

## PCR primers to amplify and sequence *rpb2* (RNA polymerase II second largest subunit) in the Basidiomycota (Fungi)

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We perform three separate PCR amplifications to obtain *rpb2* 3-11 products (ca. 2800 bp) or two PCR amplifications to get *rpb2* 5-11 products (ca. 2100 bp) in our studies of basidiomycete phylogeny (Matheny et al. 2007a; 2007b). For the study of closely related species, sequences of the most variable region of *rpb2* between domains 6 and 7 have proven sufficient (Frøslev et al. 2005; Matheny 2005). In our study on the Agaricales, we utilized 1100 bp between domains 5 and 7 (Matheny et al. 2006).

The following **PCR primer pairs** are recommended. Primer sequences are provided at the end of this document.

### *rpb2* 3-11 products

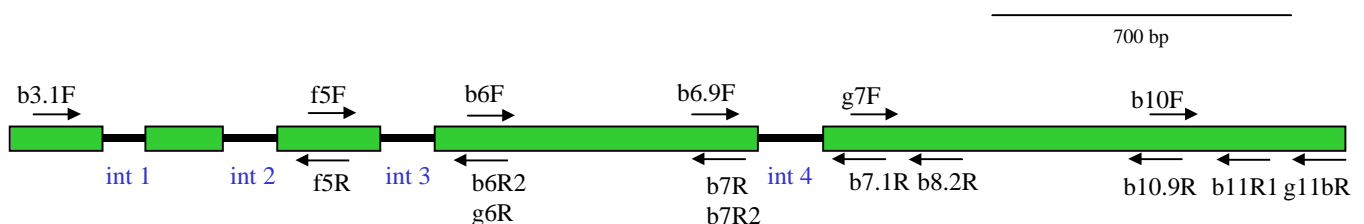
1. b3.1F and b6R2
2. f5F and b7.1R or b7R2 or b7R
3. b6.9F and b11R1 or g11bR

### *rpb2* 5-11 products

1. f5F and b7.1R or b7R2 or b7R
2. b6.9F and b11R1 or g11bR

Primer nomenclature is as follows: *b* and *f* refer to “basidiomycete” and “fungal” specific, respectively. The *g* refers to a “general” primer and is not fungal specific. A number, for example, 5, refers to the conserved domain in which the primer sequence is located. Numbers of domains increase consecutively going downstream in the 3' direction. Uppercase *F* and *R* refer to “forward” and “reverse”.

Below is a general schematic that maps *rpb2* primer positions of primers we most frequently use. Thick bars represent exons and thin lines represent introns.



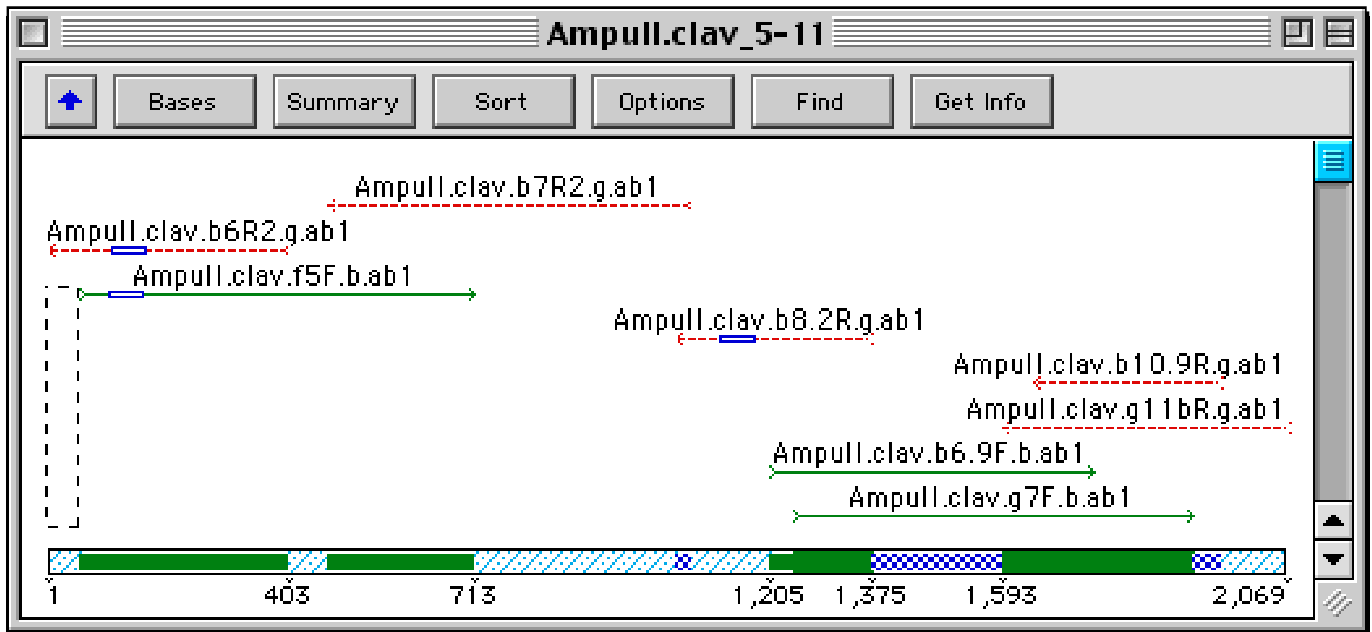
Presence or absence of introns varies depending on the group being studied but is generally conserved across the Basidiomycota. Several exceptions are noteworthy, however. For example, the Agaricales, Boletales, *Ustilago maydis* (corn smut), and *Cryptococcus neoformans* do not possess intron 2. *Ustilago*, *Malassezia* (a yeast relative of *Ustilago*), and *Cryptococcus* also lack intron 3. Exemplars of the Trichosporonales share a unique intron with varying phase insertions in domain 6, which disrupts the b6F primer binding site. In addition, *Ustilago*, *Rhodotorula hordea* (a yeast relative of rusts), *Coniophora arida* (Boletales), and *Megacollybia platyphylla* (Agaricales) lack intron 4. Furthermore, *Ustilago* and additional smut fungi, as well as the unrelated *Rhodotorula hordea* possess uniquely placed introns downstream of the g7F primer site. Our data stem primarily from the 5 to 11 region of *rpb2*, so distribution of introns 1 and 2 across the Basidiomycota is still not well known. The rust *Phragmidium* however, shares a similar *rpb2* gene structure with several major homobasidiomycete lineages (Cantharellales, Phallomycetidae, Hymenochaetales, Russulales, and Polyporales, for example). See the study by Matheny et al. (2007b) for additional details on intron distribution and intron evolution in the Basidiomycota.

**Successful PCR programs of varying stringency used to amplify *rpb2* fragments:**

- (1) 95.0 C for 4 min
- (2) 95.0 C for 1 min
- (3) 50.0 C for 1 min
- (4) ramp 0.3 C per sec to 72 C
- (5) 72.0 C for 1 min
- (6) 34 times to step 2
- (7) 72 for 10 min
- (8) 15.0 C for 0:00
- (9) END

- (1) 95.0 for 1 min
- (2) 95.0 C for 1 min
- (3) 55.0 C for 1 min
- (4) 1 min 72.0 C + 1 sec/cycle
- (5) 34 times to step 2
- (6) 72.0 C for 10 min
- (7) 15.0 C for 0:00
- (8) END

This overview of an *rpb2* 5-11 project file for *Ampulloclitocybe clavipes* (Agaricales) shows the degree of PCR and internal sequencing primer overlap. Blue boxes represent introns 3 and 4.



## ***rpb2* PCR and sequencing primers**

Primer Name	Sequence 5' – 3'	Amino acid sequence	Source
bRPB2-3.1F	ATYGCYCAAGARMGNATGGC	TAQERMA	<a href="http://faculty.washington.edu/benhall/Matheny%20et%20al.%20(2007b)">http://faculty.washington.edu/benhall/Matheny et al. (2007b)</a>
fRPB2-5F	GAYGAYMGWGATCAYTTYGG	DDRDHFG	Liu, Whelen, & Hall (1999)
fRPB2-5R	CCRAARTGATCWCKRTRC	DDRDHFG	Liu, Whelen, & Hall (1999)
bRPB2-6F	TGGGGYATGGTNTGYCCYGC	WGMVCPA	<a href="http://faculty.washington.edu/benhall/Matheny%20(2005)">http://faculty.washington.edu/benhall/Matheny (2005)</a>
gRPB2-6R	GCAGGRCARACCAWMCCCCA	WGM/LVCPA	Liu, Whelen, & Hall (1999)
bRPB2-6R2	GGRCANACCATNCCCCARTG	HWGMVCP	Matheny et al. (2007b)
bRPB2-6.3F	GTYATYGGTGTNTGGATGGG	WGMVCPA	Liu, Whelen, & Hall (1999)
bRPB2-6.9F	TGGACNCAYTGYGARATYCAACC	WTHCEIHP	Matheny et al. (2007b)
gRPB2-7F	ATGGGKAAGCARGCWATGGG	MGKQAMG	Liu, Whelen, & Hall (1999)
bRPB2-7R	GAYTGRTRTRGRTCRGGAAVGG	PFPDHNQS	<a href="http://faculty.washington.edu/benhall/Matheny%20(2005)">http://faculty.washington.edu/benhall/Matheny (2005)</a>
fRPB2-7cF	ATGGGYAARCAAGCYATGGG	MGKQAMG	Liu, Whelen, & Hall (1999)
fRPB2-7cR	CCCATRGCTTGYTTRCCCAT	MGKQAMG	Liu, Whelen, & Hall (1999)
bRPB2-7R2	ACYTGRTRTRGRTCNNGRAANGG	PFPDHNQgt	Matheny et al. (2007b)
bRPB2-7.1R	CCCATRGCTGYTTMCCCATDGC	AMGKQAMG	<a href="http://faculty.washington.edu/benhall/Matheny%20(2005)">http://faculty.washington.edu/benhall/Matheny (2005)</a>
bRPB2-8.2R	CTNCGGAANAGRCCRCGRTC	DRGLFRS	Matheny et al. (2007b)
bRPB2-10.9R	GTRAASGGYGTGGCRTCYCC	GDATPFT	<a href="http://faculty.washington.edu/benhall/Matheny%20et%20al.%20(2007b)">http://faculty.washington.edu/benhall/Matheny et al. (2007b)</a>
bRPB2-11R1	TGGATYTTGTCRTCCACCAT	MVDDKIH	Matheny et al. (2007b)
fRPB2-11aR	GCRTGGATCTTRTCRTCSACC	MVDDKIHA	Liu, Whelen, & Hall (1999)
gRPB2-11bR	CAATCWCGYTCCATYTCWCC	GEMERD	Liu, Whelen, & Hall (1999)

## **References for *rpb2* used in 32 fungal phylogenetic studies to date.**

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