

March 2011

## **L.A.B. Fish Barcoding PCR Protocol**

### **Primers (for PCR and sequencing)**

CO1FishF1 (also known as Fish-BCL): 5'-TCAACYAATCAYAAAGATATYGGCAC-3'

CO1FishR1 (also known as Fish-BCH): 5'-ACTTCYGGGTGRCCRAARAATCA-3'

### **PCR Cocktail**

10  $\mu$ L volume:

0.1  $\mu$ L Bioline Taq polymerase (Bioline USA Inc., Taunton, MA)

1.0  $\mu$ L 10X PCR Buffer (Bioline)

0.5  $\mu$ L 50mM MgCl<sub>2</sub>

0.5  $\mu$ L 10 mM dNTPs

0.3  $\mu$ L 10mM each primer

6.3  $\mu$ L molecular grade water

1  $\mu$ L DNA

### **PCR Cycling Parameters**

5 minutes at 95° C

35 cycles of:

30 seconds at 95° C

30 seconds at 52° C

45 seconds at 72° C

Ending with 5 minutes at 72° C

PCR products are purified before sequencing using the ExoSAP-IT protocol (Amersham Biosciences, Piscataway NY).

### **Sequencing Reaction Cocktail**

BigDye Terminator (version 3.1) Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, Calif.) and the following mix: a

10 $\mu$ L reaction volume:

1.75 $\mu$ L 5X Big Dye terminator buffer

6.25 $\mu$ L nuclease free water

0.5 $\mu$ L 10mM primer

0.5 $\mu$ L Big Dye (version 3.1)

1 $\mu$ L purified PCR product

### **Sequence Reaction Cycling Parameters**

30 cycles of:

30 seconds at 95° C

30 seconds at 50° C

4min at 60° C

Sequencing products were cleaned before running on the capillary sequencer using a Sephadex centrifugation protocol (Millipore MAHVN4550 plates).