

## ***P. infestans* Mitochondrial DNA Haplotyping by Sequencing**

Reference: G.W. Griffith and D.S. Shaw (1998) Appl. Environ. Microbiol. 64:4007-4014

### **Primers**

| <b>Region</b>              | <b>Primer</b>    | <b>Sequence</b>                                                        |
|----------------------------|------------------|------------------------------------------------------------------------|
| P2 (nad4) (Ib/not Ib)      | P2F4<br>P2R4     | ACCAATTGTTGCGAAAACAG<br>ATTACGGCGGTTTAGCACAT                           |
| P3 (rpl5) (Type I/Type II) | P3F1<br>P3R1     | TTCAAAATGTCTTACAGTTTTTCG<br>GCAAGGTTATACTCTACCATTGAGC                  |
| P4 (cox1) (Type I/Type II) | P4F2<br>P4R3     | GGAATGCTGTAAGTAGTTTTGGTT<br>TTTAAGATCGTGGTATTAAATTAT                   |
| P2 (nad4) (IIa/IIb)        | P2F5<br>P2R6     | CGTCAATAGGTTGTCCTAAAGC<br>GAACCTGATGTTGTTGGTGTG                        |
| nad11 (Herb-1/not Herb-1)  | nad11F<br>nad11R | AAAGTTAATCCTAAATGTTTTATAGGGTAAGTG<br>TACTTTAAAATCTATTGAAGAATCTGATCTTTG |

### **Master Mix:**

| <b>Reagent</b>           | <b>Volume per Reaction (µl)<br/>(1X)</b> | <b>___X</b> |
|--------------------------|------------------------------------------|-------------|
| ddH <sub>2</sub> O       | 35.25                                    |             |
| 10X PCR buffer           | 5                                        |             |
| dNTP mix (2mM each)      | 2.5                                      |             |
| ITS4 (10µM)              | 2                                        |             |
| ITS5 (10µM)              | 2                                        |             |
| MgCl <sub>2</sub> (50mM) | 1.8                                      |             |
| BSA (20mg/ml)            | 0.25                                     |             |
| Taq (5U/µl)              | 0.2                                      |             |

Combine 49µl of master mix with 1-2µl DNA.

### **Thermal cycling protocols:**

#### **nad11**

|                       |     |         |
|-----------------------|-----|---------|
| Initial denaturation: | 94C | 2 min   |
| 35 cycles:            | 94C | 15 sec  |
|                       | 56C | 15 sec  |
|                       | 72C | 15 sec  |
| Final extension       | 72C | 5 min   |
| Hold                  | 4C  | Forever |

#### **For all other loci**

|                       |     |         |
|-----------------------|-----|---------|
| Initial denaturation: | 96C | 90 sec  |
| 35 cycles:            | 96C | 40 sec  |
|                       | 50C | 1 min   |
|                       | 72C | 90 sec  |
| Final extension       | 72C | 10 min  |
| Hold                  | 4C  | Forever |

Clean up and sequence successful amplicons according to your sequencing lab's protocols. Relevant mutations are provided on the following page.



