Oligonucleotides

(Manfred Binder and David Hibbett 9/18/2003)

Resuspending freeze-dried oligonucleotides

Oligonucleotides are usually shipped in dry form. The dried DNA pellet becomes dislodged from the bottom of the tube during shipping and it can easily fly out of the tube when first opened, particularly as electrostatic attraction is present. For this reason:

Always briefly centrifuge oligos before opening for the first time.

We dissolve the stock oligo in sterile dH_2O which must be freshly autoclaved. Alternatively, TE buffer (10 mM Tris pH 8.0, 1 mM EDTA) can be used.

For convenience, make a freezer stock at 100 μM concentration (which should be thawed infrequently). Adding a volume of dH_2O (μL) equal to ten times the number of nanomoles of DNA present in the tube (as noted on the spec sheet provided with the oligo) will produce a stock solution at this concentration. [1 μM = 1 μmole/L or 1 pmole/μL].

For example, dissolve 50 nmoles (= quantity) of oligo in 500 μL dH_2O to make a 100 μM stock solution (= concentration). Dilute from this stock 1:10 in dH_2O (1 part 100 μM oligo solution, 9 parts dH_2O) to make a working solution at 10 μM for use in setting up PCR reactions.

Most PCR reactions use 0.1 - 0.5 μM primer. Addition of 1 μL of the 10 μM primer to a 20 μl PCR reaction (total volume) will result in a final primer concentration of 0.5 μM, or a 10 picomoles quantity of the oligo in a 20 μl volume.

Oligos used in sequencing reactions have lower concentrations at 2 pmoles/μl. For example, use a 10 μM stock and prepare a 1:5 dilution. We use up to 3 picomoles of primer in 12 μl sequencing reactions.

Primer sequences:

The conserved rDNA primers that we use for PCR and sequencing have been mostly developed in the Bruns lab and the Vilgalys lab. Visit their web pages for additional information and for a greater choice of primer sequences. The DeepHypha web page provides several primer links and is also summing-up primer sequences for protein coding genes like *atp6* (Bruns lab), RPB1 and RPB2 (Hall lab), EF-1α (Steve Rehner), and new primers developed for the AFTOL project, partly including appendant protocolls.

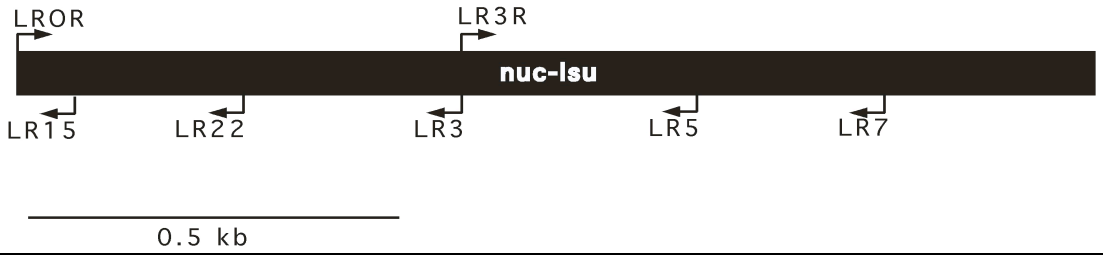
DeepHypha	http://ocid.nacse.org/research/aftol/primers.php
The Bruns lab	http://plantbio.berkeley.edu/~bruns/primers.html
The Hall lab	http://protist.biology.washington.edu/bio2/people/bio.html?parecID=142
The Vilgalys lab	http://www.biology.duke.edu/fungi/mycolab/primers.htm
AFTOL	https://aftol.biology.duke.edu/pub/primers/viewPrimers

The following is a list of primers currently in use in the Hibbett lab. *Check the protocol page for* ***updates*** *on primers of protein-coding regions.* Primer sequences (5″—3′), hybridization regions and their relative position are given where applicable. It is also indicated which primers are used for PCR and which primers are used for sequencing (SEQ) purpose.

Nuclear large subunit rDNA(nuc-lsu, 25S, 28S)

Name	Sequence 5′-3′	Position	Notes
LROR	ACC CGC TGA ACT TAA GC	26-42	Vilgalys lab
LR15	TAA ATT ACA ACT CGG AC	154-138	
LR22	CCT CAC GGT ACT TGT TCG CT	364-344	
LR3	CCG TGT TTC AAG ACG GG	651-635	
LR3R	GTC TTG AAA CAC GGA CC	638-654	
LR5	TCC TGA GGG AAA CTT CG	964-948	
LR7	TAC TAC CAC CAA GAT CT	1418-1432	

nuc-lsu primer map

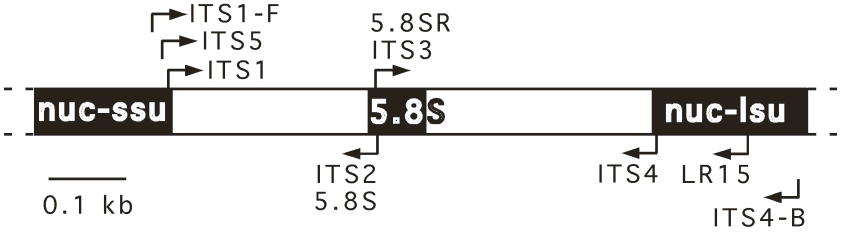


PCR: LR0R—LR5 (LR7)
SEQ: LR0R, LR22, LR3, LR3R, LR5, (LR7)

Internal transcribed spacer region (ITS region, including the 5.8S gene)

ITS1	TCC GTA GGT GAA CCT GCG G	1773-1791 (18S)	White et al. 1990
ITS1-F	CTT GGT CAT TTA GAG GAA GTA A	1735-1756 (18S)	Gardes & Bruns 1993
ITS2	GCT GCG TTC TTC ATC GAT GC	53-34	White et al. 1990
ITS3	GCA TCG ATG AAG AAC GCA GC	34-53	White et al. 1990
ITS4	TCC TCC GCT TAT TGA TAT GC	57-38 (25S)	White et al. 1990
ITS4-B	CAG GAG ACT TGT ACA CGG TCC AG	194-172 (25S)	Gardes & Bruns 1993
ITS5	GGA AGT AAA AGT CGT AAC AAG G	1749-1770 (18S)	White et al. 1990
5.8S	CGC TGC GTT CTT CAT CG	54-38	Vilgalys lab
5.8SR	TCG ATG AAG AAC GCA GCG	37-54	Vilgalys lab

ITS primer map

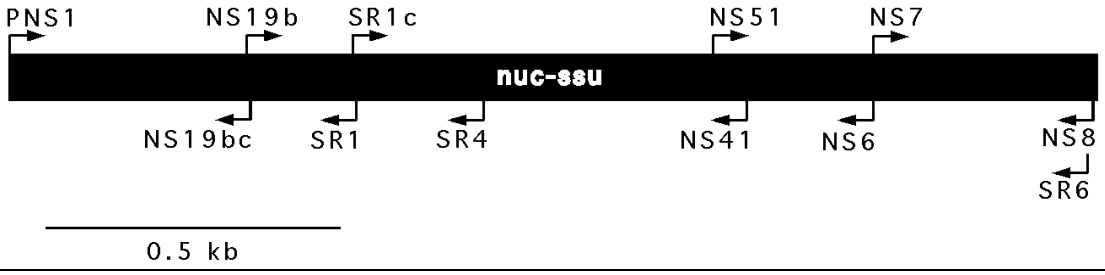


PCR: ITS1 (ITS1F, ITS5)—ITS4 (ITS4, LR15, ITS4-B) alternatives in parentheses.
SEQ: see above. It is recommended using 5.8SR (ITS3) and 5.8S (ITS2) to sequence larger products (> 800 bp).

Nuclear small subunit rDNA (nuc-ssu, 18S)

PNS1	CCA AGC TTG AAT TCG TAG TCA TAT GCT TGT C	1-31	K. O'Donnell (s. Hibbett 1996)
NS19bc	GTT TCT CAG GCT CCC TCT CCG G	399-378	Bruns lab
NS19b	CCG GAG AGG GAG CCT GAG AAA C	378-399	
NS41	CCC GTG TTG AGT CAA ATT A	1200-1182	
NS51	GGG GGA GTA TGG TCG CAA GGC	1108-1128	
NS6	GCA TCA CAG ACC TGT TAT TGC CTC	1439-1416	White et al. 1990
NS7	GAG GCA ATA ACA GGT CT GTG ATG C	1416-1439	
NS8	TCC GCA GGT TCA CCT ACG GA	1792-1773	
SR1	ATT ACC GCG GCT GCT	578-564	Vilgalys lab
SR1c	AGC AGC CGC GGT ATT	564-578	
SR4	AAA CCA ACA AAA TAGA A	838-820	
SR6	TGT TAC GAC TTT TACT T	1760-1744	

nuc-ssu primer map



PCR: 1) PNS1—NS41 and 2) NS19b—NS8; alternatively use PNS1—NS8.
SEQ: for product 1) PNS1, NS19bc, NS41 and 2) NS19b, NS51, NS7, NS8. SR primers and NS6 are alternatives for sequencing.

Mitochondrial large subunit rDNA (mt-lsu)

ML5	CTC GGC AAA TTA TCC TCA TAA G		White et al. 1990
ML6	CAG TAG AAG CTG CAT AGG GTC		
MLIN3	CGA CAC AGG TTC GTA GGT AG		Bruns lab (see web page for intron sites)
CML7.5	CCG CCC CAG TCA AAC TGC C		

mt-lsu primer map

PCR and SEQ: ML5—ML6. MLIN3 and CML7.5 are alternatives

Mitochondrial small subunit rDNA (mt-ssu)

MS1	CAG CAG TCA AGA ATA TTA GTC AAT G		White et al. 1990
MS2	GCG GAT TAT CGA ATT AAA TAA C		
U1	TAA TTT TGG TGC CGA TTG AAC G		Bruns lab
CU6	TGT GGC ACG TCT ATA GCC CA		

mt-ssu primer map

PCR and SEQ: MS1—MS2. U1 and CU6 are alternatives.

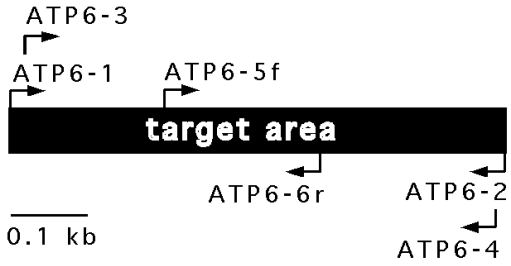
Mitochondrial ATPase subunit 6, *atp6*

ATP6-1	ATT AAT TSW CCW TTA GAW CAA TT		Kretzer & Bruns 1999
ATP6-3	TCT CCT TTA GAA CAA TTT GA		
ATP6-2	TAA TTC TAN WGC ATC TTT AAT RTA		
ATP6-4	AAG TAC GAA WAC WTG WGM TTG		

In experimental stage

ATP6-5f	WAT RGT WAG AGA WCA AWT AGG		Binder unpublished, Hibbett lab
ATP6-6r	AAC TAA TAR AGG AAC TAA AGC TA		

***atp6* primer map**



PCR and SEQ: ATP6-1 (ATP6-3)—ATP6-2 (ATP6-4) in any combination.
Note: ATP6-5f and ATP6-6r are not yet extensively tested but they work fine as SEQ primers.

Cytochrome oxidase subunit 3, *cox3*

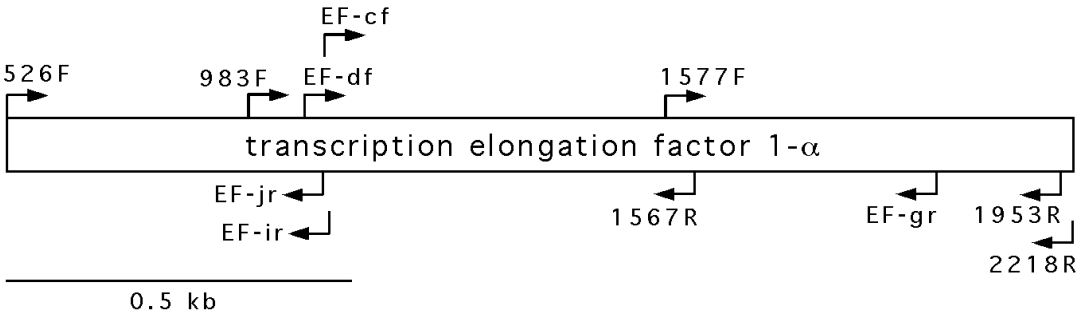
COX3-1	CAT TTA GTA TCG CCT TCA CCA TGG CC		Kretzer & Bruns 1999
COX3-2	AAC AAC CAA ACA ACA TCT ACA AAG TG		

***cox3* primer map**

Translation elongation factor 1α, EF-1α

EF1-526F	GTC GTY GTY ATY GGH CAY GT	1-20	Rehner, (DeepHypha web page)
EF1-983F	GCY CCY GGH CAY CGT GAY TTY AT	336-358	
EF1-1577F	CAR GAY GTB TAC AAG ATY GGT GG	908-930	
EF1-1567R	ACH GTR CCR ATA CCA CCR ATC TT	942-920	
EF1-1953R	CCR GCR ACR GTR TGT CTC AT	1519-1490	
EF1-2218R	ATG ACA CCR ACR GCR ACR GTY TG	1553-1530	
Efcf	ATY GCY GCN GGT ACY GGY GAR TTC GA	408-433	
Efdf	AAG GAY GGN CAR ACY CGN GAR CAY GC	447-472	
Efgr	GCA ATG TGG GCR GTR TGR CAR TC	1311-1289	
Efir	GCR TGY TCN CGR GTY TGN CCR TC	472-450	
Efjr	TGY TCN CGR GTY TGN CCR TCY TT	469-447	

EF-1α primer map



PCR: 1) 526F—1567R, 2) EF-df—2218R, 3) 983F—1953R
SEQ: 1) 526F, EF-ir, 1567R; 2) EF-df, 1577F,EF-gr, 2218R; 3) 983F, 1953R

Note: the suggestions made above are the most reliable combinations in our experience to create overlapping sequences. The whole gene, however, can be amplified in one or two pieces, while additional PCR products occur more frequently.

Laccase

Lac 1F	AGC AYT GGC AYG GCT TYT TYC		Omon Isikhuemhen, Vilgalys lab, (*Pleurotus, Lentinus, Ganoderma*)
Lac 3R	AGA CCR TCA CAR TAY TGR GTG G		
Lac 4R	ATA TCG AAG RAT GRC RGA ATT GAT		

Note: PCR conditions and primer combinations are currently being improved, suggestions will follow later.

Manganese dependant peroxidases and Lignin peroxidases (MnP, LiP)

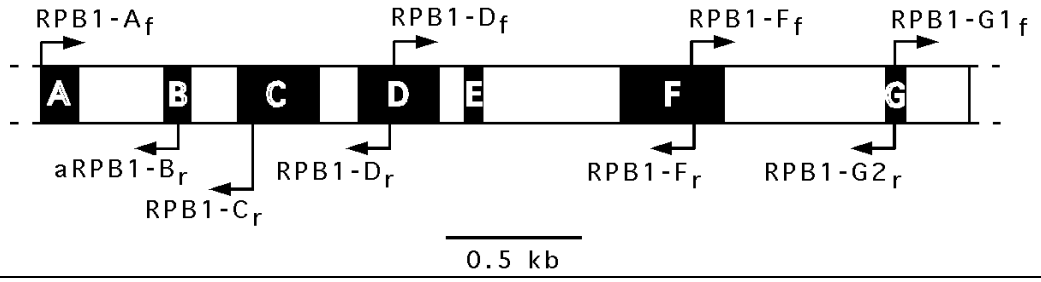
E2FB	GAC CTS CAG AAG AAC CTG TTC SA		Omon Isikhuemhen, (*Pleurotus*)
E8R	CGG AGY TGS GTC TCG ATG AAG A		

Note: PCR conditions and primer combinations are currently being improved, suggestions will follow later.

DNA-directed RNA polymerase II subunit 1, RPB1

RPB1-A_f	GAR TGY CCD GGD CAY TTY GG		Hall lab
RPB1-C_f	CCN GCD ATN TCR TTR TCC ATR TA		
RPB1-D_f	TAC AAT GCY GAY TTY GAY GG		
RPB1-D_r	TTC ATY TCR TCD CCR TCR AAR TC		
RPB1-F_f	CAY GCD ATG GGD GGD MGD GAR GG		
RPB1-F_r	CCY TCN CKW CCW CCC ATD GCR TG		
RPB1-$G1_f$	TGR AAD GTR TTD AGD GTC ATY TG		
RPB1-$G2_r$	GTC ATY TGD GTD GCD GGY TCD CC		
aRPB1-B_r	TCC GCR CCY TCT TCY TTG G		Matheny et al. 2002

RPB1 primer map



Note: PCR conditions and primer combinations are currently being improved, suggestions will follow later.

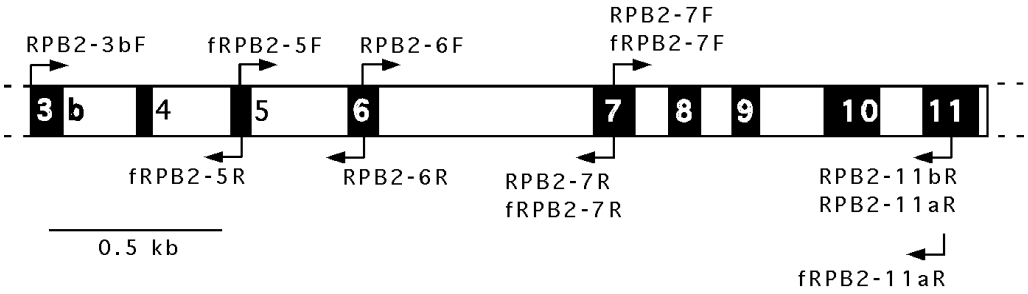
DNA-directed RNA polymerase II subunit 2, RPB2

RPB2-3bF	GGW GGW TAY TTY ATY ATY AAT GG		Hall lab, general
RPB2-6F	TGG GGK WTG GTY TGY CCT GC		
RPB2-6R	GCA GGR CAR ACC AWM CCC CA		
RPB2-7F	ATG GGK AAG CAR GCW ATG GG		
RPB2-7R	CCC ATW GCY TGC TTM CCC AT		
RPB2-11aR	GTG WAT YTT RTC RTC MAC C		
RPB2-11bR	CAA TCW CGY TCC ATY TCW CC		
fRPB2-5f	GAY GAY MGW GAT CAY TTY GG		Hall lab, fungal specific
fRPB2-5R	CCR AAR TGA TCW CKR TCR TC		
fRPB2-7cF	ATG GGY AAR CAA GCY ATG GG		
fRPB2-7cR	CCC ATR GCT TGY TTR CCC AT		
fRPB2-11aR	GCR TGG ATC TTR TCR TCS ACC		
bRPB2-3.1F	ATY GCY CAA GAR MGN ATG GC		Hall lab, basidiomycetes specific
bRPB2-6F	TGG GGY ATG GTN TGY CCY GC		
bRPB2-6.3F	GTY ATY GGT GTN TGG ATG GG		
bRPB2-7R	GAY TGR TTR TGR TCR GGG AAV GG		
bRPB2-7.1R	CCC ATR GCY TGY TTM CCC ATD GC		
bRPB2-10.9R	GTR AAS GGY GTG GCR TCY CC		

In experimental stage

RPB2-3F1	AAR GTY YTK ATY GCM CAR GAG CG		Y.W. Lim unpublished, polypore specific, Hibbett lab
RPB2-6F1	CAC AAY CAN CAY TGG GGW ATG GT		
RPB2-7F1	ATG GAT ACN ATG GCS AAY AT		
RPB2-6R1	ACC ATW CCC CAR TGN TGR TTG TG		
RPB2-7R1	ATR TTG GCC ATN GTR TCC AT		
RPB2-10R1	ACC CTT YTG MCC RTG ACR AGA		

RPB2 primer map



Note: PCR conditions and primer combinations are currently being improved, suggestions will follow later. ***See P. Brandon Matheny's recently posted updates on RPB2.***

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