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Conventional PCR primers for the detection of grapevine pathogens disseminated by propagating material

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Summary: Polymerase chain reaction driven by sequence specific primers has become the most widely used diagnostic method to detect and identify plant pathogens. The sensitive and cost-effective pathogen detection is exceptionally important in the production of propagating material. In this paper we have collected primer sequence data from the literature for the detection of the most important grapevine pathogens disseminated by propagating stocks by conventional polymerase chain reaction. Basic protocols to obtain template nucleic acids have also been briefly rewieved.

Keywords:bacteria, fungi, phytoplasmas, polymerase chain reaction, primer sequences, viroids, viruses, *Vitis vinifera*

Grapevine cultivation is endangered by several pathogens disseminated in latent form by propagating material. These pathogens include several viroids, viruses, phytoplasmas, bacteria and fungi (*Bisztray* et al. 2012, *Martelli* 2014). Their presence in the plant frequently cannot be recognized by symptoms in the plantations, but disease outbreak may take place in the later stage of infection or in the new plantations under favourable environmental conditions. Therefore the use of healthy planting material has a vital importance. Pathogen-free grapevine stocks can be selected from the existing plantations by various diagnostic protocols or produced by various curative (e. g., hot water) treatments and *in vitro* shoot tip and/or apical meristem cultures (*Bisztray* et al. 2012). The pathogen-free status of hot water treated and micropropagated plants should also be tested by appropriate diagnostic methods.

During the recent decades the polymerase chain reaction (PCR) has become the most common diagnostic protocol in plant pathology. The introduction of the conventional PCR (including multiplex, nested and reverse trascription PCR) has opened wide application possibilities due to its simplicity and cost effectiveness. Later, it was followed by the more sensitive, but also more costly, quantitative real-time PCR (qRT-PCR). Quantitative real-time PCR protocols have already been developed for several viroids (*Papayiannis* 2014, *Sun* et al. 2014) , viruses (*Harper* et al. 2011, *Osman* & *Rowhani* 2008, *Pacificio* et al. 2011), phytoplasmas (*Angelini* 2010, *Fahrentrapp* et al. 2013, *Pelletier* et al. 2009), bacteria (*Dreo* et al. 2007, *Harper* et al. 2010, *Johnson* et al. 2013) and fungi (*Martin* et al. 2012, *Schena* et al. 2004).

These protocols are based on the amplification of given nucleic acid sequences derived from plants or isolated microbes. Since viroids, viruses and phytoplasmas are closely associated with plant cells they are detected from total plant nucleic acid (RNA or DNA) preparations. Bacteria and fungi can be recovered from grapevine bleeding sap or from plant tissues on appropriate culture media thus they can be detected and identified both from total plant DNA and pure cultures. Recently, a medium for recovery of axenic phytoplasma cultures has also been developed (*Contaldo* et al. 2012) that will allow the identification of phytoplasmas from isolated colonies as well in the future.

For nucleic acid (RNA or DNA) isolation several protocols have been developed during the last decades. For rapid field sampling the use of FTA cards (Whatman) can be proposed that allows a simple collection, transport and storage of nucleic acids derived from tissue sap or from homogenized tissues. Such samples then can be processed in PCR (*Grund* et al. 2010) to detect plant pathogens. Kits specifically developed for plant nucleic acids isolation (e. g., Spectrum™ Plant Total RNA Kit produced by Sigma-Aldrich and RNeasy Plant RNA Mini Kit produced by Qiagen) are also widely used for diagnostic purposes (*Cseh* 2012, *Gambino* & *Gribaudo* 2006, *Li* et al. 2011, *Nassuth* et

al. 2000, *Ragozzino* et al. 2004). Conventional nucleic acid isolation protocols include the use of SDS/phenol extraction (*Franke* et al. 1995, *Ragozzino* et al. 2004), CTAB (*Gambino* et al. 2008, *Li* et al. 2008, *Lodhi* et al. 1994, *Steenkamp* et al. 1994, *Zhang* et al. 2012), guanidine isothiocyanate (*Gambino* et al. 2006, *MacKenzie* 1997, *Vasanthaiah* et al. 2008), and silica-based methods (*Digiaro* 2007, *Gambino* 2006, *Hajizadeh* et al 2012, *Sun* et al. 2014). These protocols are also suitable to isolate nucleic acids from bacteria or fungi (*Aroca* & *Raposo* 2007, *Botha* et al. 2001, *Hamelin* et al. 1996, *Neuhauser* et al. 2009, *Rodrigues* et al. 2003, *Szegedi* 2003). For PCR analysis of pure bacterial cultures a simple triton/sodium-azide lysis (*Abolmaaty* et al. 2002) was found highly appropriate (*Szegedi* & *Bottka* 2002). Specific nucleic acid sequences can be enriched by magnetic capture hybridization for subsequent PCR analysis (*Johnson* et al. 2013).

Primer sequence data for a given pathogen group have already been published by several authors (e. g., *Angelini* 2010, *Constable* et al. 2010, *Ghignone* & *Migheli* 2005, see also: http://www.sppadbase.ipp.cnr.it, OEEP/EPPO 2005, *Palacio-Bielsa* et al. 2009, *Thompson* et al. 2014). In the recent paper we collected a dataset of primers for conventional PCR (and reverse-transcription PCR for viroids and viruses) from the literature for the detection of various grapevine pathogens which have or may have significant importance in the production of propagating stocks. These groups of pathogens include viroids (Table 1.), the most common viruses including the newly descibed ones that may have potential importance in the future (Table 2.), phytoplasmas (Table 3.), bacteria (Table 4.) and fungi causing wooden infection frequently followed by trunk death (Table 5.). For the direct practical use of data described in this paper we list the primer sequences and the gene they are specific for, their published annealing temperatures and the length of the amplified fragments. The aim of our work is to provide comprehensive data for grapevine research and quarantine laboratories that work in studying or controlling grapevine pathogens and the plant health status of the propagation material stocks.

Table 1. Viroid-specific primers

Viroid	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Reference
AGVd	c2- h2+	TTCGGTGAGTACCACAGGAAC ACTCGTCCCAGCGGTCCCAAC	55	130	Wan Chow Wah & Symons, 1997
AGVd	Fw Rev	GTCGACGAAGGGTCCTCAGCAGAGCACC GTCGACGACGAGTCGCCAGGTGAGTCTT	60	369	Eiras et al. 2006
AGVd	AGVd-P7 AGVd-P8	ACCTGCAGGGAAGCTAGCTGGGTC CCCTGCAGGTTTCGCCAGCAAGCGC	56	369	Jiang et al. 2009b
AGVd	AGVd-mF AGVd-mR	GTCCTCAGGAGAGCACCGG GAAAACTGGTTGGGACCGCTG	60	195	Hajizadeh et al. 2012
CEVd	CEVd-Fw CEVd-Rev	GGAAACCTGGAGGAAGTCG CCGGGGATCCCTGAAGGA	60	375 (fl)	Eiras et al. 2006
CEVd	CEVd-mF CEVd-mR	GTGTCCTTCCTTTGGCTGCTG TGGCCCGGAGAACAGTGAAG	51	153	Hajizadeh et al. 2012
GHVd	F1/R1	TTACGGAATTCTACCCCCTCCCCAGCAGATGAGATCTTTAAGTTTCGTCC TTTTGGACTCGTCAGGAGCACCACA	nd	377	Wu et al. 2012
GHVd	F2/R2	CACGAAGTTTAACCTTCGTAAGTCGGGCACTGTGTGGTG ACTTCGTGGGAAAAGGTTACCGGCTAAGGCTTGGACCGG	nd	382	Wu et al. 2012
GHVd	F3/R3	CTCTGGCAGATTCGCTCCTAGGCTAGAACCGGTCCA CAGAGCTCCAACCTCAAGAATGGCAGCTAACCTTCCCC	nd	379	Wu et al. 2012
GYSVd-1	GV1M GV2P	GCGGGGGTTCCGGGGATTGC TAAGAGGTCTCCGGATCTTCTTGC	66	365-370 (fl)	Polivka et al. 1996
GYSVd-1	PBCVd100C PBCVd194H	AGACCCTTCGTCGACGACGA TGTCCCGCTAGTCGAGCGGA	56	220	Nakaune & Nakano 2006
GYSVd-1	Fw Rev	GAGGTCCTCGGATCAC AGAGCGCAATGCTGAATAGGC	60	222	Eiras et al. 2006
GYSVd-1	GYSVd-PF GYSVd-PR	TTGGATCCCACCTCGGAAGGCCGCC TTGGATCCTAACCACAGGAACCACA	56	365-370 (fl)	Jiang et al. 2009a
GYSVd-1	GYSVd-1-mF GYSVd-1-mRl	CAAAGCCCTTTTTCTTTCAACTGAG CCCAGAGCAGCGTGGTCC	52	249	Hajizadeh et al. 2012
GYSVd-2	cl- h3+	ACCGGCTTCGGAGATAGAAG AATGAGCCTCGTCGTCGA	56	123	Wan Chow Wah & Symons, 1997

Viroid	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Reference
GYSVd-2	c2- hl+	CCGAGGTGTAACCACAGGAACC TTGAGGCCCGGCGAAACGC	55	194	Wan Chow Wah & Symons, 1997
GYSVd-2	Fw Rev	TTGAGGCCCGGCGAAACGC ACCGGCTTCGGAGATAGAAG	60	363 (fl)	Eiras et al. 2006
GYSVd-2	GYSVd-2-P1 GYSVd-2-P2	ACTAGTACTTTCTTCTATCTCCGAAGC ACTAGTCCGAGGACCTTTTCTAGCGCTC	56	363 (fl)	Jiang et al. 2009c
GYSVd-3 (=CGVd)	GYSVd-PF GYSVd-PR	TTGGATCCCACCTCGGAAGGCCGCC TTGGATCCTAACCACAGGAACCACA	56	366 (fl)	Jiang et al. 2009a
HSVd	HV1M HV2P	GGCTCAAGAGAGGATCCGCG CCTCTGGGGAATTCTCGAGTTGC	65	295-300 (fl)	Polivka et al. 1996
HSVd	F R	CTGGGGAATTCTCGAGTTGCC AGGGGCTCAAGAGAGGATCCG	50	297 (fl)	Farkas et al. 1999
HSVd	HSV-83M HSV-78P	AACCCGGGGCTCCTTTCTCA AACCCGGGGCAACTCTTCTC	50 56	~300 (fl)	Sano et al. 2001
HSVd	Fw Rev	ACTCTTCTCAGAATCCAGCGAG TGCCCCGGGGCTCCTTTCTCAGGT	60	297-302 (fl)	Eiras et al. 2006

AGVd = Australian grapevine viroid, CEVd = Citrus exocortis viroid, CGVd = Chinese grapevine viroid, HSVd = Hop stunt viroid, GYSVd-1, -2 and -3 = Grapevine yellow speckle viroid-1, -2 and-3, and GHVd = Grapevine hammerhead viroid, nd = no data, fl = proper full length viroid could be amplified
*published annealing temperatures

Table 2. Virus-specific primers

Virus*	Primer name	Primer sequence (5'-3')	At/Tm (°C)**	Fragment length (nt)	Gene	Reference
GFLV	GFLV H2999 GFLV C3310	TCGGGTGAGACTGCGCAACTTCCTA GATGGTAACGCTCCCGCTGCTCTT	52	311	RNA2-polyprotein	MacKenzie et al. 1997
GFLV	GFLVFw GFLVRev	ATGCTGGATATCGTGACCCTGT GAAGGTATGCCTGCTTCAGTGG	56	118	RNA1-polyprotein	Gambino & Gribaudo 2006
GFLV	F CPS R EV00N1	TTGTGCGCCCAGATCTCTCTTTACCA GACTACACATATATACACTTGGGTCTTTTAA	55	555	RNA2-polyprotein	Demangeat et al. 2004
GFLV	GFLV V1/F GFLV C1/R	ACCGGATTGACGTGGGTGAT CCAAAGT0TGGTTTCCCAAGA	nd	322	RNA2-polyprotein	Sanchez et al. 1991
GFLV	GFLVpoly5238FwGFLV poly6048Rev	ATCGATACTCCTGGTACG ATAAAGCTCGACAAGTGC	52	828	RNA1-polyprotein	Pacifico et al. 2011
GFLV	GFLV CP 433V GFLV CP 912C	GAACTGGCAAGCTGTCGTAGAAC GCTCATGTCTCTCTGACTTTGACC	58	711	RNA2-polyprotein	Osman & Rowhani 2006
GFLV	GFLV-sp s Nepo-A a	TCAGATTTTAAGGGCGTCCA TGDCCASWVARYTCYCCATA	50	260	RNA2-polyprotein	Digiaro et al. 2007
ArMV	ArMVFw ArMVRev	TGACAACATGGTATGAAGCACA TATAGGGCCTTTCATCACGAAT	56	402	RNA1-polyprotein	Gambino & Gribaudo 2006
ArMV	H1124 C1642	CAGCGGATTGGGAGTTCGT TTGGCCCAGATATAGCGTAAAAAT	50	517	RNA2-polyprotein	MacKenzie et al. 1997
ArMV	H428 C867	GCGGCGGATTGGGAGTT CGATGGTAGGGGGAGCGTATT	54	440	RNA2-polyprotein	Nassuth et al. 2000
ArMV	ArMV-sp s Nepo-A a	GCGGGAATATATCTGAAA TGDCCASWVARYTCYCCATA	60	301	RNA2-polyprotein	Digiaro et al. 2007
ArMV	Upper Lower	CGGATTGGGAGTTCGTTGTCG CCGTTCCATTCACTAACAACTC	50	340	RNA2-polyprotein	Bertolini et al. 2001
ArMV	ArMV-CP1202F ArMV CP1313R	CTGTGCCATCCTTCCCCAATGAT GAGATGCTCCATCCATGCCAGT	58	294	RNA2-polyprotein	Osman & Rowhani 2006
ToRSV	TRSV-sp s Nepo-A a	CAGTGAGGATGCACGCCCC TGDCCASWVARYTCYCCATA	50	202	RNA2-polyprotein	Digiaro et al. 2007
ToRSV	ToRSV5(fw) ToRSV6(rev)	AGGTAGGACGCYATTGTTCCAGG AGTCTCAACTTAACATACCACTAC	54	428	RNA1-polyprotein 3'UTR	Li et al. 2011

Virus*	Primer name	Primer sequence (5'-3')	At/Tm (°C)**	Fragment length (nt)	Gene	Reference
ToRSV	ToRSV g-V/F ToRSV g-C/R	GTCACTACTCTTAACGCTAACC CCACTCATACCTCCAGTCATC	58	444	RNA2-polyprotein	Osman et al. 2008
NepoA	Nepo-A s Nepo-A a	GGHDTBCAKTMYSARRARTGG TGDCCASWVARYTCYCCATA	50	256	RNA2-polyprotein	Digiaro et al. 2007
GCMV	GCMV 5'3140 GCMV 3'3831	CATGGTCTAGCCACTAGGAG GTAGTGGCACACATGATGGC	54	692	RNA2-polyprotein	Brandt & Himmler 1995
GCMV	Nepo-B s GCMV-sp-a	ATGTGYGCHACYACWGGHATGCA CTGCAAGGGAACTTGATAAGGG	50	187	RNA2-polyprotein	Digiaro et al. 2007
TBRV	Nepo-B s TBRV-sp-a	ATGTGYGCHACYACWGGHATGCA ATGACACTCTAGAAGAAAGTTG	50	486	RNA2-polyprotein	Digiaro et al. 2007
NepoB	Nepo-B s Nepo_B a	ATGTGYGCHACYACWGGHATGCA TTCTCTDHAAGAAATGCCTAAGA	50	391	RNA2-polyprotein	Digiaro et al. 2007
GBLV	GBLV-CPs GBLV-CPa	AGTGCCCTTTTAGCCGATACCAG TCACTTAAGTGGCGTTACGCT	58	1565	RNA2-polyprotein	Elbeaino et al. 2011
GLRaV-1	LR1CPF1 LR1CPR1	CTAGCGTTATATCTCAAAATGA CCCATCACTTCAGCACATAAA	nd	502	coat protein	Engel et al. 2010
GLRaV-1	GLRaV-1Fw GLRaV-1Rev	TCTTTACCAACCCCGAGATGAA GTGTCTGGTGACGTGCTAAACG	56	232	coat protein	Gambino & Gribaudo 2006
GLRaV-1	GLRaV1poly230Fw GLRaV1poly1227Rev	CCACGATAAGYGACAACTTCCCGA CGTTAACTTGAGRTCGAACCCAA	50	998	RNA dependent RNA polymerase	Pacifico et al. 2011
GLRaV-1	HSP70-417 F HSP70-737 R	GAGCGACTTGCGACTTATCGA GGTAAACGGGTGTTCTTCAATTCT	58	320	HSP70-like protein	Osman et al. 2007
GLRaV-1	LQV1-H47 LEV1-C447	GTTACGGCCCTTTGTTTATTATGG CGACCCCTTTATTGTTTGAGTATG	60	401	coat protein	Osman & Rowhani, 2006
GLRaV-1	LR1 HSP70-149 f LR1 HSP70-293 r	ACCTGGTTGAACGAGATCGCTT GTAAACGGGTGTTCTTCAATTCTCT	60	145	HSP70-like protein	Osman et al. 2007
GLRaV-1	LR1-9727U LR1-10019D	TCGTAACGGCCGCTTCAGTA GTTCGTAACGTGCACGGAAG	56	303		Nakaune & Nakano 2006
GLRaV-2	LR2 12474U LR2 12806D	TTGACAGCAGCCGATTAAGCG CTGACATTATTGGTGCGACGG	56	333	HSP90-like protein	Nakaune & Nakano 2006
GLRaV-2	LR2 V2dCPf2 LR2 V2CPr1	ACGGTGTGCTATAGTGCGTG GCAGCTAAGTACGAATCTTC	nd	514	minor and major coat protein	Bertazzon & Angelini 2004
GLRaV-2	GLRaV-2F GLRaV-2R	GGTGATAACCGACGCCTCTA CCTAGCTGACGCAGATTGCT	56	543	major coat protein	Gambino & Gribaudo 2006
GLRaV-2	LR2-U2 LR2-L2	ATAATTCGGCGTACATCCCCACTT GCCCTCCGCGCAACTAATGACAG	52	331	HSP70-like protein	Bertazzon & Angelini 2004
GLRaV-2	CPV F CPC R	TGGAGTTGATGTCCGACAGC ACGACCGAACGTTCTAAGTT	58	338	major coat protein	Osman et al. 2007
GLRaV-2	RGHSP227V/F RGHSP777C/R	GCGACTCCAGCAACTTTAGTGA GTCTAACGAAAGATCGGGTTCTAAG	58	551	HSP70-like protein	Osman et al. 2008
GLRaV-3	LR3 LC1F LR3 LC2R	CGCTAGGGCTGTGGAAGTATT GTTGTCCCGGGTACCAGATAT	52	546	HSP70-like protein	Turturo et al. 2005
GLRaV-3	GLRaV-3F GLRaV-3R	TACGTTAAGGACGGGACACAGG TGCGGCATTAATCTTCATTG	56	336	coat protein	Gambino & Gribaudo 2006
GLRaV-3	LR3-H330 LR3-C629	GATGCTTTCGCGTATTTCTTG CGGCACGATCGTACTTTCTAA	54	300	HSP90-like protein	Mackenzie et al. 1997

Virus*	Primer name	Primer sequence (5'-3')	At/Tm (°C)**	Fragment length (nt)	Gene	Reference
GLRaV-3	CP-111 F CP-722 R	AAAGTAGGTTAAGGACGGGACACA AGGGTCGCCGTGATGAAG	58	612	coat protein	Osman et al. 2007
GLRaV-3	GLRaV3-56f GLRaV3-285r	AAGTGCTCTAGTTAAGGTCAGGAGTGA GTATTGGACTACCTTTCGGGAAAAT	60	230	HSP70-like protein	Bertolini et al. 2010
GLRaV-3	GLRaV3poly7209Fw GLRaV3poly8145Rev	GGGTTGTGACGACTCTGTGGCGCAT GATATCTGAAGTTTTGGAAGCT	52	958	RNA-dependent RNA polymerase	Pacifico et al. 2011
GLRaV-3	LR3-8033U LR3-8408D	TTACGGCACAAACGCTACCAG CTGGTGTGGTAGAGTAGTTCC	56	376	HSP90-like protein	Nakaune & Nakano 2006
GLRaV-3	H587(Fw) C547 (Rev)	ATAAGCATTCGGGATGGACC ATTAACTTGACGGATGGCACGC	62	340	RNA-dependent RNA polymerase	Minafra & Hadidi 1994
GLRaV4	LR4 HSPV-F LR4 HSPV-R	ACATTCTCCACCTTGTGCTTTT CATACAAGCGAGTGCAATTACA	58	321	HSP70-like protein	Osman et al. 2007
GLRaV5	HPPV F HSPV R	AACACTCTGCTTTTCTGCTGGCA TCTCCAGAAGACGGACCAATGTAA	58	273	HSP70-like protein	Osman & Rowhani 2006
GLRaV5	HSP70-29F HSP70-634R	CACTCTGCTTTTCTGCTGGCA CGACGCACAGACGTAGGATGA	58	600	HSP70-like protein	Osman et al. 2007
GLRaV4-5	HSP45A HSP45B	GTATCTYATGTACCAACAGAT GGTATGAACAARTTCAATGC	53	370	HSP70-like protein	Routh et al. 1998
GLRaV7	LR7F LR7R	TATATCCCAACGGAGATGGC ATGTTCCTCCACCAAAATCG	nd	502	HSP70-like protein	Engel et al. 2008
GLRaV9	LR9F LR9R	CGGCATAAGAAAAGATGGCAC TCATTCACCACTGCTTGAAC	58	393	HSP70-like protein	Alkowni et al. 2004
GVA	GVA Fw GVA Rev	GAGGTAGATATAGTAGGACCTA TCGAACATAACCTGTGGCTC	56	272	coat protein	Gambino & Gribaudo 2006
GVA	GVA 6540U GVA 6880D	TTTGGGTACATCGCGTTGGT TCTAAGCCCGACGCGAAGT	56	341	coat protein	Nakaune & Nakano 2006
GVA	GVAH587 V1/F GVAC995 C1/R	GACAAATGGCACACTACG AAGCCTGACCTAGTCATCTTGG	62	430	coat protein	Minafra & Hadidi 1994
GVA	GVADeg7Fw GVADeg4Rev	MRNCCMGARTAYGATGC TRTARAABGCYACCTC	52	652	replicase	Pacifico et al. 2011
GVA	GVA-H7038 GVA-C7273	AGGTCCACGTTTGCTAAG CATCGTCTGAGGTTTCTACTAT	54	240	putative RNA binding protein	MacKenzie 1997
GVB	GVB H28 GVB C410	GTGCTAAGAACGTCTTCACAGC ATCAGCAAACACGCTTGAACCG	56	460	putative RNA binding protein	Minafra & Hadidi 1994
GVD	GVDORF2-F1 GVDORF2-R1	TAATAGGGCCTAAGTC GGGCGTTGAATACACCTTTAGC	50	371	coat protein	Lebas & Ward 2012
GVD	D_ORF/F D_ORF/R	CTTAGGACGCTCTTCGGGTACA CTGCTCTCCAACCGACGACT	58	465	coat protein	Abou-Ghanem et al. 1997
GVD	GVDmu-554f GVDmu-661r	AGGTGTATTCAACGCCCAGTC GCCCTACGCTTCTTAGCATAACTAC	57	108	coat protein and RNA binding protein	Osman et al. 2013
GVA, GVB, GVD	VT-165 F1 VT-594 R1	ACYCTCTTYGGGTACATHGC GCBCCYTCHGTVCGAAAGAG	55	429	coat protein	Osman et al. 2013
GVE	GVE-1For GVERev	AATGGAGTCAAAAGCGATCC GTAGGGTCAATCAACCAACA	55	992	coat protein	Coetzee et al. 2010
GFkV	GFkVF GFkVR	TGACCAGCCTGCTGTCTCTA TGGACAGGGAGGTGTAGGAG	56	179	coat protein	Gambino & Gribaudo 2006

Virus*	Primer name	Primer sequence (5'-3')	At/Tm (°C)**	Fragment length (nt)	Gene	Reference
GFkV	RD1 RD2	CYCARCAYAARGTVAACGA GCGCATGCABGTSAGRGGG	56	386	replicase	Nakaune & Nakano 2006
GFkV	GFk V1/F GFk C1/R	GGTCCTCGGCCCAGTGAAAAAGTA GGCCAGGTTGTAGTCGGTGTTGTC	58	352	replicase	Osman et al. 2008
GFkV	FL CP V FL CP C	CCTCGTGTAAGCATCCATCT CCGAAGACGGAGAGGATCTC	58	260	coat protein	Osman & Rowhani, 2006
GFkV	GFkV OB F GFkV OB R	CGAGAACTCTCTTTTCACCTC CCGGCGTGGATGTAGAG	60	147	replicase	Bertolini et al. 2010
GFkV	GFkVrep4944Fw GFkC1m/R	GCGYATCCTYGACRGCCG GCCAGGYTGTAGTCGGTGTTGT	52	324	replicase	Pacifico et al. 2011
GRSPaV	RSP-up1 RSP-do2	TGAGATGGTYGCTAATATCG CTATTAGTACGGTATTCCAG	56	242	coat protein + 3'UTR	Nakaune & Nakano 2006
GRSPaV	RSPaVFw RSPaVRev	GGGTGGGATGTAGTAACTTTTGA GCAAGTGAAATGAAAGCATCACT	56	155	replicase	Gambino & Gribaudo 2006
GRSPaV	48V 49C	AGCTGGGATTATAAGGGAGGT CCAGCCGTTCCACCACTAAT	52	330	coat protein	Lima et al. 2006
GRSPaV	RSP-H5638 RSP-C5992	AGGGATTGGCTGTTAGATGTT CTTCAGGCAACCCCAAAAA	54	355	replicase	MacKenzie, 1997
GPGV	GPgV5619f GPgV6668r	TGGGAGGATTGCAACAGGGGCT ATTCGCCTCGCTCAACTTTGCGA	nd	1049	movement protein	Giampetruzzi et al. 2012
GPGV	GPGVFw GPGVRev	ATGTCGATTCGTCAGGAGCTG CTACATACTAAATGCACTC	nd	588	coat protein	Cho et al. 2013
GPGV	GPG-5637F GPG5939R	ATTGCGGAGTTGCCTTCAAAG CTGAGAAGCATTGTCCCA	56	303	movement protein	Glasa et al. 2014
GPGV	GPG6609F GPG7020R	GAGATCAACAGTCAGGAGAG GACTTCTGGTGCCTTATCAC	56	412	coat protein	Glasa et al. 2014
GRVFV	GVFVC105F1 GVFVC105R2	CCTGTCGCTTCCTTCTCATCT CATCTTCCATGCCCATTTCTTG	nd	323	polyprotein1	Al Rwahnih et al. 2009
GSyV-1	Det-F Det-R	CAAGCCATCCGTGCATCTGG GCCGATTTGGAACCCGATGG	nd	296	putative movement protein	Al Rwahnih et al. 2009
GAMaV	GAMaVF3 GAMaVR2	GCATCTCCAATCTGTCCCAT AGTACGAGGTCTCGGTGGTG	nd	479	replicase-associated polyprotein	Al Rwahnih et al. 2009
GVCV#	GVCV-F1 GVCV-R1	CACGTTTCAAAGAAAGATGGAC ATCCKTCCATGCAWCCGTCAG	52	526	polyprotein ORF3	Zhang et al. 2011
GRBaV#	GRBaVF1 GRBaVR1	CTCGTCGCATTTGTAAGA ACTGACAAGGCCTACTACG	60	557	V2 protein	Al Rwahnih et al. 2013

*ArMV: Arabis mosaic virus, GAMaV: Grapevine asteroid mosaic-associated virus, GBLV: Grapevine Bulgarian latent virus, GCMV: Grapevine chrome mosaic virus, GFLV: Grapevine fanleaf virus, GFkV: Grapevine fleck virus, GLRaV-1, -2, -3, -4, -5, -7 and -9: Grapevine leafroll-associated virus-1, -2, -3, -4, -5, -7 and -9, respectively, GPGV: Grapevine pinot gris virus, GRBaV: Grapevine red blotch-associated virus, GRSPaV: Grapevine rupestris stem pitting-associated virus, GRVFV Grapevine rupestris vein-feathering virus, GSyV-1: Grapevine Syrah virus-1, GVCV: Grapevine vein clearing virus, GVA: Grapevine virus A, GVB: Grapevine virus B, GVD: Grapevine virus D, GVE: Grapevine virus E, Nepo A: Nepovirus subgroup A, Nepo B: Nepovirus subgroup B, TBRV: Tomato black ring virus, ToRSV: Tomato ringspot virus

**published annealing temperatures, nd = no data

#: GRBaV and GVCV are DNA viruses.

Table 3. Phytoplasma specific primers

Phytoplasma	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Target gene	Reference
Universal	P1 P7	AAGAGTTTGATCCTGGCTCAGGATT CGTCCTTCATCGGCTCTT	50	1850	16S and intergenic 16S–23S and a small part of 23S rRNA gene	Deng and Hiruki 1991, Schneider et al. 1995
Universal	fU5 rU3	CGGCAATGGAGGAAACT TTCAGCTACTCTTTGTAACA	55	880	16S rRNA gene	Lorenz et al., 1995

Phytoplasma	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Target gene	Reference
Universal	R16F2n R16R2	GAAACGACTGCTAAGACTGG TGACGGGCGGTGTGTACAAACCCCG	55	1250	16S rRNA gene	Lee et al., 1998
Flavescence dorée (FD) (16SrV-C, D)	FD9f1 FD9r1	GAATTAGAACTGTTTGAAGACG TTTGCTTTCATATCTTGTATCG	55	1300	Non ribosomal DNA	OEEP/EPPO 2007**, Daire et al., 1997
Flavescence dorée (16SrV-C, D)	FD9f3b FD9r2	TAATAAGGTAGTTTTATATGACAAG GACTAGTCCCGCCAAAAG	55	1150	Non ribosomal DNA	OEEP/EPPO 2007**, Clair et al., 2003a, 2003b, Angelini et al., 2001
Bois noir (BN, Stolbur) (16SrXII-A)	STOL11f2 STOL11r1	TATTTTCCTAAAATTGATTGGC TGTTTTTGCACCGTTAAAGC	55	830	Non ribosomal DNA	OEEP/EPPO 2007**, Daire et al., 1997, Clair et al. 2003a, 2003b
Bois noir	STOL11f3 STOL11r2	ACGAGTTTTGATTATGTTCAC GATGAATGATAACTTCAACTG	55	720	Non ribosomal DNA	OEEP/EPPO 2007**, Clair et al. 2003b
16S group I,II,XII,XV specific	R16(I)F1 R16(I)R1	TAAAAGACCTAGCAATAGG CAATCCGAACTAAGACTCT	50	1100	16S rRNA gene	Lee et al., 1994
16S group III specific	R16(III)F1 R16(III)R1	AAGAGTGGAAAAACTCCC TTCGAACTGAGATTGA	50	800	16S rRNA gene	Lee et al., 1994
16S group V specific	R16(V)F1 R16(V)R1	TTAAAAGACCTTCTTCGG TTCAATCCGTACTGAGACTACC	50	1100	16S rRNA gene	Lee et al., 1994

*published annealing temperatures, **see also papers cited therein

Table 4. Bacterium specific primers

Species	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Target gene	Reference
Agrobacterium spp.	UF f (fw) B1R r (rev) B2R r (rev) AvR r (rev) ArR r (rev)	GTAAGAAGCGAACGCAGGGAACT GACAATGACTGTTCTACGCGTAA TCCGATACCTCCAGGGCCCCTCACA AACTAACTCAATCGCGCTATTAAC AAAACAGCCACTACGACTGTCTT	67	184, 1066, 478, 1006	23S RNA gene	Puławska et al. 2006
Agrobacterium tumefaciens	VCF (fw) VCR (rev)	ATCATTTGTAGCGACT AGCTCAAACCTGCTTC	55	730	*virC* gene	Sawada et al. 1995
A. tumefaciens	virD2A (fw) virD2C(rev) virD2E(rev)	ATGCCCGATCGAGCTCAAGT TCGTCTGGCTGACTTTCGTCATAA CCTGACCCAAACATCTCGGCTGCCCA	50	224, 338	*virD2* gene	Haas et al. 1995
Agrobacterium spp.	VCF3 (fw) VCR3 (rev)	GGCGGGCGYGCYGAAAGRAARACYT AAGAACGYGGNATGTTGCATCTYAC	60	414	*virC1-virC2*	Kawaguchi et al. 2005
Agrobacterium vitis	PGF (fw) PGR (rev)	GGGGCAGGATGCGTTTTTGAG GACGGCACTGGGGCTAAGGAT	54-58	466	poligalacturo-nase gene	Szegedi & Bottka 2002
A.vitis	Ab3-F3 (fw) Ab3-R4(rev)	ATGACGGTAGTCGGAGAAGAAGCC CTGTCTCTGTGTCCCCGAAAGG	62	570	16S rDNA gene	Kawaguchi et al. 2005
A. tumefaciens, A. vitis	iaaH-F10 (fw) iaaH-R10 (rev)	GGAAACATGCATGAGTTATCGTT CCACATCAGCATCAAGGTCATC	54	424	oct, nop pTi-*iaaH*	Bini et al. 2008
A. vitis	S4iaaM5 (fw), S4iaaM3 (rev)	CGCGTCCCCGTTTACACTA CGAGATCGCGCTTCAAGAT	54	800	vitopin pTi *iaaM*	Bini et al. 2008
Xylophilus ampelinus	S3 (fw) S4 (rev)	GGTGTTAGGCCGAGTAGTGAG GGTCTTTCACCTGACGCGTTA	55	277	ITS	Botha et al. 2001
X. ampelinus	Xamp1.27A (fw) Xamp1.27B(REV)	GATCGCAAGAAATCCCGATG AAATTCCCTTCGTTGTTCTG	60	310	nd**	Manceau et al. 2000
Xylella fastidiosa	RST31(fw), RST33 (rev)	GCGTTAATTTTCGAAGTGATTCGATTGC CACCATTCGTATCCCGGTG	55	733	Specific EcoRI fragment	OEEP/EPPO 2004

Species	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Target gene	Reference
X. fastidiosa	S19 (fw) S21 (fw) A19 (rew) A21 (rew)	CGGCAGCACATTGGTAGTA GCAAATTGGCACTCAGTATCG CTCCTCGCGGTTAAGCTAC CGATACTGAGTGCCAATTTGC	55	600-1350	*16S rDNA*	Rodrigues et al. 2003
X. fastidiosa	FXYgyr499 (fw) RXYgyr907 (rev)	CAGTTAGGGGTGTCAGCG CTCAATGTAATTACCCAAGGT	54	429	*gyrase b*	Rodrigues et al. 2003

*published annealing temperatures, **nd: no data

Table 5. Fungus specific primers

Species	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Target gene	Reference
Universal	ITS1F(fw) ITS5 (fw), ITS4(rev)	CTTGGTCATTTAGAGGAAGTAA GGAAGTAAAAGTCGTAACAAGG TCCTCCGCTTATTGATATGC	55, 50	584, 620	ITS (5.8S-28S rDNA)	Hamelin et al. 1996 Tegli et al. 2000
Phaeomoniella chlamydospora	MONO1475 (fw) MONO1962 (rev)	GATCAAACGCCTTGGTGGTCC ATTGCATCTTGCAAAGGGAC	52	489	SCAR	Rigdway et al. 2005
Phaeoacremonium chlamydosporum (Pch)	Pal1N(fw), Pal2 (rev)	AGGTCGGGGGCCAAC AGGTGTAAACTACTGCGC	50	400	ITS	Tegli et al. 2000
Phaeoacremonium aleophilum (Pal)	Pch1 (fw), Pch2 (rev)	CTCCAACCCTTTGTTTATC TGAAAGTTGATATGGACCC	50	360	ITS	Tegli et al. 2000
Phaeoacremonium spp.	Pm1 (fw) Pm2 (rev)	CTCCAAACCCTTTGTGAACAT CGAGCCCGCCACTGACTT	52	415	ITS	Aroca & Raposo 2007
Botryosphaeria spp.	ITS1 NL4	TCCGTAGGTGAACCTGCGG GGTCCGTGTTTCAAGACGG	50	1200	ITS and rDNA regions	Alves et al. 2005
Cylindrocarpon destructans	Dest1(fw) Dest4 (rev)	TTGTTGCCTCGGCGGTGCCTG GGTTTAACGGCGTGGCCGCGCTGTT	60	400	ITS1-ITS2	Hamelin et al. 1996 Nascimento et al. 2001
Rosellinia necatrix	R1(fw) R2(fw) R3(fw) R5(fw) R10(fw), and R7(rev) R8(rev) R11(rev)	ATAACTCCCAAAACCCATGTGA CAAAACCCATGTGAACATACCA CGAAGTGCCCTACCCTGTTA CACGAAACTCTGTTTAGCATTGA CCCCTGTTGCTTAGTGTTGG AACCATAGGCGAGATGAGAAAT CCGAGGTCAACCTTTGGTATAG CACAACCATAGGCGAGATGA	60	112-500	ITS1-ITS2	Schena et al. 2002
Roesleria subterranea	Rs1R(fw) Rs2F(rev)	TCCGGAACGTCTATAGCGAGGAGA TCGCGGGCAACCGGCTCACGC	60	360	ITS1-ITS2	Neuhauser et al. 2009

*published annealing temperatures

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