Species:	*Venturia inaequalis*
Product Class(es):	Qo-Inhibitors
Method type described:	Molecular genetic detection of G143A mutation conferring QoI resistance in *Venturia inaequalis* in apple leaves.
Date of protocol:	2015-05
Version	1
comments	Proven for the detection of the mutation G143A in cytochrome *b*

Method:

1. Sampling and DNA extraction: From each sample 20 lesions from dried apple leaves with *Venturia inaequalis* symptoms are cut out and used for DNA extraction. DNA is extracted using Nucleo Spin Plant Kit (Macherey-Nagel) following manufactures' instructions.

2. PCR: In a first step a cytochrome *b* gene fragment containing the target sequence is amplified in a PCR reaction with a final volume of 100 µl using 10 µL PCR-buffer, 3 µL $MgCl_2$ (50 mM), 2 µl dNTP´s (10 mM), 0.5 µl Taq-Polymerase (Life Technologies), 5 µl of each primer (ANK 10 5´ CTGTTGTTAGGCTCTTCAATG 3´ and ANK 283 5´CTGTAGTTGAAAGGCTATTAG 3´, final concentration 500 nM), 5 µl DNA and 69.5 µL Aqua bidest under the following conditions: An initial heating step for 3 min at 94°C is followed by 35 cycles with 45 sec at 95°C, 30 sec at 58°C and 90 sec at 72°C and with a final step of 10 min at 72°C.
   PCR-products are cleaned up with NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel) following manufactures' instructions. The purified PCR-product is eluted in 20 µl elution buffer.
   The subsequent digestion reactions are performed with a final volume of 30 µl using 10 µl purified PCR-product, 0.5 µl *Tse*I (New England Biolabs, 5000 U/ml), 3 µl corresponding buffer and 16.5 µl Aqua bidest followed by an incubation at 65°C at least for 2 hours for complete digestion. Afterwards agarose gel electrophoresis is performed with a 2% agarose gel stained with ethidium bromide.

3. Analysis: After running electrophoresis, the gel is analysed regarding the number and size of fragments. Following pattern can show up:

   1 fragment (size: 413 bp): sensitive (wild type)

   2 fragments (size: 112 bp and 301 bp): resistant (G143A)

   3 fragments (size: 112 bp, 301 bp and 413 bp), mixture of sensitive (wild type) and resistant (G143A)

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