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A nomenclature for fungal PCR primers with examples from intron-containing SSU rDNA

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Abstract: We present a compilation of polymerase chain reaction primers (oligonucleotides) used to amplify and sequence the small subunit of nuclear ribosomal DNA. To simplify use of these primers we designed a nomenclature that defines the location of each relative to *Saccharomyces cerevisiae*. With these primers we have developed strategies for selectively amplifying the diverse ribosomal DNA repeat types found in lichens; either between symbionts or between ribosomal DNA repeats (often the product of optional group I introns) within an individual fungus. A diversity of primers allows a choice of which small subunit ribosomal DNA repeat is amplified or sequenced from a complex extract such as that derived from lichens.

Key Words: 18S rDNA, Eumycota, fungi

The polymerase chain reaction (PCR) has made possible rapid and selective amplification of any gene with known and conserved nucleotide sequence. Using these conserved sequences, the oligonucleotide primers essential for PCR amplification can be designed for universal or specific applications. The positioning of PCR primers within a gene is critical to obtaining appropriate DNA fragments for sequencing and analysis. In particular, PCR has made possible the amplification of small subunit ribosomal DNA (SSU rDNA), which has consequently come into widespread use for studies on fungal molecular systematics and evolution (see references in Gargas et al., 1995a). The large number of primers now available for the SSU rDNA has led to a confusing array of primer names and nomenclature systems. Many primer names give no details of primer location within the gene, or whether the primer anneals to the coding or noncoding DNA strand. To provide each primer with a name which unambiguously describes its actual position, we designed a standard system of nomenclature for primers, with examples for eukaryotic SSU rDNA (TABLE I). The code 'nu-SSU' denotes that these primers anneal to the *nu*clear *S*mall *SU*bunit of ribosomal DNA. The −5′ or −3′ specifies whether each primer anneals to the coding or noncoding strand, respectively. A number in the primer name denotes the location of the 5′ end of the primer in reference to a *Saccharomyces cerevisiae* Meyen *ex* Hansen sequence standard (Mankin et al., 1986; Rubtsov et al., 1980). This number contains the same number of digits as the entire gene, i.e. the primer at nucleotide 21 is designated 0021. This numbering system allows an estimation of product size (minus the sum of the primers' lengths) by simply subtracting the numbers of the −5′ and the −3′ primers. To distinguish between two primers of different lengths which begin at the same 5′ nucleotide we would add a dash, and then the size of the primer in nucleotides.

This system may be adapted to other genes, for example primers for *nu*clear *L*arge *SU*bunit rDNA by using the prefix 'nu-LSU', or for protein genes using standard prefixes. For sequences that are optional among even closely related organisms (i.e. transposable elements, retroviruses, or introns) it is important to note the gene in which they reside, the location of the optional sequence within the gene, and the organism that serves as a reference for the location. In addition, each primer's position within the optional sequence must be denoted as described above. For example, primers designed to anneal to intron sequences in SSU rDNA are denoted with the following: nu-SSI to indicate *nu*clear *S*mall *S*ubunit of the ribosomal DNA, I for *I*ntron, with a number indicating the insertion position (relative to an *Escherichia coli* (Migula) Castellani and Chalmers standard, following Gargas et al., 1995b), in parentheses. This is followed by a dash and then a number denoting the 3′ end of the primer within an intron, −5′ or −3′ as above, a dash and then the species for which the

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TABLE I. Primers for PCR amplifying and sequencing small subunit ribosomal DNA

Primer	Other names	5′ end	3′ end	Primer sequence	Primer notes[a]	Reference
nu-SSU Primers						
nu-SSU-0021-5′		0004	0021	CTGGT TGATT CTGCC AGT		This study
nu-SSU-0038-5′	NS1	0020	0038	GTAGT CATAT GCTTG TCTC		White et al., 1990
nu-SSU-0072-5′	NS17UCB	0054	0072	CATGT CTAAG TTTAA GCAA	non-green algal	Gargas and Taylor, 1992
nu-SSU-0305-3′	CNS26	0323	0305	TCGAA AGTTG ATAGG GCAG		Gargas et al., 1995b
nu-SSU-0402-5′	NS19UCB	0381	0402	CCGGA GAAGG AGCCT GAGAA AC	spans intron position 323 non-green algal	Gargas and Taylor, 1992
nu-SSU-0497-3′	NS18UCB	0516	0497	CTCAT TCCAA TTACA AGACC	non-green algal	Gargas and Taylor, 1992
nu-SSU-0573-5′	NS3	0553	0573	GCAAG TCTGG TGCCA GCAGC C	spans intron position 516	White et al., 1990
nu-SSU-0553-3′	NS2	0573	0553	GGCTG CTGGC ACCAG ACTTG C	spans intron position 516	White et al., 1990
nu-SSU-0819-5′	NS21UCB	0802	0819	GAATA ATAGA ATAGG ACG	non-green algal	Gargas and Taylor, 1992
nu-SSU-0852-3′	NS20UCB	0871	0852	CGTCC CTATT AATCA TTACG	non-green algal	Gargas and Taylor, 1992
nu-SSU-1150-5′	NS5	1128	1150	AACTT AAAGG AATTG ACGGA AG		White et al., 1990
nu-SSU-1131-3′	NS4	1150	1131	CTTCC GTCAA TTCCT TTAAG		White et al., 1990
nu-SSU-1203-5′	NS23UCB	1184	1203	GACTC AACAC GGGGA AACTC	non-green algal	Gargas and Taylor, 1992
nu-SSU-1184-3′	MB2	1203	1184	GAGTT TCCCC GTGTT GAGTC		Gargas et al., 1995b
nu-SSU-1258-5′	SR12R	1241	1258	GCTCT TTCTT GATTT TGT		DePriest, 1993
nu-SSU-1293-3′	NS22UCB	1312	1293	AATTA AGCAG ACAAA TCACT	non-green algal	Gargas and Taylor, 1992
nu-SSU-1427-5′	SR14R	1410	1427	TTTGA GGCAA TAACA GGT		DePriest, 1993
nu-SSU-1431-5′	NS7R	1413	1431	GAGGC AATAA CAGGT CTGT	spans intron position 1199	White et al., 1990
nu-SSU-1436-5′	NS7	1413	1436	GAGGC AATAA CAGGT CTGTG ATGC	spans intron position 1199	White et al., 1990
nu-SSU-1412-3′	NS6	1436	1412	GCATC ACAGA CCTGT TATTG CCTC	spans intron position 1199	White et al., 1990
nu-SSU-1428-3′	SR15	1445	1428	CATCT AAGGG CATCA CAG	spans intron position 1210	DePriest, 1993
nu-SSU-1465-3′	SR16	1482	1465	TTGTC TCTGT CAGTG TAG		DePriest, 1993
nu-SSU-1583-5′	SR18R	1566	1583	CAACG AGGAA TTCCT AGT		DePriest, 1993
nu-SSU-1580-3′	SR17	1597	1580	GATGA CTCGC GCTTA CTA		DePriest, 1992
nu-SSU-1626-5′	SR11R	1609	1626	ATTAC GTCCC TGCCC TTT	spans intron position 1389	DePriest, 1992
nu-SSU-1627-3′	BMB C	1641	1627	ACGGG CGGTG TGTRC		Lane et al., 1985
nu-SSU-1766-5′	ITS5	1744	1766	GGAAG TAAA GTCGT AACAA GG		White et al., 1990
nu-SSU-1722-3′		1747	1722	TCCTC TAAAT GACCA AGTTT GWCCA A		This study
nu-SSU-1776-5′		1750	1776	TAAAA GTCGT AACAA GGTTT CCGTA GG	spans intron positions 1506, 1512	This study
nu-SSU-1750-3′	NS24UCB	1769	1750	AAACC TTGTT ACGAC TTTTA	spans intron position 1506, non-green algal	Gargas and Taylor, 1992
nu-SSU-1787-5′	ITS1	1769	1787	TCCGT AGGTG AACCT GCGG	spans intron positions 1512, 1516	White et al., 1990
nu-SSU-1769-3′	NS8	1788	1769	TCCGC AGGTT CACCT ACGGA	spans intron positions 1512, 1516	White et al., 1990
nu-SSU-1780-3′	PD9rev, SR13	1797	1780	AATGA TCCTT CCGCA GGT		DePriest, 1993
Intron-specific Primers						
nu-SSI(1046)-154-5′-Ccg		128	154	GGYCT CAGTT CGAGA TATAG TCGAG CC	within introns at position 1046	This study
nu-SSI(1046)-164-3′-Ccg		184	164	TCAGC CCATC ACTCG CCTAG TCTGT GA	within introns at position 1046	This study
nu-SSI(1516)-194-5′-Ccg		168	194	CACTT CGGTG GTTGA GATAT GATCG GC	within introns at position 1516	This study
nu-SSI(1516)-146-3′-Ccg		172	146	AAGTG GCCGC TATCG TTTGA CCTGT GA	within introns at position 1516	This study

[a] Intron position numbers are relative to location within *Escherichia coli* (Gargas et al., 1995b).

primer was designed denoted at the end using *G*enus *s*pecies and optional subspecific designation (Ccg = *Cladonia chlorophaea* (Flörke *ex* Sommerf.) Sprengel *g*rayii chemotype, as in DePriest and Been, 1992).

The primers listed in TABLE I have been used for PCR amplification, and for sequencing by manual and automated methods. We have used each successfully for PCR amplifications of fungal DNA, although many also work well for plant or animal DNA. A selection of primers placed throughout the SSU rDNA is valuable for PCR amplification and sequencing because primers do not generate consistent results for every organism. Minor sequence variations have been exploited to design primers that differentiate between the fungal and algal partners of a lichen association (Gargas and Taylor, 1992); primers that preferentially amplify the fungal partner are designated 'non-green-algal' in TABLE I. Additionally, the SSU rDNA from lichen-forming and other ascomycetous fungi frequently contains group I introns in conserved regions (Gargas et al., 1995b)—the same regions considered optimal for universal primers. In fact, of ten universal rDNA primers described in White et al. (1990), six span intron sites known from some fungi. For such intron-spanning primers, the nucleotide mismatch will thwart PCR amplification—a phenomenon common with PCR amplification of intron-containing organisms (FIG. 1a). A primer pair flanking an intron will produce a larger PCR product than that predicted from homologous genes lacking the intron (FIG. 1b). Each intron increases the length of the PCR product (100, 200, or more nt) and may produce amplification products longer than the optimum length for sequencing. Furthermore, primers have been designed that anneal to intron sequences, allowing selective amplification of introns and intron-containing repeats (FIG. 1c).

Ribosomal introns and insertions may be the rule rather than the exception for some ascomycetes—as many as eight insertions may interrupt a single SSU rDNA (Gargas et al., 1995b). These insertions vary in occurrence within groups and species, and even within an individual (DePriest, 1993). Specifically, rDNA copies within a tandem array may vary in the presence and absence of introns (DePriest, 1993). In light of this observed variation, rDNA extracted from a single organism must be examined for rDNA repeats that vary in length. PCR amplifications from such rDNA would result in multiple superimposed fragments, and produce multiple sequences. In these situations PCR primer selection is critical in determining whether copies with or without introns are amplified and sequenced from an individual. Choosing primers which span an intron position, or are intron-specific, allows selective amplification of intron-lacking, or intron-containing templates. In TABLE I we indicate primers useful for sequencing rDNA from potentially intron-containing organisms, and whether each primer spans known intron positions.

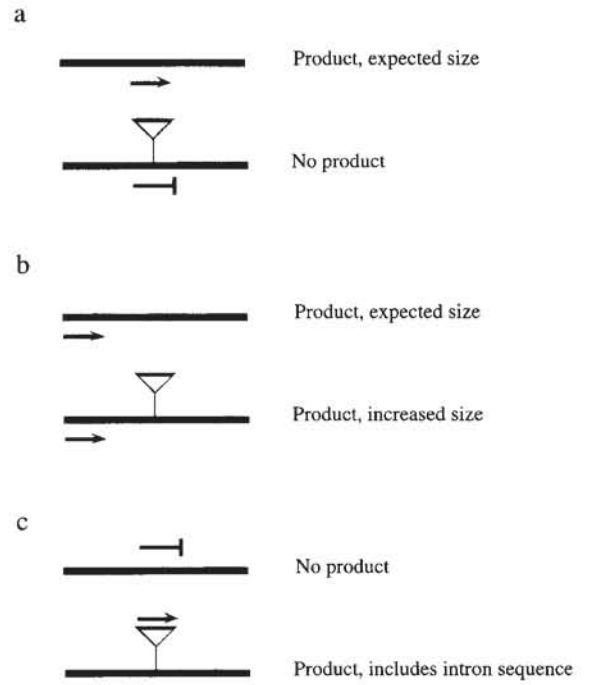

FIG. 1. Primer placement allowing selective PCR amplification and sequencing of intron-containing and intron-lacking SSU rDNA repeats. a. Intron-spanning primers allow selective amplification and sequencing of intron-lacking rDNA repeats by blocking of annealing to intron-containing rDNA repeats. b. Primers flanking introns allow amplification of both intron-lacking and intron-containing repeats. However, the resulting PCR products will differ in size. c. Primers annealing to intron sequences allow amplification and sequencing of intron-containing rDNA repeats, since they will not amplify or sequence intron-lacking rDNA repeats. Straight lines represent the SSU rDNA repeats. Triangles denote introns, with a stem to their position in the rDNA repeat. Bold arrows indicate primers that anneal. Short lines with blunt ends indicate primers that fail to anneal. For each situation the expected product is described.

TABLE I demonstrates the new primer nomenclature we designed for our research on SSU rDNA, SSU rDNA introns, and the adjoining intergenic spacer (ITS) regions. This list includes three new SSU rDNA primers, and four new SSU rDNA intron-specific primers. We have included original names and citations for previously published primers where appropriate, the nucleotide position of their 5′ and 3′ ends relative to a reference species, and their nucleotide sequences. For many of the primers, we have denoted special applications (intron-spanning, intron-specific, or non-green algal) in the notes. We hope that this

simplified and predictive nomenclature will be applied broadly to primers for PCR amplifications and sequencing, and the creative use of SSU rDNA and intron-specific primers will encourage further research on rDNA variability.

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LITERATURE CITED

DePriest, P. T. 1992. Molecular genetic analysis of ribosomal DNA polymorphism in the *Cladonia chlorophaea* complex. Ph. D. Thesis, Duke Univ., Durham, North Carolina.

———. 1993. Small subunit rDNA variation in a population of lichen fungi due to optional group I introns. *Gene* 134: 67–74.

———, and M. Been. 1992. Numerous group I introns in the ribosomal DNA of a lichen fungus. *J. Molec. Biol.* 228: 315–321.

Gargas, A., P. T. DePriest, M. Grube and A. Tehler. 1995a. Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny. *Science* 268: 1492–1495.

———, ———, and J. W. Taylor. 1995b. Positions of multiple insertions in SSU rDNA of lichen-forming fungi. *Molec. Biol. Evol.* 12: 208–218.

———, and J. W. Taylor. 1992. Polymerase chain reaction (PCR) primers for amplifying and sequencing nuclear 18S rDNA from lichenized fungi. *Mycologia* 84: 589–592.

Lane, D. J., B. Pace, G. J. Olsen, D. A. Stahl, M. L. Sogin, and N. R. Pace. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc. Natl. Acad. Sci. USA* 82: 6955–6959.

Mankin A. S., K. G. Skryabin, and P. M. Rubtsov. 1986. Identification of ten additional nucleotides in the primary structure of yeast 18S rRNA. *Gene* 44: 143–143.

Rubtsov, P. M., M. M. Musakhanov, V. M. Zakharyev, A. S. Krayev, K. G. Skryabin, and A. A. Bayev. 1980. The structure of the yeast ribosomal RNA genes. I. The complete nucleotide sequence of the 18S ribosomal RNA gene from *Saccharomyces cerevisiae*. *Nucl. Acids Res.* 8: 5779–5794.

White, T. J., T. D. Bruns, S. B. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–321. *In: PCR protocols: A guide to methods and applications*. Eds., M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White. Academic Press, New York.