

Quantitative multiplex detection of pathogens using PRI-lock probes and ultra-high-throughput Q-PCR on OpenArrays™

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Introduction

Current technologies for multiplex, quantitative analyses frequently suffer from compromises between the level of multiplexing, through-put and accuracy of quantification. In general, for the detection of nucleic acids, microarrays provide a very high level of multiplexing, but less accurate quantification and usually low throughput. Recently, a conceptually new, ultra-high-throughput OpenArray™ platform has become available for real-time PCR, capable of accommodating more than 3000 reactions per array.

Plant Research International has developed PRI-lock probes for multiplex detection which provide flexibility in target specific recognition and high-throughput amplification. PRI-lock probes are long circularizable oligonucleotides with artificially selected unique primer pairs and an universal TaqMan probe region, flanked by target complementary regions (fig 1).

In this study, we have characterized the quantification power of circularizable ligation probes and the development of a high-throughput, quantitative multiplex diagnostic assay.

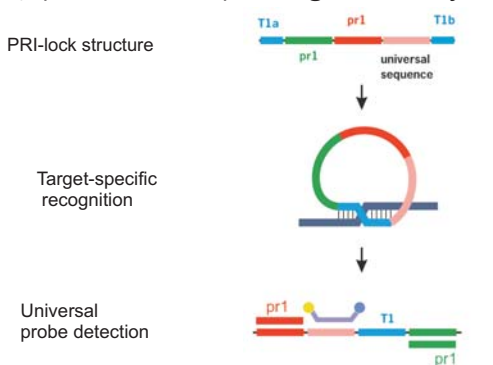


Fig. 1 Schematic PRI-lock principle for the detection of one target

OpenArray™ Anatomy

Each slide consists of 48 subarrays. Every subarray has 64 through-holes of 33 nl, each in an 8x8 pattern. Primers are pre-loaded into the holes, while the sample along with the reagents are auto-loaded due to surface tension, provided by the hydrophilic coating of the holes and the hydrophobic surface of the array (fig2).

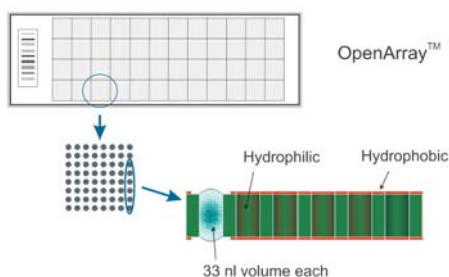


Fig. 2 OpenArray™ anatomy

Multiplex quantitative target detection

Real-time quantification for multiple targets is performed in an OpenArray™. Multiplex PLP ligation is followed by single-plex amplification using unique primer pairs and an universal TaqMan probe in nano-liter PCR arrays (fig 3, 4 and 5).

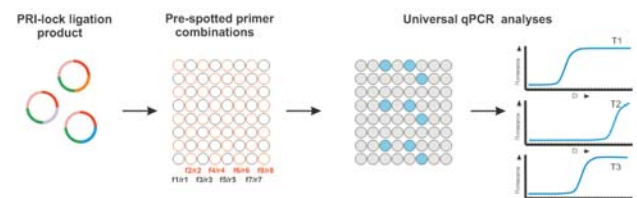


Fig. 3 Schematic overview of the PRI-lock principle combined with the OpenArray™ technology for multiplex detection of three different targets

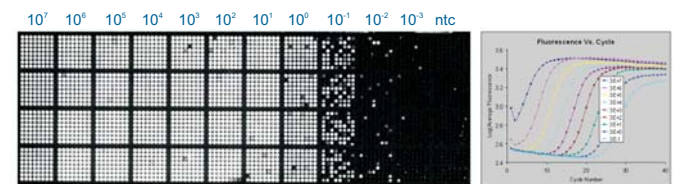


Fig. 4 Real-time detection and quantification of a single target in serial dilutions within an OpenArray™ slide

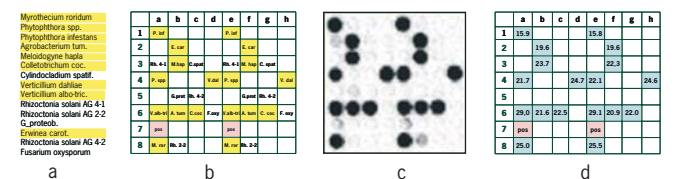


Fig. 5 Real-time multiplex detection and quantification of 9 different plant pathogens in an OpenArray™ slide. a) Total number of used PRI-locks, targets in yellow were applied to the sample. b) Spotting pattern of the through-hole subarray. c) Subarray picture after 32 cycles. d) Ct values for the found targets

Discussion

Preliminary PRI-lock / OpenArray data indicate:

- High specificity
- High level of multiplexing
- Universal downstream processing after ligation
- Flexible and easily adaptable
- High-throughput format with universal TaqMan probe
- Quantitative multiplexing

Reference

Szemes *et al.* Diagnostic application of Padlock Probes - Multiplex Detection of Plant Pathogens using universal microarray. Nucleic Acids Research, 2005, Vol. 33, No. 8 e70