RAHI-2

June 8, 2010

**Mystery Animal PCR Lab**

Today you will perform PCR on the sample you extracted yesterday. This PCR reaction will amplify the ND2 gene. ND2 is a highly **polymorphic** gene which is often used to identify different species of bird. NADH dehydrogenase subunit 2 gene is an enzyme located in the inner mitochondrial membrane that catalyzes the transfer of electrons from NADH to coenzyme Q (CoQ). In terms of amino acid sequence, ND2 is the 3rd most variable gene after ATPase 8, which is very short (~165-168 bp) and therefore provides relatively little information, and ND6, which is also relatively short (~519-522 bp) and is generally more difficult to amplify and sequence, given its unusual base composition and location near the control region. In contrast, the complete ND2 gene can be amplified in either one or two pieces with primers that have worked well on essentially all birds (L5216-H6313 for the whole gene; L5216-H5766 and L5758-H6313 for two pieces).

Reaction Mixture

|  |  |
| --- | --- |
| 10x PCR Buffer\* | 2 |
| dNTP mix (10mM ea) | 1 |
| Forward Primer | 1 |
| Reverse Primer | 1 |
| DNA template | 1 |
| HotStarTaq DNA Polymerase | 0.1 |
| ddH2O | 14.5 |
| TOTAL | 20ul |

We will be using a technology called Hot Start. To prevent the primers from binding to each other (a common problem) the primers will not function until they are activated by being heated for 15m at a very high temperature.

This is the thermocycler profile we will be using:

* Initial activation step: 15m at 95C
* 30 cycles of
	+ Denaturation: 25 seconds at 95C
	+ Annealing: 30 seconds at 55C
	+ Extension: 1 minute at 72C
* Final extension of 10 minutes at 72C

It can also be written as : 15m@95C, 30x [25s @ 95, 1.5m@72C], 10m@72C, hold at 4C

Primer sequences and thermocycler profile from

MD Sorenson et al. 1999. Primers for a PCR-Based Approach to Mitochondrial Genome

Sequencing in Birds and Other Vertebrates. Molecular Phylogenetics and Evolution. 12(2): 105-114.

AM Kearns, et al. 2008. Inferring the phylogeography and evolutionary history of the splendid fairy-wren Malurus splendens from mitochondrial DNA and spectrophotometry. Avian Biology. 40(1): 7-17.