**Additional file 3. Nested PCR Reaction Mixtures for Genes *Pfhrp2*, *Pfhrp3*, *Pfmsp1* and *Pfmsp2*.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Reaction Mix for nested PCR** | ***Pfhrp2* (exon 1-2) primary rxn** | ***Pfhrp2* (exon 1-2) secondary rxn** | ***Pfhrp3* (exon 1-2) primary rxn** | ***Pfhrp3* (exon 1-2) secondary rxn** |
| **10x HF\* buffer** | 2 µl | 2 µl | 2 µl | 2 µl |
| **dNTPs** | 2 µl | 2 µl | 2 µl | 2 µl |
| **forward primer (15µM)** | 1 µl | 1 µl | 1 µl | 1 µl |
| **reverse primer (15µM)** | 1 µl | 1 µl | 1 µl | 1 µl |
| **HF\* enzyme** | .2 µl | .2 µl | .2 µl | .2 µl |
| **Nuclease- free water** | 11.8 µl | 11.8 µl | 11.8 µl | 11.8 µl |
| **DNA** | 2 µl | 2 µla | 2 µl | 2 µla |
| **Total rxn** | **20 µl** | **20 µl** | **20 µl** | **20 µl** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Reaction Mix for nested PCR** | ***Pfhrp2* (exon 2) primary rxn** | ***Pfhrp2* (exon 2) secondary rxn** | ***Pfhrp3* (exon 2) primary rxn** | ***Pfhrp3* (exon 2) secondary rxn** |
| **10x HF\* buffer** | 2 µl | 2 µl | 2 µl | 2 µl |
| **dNTPs** | 2 µl | 2 µl | 2 µl | 2 µl |
| **forward primer (15µM)** | 1 µl | 1 µl | 1 µl | 1 µl |
| **reverse primer (15µM)** | 1 µl | 1 µl | 1 µl | 1 µl |
| **HF\* enzyme** | .2 µl | .2 µl | .2 µl | .2 µl |
| **Nuclease- free water** | 11.8 µl | 11.8 µl | 11.8 µl | 11.8 µl |
| **DNA** | 2 µl | 2 µlb | 2 µl | 2 µlb |
| **Total rxn** | **20 µl** | **20 µl** | **20 µl** | **20 µl** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Reaction Mix for nested PCR** | ***Pfmsp1* primary rxn** | ***Pfmsp1* secondary rxn** | ***Pfmsp2* primary rxn** | ***Pfmsp2* secondary rxn** |
| **10x HF\* buffer** | 2 µl | 2 µl | 2 µl | 2 µl |
| **dNTPs** | 2 µl | 2 µl | 2 µl | 2 µl |
| **forward primer (15µM)** | 1 µl | 1 µl | 1 µl | 1 µl |
| **reverse primer (15µM)** | 1 µl | 1 µl | 1 µl | 1 µl |
| **HF\* enzyme** | .2 µl | .2 µl | .2 µl | .2 µl |
| **Nuclease- free water** | 11.8 µl | 12.8 µl | 11.8 µl | 12.8 µl |
| **DNA** | 2 µl | 1 µl | 2 µl | 1 µl |
| **Total rxn** | **20 µl** | **20 µl** | **20 µl** | **20 µl** |

\* HF: high-fidelity reagents from Sigma Aldrich kit; a2µL of a 1:10 dilution of the primary product; b2µL of a 1:200 dilution of the primary product.