Simultaneous detection & identification of pathogenic fungi in wheat using a DNA macroarray

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Institute of Natural Resource Sciences

The power of the DNA macroarray


© dpa
Quick


© victorinox
Versatile


© softwarethinktank
Accurate


© allthingsd
Cost-effective

Key Facts

The detection of economically important pathogens is a key element in sustainable wheat production and a prerequisite for crop protection. The objective of the project was to develop a DNA macroarray for fast and cost-effective detection of nine pathogenic fungi in wheat: *Fusarium graminearum, F. culmorum, F. poae, Microdochium nivale var. majus, M. nivale var. nivale, Puccinia recondita, Septoria tritici, S. nodorum and Pyrenophora tritici-repentis*.

The macroarray is sensitive enough to detect single nucleotide polymorphisms (SNPs). Sample analysis is simple, fast, cost-effective and suitable for high throughput screening.


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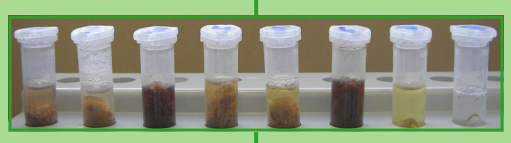
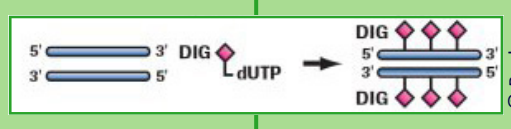
Methods

1. Spotting
- Species-specific oligonucleotides are covalently bound onto the surface of a microtiter plate. Each well contains a different probe.

2. DNA-Extraction
- The total DNA is isolated from wheat using DNeasy Plant Mini Kit (Qiagen).

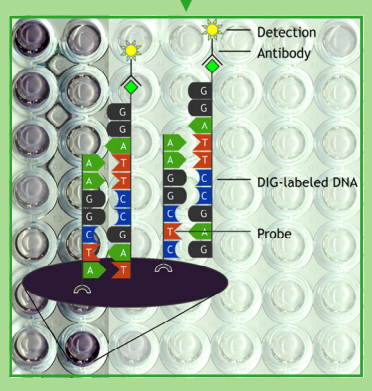


3. Labeling
- β-tubulin and succinate dehydrogenase region for fungi are amplified by PCR and simultaneously labeled with digoxigenin, DIG (Roche).



© Roche

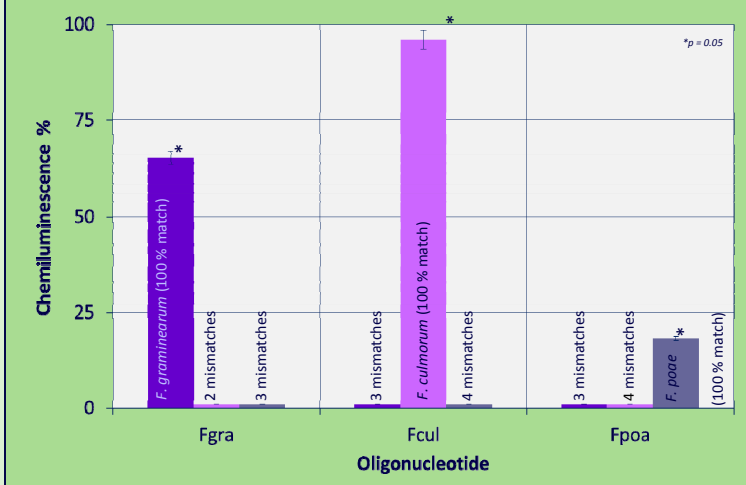
4. Hybridization
- The DIG-labeled PCR product is hybridized to the surface-bound capture probe.



5. Detection
- The DIG-labeled DNA is detected by chemiluminescence using anti-DIG antibodies conjugated with alkaline phosphatase (Roche).

Results within 6 hours

Results



The figure shows the specifity of the DNA macroarray. Three species-specific oligonucleotides (Fgra, Fcul, Fpoa) were spotted on the array and hybridized with DIG-labeled PCR products of genomic DNA from *Fusarium graminearum, F. culmorum* and *F. poae*.
The oligonucleotides could not hybridize with a non target DIG-labeled PCR product when there were more than two mismatches on the binding side.

Applications & benefits

⇒ All-in-one solution for the entire plant production cycle

⇒ Precise pathogen identification guarantees efficient treatment

⇒ Risk management tailored to individual customer needs

⇒ Improved financial security

⇒ Improvement of product quality

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