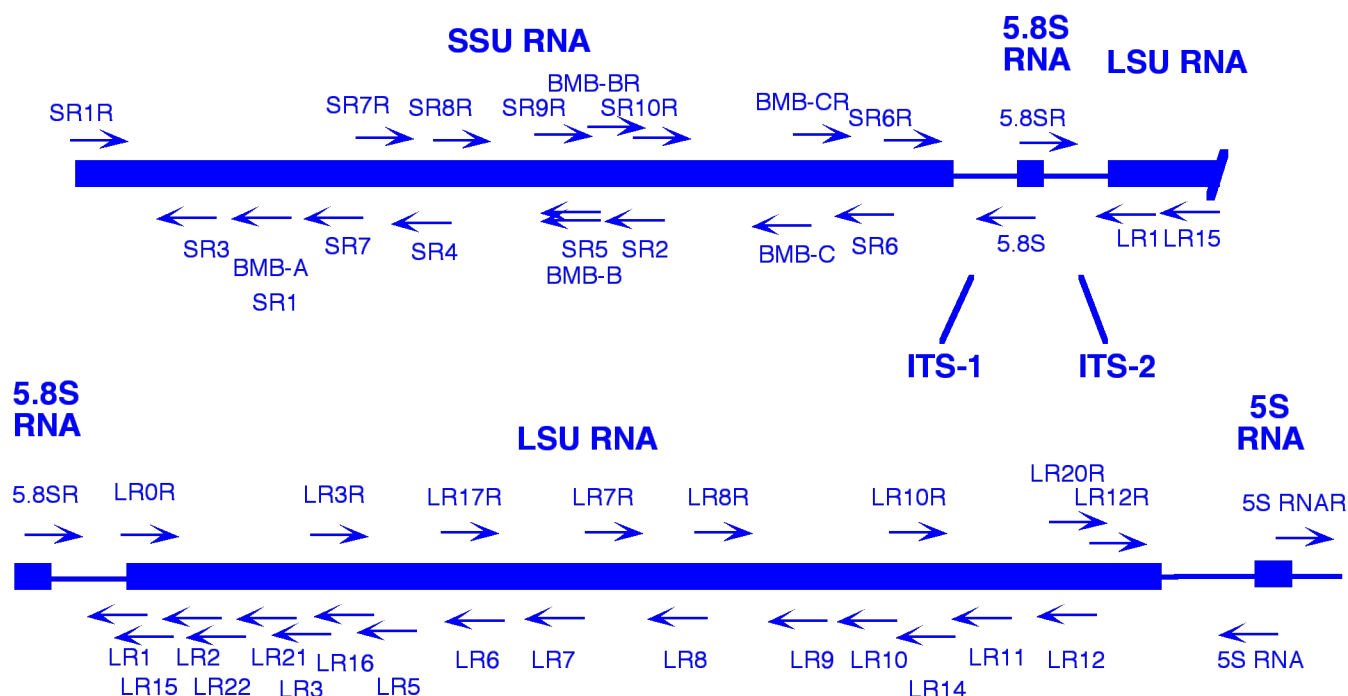


Conserved primer sequences for PCR amplification and sequencing from nuclear ribosomal RNA
(Vilgalys lab, Duke University, updated Feb 3, 1992)

We have compiled the following list of conserved primer sequences useful for amplification and sequencing of nuclear rDNA from most major groups of fungi (primarily Eumycota), as well as other eukaryotes. All of these primers were identified and tested by our own lab based on consensus between the published large and small subunit RNA sequences from fungi, plants and other eukaryotes; sources of other useful primer sequences from published literature are also indicated. Together, these primers span most of the nuclear rDNA coding region (see figure), permitting amplification of any desired region. Standard symbols are used for the four primary nucleotides; variable positions are indicated as follows: P=A,G / Q=C,T / R=A,T / V=A,C / W=G,T. Primers ending with "R" represent the coding strand (same as RNA). All other primers are complementary to the coding strand. This information is provided freely and may be passed on to anyone who wants to use it.



Small subunit RNA (SR) primer sequences:

Primer name	Sequence (5'-->3')	Position within <i>S. cerevisiae</i> 17S RNA
BMB-'A' ¹	GRATTACCGCGGCWGCTG	580-558
BMB-'B' ¹	CCGTCAATTCVTTTPAGTTT	1146-1127
BMB-'C' ¹	ACGGGCGGTGTGTPC	1638-1624
BMB-BR ¹	CTTAAAGGAATTGACGGAA	1130-1148
BMB-CR ¹	GTACACACCGCCCGTCCG	1624-1640
SR1R	TACCTGGTTGATQCTGCCAGT	1-21
SR1	ATTACCGCGGCTGCT	578-564
SR2	CGGCCATGCACCACC	1277-1263
SR3	GAAAGTTGATAGGGCT	318-302
SR4	AAACCAACAAAATAGAA	838-820
SR5	GTGCCCTTCCGTCAATT	1146-1130
SR6	TGTTACGACTTTTACTT	1760-1744
SR6R	AAGWAAAAGTCGTAACAAGG	1744-1763
SR7	GTTCAACTACGAGCTTTTTAA	617-637
SR7R	AGTTAAAAAGCTCGTAGTTG	637-617
SR8R	GAACCAGGACTTTTACCTT	732-749
SR9R ⁺	QAGAGGTGAAATTCT	896-910

SR10R ⁺	TTTGA CTCAACACGGG	1181-1196
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¹Primers originally developed by Lane et al., 1985. PNAS USA 82:6955-6959; ⁺Based upon primers used by Elwood et al., 1985. Mol. Biol. Evol. 2: 399-410

Large subunit RNA (LR) and 5.8S RNA primer sequences:

Primer name	Sequence (5'-->3')	Position within <i>S. cerevisiae</i> rRNA
5.8S	CGCTGCGTTCTTCATCG	51-35 (5.8S RNA)
5.8SR	TCGATGAAGAACGCAGCG	34-51 (5.8S RNA)
LR0R	ACCCGCTGAACTTAAGC	26-42
LR1	GGTTGGTTTCTTTTCCT	73-57
LR2	TTTTCAAAGTTCTTTTC	385-370
LR2R	AAGAACTTTGAAAAGAG	374-389
LR3	CCGTGTTTCAAGACGGG	651-635
LR3R	GTCTTGAACACGGACC	638-654
LR4	ACCAGAGTTTCTCTGG	854-838
LR5	TCCTGAGGGAAACTTCG	964-948
LR6	CGCCAGTTCTGCTTACC	1141-1125
LR7	TACTACCACCAAGATCT	1448-1432
LR7R	GCAGATCTTGGTGGTAG	1430-1446
LR8	CACCTTGGAGACCTGCT	1861-1845
LR8R	AGCAGGTCTCCAAGGTG	1845-1861
LR9	AGAGCACTGGGCAGAAA	2204-2188
LR10	AGTCAAGCTCAACAGGG	2420-2404
LR10R	GACCCTGTTGAGCTTGA	2402-2418
LR11	GCCAGTTATCCCTGTGGTAA	2821-2802
LR12	GACTTAGAGCGTTCAG	3124-3106
LR12R	CTGAACGCCTCTAAGTCAGAA	3106-3126
LR13	CGTAACAACAAGGCTACT	3357-3340
LR14	AGCCAACTCCCCACCTG	2616-2599
LR15	TAAATTACAACCTCGGAC	154-138
LR16	TTCCACCCAAACACTCG	1081-1065
LR17R	TAACCTATTCTCAAACCT	1033-1050
LR20R	GTGAGACAGGTTAGTTTTACCCT	2959-2982
LR21	ACTTCAAGCGTTTCCCTTT	424-393
LR22	CCTCACGGTACTTGTTTCGCT	364-344

5S RNA PRIMER SEQUENCES

In addition we have also identified two complementary 5S RNA PRIMER SEQUENCES which are largely specific for Basidiomycetes, and which may be used to amplify a portion of the intervening rDNA sequence beyond the large subunit RNA coding region:

5SRNA ATCAGACGGGATGCGGT (complementary to 5S RNA positions 46-26)

5SRNAR ACQGCATCCCGTCTGAT (=5S RNA positions 26-46)

OTHER PRIMER SEQUENCES USEFUL FOR AMPLIFICATION OF RDNA FROM FUNGI:

ITS1 [#]	TCCGTAGGTGAACCTGCGG	
ITS2 [#]	GCTGCGTTCTTCATCGATGC (is similar to 5.8S above)	
ITS3 [#]	GCATCGATGAAGAACGCAGC (is similar to 5.8SR above)	
ITS4 [#]	TCCTCCGCTTATTGATATGC	
ITS5 [#] R	GGAAGTAAAAGTCGTAACAAGG (similar to SR6R)	
NS1 [#] R	GTAGTCATATGCTTGTCTC	
NS2 [#]	GGCTGCTGGCACCAGACTTGC	
NS3 [#] R	GCAAGTCTGGTGCCAGCAGCC	
NS4 [#]	CTTCCGTCAATTCCTTTAAG (similar to BMB-B)	
NS5 [#] R	AACTTAAAGGAATTGACGGAAG (is similar to BMB-BR)	
NS6 [#]	GCATCACAGACCTGTTATTGCCTC	
NS7 [#] R	GAGGCAATAACAGGTCTGTGATGC	1413-1431
NS8 [#]	TCCGCAGGTTACCTACGGA	

[#]White et al., 1990. pp 315-322 in PCR Protocols: A Guide to Methods and Applications. Academic Press, Inc.

GUADET P1*	GAGTCGAGTTGTTTGGGAATGCAGCT	681-657
GUADET P2*	GAAAAGAAGCTTTGAAAGAGAGTGAA	786-761
GUADET P3*	CCCGTCTTGAAACACGGACCAAGGA	1049-1027

*Guadet et al., 1989. Mol. Biol. Evol. 6: 227-242.

