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Primers for bark beetles and associated fungi

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Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes the primers used for bark beetles and associated fungi.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

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Diluting primers

Reconstituting lyophilized primers to create 100X stock:
Resuspend to 100 uM/L concentration: for 28.5 nM of lyophilized primer, add 285 ul water (10 times more, in uL).
Dilute to make working solution :

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To 10uL of the working stock above, add 90 uL of water, to make 10 uM/L (or 10 pM/ul) solution.
Working concentration: Use 1uL of the working solution above in 25uL reaction (10/25=0.4pM per rxn).

Primers we use

GENE	PRIMER CODE	SEQ	REFERENCE
16S	1492R	GGTTACCTTGTTACGACTT	
16S	27F	AGAGTTTGATCCTGGCTCAG	
16S bact rev	338R	TGCTGCCTCCCGTAGGAGT	Amann et al. 1995. Microbiol. Rev. 59, 143–169.
16S bact+archaea for	515F	GTGCCAGCMGCCGCGGTAA	Bergmann et al 2011 (Fierer lab)
16S bact+archaea rev	806R	GGACTACVSGGGTATCTAAT	Bergmann et al 2011 (Fierer lab)
28S	A4053 (alt_name: A1)	TCCKGTKTTCAAGACGGGGTC	Whiting et al. 1997
28S	A4221 (alt_name: A160)	CGCCTCTTCTCGCAATGAGA	Sequeira et al, 2000
28S	A4285 (alt_name: A247)	CCTGACTTCGTCCTGACCAGGC	Sequeira et al, 2000
28S	A4394 (alt_name: A335)	TCGGARGGAACCAGCTACTA	Whiting et al. 1997
28S	D2F1	ACTGTTGGCGACGATGTTCT	Jordal etal. (2008)
28S	D3R2	TCTTCGCCCCTATACCC	Jordal etal. (2008)
28S	f1094	GGATCGTCGCAAGACGGACAGAAG	Sequeira et al, 2000
28S	f116 (alt_name: e)	CTGGTTGATCCTGCCACGT	Hamby & Zimmer 1988
28S	f1403	CGGAAGGATTGACAGATTGAGAG	Sequeira et al, 2000
28S	f420	GGCGACGCATCTTTCAAATGTCTG	Sequeira et al, 2000
28S	r1094 (alt_name: P)	CGCTTTCGTAAACGGTT	Hamby & Zimmer 1988
28S	r1138	CGCCTTCGAACCTCTAAC	Sequeira et al, 2000
28S	r1626	GGCATCACAGACCTGTTATTGCTCAATCTC	Sequeira et al, 2000
28S	r1856 (alt_name: Q)	CAGCGAGGATGGCTAACTTA	Hamby & Zimmer 1988
28S	r803	CCACCGGCAGGACGTCTC	Sequeira et al, 2000
28S	S3690F	GAGAGTTMAASAGTACGTGAAAC	Dowton & Austin 1998
28S	S4072 (alt_name: S1)	GACCCGTCTTGAAMCAMGGA	Whiting et al. 1997
Ap1098rev2		ATATTRTTKGGCARYTGDCCKCCC	[note: TmD 61.1 °C] [primer_reference:Danforth et al. 2006]
Ap787for2		TGCTTYGARCCDAGYCTHGATTAYTG	[note: TmD 60.0 °C] [primer_reference:Danforth et al. 2006]
Ap835rev1		GCATHACYTCHCCCACRCTYTTC	[note: TmD 59.8 °C] [primer_reference:Danforth et al. 2006]
ApCADfor1		GGWTATCCCGTDATGGCBMGWGC	[note: TmD 62.1 °C] [primer_reference:Danforth et al. 2006]

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ApCADfor2mod		GATGGGAYCTNRGNAARTTYC	[note: TmD 53.1 °C] [primer_reference:Danforth et al. 2006]
ApCADfor3		CTCHGTKGARTTYGATTGGTGYGC	[note: TmD 60.9 °C] [primer_reference:Danforth et al. 2006]
ApCADfor4		TGGAARGARGTBGARTACGARGTGGTYCG	[note: TmD 63.1 °C] [primer_reference:Danforth et al. 2006]
ApCADfor4		TGGAARGARGTBGARTACGARGTGGYCG	This is not good, do not use.
ApCADrev1mod		GCCATYRCYTCBCCYACRCTYTTCAT	[note: TmD 62.2 °C] [primer_reference:Danforth et al. 2006]
ApCADrev1mod		GCCATYRCTCBCCTACRCTYTTCAT	This is not good, do not use.
ApCADrev4a		GGCCAYTGNGCNGCCACYGTGTCTATYTGYTTNACC	[note: TmD 68.6 °C] [primer_reference:Danforth et al. 2006]
ArgK	ArgK-forB2	GAYTCCGGWATYGGWATCTAYGCTCC	Dole et al. 2010
ArgK	ArgK-revB1	TCNGTRAGRCCCATWCGTCTC	Danforth et al. 2005
ArgK	ArgK-revB2	GTATGYTCMCCRCGRGTACCACG	Dole et al. 2010
cellobiohydrolase	fungCBH1f		
cellobiohydrolase	fungCBH1r		
CO1	1495b	AACAAATCATAAAGATATTGGRAC	Smith and Cognato, 2014
CO1	A2237R	CCGAATGCTTCTTTTTTACCTCTTTCTTG	
CO1	A2411R	GCTAATCATCTAAAAACTTTAATTCCWGTWG	
CO1	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Hebert et al. 2003
CO1	LCO1490	GGTCAACAAATCATAAAGATATTGG	Hebert et al. 2003
CO1	LepF1	ATTCAACCAATCATAAAGATATTGG	Hebert et al. 2004
CO1	LepR1	TAAACTTCTGGATGTCCAAAAAATCA	Hebert et al. 2004
CO1	r750	GAAATTATNCCAATTCCTGG	Smith and Cognato, 2014
CO1	S1718F	GGAGGATTTGGAAATTGATTAGTTCC	
EF1a	A1043	GTATATCCATTGGAAATTTGACCNGGRTGRTT	Normark et al. 1999
EF1a	S149	ATCGAGAAGTTCGAGAAGGAGGCYCARGAAATGGG	Normark et al. 1999
efTu	52F		Ludwig et al
ITS (end of SSU)	ITS1	TCCGTAGGTGAACCTGCGG	
ITS (in 5.8S, for)	ITS3	GCATCGATGAAGAACGCAGC	
ITS (in 5.8S, rev)	ITS2	GCTGCGTTCTTCATCGATGC	
ITS (rev, start of LSU)	ITS4	TCCTCCGCTTATTGATATGC	

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ITS fungal (like ITS1, basidio)	ITS1-F	CTTGGTCATTTAGAGGAAGTAA	
LSU (28S)	JH-LSU-91	AGTAACGGCGAGTGAAG	probably universal for fungi, not for insect, position 91 in S. cerevisiae 28S
LSU (28S)	LR0R	ACCCGCTGAACTTAAGC	identical in insect and fungi
LSU (28S) rev	JH-LSU-377	TTTTCAAAGTGCTTTTC	almost identical to LR2 but with one bp more specific to filamentous ascos, position 377 in S. cerevisiae; doesn't work very well
LSU (28S) rev	JH-LSU-638	GGTCCGTGTTTCAAGAC	reverse complement to Vilgalis's LR3R (which is not the same as reverse complement of LR3)
LSU (28S) rev	JH-LSU-643	CTCCTTGGTCCGTGTTTC	tested with Raffaelea spp., inconsistent product
LSU (28S) rev	JH-LSU-830	GTTTCCTCTGGCTTCACCC	tested with Raffaelea spp., inconsistent product
LSU (28S) rev	LR2	TTTTCAAAGTTCTTTTC	White et al., from yeast, 1bp difference from filamentous ascos
LSU (28S) rev	LR3	CCGTGTTTCAAGACGGG	identical for beetles and fungi
SSU (18S) general	NS1	GTAGTCATATGCTTGTCTC	eukaryotes?
SSU (18S) general	NS4	CTTCCGTCAATTCCTTTAAG	eukaryotes?
SSU fungal	AU2	TTTCGATGGTAGGATAGDGG	Vandenkoornhuyse et al. 2002, Science 295:2051
SSU fungal	AU4	RTCTCACTAAGCCATTC	Vandenkoornhuyse et al. 2002, Science 295:2051
SSU fungal	JH-SSU-304	CCCTATCAACTTTCGATG	upstream of AU2, 2bp difference from Curculionidae, can use with NS2 for 240bp conserved product or with JH-SSU-822 for ~500bp of variable product
SSU fungal (rev)	JH-SSU-1646	CTAAGCCATTCAATCGGTA	6bp upstream of AU4, 2bp difference from Curculionidae
SSU fungal (rev)	JH-SSU-822	CTAGAAACCAACAAAATAG	2bp upstream of SR4, 4bp difference from Curculionidae
SSU rev	NS2	GGCTGCTGGCACCAGACTTGC	550 in S. cerevisiae; eukaryotes?

R= A G (purine) D= G A T

Buying primers

ITS1 example CType: DNA Oligos – modified & non-modified
Oligo Name: …
Synthesis Scale: 0.025 µmol
Purification: Desalt
Sequence (5' to 3'): …
Format: Lab-ready

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Number of Tubes per Oligo: 1
Concentration (μM): NA

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