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🌐 Primers for bark beetles and associated fungi

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Protocols Bark Beetle Mycobiome

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, Selmann 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
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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 :

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	GGTT ACCT TGTT ACGA CTT	
16S	27F	AGAG TTTG ATCC TGGC TCAG	
16S bact rev	338R	TGCT GCCT CCCG TAGG AGT	Aman n et al. 1995. Micro biol. Rev. 59, 143– 169.
16S bact+ archa ea for	515F	GTGC CAGC MGC CGCG GTAA	Berg mann et al 2011 (Fiere r lab)
16S bact+ archa ea rev	806R	GGAC TACV SGGG TATC TAAT	Berg mann et al 2011 (Fiere r lab)
28S	A4053 (alt_name: A1)	TCCK GTKT TCAA GACG GGGT C	Whitin g et al. 1997
28S	A4221 (alt_name: A160)	CGCC TCTT CTCG CAAT GAGA	Sequ eira et al, 2000
28S	A4285 (alt_name: A247)	CCTG ACTT CGTC CTGA CCAG GC	Sequ eira et al, 2000
28S	A4394 (alt_name: A335)	TCGG ARGG AACC AGCT ACTA	Whitin g et al. 1997

28S	D2F1	ACTG TTGG CGAC GATG TTCT	Jordal etal. (2008)
28S	D3R2	TCTT CGCC CCTA TACC C	Jordal etal. (2008)
28S	f1094	GGAT CGTC GCAA GACG GACA GAAG	Sequ eira et al, 2000
28S	f116 (alt_n ame: e)	CTGG TTGA TCCT GCCA CGT	Hamb y & Zimm er 1988
28S	f1403	CGGA AGGA TTGA CAGA TTGA GAG	Sequ eira et al, 2000
28S	f420	GGCG ACGC ATCT TTCA AATG TCTG	Sequ eira et al, 2000
28S	r1094 (alt_n ame: P)	CGCT TTCG TAAA CGGT T	Hamb y & Zimm er 1988
28S	r1138	CGCC TTCG AACC TCTA AC	Sequ eira et al, 2000
28S	r1626	GGCA TCAC AGAC CTGT TATT GCTC AATC TC	Sequ eira et al, 2000
28S	r1856 (alt_n ame: Q)	CAGC GAGG ATGG CTAA CTTA	Hamb y & Zimm er 1988
28S	r803	CCAC CGGC AGGA CGTC TC	Sequ eira et al, 2000

28S	S3690F	GAGA GTTM AASA GTAC GTGA AAC	Dowt on & Austi n 1998
28S	S4072 (alt_name: S1)	GACC CGTC TTGA AMC AMG GA	Whitin g et al. 1997
Ap10 98rev 2		ATAT TRTT KGGC ARYT GDCC KCCC	[note: TmD 61.1 °C] [prim er_ref erenc e:Dan forth et al. 2006]
Ap78 7for2		TGCT TYGA RCCD AGYC THGA TTAY TG	[note: TmD 60.0 °C] [prim er_ref erenc e:Dan forth et al. 2006]
Ap83 5rev1		GCAT HACY TCHC CCAC RCTY TTC	[note: TmD 59.8 °C] [prim er_ref erenc e:Dan forth et al. 2006]
ApCA Dfor1		GGW TATC CCGT DATG GCB MGW GC	[note: TmD 62.1 °C] [prim er_ref erenc e:Dan forth et al. 2006]

ApCA Dfor2 mod		GATG GGAY CTNR GNAA RTTY C	[note: TmD 53.1 °C] [prim er_ref erence: Dan forth et al. 2006]
ApCA Dfor3		CTCH GTKG ARTT YGAT TGGT GYGC	[note: TmD 60.9 °C] [prim er_ref erence: Dan forth et al. 2006]
ApCA Dfor4		TGGA ARGA RGTB GART ACGA RGTG GTYC G	[note: TmD 63.1 °C] [prim er_ref erence: Dan forth et al. 2006]
ApCA Dfor4		TGGA ARGA RGTB GART ACGA RGTG GYCG	This is not good, do not use.
ApCA Drev1 mod		GCCA TYRC YTCB CCYA CRCT YTTC AT	[note: TmD 62.2 °C] [prim er_ref erence: Dan forth et al. 2006]
ApCA Drev1 mod		GCCA TYRC TCBC CTAC RCTY TTCA T	This is not good, do not use.

ApCA Drev4 a		GGCC AYTG NGCN GCCA CYGT GTCT ATYT GYTT NACC	[note: TmD 68.6 °C] [prim er_ref erence: Dan forth et al. 2006]
ArgK	ArgK- forB2	GAYT CCGG WATY GGW ATCT AYGC TCC	Dole et al. 2010
ArgK	ArgK- revB1	TCNG TRAG RCCC ATW CGTC TC	Danfo rth et al. 2005
ArgK	ArgK- revB2	GTAT GYTC MCC RCGR GTAC CACG	Dole et al. 2010
cellob iohydr olase	fungC BH1f		
cellob iohydr olase	fungC BH1r		
CO1	1495b	AACA AATC ATAA AGAT ATTG GRAC	Smith and Cogn ato, 2014
CO1	A223 7R	CCGA ATGC TTCT TTTT TACC TCTT TCTT G	
CO1	A241 1R	GCTA ATCA TCTA AAAA CTTT AATT CCW GTW G	

C01	HC02198	TAAA CTTC AGGG TGAC CAAA AAAT CA	Hebert et al. 2003
C01	LC01490	GGTC AACA AATC ATAA AGAT ATTG G	Hebert et al. 2003
C01	LepF1	ATTC AACC AATC ATAA AGAT ATTG G	Hebert et al. 2004
C01	LepR1	TAAA CTTC TGGA TGTC CAAA AAAT CA	Hebert et al. 2004
C01	r750	GAAA TTAT NCCA ATTC CTGG	Smith and Cognato, 2014
C01	S1718F	GGAG GATT TGGA AATT GATT AGTT CC	
EF1a	A1043	GTAT ATCC ATTG GAAA TTTG ACCN GGRT GRTT	Normark et al. 1999
EF1a	S149	ATCG AGAA GTTC GAGA AGGA GGCY CARG AAAT GGG	Normark et al. 1999
efTu	52F		Ludwig et al

ITS (end of SSU)	ITS1	TCCG TAGG TGAA CCTG CGG	
ITS (in 5.8S, for)	ITS3	GCAT CGAT GAAG AACG CAGC	
ITS (in 5.8S, rev)	ITS2	GCTG CGTT CTTC ATCG ATGC	
ITS (rev, start of LSU)	ITS4	TCCT CCGC TTAT TGAT ATGC	
ITS fungal (like ITS1, basidi o)	ITS1- F	CTTG GTCA TTTA GAGG AAGT AA	
LSU (28S)	JH- LSU- 91	AGTA ACGG CGAG TGAA G	proba bly univer sal for fungi, not for insect , posi on 91 in S. cerevi siae 28S
LSU (28S)	LR0R	ACCC GCTG AACT TAAG C	identi cal in insect and fungi

LSU (28S) rev	JH- LSU- 377	TTTT CAAA GTGC TTTT C	almo st identi cal to LR2 but with one bp more specif ic to filame ntous ascos , posi on 377 in S. cerevi siae; doesn 't work very well
LSU (28S) rev	JH- LSU- 638	GGTC CGTG TTTC AAGA C	revers e compl emen t to Vilgali s's LR3R (whic h is not the same as revers e compl emen t of LR3)
LSU (28S) rev	JH- LSU- 643	CTCC TTGG TCCG TGTT TC	teste d with Raffa elea spp., incon sisten t produ ct

LSU (28S) rev	JH- LSU- 830	GTTT CCTC TGGC TTCA CCC	teste d with Raffa elea spp., incon sisten t produ ct
LSU (28S) rev	LR2	TTTT CAAA GTTC TTTT C	White et al., from yeast, 1bp differ ence from filame ntous ascos
LSU (28S) rev	LR3	CCGT GTTT CAAG ACGG G	identi cal for beetle s and fungi
SSU (18S) gener al	NS1	GTAG TCAT ATGC TTGT CTC	eukar yotes ?
SSU (18S) gener al	NS4	CTTC CGTC AATT CCTT TAAG	eukar yotes ?
SSU fungal	AU2	TTTC GATG GTAG GATA GDGG	Vand enkoo rnhuy se et al. 2002, Scien ce 295:2 051
SSU fungal	AU4	RTCT CACT AAGC CATT C	Vand enkoo rnhuy se et al. 2002, Scien ce 295:2 051

SSU fungal	JH-SSU-304	CCCT ATCA ACTT TCGA TG	upstream of AU2, 2bp difference from Curculionidae, can use with NS2 for 240bp conserved product or with JH-SSU-822 for ~500 bp of variable product
SSU fungal (rev)	JH-SSU-1646	CTAA GCCA TTCA ATCG GTA	6bp upstream of AU4, 2bp difference from Curculionidae
SSU fungal (rev)	JH-SSU-822	CTAG AAAC CAAC AAAA TAG	2bp upstream of SR4, 4bp difference from Curculionidae
SSU rev	NS2	GGCT GCTG GCAC CAGA CTTG C	550 in <i>S. cerevisiae</i> ; 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

Number of Tubes per Oligo: 1

Concentration (μM): NA