

Multiplex detection of plant pathogens by micro-arrays: an innovative tool for plant health management

C. Schoen¹, R. van Beuningen², M. de Weerd¹, R. Hilhorst², A. Chan², P. Boender² and P. Bonants¹
¹Plant Research International B.V., Wageningen, The Netherlands, ²PamGene B.V., Den Bosch, The Netherlands

Introduction

Detection of harmful quarantine (Q) organisms in plant propagation material is necessary in order to ensure safe and sustainable agriculture. The use of micro-arrays will provide the next generation of inexpensive DNA diagnostics to measure different pathogens in a massively parallel manner.

Recently PamGene has developed a revolutionary porous capillary solid phase micro-array system which eliminates the rate limiting diffusion of the sample molecules (the target) towards the capture probes at specific positions (the spot) like in flat surface micro-array hybridizations (figure 1). Target molecules in the **PamChip™** are forced through the (porous) array by flow, resulting in diffusion distances in the flow-through array in the order of 100 nm. The hybridization process is therefore fully reaction rate limited and results can be obtained within a few minutes.

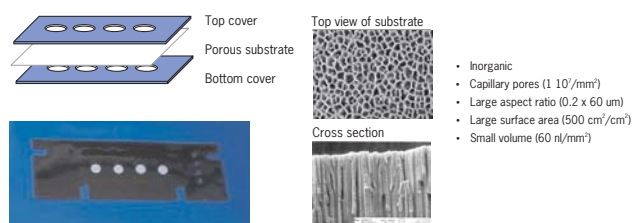


Figure 1: Left PamChip™ single test area containing 400 individual test spots, right side the porous substrate of the PamChip™ showing the capillary structure

Hybridization

Attachment of the oligonucleotides onto the porous support is done by covalent binding. The incubation with a sample containing a fluorescein labeled target molecule is performed by pumping the sample several times through the porous support at the position of the array (figure 2).

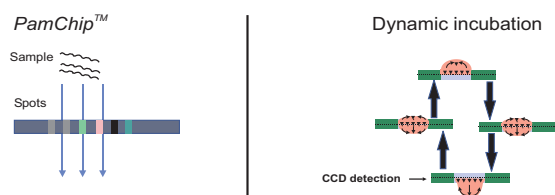


Figure 2: Schematic illustration of the dynamic incubation

Array Analysis

The dynamics of the hybridization reaction of the target molecules with all spots on the array was measured simultaneously by using a CCD camera mounted on a microscope; data analysis is performed using the PamChip Analyzer (figure 3).



Figure 3: Setup of microscope CCD detection system, thermostated hybridization chamber and PamChip Analyzer Data Analysis package

Real-time kinetics

Specificity was shown on an oligo-oligo hybridization array with several mismatches and a perfect match shown in blue (figure 4). The result of the hybridization is shown after 0, 1, 2, 9, 16, 31, 38, 44, 50 and 55 minutes. The signal increases from 0 to 31 minutes as a function of time. Specificity of the binding was achieved by changing the hybridization temperature from RT to 48, 54, 60 and 63 °C at respectively 38, 44, 50, and 55 minutes of incubation. Single mismatches can be visualized.

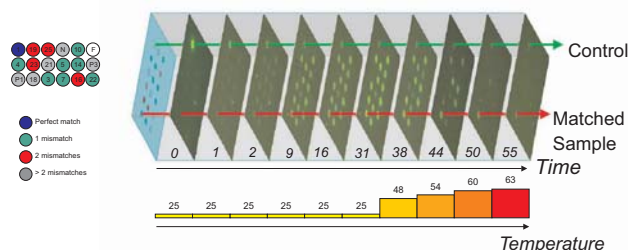


Figure 4: Results of dynamic incubation of an oligo-oligo mismatch array with increased stringency sliced in 10 pictures (right side). The setup of the array is seen on the left side. F indicates a control fluorescent sample used for focussing

Multiplex detection of Phytophthora sp.

Generic amplification of the ITS-1 region of several plant pathogenic **Phytophthora sp.** with primers I1 and APH2F followed by hybridization on **PamChip™** micro-arrays (figure 5).

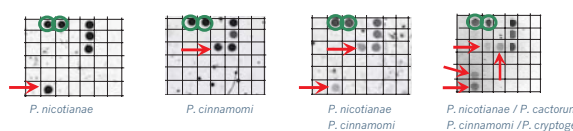


Figure 5: PCR products from different *Phytophthora sp.* were hybridized to the arrays. Species specific probe hybridizations are indicated by an arrow. Spots ringed in green are used for orientation

Conclusions

Advantages of the flow-through **PamChip™** micro-array

- Fast and sensitive reaction analysis
- Binding kinetics can be directly measured
- Broad area of application