

SUPPLEMENTARY INFORMATION

Transferability of PCR-based diagnostic protocols: An international collaborative case study assessing protocols targeting the quarantine pine pathogen *Fusarium circinatum*

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Supplementary information 1

Protocol 8: Real-time PCR assay parameters and conditions targeting *Fusarium circinatum*

Primers and probe: The primers and probe were designed based on *F. circinatum* SCAR marker sequence (Baskarathevan et al. unpublished)

FC-2F (Forward primer) : 5'-GGATCACATGTAACATAATGTG-3'

FC-3R (Reverse primer) : 5'-TAAGCTCAGACTATCACCT-3'

FC-3P (Probe) : 5'-**FAM**-CGGGATTCTCCCCGATGGTGTTC**A-BHQ1**-3'

PCR reaction (20 µL Volume):

Reagents	Volume per reaction (µL)
Sterile water	5.0
Reaction Mix (x2)	10.0
5 µM FC-2F (300 nM)	1.2
5 µM FC-3R (300 nM)	1.2
5 µM FC-3P (150 nM)	0.6
DNA template	2.0
Total Volume	20.0

Note: This assay has been tested with PerfeCTa™ qPCR ToughMix™ (Quanta Biosciences) and LightCycler 480 Probes Master (Roche). Any alternative qPCR master mix can be used. However, the test should be validated with relevant positive controls.

PCR cycling conditions

Step	Time	Temp	Cycles
Initial denaturation	5 min	95°C	Hold
Denaturation	15 sec	95°C	} x 40 cycles
Annealing and Extension	40 sec	62°C	

Result

A Cq value of <36 is considered as positive detection of *F. circinatum*.

Supplementary information 2

Recommended amplification conditions of each protocol for the detection of *F. circinatum* according to the original publication

Protocol ^a	Forward/ reverse primers	Probe	Recommended DNA Polymerase / master mix ^b	Final concentrations			Amplification parameters [T(°C)/time(S)]					Nb Cycles
				Primers (μ M)	Probe (nM)	MgCl ₂ (mM)	ID ^c	denaturati on	Anneali ng	Extensi on	Final elongation	
p1 ²³	10Fcirc-F / 10Fcirc-R 30Fcirc-F / 30Fcirc-R	n.a.	Taq DNA Polymerase (Roche Applied Science)	0.5	n.a.	1.5	94/120	94/30	60/30	72/60	72/420	30
p2 ²⁴	FCIR-F / FCIR-R	FCIR-P	qPCR core kit no rox (Eurogentec)	0.3	0.1	5	95/600	95/15	70/55		n.a.	40
p3 ²⁷	Fcircinatum_F315 / Fcircinatum_R775	Fcircinatum_T717	Quantitect Multiplex NoRox	0.6	0.1	Unkn.	95/900	95/15	60/90		n.a.	50
p4 ²²	CIRC1A / CIRC4A	n.a.	Platinum Taq Polymerase (Invitrogen) + SYBR Green	0.5	n.a.	5	94/180	94/35	66/55	72/50	72/720 ^e	45
p5 ²⁶	CIRC1A / CIRC4A	n.a.	SoFast EvaGreen Supermix	0.1	n.a.	Unkn.	-	95/10	65/15	72/30	- ^e	44
p6 ²⁵	CIRC1L / CIRC4L	n.a.	Platinum Taq Polymerase (Invitrogen) + SYBR Green	0.4		Unkn.	95/180	95/35	66/55	72/50	- ^e	45
p7 ²⁸	Fcrc-F Fcrc-R	Fcrc-P	TaqMan Universal Master mix (Appl. Biosystems)	0.3	0.2	Unkn.	94/600	95/30	60/60	-	-	50
p8 ^{5F1}	FC-2F / FC-3R	FC-3P	Perfecta qPCR ToughMix, quanta biosciences	0.3	0.15	Unkn.	95/300	95/15	62/40	-	-	40
p9 ¹³	CIRC1A / CIRC4A	-	Not specified ^d	0.5	-	2 mM	94/180	94/35	66/55	72/50	72/720	45

^a Reference

^d Brand / type recommended in the original paper but study participants were free to use others

^c Initial denaturation

^d Not specified in EPPO (2009), but the original work of Schweigkofler *et al.* (2004) reports the use of Platinum Taq Polymerase (Invitrogen)

^e Melt curve analysis required

Supplementary Information 3

Thermal cycler model and reagent details used by each of the participating laboratories

Laboratory	Reagent PCR	Reagent Sybr-Green PCR	Reagent hydrolysis probe PCR	PCR equipment	Real-time PCR equipment
Lab1	n.a.	KAPA BIOSYSTEMS SYBR FAST Universal qPCR kit (Roche)	n.a.	n.a.	QuantStudio 6 Flex System (Applied Biosystems)
Lab2	5x HOT FIREPol Blend Master Mix with 7,5 mM MgCl ₂ (Solis BioDyne)	5x HOT FIREPol Probe qPCR Mix Plus, no rox (Solis BioDyne)	5x HOT FIREPol Probe qPCR Mix Plus, no rox(Solis BioDyne)	T Professional (Biometra)	Rotor-Gene Q (Qiagen)
Lab3	Taq DNA Polymerase (Qiagen)	n.a.	qPCR Core Kit No ROX (Eurogentec)	T Professional (Biometra)	StepOnePlus (Applied Biosystems)
Lab4	KAPATaq Ready Mix (Roche)	n.a.	n.a.	Techne Prime Thermal Cycler (Cole-Parmer)	n.a.
Lab5	Taqmix BioMIX (Biolin)	n.a.	n.a.	MJ Mini thermal cycler (MJ Research)	n.a.
Lab6	HotBegan Taq DNA polymerase (Canvax Biotech SL)	Sybr Premix Ex Taq (Takara)	Premix Ex Taq -Probe qPCR (Takara)	Verity Thermal Cycler (Applied Biosystems)	Rotor-Gene Q (Qiagen)
Lab7	HGS Diamond Taq (Eurogentec)	Mesa green master mix (Eurogentec)	Core kit no ROX (Eurogentec)	GeneAmp 9700 (Applied Biosystems)	Rotor-Gene Q (Qiagen)

Laboratory	Reagent PCR	Reagent Sybr-Green PCR	Reagent hydrolysis probe PCR	PCR equipment	Real-time PCR equipment
Lab8	n.a.	n.a.	Lumino Ct qPCR Ready Mix (Sigma-Aldrich)	n.a.	7500 Real Time PCR System (Applied Biosciences)
Lab9	n.a.	n.a.	TaqMan Universal PCR Master Mix (Applied Biosystems)	n.a.	ABI 7500 Fast Realtime (Applied Biosystems)
Lab10	Platinum qPCR SuperMix-UDG (Invitrogen)	Platinum SYBRGreen qPCR supermix (Invitrogen)	Maxima Probe/ROX qPCR Master Mix 2X (ThermoFisher Scientific)	Rotor-Gene Q (Qiagen)	Rotor-Gene Q (Qiagen)
Lab11	n.a.	POWER SYBR GREEN PCR MASTER MIX (Applied Biosystems)	Taq-Man Universal MasterMix (Applied Biosystem)	n.a.	StepOne Plus Real-Time PCR System (Applied Biosystems)
Lab12	n.a.	Lightcycler 480 SYBR Green I Master (Roche)	Lightcycler 480 Probes Master (Roche)	n.a.	Lightcycler 480 II (Roche)
Lab13	n.a.	Evagreen qPCR Master Mix (Biotium)	n.a.	n.a.	Step one plus (Applied Biosystems)
Lab14	n.a.	Power sybr green mastermix (Applied Biosystem)	n.a.	n.a.	Quantstudio 3 (Thermo)

Laboratory	Reagent PCR	Reagent Sybr-Green PCR	Reagent hydrolysis probe PCR	PCR equipment	Real-time PCR equipment
Lab15	Taq DNA Polymerase (Roche)	SenseFast SYBR mix (Bioline)	n.a.	Mastercycler nexus gradient (Eppendorf)	CFX96 (Bio-Rad)
Lab16	n.a.	KAPA SYBR qPCR Kit (Roche)	KAPA PROBE FAST qPCT Kit (Roche)	n.a.	iQ5 (Bio-rad)
Lab17	n.a.	n.a.	PerfeCTa qPCR ToughMix (Quanta Biosciences)	n.a.	Bio-Rad, CFX96 Touch™ (Bio-Rad)
Lab18	n.a.	n.a.	QuantiNova Probe PCR Master Mix (Qiagen)	n.a.	CFX96 (Bio-Rad)
Lab19	n.a.	n.a.	Perfecta QPCR Toughmix, ROX (Quanta Biosciences)	n.a.	QuantStudio 6 Flex (Applied Biosystems)
Lab20	TrueStart Hot Start Taq DNA Polymerase (Thermo Scientific)	n.a.	n.a.	Mastercycler Nexus GSX1 (Eppendorf)	n.a.
Lab21	VWR Taq DNA Polymerase (VWR)	n.a.	n.a.	Hybaid PX2 thermal cycler (Thermo Scientific)	n.a.

Laboratory	Reagent PCR	Reagent Sybr-Green PCR	Reagent hydrolysis probe PCR	PCR equipment	Real-time PCR equipment
Lab22	Taq DNA Polymerase with Standard Taq (Mg-free) Buffer (New England Biolabs)	n.a.	n.a.	Mj Mini (Bio-rad)	n.a.
Lab23	n.a.	n.a.	TaqMan universal Master Mix (Applied Biosystems)	n.a.	iCycler iQ (Bio-rad)

n.a: not applicable