Barcoding Notes from the Smithsonian Institution's Laboratories of Analytical Biology (L.A.B.)

We thought it might be useful to share our experiences with primers and protocols. We have been working on barcoding most of the marine phyla and vertebrates. Many of the primers we used were developed by researchers from the CCDB, so there is a lot of overlap of experience. I include them in this document for the sake of completion. We have been working for six years on many different taxa, some of which are "easy" (e.g. birds) and some of which are "difficult" (e.g. nematodes). We hope you can benefit from the knowledge we have gained through our struggles. Below are the primer combinations we use for different groups. We have experimented with many more phyla, but have not processed significant numbers of specimens to draw any firm conclusions and so I have not detailed that work here.

MARINE INVERTEBRATES

The Folmer (LCO1490/HCO2198) and degenerate Folmer (dgLCO-1490/dgHCO-2198) primers amplify CO1 reasonably well across a number of taxa. We use the degenerate primers more often than the originals for most marine phyla. We usually have better success with the degenerates, but not always (and this is unpredictable too). We have used these to generate barcode sequences from most of the mraine phyla, but have produced a significant number of barcodes with these primers from the following: Cnidarians (Hydroids), Nemerteans, Crustaceans (Isopods, Decapods, Amphipods, Tanaids), Annelids, Molluscs (Gastropods, Bivalves), Gastrotrichs. We know that these primers work poorly in Nematodes and Sponges. For crustaceans, we have lately found better amplification success with Simon's (1994) "Nancy" as the reverse primer with LCO1490 as forward.

Sponges are difficult to barcode. The Sponge Tree of Life project provided the primers below. Many different primer combinations are needed to amplify CO1 and it may take much trial and error to succeed. Some of the primers in the table span the entire length of CO1, as this is a gene commonly used in sponge phylogenetics.

INSECTS AND SPIDERS

Our primary experience with insect barcoding is in the orders Coleoptera, Diptera, Ephemeroptera, and Trichoptera and we generally begin with the standard Folmer primers. We have had additional success, particularly with the Trichoptera, using the LepF1 and LepR1 primers (from the CCDB) in together or in combination with the standard Folmer primers. In addition, we find that the primers ubc6/ubc9(=Nancy) work very well on many insect taxa, although the fragment amplified is not the entire barcode fragment. However, this allows us to obtain sequence data from which we can design additional primers. For spiders we've used the standard Folmer primers, as well as the "Chelicerate" primers (from the CCDB), but we've handled <200 spider specimens to date.

VERTEBRATES
Fish, including some sharks and rays, but excluding some peculiar eels, amplify extremely well with the Fish-BCL/Fish-BCH combo. We never use anything else.
Amphibians, particularly frogs and salamanders usually amplify well with the standard or degenerate Folmer primers. While we've handled only a few caecilians, they did not amplify with these primers.
Reptiles, except turtles, amplify well with the standard Folmer primers. Turtles are more difficult to amplify, but the standard primers will work for some species.
Mammals amplify well with the CCDB's VF1d/VR1d combination. We have primarily processed large numbers of bats and small rodents.
Birds amplify very well with the CCDB's BirdF1/CO1birdR1 combination. We have never used anything else.

We use a very standard PCR protocol. For amplification of CO1 from most marine phyla, we add BSA to the recipe. This appears to decrease the effects of PCR inhibitors. We clean PCR products prior to sequencing with the Exo-SAP-IT protocol (Amersham Biosciences, Piscataway, NY).

Our standard invertebrate cocktail and cycling parameters:
PCR Cocktail:

- 10 uL reaction volume
- 2.5 mM $MgCL_2$
- 0.3 uL each 10mM primer
- 0.5 uL 10 mM dNTPs
- 0.25 uL BSA (10mg/mL)
- 0.1 uL Bioline Taq polymerase

Cycling parameters:

- 95° 30 secs
- 48° 30 secs
- 72° 45 secs
- 40 cycles

Our standard vertebrate cocktail and cycling parameters:
PCR Cocktail:

- 10 uL reaction volume
- 0.5 uL 50 mM $MgCl_2$
- 0.3 uL each 10 mM primer
- 0.5 uL 10 mM dNTPs
- 0.1 uL Bioline Taq Polymerase

Cycling Parameters:

- 95° 30 secs
- 52° 30 secs
- 72° 45 secs