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Primers for bark beetles and associated fungi

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Bark Beetle Mycobiome Research Coordination Network Bark

Beetle

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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.

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 :
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	Bergman et al 2011 (Fierer lab)
	16S bact +arc	806R	GGACTACVS	Bergman et

	haea rev		GGG TAT CTA AT	al 2011 (Fier er lab)
	28S	A40 53 (alt_ nam e: A1)	TCC KGT KTT CAA GAC GGG GTC	Whit ing et al. 1997
	28S	A42 21 (alt_ nam e: A160)	CGC CTC TTC TCG CAA TGA GA	Seq ueira et al, 200 0
	28S	A42 85 (alt_ nam e: A24 7)	CCT GAC TTC GTC CTG ACC AGG C	Seq ueira et al, 200 0
	28S	A43 94 (alt_ nam e: A33 5)	TCG GAR GGA ACC AGC TAC TA	Whit ing et al. 1997
	28S	D2F1	ACT GTT GGC GAC GAT GTT CT	Jord al etal. (200 8)
	28S	D3R 2	TCT TCG CCC CTA TAC CC	Jord al etal. (200 8)
	28S	f109 4	GGA TCG TCG CAA GAC GGA CAG AAG	Seq ueira et al, 200 0

	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	CCACCGGCA	Sequeira et al,

			GGACGTCTC	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		GCATHACYTCHCCACRCTYTTC	[note: TmD 59.8 °C] [primer_reference :Danforth et al.

				2006]
	ApCADfor1		GGWTATCCCGTDATGGCBMGWGC	[note: TmD 62.1 °C] [primer_reference :Danforth et al. 2006]
	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	CCGAATGCTTCTTTTTACCTCTTTCTTG	
	CO1	A2411R	GCTAATCATCTAAAACTTTAATTCCWGTWG	
	CO1	HCO2198	TAAACTTCA	Hebert et al.

			GGGTGACCAAAAATCA	2003
	CO1	LCO1490	GGTCAACAAATCATAAAGATATTGG	Hebert et al. 2003
	CO1	LepF1	ATTCAACCAATCATAAAGATATTGG	Hebert et al. 2004
	CO1	LepR1	TAAACTTCTGGATGTCCAAAAATCA	Hebert et al. 2004
	CO1	r750	GAAATTATNCCAATTCCTGG	Smith and Cognato, 2014
	CO1	S1718F	GGAGGATTTGGAAATTGATTAGTTCC	
	EF1a	A1043	GTATCCATTGGAATTTGACCN	Normark et al. 1999

			GGRTGRTT	
	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	
	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	TTTTCAAGTGCTTTTC	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	JH-LSU	GGTCCG	reverse

	) rev	-638	TGTTTCAAGAC	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	TTTTCAAGTTCTTTTC	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 240 bp 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	CTAGAACCAACAAAATAG	2bp upstream of SR4, 4bp difference from Curculionidae
	SSU rev	NS2	GGCTGCTGGCACCAG	550 in S. cerevisiae; euka

			ACT TGC	ryot es?

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