

Reaction conditions used in the CBOL Plant Working Group paper (CBOL Plant Working Group (2009) A DNA barcode for land plants. Proceedings of the National Academy of Sciences, USA, 106: 12794-12797)

rpoC1 (Protocol provided by Robyn Cowan, R.Cowan@kew.org;
<http://www.kew.org/barcoding/protocols.html>)

Primers

2f GGCAAAGAGGGAAGATTTTCG
4r CCATAAGCATATCTTGAGTTGG

PCR profile:

94°C 1min 1 cycle

94°C 30sec

53°C 40sec 35 cycles

72°C 40sec

72°C 5min 1 cycle

PCR mix:

Buffer	X 1
MgCl ₂	1.5mM
dNTPs	0.2mM
Forward primer	1 µM
Reverse primer	1 µM
Taq polymerase (Platinum Taq, Invitrogen)	2 units
BSA	0.1mg/ml
Template DNA	1 µl
H ₂ O	to 20 µl

rpoB (Protocol provided by Robyn Cowan, R.Cowan@kew.org;
<http://www.kew.org/barcoding/protocols.html>)

Primers (2+3)

2f ATGCAACGTCAAGCAGTTCC
3r CCGTATGTGAAAAGAAGTATA

PCR profile:

94°C 1min 1 cycle

94°C 30sec

48°C 40sec 35 cycles

72°C 40sec

72°C 5min 1 cycle

PCR mix:

Buffer	X 1
MgCl ₂	1.5mM
dNTPs	0.2mM
Forward primer	1 µM
Reverse primer	1 µM
Taq polymerase (Platinum Taq, Invitrogen)	2 units
BSA	0.1mg/ml
Template DNA	1 µl
dH ₂ O	to 20 µl

matK (primer sequences provided by Ki-Joong Kim; kimkj@KOREA.AC.KR)

Primers

3F_KIM f CGTACAGTACTTTTGTGTTTACGAG

1R_KIM r ACCCAGTCCATCTGGAAATCTTGGTTC

PCR profile:

94°C 1min

94°C 30sec

52°C 20sec 35 cycles

72°C 50sec

72°C 5min

PCR mix:

Buffer	X 1
MgCl ₂	1.5mM
dNTPs	0.2mM
DMSO	4% of the total reaction volume
Forward primer	1 μM
Reverse primer	1 μM
Taq polymerase (Platinum Taq, Invitrogen)	2 units
BSA	0.1mg/ml
Template DNA	1 μl
dH ₂ O	to 20 μl

Sequencing mix:

Add DMSO to sequencing reaction mix (4% total reaction volume)

atpF-atpH (primer sequences provided by Ki-Joong Kim; kimkj@KOREA.AC.KR)

Primers:

atpF f ACTCGCACACACTCCCTTTCC

atpH r GCTTTTATGGAAGCTTTAACAAT

PCR profile:

94°C 5min

94°C 30sec

50°C 30sec 35 cycles

72°C 40sec

72°C 5min

PCR mix:

Buffer

X 1

MgCl₂

1.5mM

dNTPs

0.2mM

Forward primer

1 μM

Reverse primer

1 μM

Taq polymerase (Platinum Taq, Invitrogen)

2 units

BSA

0.1mg/ml

Template DNA

1 μl

dH₂O

to 20 μl

psbK-psbI (primer sequences provided by Ki-Joong Kim; kimkj@KOREA.AC.KR)

Primers:

psbK f TTAGCCTTTGTTTGGCAAG
psbI r AGAGTTTGAGAGTAAGCAT

PCR profile:

94°C 5min

94°C 30sec

50°C 30sec 35 cycles

72°C 40sec

72°C 5min

PCR mix:

Buffer	X 1
MgCl ₂	1.5mM
dNTPs	0.2mM
Forward primer	1 μM
Reverse primer	1 μM
Taq polymerase (Platinum Taq, Invitrogen)	2 units
BSA	0.1mg/ml
Template DNA	1 μl
dH ₂ O	to 20 μl

rbcL (Protocol provided by David Erickson; ERICKSOND@si.edu)

Primers

rbcLa_R GTAAAATCAAGTCCACCRCG
rbcLa_F ATGTCACCACAAACAGAGACTAAAGC

PCR profile:

95°C 4 min

94°C 30 sec

55°C 1 min 35 cycles

72°C 1 min

72°C 10 min

10°C ∞

PCR mix:

Bioline 10x biolase buffer	2.0µl [1x final]
50mM MgCl ₂	1.0µl [2.5mM final]
10 mM dNTPs	0.8µl [0.4 mM final]
100 µM Forward Primer	0.1µl [0.5 µM final]
100 µM Reverse Primer	0.1µl [0.5 µM final]
Bioline Biolase taq (5U/ul)	0.2µl [1 unit final]
DNA	1.0µl
dH ₂ O	14.8µl

trnH-psbA (Protocol provided by David Erickson ERICKSOND@si.edu)

Primers

psbA3' f GTTATGCATGAACGTAATGCTC
trnHf_05 CGCGCATGGTGGATTCAACAATCC

PCR profile:

95°C 4 min

94°C 30 sec

55°C 1 min 35 cycles

72°C 1 min

72°C 10 min

10°C ∞

PCR mix:

Bioline 10x biolase buffer	2.0µl [1x final]
50mM MgCl ₂	1.0µl [2.5mM final]
10 mM dNTPs	0.8µl [0.4 mM final]
100 µM Forward Primer	0.1µl [0.5 µM final]
100 µM Reverse Primer	0.1µl [0.5 µM final]
Bioline Biolase taq (5U/ul)	0.2µl [1 unit final]
DNA	1.0µl
dH ₂ O	14.8µl