L.A.B. Barcoding Protocol
Field to lab – marine invertebrates

EXTRACTION

1. Specimens were collected into 150 μL extraction buffer M2 (part of the Autogen automated extraction kit; essentially typical extraction buffer without the prot-k) in the field. Upon return to the LAB, they were frozen at -20° C until processed.
2. The second part of the extraction buffer, containing proteinase-K, was added to the specimens. Specimens were incubated overnight, with shaking, at 60° C.
3. Digested samples were placed on the Autogen Automated Extractor (a phenol-chloroform extraction and ethanol precipitation protocol) and processed using the Animal Protocol. Resuspension volume of the precipitated product was 50 μL.

PCR

PCR – 18S

The "universal" 18S primers EukF (Sands et al., BMC Ecol. 2008) and SR7 (I think this is: Vilgalys & Bun, PNAS 1994) were used for amplification.
We run 10 μL reactions and use 1 μL undiluted DNA as template.

Specifics of the reaction:
2.5 mM MgCl2 (0.4 μL of 50 mM stock)
0.3 μL of each 10mM primer
0.1 μL Bioline Taq polymerase

Cycling conditions:
Melt 95° 30 secs
Anneal 52° 30 secs
Extend 72° 45 secs
35 cycles

PCR – 16S

The "universal animal" 16S primer 16Sar and 16Sbr (Palumbi in Hillis and Moritz, Mol Phylogenetics, 1996) were used for amplification.
The reaction recipe and cycling parameters are the same as for 18S, but the annealing temperature used was 48° C.

PCR – CO1

Newly designed and unpublished primers by John Geller (Moss Landing) were used for amplification of CO1.
We ran 10 μL reactions with 1 μL undiluted DNA as template.
Recipe is the same as for both 16S and 18S, but with the addition of 0.25 BSA (10 mg/mL).
Annealing temp was 48°C and 40 cycles of PCR were run.

POST-PCR

-Amplified samples were "cleaned up" using Exo-SAP and then sequenced with Big Dyes.
Sequencing reactions were cleaned up via the Sephadex method and run on an ABI 3730xl.