix into PCR tubes (12 µL for this 12.5µL reaction).
3. Add 0.5 µL of sample DNA to each experimental PCR tube. Use Milli-Q water for negative controls.
4. Mix and spin down, and run the thermocycler protocol 'GreenSymbiodinium':

Temperature (°C)	Time	
94	5 min.	
94	40 s	} repeat 35x
59	120 s	
72	60s	
72	5 min	

5. Run on gel to verify amplification & purity, and pool replicates.
6. Perform second PCR without triplicates, this time adding 12.5 µL ToughMix and 9.5 µL water directly to each well, then 1 µL of each of the appropriate index primers directly to each well, and finally 1 µL of the PCR product from the first amplification. Run the thermocycler program Adaptor -> Indexed.
7. Run on gel to verify amplification & purity (the intermediate amplicon can be diluted 1:25 and run alongside these products as a negative control), and pool replicates.
8. Clean up samples individually using standard bead protocol.
9. Quantify using Qubit and pool samples at equimolar ratios.