

Confirmation of resistant phenotypes by the proof of *cytb* mutations (F129L, G143A) in *Alternaria* isolates

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Detection of the F129L mutation in *A. solani*

Genomic DNA of *A. solani* isolates was extracted from mycelia cultivated on V8 medium under near-UV light for 14 days at 21°C. Mycelium and spores were carefully scraped off with a spatula and ground in liquid nitrogen. Genomic DNA extraction was carried out using the Wizard Genomic DNA Purification Kit (Promega Corp.) according to the manufacturer's instructions for isolating genomic DNA from plant tissue, followed by a phenol–chloroform extraction. The DNA was resuspended in sterile H₂O. The DNA content was measured with a NanoDrop Spectrophotometer (ND-1000) and adjusted to 2.5 ng L⁻¹.

Mastermix:

	µl
H ₂ O	14,8
10x buffer with 15mM MgCl ₂	2,5
10 µM Primer As-Gf	1,5
10 µM Primer As-Gr	1,5
2 mM dNTPs	2,5
Taq	0,2
Sample	2
Total	25

Cycling conditions:

Preincubation:	95°C, 10 min	} 30x
Amplification:	94°C, 1 min	
	54°C, 30 sec	
	72°C, 30 sec	
last step	72°C, 10 min	

Primer:

As-Gf: CGGGGACTAATATTTTGATA

As-Gr: TGTTATTTAACCAAGAATGAAA

207 bp product

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