12-plex Microsatellite (SSR) Genotyping of *Phytophthora infestans*

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Microsatellites can be used for genotyping lineages of *P. infestans*. Li and Cooke (2013) have developed a protocol that multiplexes 12 diagnostic SSR primer sets in a single tube for more rapid analysis and genotyping. The protocol uses fluorescently labeled primers, which can then be read by a capillary analyzer for analysis. This protocol is optimized for use with an ABI 3730xl DNA analyzer with a 5 dye set (6-FAM, VIC, NED, PET, and LIZ size standard). The following protocol is from Li and Cooke (2013), with modifications implemented by the lab of Bill Fry at Cornell University and Anne Njoroge.

Primers (5' – 3')

Locus	Dye	Product size range (bp)	Primer sequence
PiG11	NED	130-206	FwdNED-TGCTATTTATCAAGCGTGGG Rev-GTTTCAATCTGCAGCCGTAAGA
Pi02	NED	255-275	FwdNED-ACTTGCAGAACTACCGCCC Rev-GTTTGACCACTTTCCTCGGTTC
PinfSSR11	NED	325-360	FwdNED-TTAAGCCACGACATGAGCTG Rev-GTTTAGACAATTGTTTTGTGGTCGC
D13	FAM	100-210	FwdFAM-TGCCCCCTGCTCACTC Rev-GCTCGAATTCATTTTACAGACTTG
PinfSSR8	FAM	250-275	FwdFAM-AATCTGATCGCAACTGAGGG Rev-GTTTACAAGATACACACGTCGCTCC
PinfSSR4	FAM	280-305	FwdFAM-TCTTGTTCGAGTATGCGACG Rev-GTTTCACTTCGGGAGAAAGGCTTC
Pi04	VIC	160-175	FwdVIC –AGCGGCTTACCGATGG Rev-GTTTCAGCGGCTGTTTCGAC
Pi70	VIC	185-205	FwdVIC – ATGAAAATACGTCAATGCTCG Rev-CGTTGGATATTTCTATTTCTTCG
PinfSSR6	VIC	230-250	FwdVIC-GTTTTGGTGGGGCTGAAGTTTT Rev - TCGCCACAAGATTTATTCCG
Pi63	VIC	265-280	FwdVIC – ATGACGAAGATGAAAGTGAGG Rev-CGTATTTTCCTGTTTATCTAACACC
PinfSSR2	PET	165-180	FwdPET-CGACTTCTACATCAACCGGC Rev-GTTTGCTTGGACTGCGTCTTTAGC
Pi4B	PET	200-295	FwdPET – AAAATAAAGCCTTTGGTTCA Rev-GCAAGCGAGGTTTGTAGATT

Reference: Li, Y.; Cooke, D.E.L.; Jacobsen, E.; van der Lee, T. 2013. Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen *Phytophthora infestans*. Journal of Microbiological Methods 92: 316-322.

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Instead of individually pipetting each primer into the master mix, a 10X multiplex primer mix is made that includes all primers. The primer mix is made as follows (makes 400µl):

Primer	Volume of 100µM primer stock (µl)
PiG11F	6
PiG11R	6
Pi02F	6
Pi02R	6
PinfSSR11F	6
PinfSSR11R	6
PinfSSR4F	6
PinfSSR4R	6
Pi04F	6
Pi04R	6
Pi70F	6
Pi70R	6
PinfSSR6F	6
PinfSSR6R	6
Pi63F	6
Pi63R	6
PinfSSR2F	6
PinfSSR2R	6
D13F	6.4
D13R	6.4
PinfSSR8F	12
PinfSSR8R	12
Pi4BF	12
Pi4BR	12

Combine with 231.2µL of 10 mM Tris buffer (pH=8.0) to make 400µl of primer mix.

Reference: Njoroge, A.W. et al. 2019. Genotyping of *Phytophthora infestans* in eastern Africa reveals a dominating invasive European lineage. Phytopathology 109: 670-680.

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The master mix can be made using either the Qiagen multiplex PCR kit (Qiagen, cat. No 206145) or the Qiagen Type-it Microsatellite PCR kit (Qiagen, cat. No. 206243). For the purposes of this protocol we use the Type-it microsatellite PCR kit.

Reagent	**Volume per reaction (µl)**
2X Type-it master mix	6.25
10X multiplex primer mix	1.3
PCR grade water	1.95
Total reaction mix volume per sample (µl)	9.5

3µl of template DNA is added to bring the total volume per sample to 12.5 µl. If more or less DNA is desired, adjust the volume of water per sample.

Thermocycling program:

1 cycle	95C	5 min.
33 cycles	95C	30 sec.
	58C	90 sec.
	72C	20 sec.
1 cycle	60C	30 min.

Before loading on a DNA analyzer, samples must be prepared with the LIZ size standard (Applied Biosystems LIZ500, cat. No. 4322682) and suspended in an appropriate loading solution. For use on an ABI 3730xl DNA analyzer we use highly deionized formamide (Hi-Di formamide, Applied Biosystems, cat. No. 4311320). Check with your local source for fragment analysis for preparation and submission protocols specific to their facilities.

Master mix for analysis preparation:

Reagent	Volume per reaction (µl)
Hi-di formamide	10
LIZ500 size standard	0.3
Total volume per sample (µl)	10.3

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Add 0.5 µl of template reaction product to bring the total volume per sample to 10.8 µl. More product may be used if desired, up to 3 µl.

An optional denaturation step can be employed after plate prep to increase peak resolution by heating the prepared plate at 95C for 3 min, then chilling on ice.