

Additional file 2 — Basic PCR Recipes (using Eppendorf MasterTaq PCR kit; Brinkman, Westbury, New York, USA) and PCR conditions

rbcL

1x Recipe:

8.5 μ l H₂O

2.5 μ l Buffer

2.5 μ l Taq Master (preheated to 65°C)

3.0 μ l Forward Primer (50 picomoles/ μ l)

3.0 μ l Reverse Primer (50 picomoles/ μ l)

1.0 μ l 25 mM MgCl₂

1.0 μ l dNTPs

0.5 μ l Taq polymerase

3.0 μ l sample (~ 25 – 50 ng/ μ l)

PCR conditions for Full Length Template¹:

1. 94°C 60 s
2. 46°C 90 s
3. 72°C 150 s
4. repeat steps 1 – 3 29 times
5. 72°C 15 min
6. 10°C until subsequent processing

matK

1x Recipe:

8.0 μ l H₂O

2.5 μ l Buffer

2.5 μ l Taq Master (preheated to 65°C)

3.0 μ l Forward Primer (50 picomoles/ μ l)

3.0 μ l Reverse Primer (50 picomoles/ μ l)

2.5 μ l 25 mM MgCl₂

1.0 μ l dNTPs

0.5 μ l Taq polymerase

3.0 μ l sample (~ 25 – 50 ng/ μ l)

PCR conditions for Full Length Template¹:

1. 94°C 60 s
2. 58°C 90 s
3. 72°C 150 s
4. repeat steps 1 – 3 29 times
5. 72°C 15 min
6. 10°C until subsequent processing

ndhF

1x Recipe:

10.0 μ l H₂O

2.5 μ l Buffer

2.5 μ l Taq Master (preheated to 65°C)

2.0 μ l Forward Primer (50 picomoles/ μ l)

2.0 μ l Reverse Primer (50 picomoles/ μ l)

1.5 μ l 25 mM MgCl₂

1.0 μ l dNTPs

0.5 μ l Taq polymerase

3.0 μ l sample (\sim 25 – 50 ng/ μ l)

PCR conditions for Full Length Template¹:

1. 94°C 60 s
2. 43°C 90 s
3. 72°C 150 s
4. repeat 1 – 3 29 times
5. 72°C 15 min
6. 10°C until subsequent processing

¹Annealing temperatures for other primer combinations were \sim 5°C below the lower melting temperature of the primer pair.