Species:	*Erysiphe necator*
Product Class(es):	Qo-Inhibitors
Method type described:	Molecular genetic detection of G143A mutation conferring QoI resistance in *Erysiphe necator* fungal material from leaves.
Date of protocol:	2016-1
Version	1
comments	

Method:

1. Sampling and DNA extraction:

   Samples are obtained by washing fungal material from infected leaf parts and/or grapes. 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 leading to the G143A change is assessed by Q-PCR. The forward ARMS primers are allele-specific: The sequence for the wild type allele (G143) is 5'-TACGGGCAGATGAGCCTATGCGG-3' and for the mutant allele (A143) 5'-TACGGGCAGATGAGCCTATGCGC-3'. The sequence of the common reverse primer is 5'-ACCTACTTAAAGCTTTAGA AGTTTCC 3'. Singleplex SYBR Green Q-PCR assays are carried out in 384 well plates on the ABI 7900 HT SDS cycler (Applied Biosystems). The reaction volume is 10 µl comprising 2 µl template plus 8 µl Mastermix [FastStart Universal SYBR Green Master (ROX), Roche, at 1x and primers at a final concentration of 300 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 30 sec and 68°C for 30 sec.

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