

SI-2. *rpoB* and PacBio primers and thermocycling conditions

Use	Primer Name	Sequence (5' to 3')
Forward Degenerate <i>rpoB</i> Primer		TCCGAYGAGGTGCTSGCNGA
Reverse Degenerate <i>rpoB</i> Primer		SGAYTTGATGGTCARCAGYTCC
PacBio round 1 Forward Primer	myco_rpoB_2572_M13_f4	TGTA AACGACGGCCAGTCCGACGAGGTGCTCGCTGA
PacBio round 1 Forward Primer	myco_rpoB_2572_M13_f6	TGTA AACGACGGCCAGTCCGACGAGGTGCTGGCCGA
PacBio round 1 Forward Primer	myco_rpoB_2572_M13_f8	TGTA AACGACGGCCAGTCCGACGAGGTGCTGGCTGA
PacBio round 1 Forward Primer	myco_rpoB_2572_M13_f10	TGTA AACGACGGCCAGTCCGATGAGGTGCTCGCCGA
PacBio round 1 Forward Primer	myco_rpoB_2572_M13_f13	TGTA AACGACGGCCAGTCCGATGAGGTGCTGGCAGA
PacBio round 1 Forward Primer	myco_rpoB_2572_M13_f14	TGTA AACGACGGCCAGTCCGATGAGGTGCTGGCCGA
PacBio round 1 Reverse Primer	myco_rpoB_3864_M13_r1	CAGGAAACAGCTATGACCGGACTTGATGGTCAACAGCTCC
PacBio round 1 Reverse Primer	myco_rpoB_3864_M13_r3	CAGGAAACAGCTATGACCGGACTTGATGGTCAGCAGCTCC
PacBio round 1 Reverse Primer	myco_rpoB_3864_M13_r5	CAGGAAACAGCTATGACCGGATTTGATGGTCAACAGCTCC
PacBio round 1 Reverse Primer	myco_rpoB_3864_M13_r9	CAGGAAACAGCTATGACCGGACTTGATGGTCAACAGCTCC
<b>Round 2 Primers - Universal</b>		
PacBio round 2 Forward Primer	Forward_Bar01_M13	TCAGACGATGCGTCATTGTAAACGACGGCCAGT
PacBio round 2 Forward Primer	Forward_Bar02_M13	CTATACATGACTCTGCTGTAAACGACGGCCAGT
PacBio round 2 Forward Primer	Forward_Bar03_M13	CGTCTATATACGTATATGTAAACGACGGCCAGT
PacBio round 2 Forward Primer	Forward_Bar04_M13	TGTGTATCAGTACATGTGTAAACGACGGCCAGT
PacBio round 2 Forward Primer	Forward_Bar05_M13	ACACGCATGACACACTTGTAAACGACGGCCAGT
PacBio round 2 Forward Primer	Forward_Bar06_M13	GATCTCTACTATATGCTGTAAACGACGGCCAGT
PacBio round 2 Forward Primer	Forward_Bar07_M13	GCGTATATCTCATGCGTGTAAACGACGGCCAGT
PacBio round 2 Forward Primer	Forward_Bar08_M13	ATGATGTGCTACATCTTGTAAACGACGGCCAGT
PacBio round 2 Reverse Primer	Reverse_Bar01_M13	CTATAGACACAGCGATCAGGAAACAGCTATGACC

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PacBio round 2 Reverse Primer	Reverse_Bar02_M13	<b>CACGCTATCAGTGAGACAGGAAACAGCTATGACC</b>
PacBio round 2 Reverse Primer	Reverse_Bar03_M13	<b>TACATGATAGACGACACAGGAAACAGCTATGACC</b>
PacBio round 2 Reverse Primer	Reverse_Bar04_M13	<b>GATGAGATCTCGTGTGCAGGAAACAGCTATGACC</b>
PacBio round 2 Reverse Primer	Reverse_Bar05_M13	<b>AGTATCATGTGTATCTCAGGAAACAGCTATGACC</b>
PacBio round 2 Reverse Primer	Reverse_Bar06_M13	<b>GCACGTCGTATATCGACAGGAAACAGCTATGACC</b>
PacBio round 2 Reverse Primer	Reverse_Bar07_M13	<b>ATATGTGCAGTCGAGACAGGAAACAGCTATGACC</b>

Bold and underlined bases in the original degenerate primers represent the changes made from the original Macheras *et al.* (2011) primers.

Blue text is the part of the primer that amplifies the *rpoB* gene.

16 bp PacBio barcodes are presented in red.

### PCR THERMOCYCLING CONDITIONS

#### PCR1

98°C x 10 s	}	2 cycles
98°C x 1 s		
54°C x 5 s		
72°C x 14 s		
98°C x 1 s	}	22 cycles
72°C x 5 s		
72°C x 14 s		
72°C x 1min		
4°C x forever		

#### PCR2

98°C x 10 s	}	1 cycle
98°C x 1 s		
52°C x 5 s	}	24 cycles
72°C x 14 s		
98°C x 1 s		
72°C x 5 s		
72°C x 14 s		
72°C x 1 min		
4°C x forever		