Species:	*Plasmopara viticola*
Product Class(es):	Qo-Inhibitors
Method type described:	Molecular genetic detection of G143A mutation conferring QoI resistance in *Plasmopara viticola* fungal material from leaves.
Date of protocol:	2016-1
Version	1
comments	The F129L mutation has been described, but is very rare. Q-PCR method used since several years

Method:

1. Sampling and DNA extraction:

   Samples are obtained by washing fungal material from infected leaves or parts of it. After centrifuging the samples and lyophilizing the pellets DNA is isolated using the MagAttract 96 DNA Plant Core Kit (Qiagen, Hilden , Germany) according to the instructions in the handbook (August 2003). Only at the disruption step RLT lysis buffer is added before the samples are shaken and thus they are not cooled in liquid nitrogen.

2. Q-PCR:

   The single nucleotide polymorphism that leads to G143A mutation is assessed by SYBR Green Q-PCR. The forward ARMS primers are allele-specific: The primer sequence for the wild type allele is 5'-CCTTGGTGACAAATGAGTTTT TGGAG-3' and for the mutant allele 5'-CCTTGGTGACAAATGAGTTTT TGGAC-3'. The reverse primer has the sequence 5'-CAACTTCTTTTCCAATT AATGGGATAG-3'. Singleplex Q-PCR assays are carried out in 384 well plates on the ABI 7900 HT SDS cycler (Applied Biosystems). The reaction volume is 12.5 µl comprising 2.5 µl template plus 10.0 µl Mastermix [Power SYBR® Green PCR Master Mix, Applied Biosystems, at 1x and primers at a final concentration of 500 nM]. Reactions are performed using the following cycling profile: 95°C for 10 min, 40 cycles of 95°C for 15 sec, 60°C for 1 min.

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