Final definitions of data standards for plant barcodes await the decision by CBOL on which loci will have official barcode status. The following notes are to provide interim information on the regions discussed in the CBOL plant working group PNAS paper.

rbcL: The section of rbcL being considered as the barcoding region is at the 5' end of the gene (see primer map below). In the Arabidopsis thaliana sequence this region is between bp1-599 (27-579 excluding primer sequences).

rbcL primer map

This region can be amplified using David Erickson's rbcLa_f primer (forward) and his modified rbcLa_rev primer. An alternative reverse primer is Aron Fazekas' rbcL ajf634R sitting about 30bp outside of this amplicon. Both of these reverse primers have shown good universality in barcoding studies.

David Erickson's forward primer (described in PLOS One 6 e508):
rbcLa_f ATGTCACCACAAACAGAGACTAAAGC

David Erickson's modified reverse primer (different from the PLOS One paper):
rbcLa_rev GTAAAATCAAGTCCACCRCG

Aron Fazekas reverse primer (PLOS One 7 e2802):
rbcL ajf634R GAAACGGTCTCTCCAACGCAT

A third reverse primer used in barcoding studies is that designed by Fay et al (1997 Kew Bulletin **52**, 111-120): 724R
TCGCATGTACCTGCAGTAGC

No special PCR conditions are required for rbcL, but the protocols used by David Erickson (Smithsonian Institute) and Damon Little (New York Botanic Garden) are provided below for convenience.

David Erickson:
PCR cycling conditions:
95°C 4 min; [5 cycles: 94°C 30 sec ; 55°C 1 min ; 72°C 1 min]; [30 cycles: 94°C 30 sec; 54°C 1 min; 72°C 1 min]; 72°C 10 min; 10°C ∞

Reagents:	1 reaction (20 µL)
Bioline 10x biolase buffer	2.0µl [1xfinal]
50 mM $MgCl_2$	1.0µl [2.5mM final]
10 mM dNTP’s	0.8µl [0.4mM final]
100 µM Forward Primer	0.1µl [0.5µM final]
100 µM Reverse Primer	0.1µl [0.5µM final]
Bioline Biolase taq (5U/ul)	0.2µl [1 unit final]
DNA	1.0µl
H_2O	14.8µl

Damon Little:
Using primer combination rbcLa_f and rbcL ajf634R

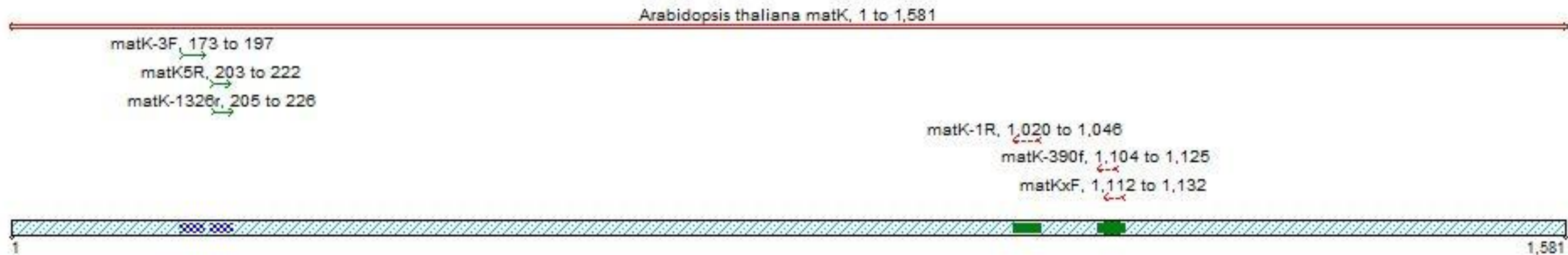
PCR cycling conditions:
95°C 2.5 min; [35 cycles: 95°C 30 s, 58°C 30 s, 72°C 30 s]; 72°C 10 min

Standard 10 x PCR buffer:
200 mM Tris-HCl pH 8.8 (25°C)
100 mM KCl
100 mM $(NH_4)_2SO_4$
20 mM $MgSO_4 \cdot 7H_2O$
1% (v/v) Triton X-100
50% (w/v) sucrose
0.25% (w/v) cresol red

Reagents:	1 reaction (15 µL)
H_2O	8.93 µL
10X buffer	1.5 µL [1x final]
dNTPs	1.2 µL [0.2 mM final]
BSA	1.5 µL [0.25 µg/µL final]
F primer	0.75 µL [0.5 µM final]
R primer	0.75 µL [0.5 µM final]
Taq	0.12 µL [0.5 unit final]
Subtotal	14.75 µL
DNA	0.25 µL

MATK
For matK, the proposed barcode region is indicated on the primer map below. It falls in the region between bp205-1046 (227-1019 excluding primer sequence) in the Arabidopsis thaliana sequence.

MatK primer map. Note that the designation of F and R varies between primer names from different sources.



For angiosperms we suggest using the primers designed by Ki-Joong Kim (detailed below) in the first instance. It is recognised that these primers will not work in all angiosperms and further primer development work is required in non-angiosperms for matK. We encourage community effort to enhance amplification strategies for matK, including the development of new primers and primer cocktails.

The protocol used in the CBOL plant working group PNAS paper, using Ki-Joong Kim's primers is:

Primers
3F_KIM f CGTACAGTACTTTTGTGTTTACGAG
1R_KIM r ACCCAGTCCATCTGGAAATCTTGGTTC
Citation Ki-Joong Kim (unpublished)

PCR cycling conditions:
94°C 1min; [35 cycles : 94°C 30sec ; 52°C 20sec ; 72°C 50sec]; 72°C 5min

Reagents:	**1 reaction (20ul)**
Buffer	X 1 final
$MgCl_2$	1.5 mM final
dNTPs	0.2 mM final
DMSO	4% of the total reaction volume
Forward primer	1 µM final
Reverse primer	1 µM final
Taq polymerase	2 units final
BSA	0.1mg/ml final
Template DNA	1 µl
dH_20	to 20 µl

Sequencing mix:
Optional: Add DMSO to sequencing reaction mix (4% of total reaction volume)

New York Botanic Garden Protocols for matK
PCR cycling conditions:
95°C 2.5 min; [10 cycles: 95°C 30 s, 56°C 30 s, 72°C 30 s]; [25 cycles: 88°C 30 s, 56°C 30 s, 72°C 30 s]; 72°C 10 min

Standard 10 x PCR buffer:
200 mM Tris-HCl pH 8.8 (25°C)
100 mM KCl
100 mM $(NH_4)_2SO_4$
20 mM $MgSO_4 \cdot 7H_2O$
1% (v/v) Triton X-100
50% (w/v) sucrose
0.25% (w/v) cresol red

Reagents:	1 reaction (15 µL)
H_2O	7.18 µL

10X buffer	1.5 µL [1x final]
dNTPs	1.2 µL [0.2 mM final]
BSA	1.5 µL [0.25 µg/µL final]
F primer	1.5 µL [1 µM final]
R primer	1.5 µL [1 µM final]
Taq	0.12 µL [0.5 u final] (for difficult reactions, up to 2 u may be used)
DNA	0.5 µL

For samples in which 3F/1R fail an alternative primer pair is:
F: CGATCTATTCATTCAATATTTC (390f)
R: TCTAGCACACGAAAGTCGAAGT (1326r)
Cuenoud et al. (2002). Amer Journal of Botany 89: 132–144.

In gymnosperms the following primer pair has been used:
F: CTGGATYCAAGATGCTCCTT (NY552)
R: GGTCTTTGAGAAGAACGGAGA (NY1150)
D. Little (unpublished)

RBG Kew matK protocol

Xf TAATTTACGATCAATTCATTC
5r GTTCTAGCACAAGAAAGTCG
Ford et al. (2009). Botanic Journal of the Linnean Society, 159: 1-11.

PCR cycling conditions:
94ºC 1min; [35 cycles: 94ºC 30sec; 46ºC 40sec ; 72ºC 40sec] ; 72ºC 5min

Reagents	**1 reaction (20**µl)
Buffer	X 1 final
$MgCl_2$	1.5 mM final
dNTPs	0.2 mM final
DMSO	4% of the total reaction volume
Forward primer	1 µM final

Reverse primer	1 µM final
Taq polymerase	2 units final
BSA	0.1mg/ml final
Template DNA	1 µl
dH_2O	to 20 µl

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

See also http://www.kew.org/barcoding/update.html