S1 Table
A. List of primers

Locus	External primers (5-3)	Length of PCR product	Internal primers (5-3)	Length of PCR product
TP0136	AACCCGTTAGCGCCCAACAT	1789 bp	AGTGTCTTCCTCGTCCGTTC	1206 bp
	TCCCAGCTCAGCCGAATCTC		CACGTGGTGGTGTCAAACTT	
TP0548	TGGGGCACTAAACCGGAAGA	1567 bp	GCGGTCCCTATGATATCGTGT	1065 bp
	TACGGGCATTTGCGGATAGG		GAGCCACTTCAGCCCTACTG	
TP0705	GGTCTATATGCAGCCCTTCTTC	1181 bp	TGCGGCTTATCCTGATGAATAG	803 bp
	GCTTGAGAACGATACCGGATAC		TATTCTGCGGCGTTGGATAG	
23S rDNA[1]	CGAAGGGAAGCAGGTGTAGT	1666 and 1658 bp	GTACCGCAAACCGACACAG	629 bp
	GCGCGAACACCTCTTTTTAC		AGTCAAACCGCCCACCTAC	
	GAACCGTCCCTGAAAACTCA			
TP0319	CTGCTCATCGGCTGCTCTA	733 bp	GAAGGTGGTGACTTCGTCGT	451 bp
	ACCACAGACTTCGACCCATC		CAAAACCCGCTTCAAAGAGA	
TP0105	TTCTGTGCTCACGTCTGGTC	637 bp	TGCGCGTGTGCGAATGGTGTGGTC	376 bp
	TGCAACCATCGTATCGAAAA		CACAGTGCTCAAAAACGCCTGCACG	

[1]Both copies of 23S rDNA gene were amplified

B. First step reaction with 1 µl of DNA

Compound	Volume (µl)	Final volume
water	16.3	25 µl
2.5 mM deoxynucleotide triphosphate (dNTP)	2	
5x PS GXL buffer	5	
Primer F (100 pmol/µl)	0.095	
Primer R (100 pmol/µl)	0.095	
DNA**	1	
PrimeSTAR GXL polymerase*	0.5	

*Takara, BioEurope, France

** DNA of TPA strain Nichols (5 pg/µl) was used as a positive control; distilled water was used as a negative control.

Phase	Step	Temperature	Time	No. of cycles
1	First denaturation	94 °C	1 min	1
2	Denaturation	98 °C	10 s	8
	Annealing	68 °C*	15 s	
	Extension	68 °C	1 min 45 s	
3	Denaturation	98 °C	10 s	35
	Annealing	61 °C	15 s	
	Extenaion	68 °C	1 min 45 s	
4	Final extension	68 °C	7 min	1

*−1.0 °C per cycle

C. First step reaction with 10 µl of DNA

Compound	Volume (µl)	Final volume
water	11.5	25 µl
10 mM dNTP mixture	0.5	
ThermoPol Reaction buffer	2.5	
Primer F (100 pmol/µl)	0.25	
Primer R (100 pmol/µl)	0.25	
DNA**	10	
Taq polymerase*	0.1	

*5,000 U/ml; New England BioLabs, Ipswich, MA

** DNA of TPA strain Nichols (5 pg/µl) was used as a positive control; distilled water was used as a negative control.

Phase	Step	Temperature	Time	No. of cycles
1	First denaturation	94 °C	1 min	1
2	Denaturation	94 °C	20 s	8
	Annealing	55 °C*	20 s	
	Extension	72 °C	1 min 45 s	
3	Denaturation	94 °C	20 s	35
	Annealing	48 °C	20 s	
	Extension	72 °C	1 min 45 s	
4	Final extension	72 °C	7 min	1

* −1.0 °C per cycle

Note: Conditions of PCR amplification of TP0319 and TP0105: 94 °C (1 min); 94 °C (30 s), 58 °C (30 s), 72 °C (1 min) for 30 cycles; and 72 °C (10 min).

D. Second step reaction

Compound	Volume (µl)	Final volume
water	20.5	25 µl
10 mM dNTP mixture	0.5	
ThermoPol Reaction buffer	2.5	
Primer F (100 pmol/µl)	0.25	
Primer R (100 pmol/µl)	0.25	
PCR product from first step	1	
Taq polymerase*	0.1	

*5,000 U/ml; New England BioLabs, Ipswich, MA

Phase	Step	Temperature	Time	No. of cycles
1	First denaturation	94 °C	1 min	1
2	Denaturation	94 °C	30 s	40
	Annealing	48 °C	30 s	
	Extension	72 °C	1 min 15 s	
3	Final extension	72 °C	7 min	1

Note: Conditions for TP0319 and TP0105: 94 °C (1 min); 94 °C (30 s), 58 °C (30 s), 72 °C (1 min) for 40 cycles; and 72 °C (10 min).