Sukhwinder Kaur & Lynn Epstein, 30 May 2022

1) EF1 for *F. oxysporum* haplotype sequencing

Bio rad Method=ef10110

Today's date	FOA EF1 PCR -30 ul volume				FOA EF1 PCR -40 ul vol for seq	
Final conc in PCR mix	working conc.	1X (ul)	#	samples	working conc.	1X (ul)
1X	2X Go taq green MM	15			2X Go taq green MM	20
0.25uM	10uM EF1	0.75			10uM EF1	1
0.25uM	10uM EF2	0.75			10uM EF2	1
	aliquot PCR mix in each 0.2 ml tube	16.5			aliquot PCR mix in each 0.2 ml tube	22
	Nuclease free SDI water+temp DNA	13.5			**Nuclease free SDI water+temp DNA**	18
	Total Vol of PCR	30 ul			Total Vol of PCR	40

Biorad thermocycler PCR
Method=ef10110

10-20 ng based on ND template DNA
2 ng DNA on qubit is OK

Ta=52C, 700 bp amplicon
PCR conditions

TEMP	TIME	#CYCLES
94C	10 MIN	1X
94C	30 SEC	
52C	30 SEC	
72C	60 SEC	40X
72C	10 MIN	1X

All shown 5' to 3'
EF1(F) -ATGGGTAAGGA(A/G)GACAAGAC
EF2(R)-GGA(G/A)GTACCAGT(G/C)ATCATGTT

2) IGS amplification for *F. oxysporum* haplotype sequencing

Today's date	FOA IGS PCR			
Final conc in PCR mix	working conc.	1X (ul)	#	samples
1X	2X Go taq green MM	12.5		
0.1uM	10uM iNL11	0.25		
0.1uM	10uM FOIGSR	0.25		
	Nuclease free SDI water	7		
	Template DNA	5(10ngDNA)		
	Total Vol of PCR	25ul		
	Biorad thermocycler PCR Method=igs121410			

Ta=57C, 970 bp amplicon
PCR conditions

TEMP	TIME	#CYCLES
94C	90 SEC	1X
94C	30 SEC	
57C	45 SEC	
72C	60 SEC	35X
72C	10 MIN	1X

All shown 5' to 3'
iNL11(F): AGGCTTCGGCTTAGCGTCTTAG
FoIGS-R: GCCGACACCGCGCCTCTTAA

3. Older *Foa* race 4 diagnostic FOA-R4-N3875 (mostly obsolete)

Race 4 primers: see list of undesired positives in "Table 5"

Final conc in PCR mix	working conc.	1X (ul)	# samples
1X	2X Go taq green MM	10	
0.2uM	10uM N3875-L2	0.4	
0.2uM	10uM N3875-R2	0.4	
200ng	20mg/ml BSA	0.2	
	Nuclease free SDI water	5	
	Template DNA(8-10ng)	4	
	Total Vol of PCR	20	
	Biorad thermocycler PCR Method=Race4specific		

Ta=60C, 189 bp amplicon

PCR conditions

TEMP	TIME	#CYCLES
94C	10 MIN	1X
94C	30 SEC	
60C	30 SEC	
72C	60 SEC	40X
72C	10 MIN	1X

All shown 5' to 3'

N3875-L2	AGGACTTGAATCACGGCTCG
N3875-R2	CCTGCCACTCGCTTTTTGAG

4. Older *Foa* race 2 diagnostic primers N4851 (mostly obsolete)

Today's date

Final conc in PCR mix	working conc.	1X (ul)	# samples
1X	2X Go taq green MM	10	
0.2uM	10uM N4851-F	0.4	
0.2uM	10uM N4851-R	0.4	
200ng	20mg/ml BSA	0.2	
	Nuclease free SDI water	5	
	Template DNA(8-10ng)	4	
	Total Vol of PCR	20	

Biorad Thermocycler
PCR Method=r2r4pcr

Ta=60C, 100 bp amplicon

PCR conditions

TEMP	TIME	#CYCLES
94C	10 MIN	1X
94C	30 SEC	
60C	30 SEC	
72C	60 SEC	40X
72C	10 MIN	1X

All shown 5' to 3'

N4851-1F	TGTTCGAGTACATTGGAGACTTTGG
N4851-1R	CAGCATAGATCGTAGGTGGATATAGAG

5. ITS 1f (fungal-specific) and ITS4 rDNA primers for sequencing

	working conc.	1X (ul)
1X	2X Go taq green MM	20
0.25uM	5uM ITS-1F	1
0.25uM	5uM ITS-4	1

aliquot 22 ul of above PCR mix in each 0.2 ml PCR tube.

NF water+templateDNA		18
Nuclease free SDI water		Variable
Template DNA(8-10ng)		Variable
Total Vol of PCR		40

Biorad thermocyclerPCR Method=its1fits4 2017

Ta 55C 550 bp amplicon

PCR conditions		# CYCLES	
95C	5 Min		1
94C	30S		
55C	30S		
72C	60S	35	
72C		7	1

All shown 5' to 3'

ITS-1F	CTTGGTCATTTAGAGGAAGTAA
ITS-4	TCCTCCGCTTATTGATATGC

6. ITS 1 and 4 rDNA primers for DNA sequencing (will amplify plant + DNA)

Today's date			
Final conc in PCR mix	working conc.	1X-ul	
1X	2X Go taq green MM	12.5	
0.4	10uM ITS-1	1	
0.4	10uM ITS-4	1	
	Nuclease free SDI water	5	Variable
	Template DNA(25-50ng)	5.5	variable
	Total Vol of PCR	25	

Biorad thermocycler PCR Method=ITS-1-4

Ta 60C 550 bp amplicon

PCR conditions			# CYCLES
95C	5 Min		1
94C	30S		
60C	30S		
72C	60S		35
72C		7	1

All shown 5' to 3'

ITS-1	TCCGTAGGTGAACCTGCGG
ITS-4	TCCTCCGCTTATTGATATGC

7. Multiplexed older Foa race 2 & race 4 diagnostic primers

Note: see list of undesired positives in "Table 5" and Phytopathology 2017; new primers in Henry et al. 2020 are, in general, better

Final conc in PCR mix	working conc.	1X (ul)
1X	2X Go taq green MM	10
0.2uM	10uM N4851-F	0.4
0.2uM	10uM N4851-R	0.4
0.2uM	10uM N3875-L2	0.4
0.2uM	10uM N3875-R2	0.4
200ng	20mg/ml BSA	0.2
	Aliquot PCR mix in each 0.2ml PCR tube	11.8
	Nuclease free SDI water	variable
	Template DNA(10-20ng)	variable
	Both NF H2O+DNA	8.2
	Total Vol of PCR	20
	Bio rad Method=Race4specific	

189 bp product Ta 60C for Race 4, N3875-2
100 bp product Ta 60C for Race 2, N4851

Use 50 bp DNA ladder, 2% agarose(0.7g in 35ml 1XTAE buffer)
if using one comb Run at 110V for 40 min(0.7)
if using two combs Run at 70V for 40 min (0.7)

PCR conditions

TEMP	TIME	#CYCLES
94C	10 MIN	1X
94C	30 SEC	
60C	30 SEC	
72C	60 SEC	40X
72C	10 MIN	1X

8. Current assay: 3-Multiplexed Foa race 2 & race 4, and Foci C21 diagnostic primers

Note: see list of undesired positives for race 4 & Foci in Henry et al. 2020

Final conc	working conc		1X-ul
1X	2X	Go Taq Green MM	10
0.2uM	10uM	R4-447F	0.4
0.2uM	10uM	R4-447R	0.4
0.2uM	10uM	R2-76kF	0.4
0.2uM	10uM	R2-76kR	0.4
0.2uM	10uM	C21-2F	0.4
0.2uM	10uM	C21-2R	0.4
200ng	20mg/ml	BSA	0.2
		PCR Mix in each tube	12.6
		TEMP DNA+Water	7.4
			20
	Biorad thermocycler PCR Method	R234 FOC2018	

FOA Race4=196bp
FOA Race2=226bp
FOCI = 374 bp
Use 50 bp DNA ladder, 2% agarose(0.7g in 35ml 1XTAE buffer)
if using one comb Run at 110V for 50 min(setting 0.8)
if using two combs Run at 70V for 40 min (setting 0.7)

94C	10 MIN	X1
94C	30 SEC	X40
63C	30 SEC	
72C	60 SEC	
72C	10 MIN	X1

All shown 5' to 3'

Primer seq	
FoaR4_SS5_447F	AACCCAAGGTCTCGACGATCTG
FoaR4_SS5_447R	ACCTTCCGTGCAGTCCTCATTC
FoaR2_207_9_76kF	TGTTGAGTTGTCGGAGTTCTGC
FoaR2_207_9_76kR	TCCTTGTGTTTCTCGGTTCCTC
Foci-2-21_2F	GTAGTATCGTGGGATTGGCGTTTG
Foci-2-21_2R	GGCCTCTTCTGAATTGTCGCATAC

9. Current assay: 2-multiplexed Foa race 2 & race 4 diagnostic primers

Note: see list of undesired positives for race 4 & Foci in "Table 5"

Final conc	working conc		1X-ul	? X
1X	2X	Go Taq Green MM	10	
0.2uM	10uM	R4-447F	0.4	
0.2uM	10uM	R4-447R	0.4	
0.2uM	10uM	R2-76kF	0.4	
0.2uM	10uM	R2-76kR	0.4	
200ng	20mg/ml	BSA	0.2	
		PCR Mix in each tube	11.8	
		TEMP DNA+Water	8.2	
			20	
	Biorad thermocycler PCR Method	R234 FOC2018		

FOA Race4=196bp
FOA Race2=226bp
Use 50 bp DNA ladder, 2% agarose(0.7g in 35ml 1XTAE buffer)
if using one comb Run at 110V for 50 min(setting 0.8)
if using two combs Run at 70V for 40 min (setting 0.7)

94C	10 MIN	X1
94C	30 SEC	X40
63C	30 SEC	
72C	60 SEC	
72C	10 MIN	X1

All shown 5' to 3'

Primer seq	
FoaR4_SS5_447F	AACCCAAGGTCTCGACGATCTG
FoaR4_SS5_447R	ACCTTCCGTGCAGTCCTCATTC
FoaR2_207_9_76kF	TGTTGAGTTGTCGGAGTTCTGC
FoaR2_207_9_76kR	TCCTTGTGTTTCTCGGTTCCTC

10. Foci gC-31 diagnostic primer

Final conc	working conc		1X-ul
1X	2X	Go Taq Green MM	10
0.2uM	10uM	FOCig-C31-F	0.4
0.2uM	10uM	FOCig-C31-R	0.4
200ng	20mg/ml	BSA	0.2
		PCR Mix in each tube	11
		TEMP DNA+Water	9
			20

Ta=60C 402bp amplicon

Biorad thermocycler PCR method=FOCi60C

94C	10 MIN	X1
94C	30 SEC	X40
60C	30 SEC	
72C	60 SEC	
72C	10 MIN	X1

All shown 5' to 3'

Amplicon size is 402 bp

Forward 5’ TGG TTC ATC TAT CCC TCA AGG AGT ATC 3’

Reverse 5’ AGC CTT TAT TCT CGT CCA TCA TAA GTT C 3’

11. Foa Race 2-Multi-Copy1-primers & probe for qPCR biomass in crowns

Final conc in pcr		Final conc in pcr-units	Working stocks	1X (ul)
	1	1X	2X IDT PRIMER MM	10
	0.5	uM	10uM-MC1-F	1
	0.5	uM	10uM-MC1-R	1
	0.2	ug	20ug/ul BSA	0.2
	0.15	uM	MC1-150nM Probe	0.3
			nf H2O	**4.5**
			DNA template	**3**
			Total PCR vol	20
			DNA+water=7.5ul	

Aliquot 12.5 ul of pcr MMix in each well and 7.5 ul of **DNA+nfwater**

stock FOA R2 DNA is 28.2ng/ul qubit quantity

well ID		ng DNA in PCR 20ul vol			Cq	slope
A1	NTC1		7.5ul nf H2O			
A2	FOA R2 stnd DNA-3dil	0.846	3ul	0.282ng/ul	17.15	
A3	FOA R2 stnd DNA-4dil	0.0846	3ul		20.64	
A4	FOA R2 stnd DNA-5dil	0.00846	3ul		24.1	
A5	FOA R2 stnd DNA-6dil	0.000846	3ul		27.56	
A6	FOA R2 stnd DNA-7dil	0.0000846	3ul		30.59	
A7	FOA R2 stnd DNA-8dil	0.00000846	3ul		35.16	
A8	FOA R2 stnd DNA-9dil	0.000000846	3ul		37.43	

153 bp product Ta 56C for Race 2

qPCR conditions

TEMP	TIME	#CYCLES
95C	3 MIN	1X
95C	15 SEC	
56C	30 SEC	
72C	60 SEC	45X
4C	until reactions are stopped	

All shown 5' to 3'

FoaR2-MC1-F	TCTTCGGACCCTAGGCTTATAG
FoaR2-MC1-R	AGGTTTAGGTTCAGGCTTCAG
MC1-probe	5'6FAM/ATATGGACG/ZEN/TTGCAGGCCCTACC/3' IABkFQ/

PrimeTime 5'nuclease probes were purchased from IDT (Coralville, IA). The probes have a 5' 6-FAM fluorescent reporter dye and two quencher dyes: an internal ZEN™ and a 3' Iowa Black forward quencher (IABkFQ). . 99 exact copies of FOA Race2 primers/probe

12. Foa Race 4-Multi-Copy1-primers & probe for qPCR biomass in crowns

Final conc in QPCR mix	conc in pcr	Final conc in pcr-units	working stocks	ul-1X
	1	1X	2X IDT PRIMER MM	10
	0.5	uM	10uM-MC2-F	1
	0.5	uM	10uM-MC2-R	1
	0.2	ug	20ug/ul BSA	0.2
	0.15	uM	MC2F-150nM Probe	0.3
			nf H2O	4.5
			DNA template	3
			Total PCR vol	20 ul
			DNA+water=7.5ul	

stock FOA R4 DNA is 73.4ng/ul qubit quantity

well ID		ng DNA in PCR	ul vol	7.5ul nf H2O	Cq	slope
D1	NTC1					
D2	FOA R4 stnd DNA-2dil	2.202	3ul	0.734ng/ul	18.14	
D3	FOA R4 stnd DNA-3dil	0.2202	3ul		21.18	
D4	FOA R4 stnd DNA-4dil	0.02202	3ul		24.79	
D5	FOA R4 stnd DNA-5dil	0.002202	3ul		27.93	
D6	FOA R4 stnd DNA-6dil	0.00022	3ul		31.59	
D7	FOA R4 stnd DNA-7dil	2.2E-05	3ul		34.71	
D8	FOA R4 stnd DNA-8dil	2.2E-06	3ul		38.09	

107 bp product Ta 56C for Race 4

qPCR conditions

TEMP	TIME	#CYCLES
95C	3 MIN	1X
95C	15 SEC	
56C	30 SEC	
72C	60 SEC	45X
4C	until reactions are stopped	

FoaR4_MC2-F	GGGTACGTGGATAGTAGGTACA
FoaR4_MC2-R	CGAAGCAAGCATTAAAGGAGAAG
MC2-probe	5'6FAM/AGGCGGGCT/ZEN/TCAAAGATGTCGTTA/3' IABkFQ/

PrimeTime 5'nuclease probes were purchased from IDT (Coralville, IA). The probes have a 5' 6-FAM fluorescent reporter dye and two quencher dyes: an internal ZEN™ and a 3' Iowa Black forward quencher (IABkFQ).