



IDENTIFICATION OF **GRAPEVINE DISEASES** USING MICROCHIP REAL-TIME PCR ANALYZER ARIADNA®

Grapevine (*Vitis vinifera*) is one of the most widely produced fruit crop in many countries around the world. Many of the grapevine pathogens cause significant losses in both yield and quality of the crop. Thus detection and identification of bacteria, phytoplasmas, fungi and viruses is very important task for the grape producers to meet the requirements of viticulture plant health control programs as well as to prevent enormous losses in the grape production. Cultural methods are long and laborious, and not always produce consistent results especially for phytoplasmas and viruses. ELISA method has relatively low sensitivity for bacteria and phytoplasmas making it useful only for the confirmation of grapevine diseases after visual symptoms appear but not for early detection before disease symptoms occur. Real-time PCR and reverse transcription PCR (RT-PCR) have an advantage of direct and specific identification of microorganisms. A microchip real-time PCR analyzer AriaDNA® and microchips allow simplifying diagnostics of grapevine diseases, to reduce analysis time and the number of manual operations. Microchips can be transported and stored at ambient temperature up to 12 months.

Lumex Instruments has tested microchip real-time PCR analyzer AriaDNA® with microchips using commercial PCR reagents for identification of several grapevine pathogens: **Flavescence dorée**, **Bois noir**, **Grapevine fleck virus**, **Grapevine fanleaf virus**, **Grapevine leaf-roll associated virus-1**, **Grapevine leaf-roll associated virus-3**, **Grapevine virus A**, **Grapevine virus B**.

Fig.1A shows an example of microchip layout for determination of **Flavescence dorée (FD)** and **Bois noir (BN)** in grapevine. On a single microchip 8 unknown samples can be analyzed along with positive, negative, and internal control samples. **Fig.1B** shows an example of microchip layout for determination of **Grapevine fleck virus (GFkV)**, **Grapevine fanleaf virus (GFLV)**, **Grapevine leaf-roll associated virus-1 (GLRaV-1)**, **Grapevine leaf-roll associated virus-3 (GLRaV-3)**, **Grapevine virus A (GVA)**, **Grapevine virus B (GVB)** in grapevine. On a single microchip 2 unknown samples can be analyzed along with positive, negative, and internal control samples.

Microchip PCR analyzer AriaDNA®

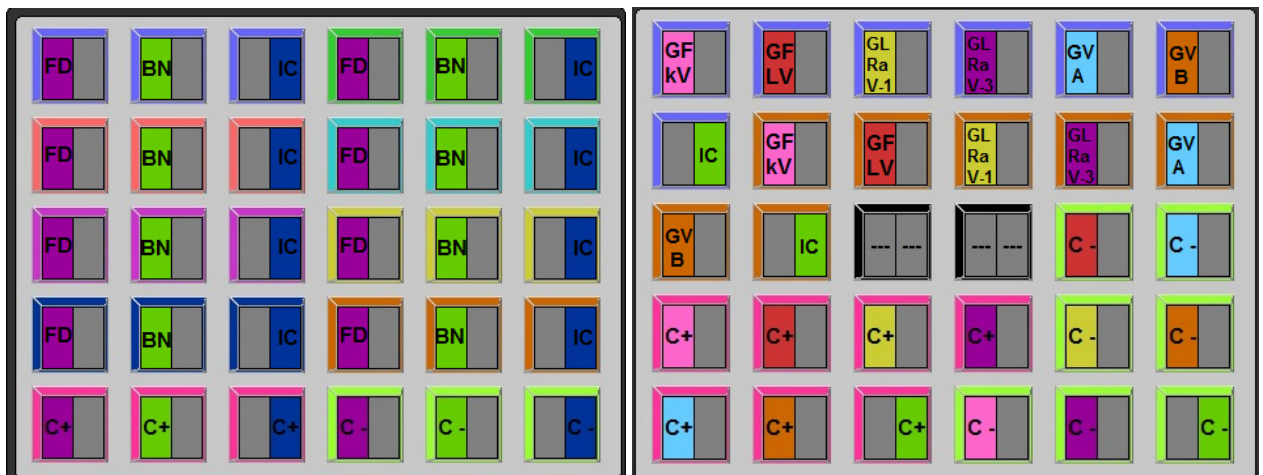


Fig. 1. A) layout for the analysis of 8 DNA samples B) layout for the analysis of 2 RNA samples
C+ positive control sample, C- negative control sample, IC internal control sample



The main advantages of the microchip-based real-time PCR for grapevine pathogen detection

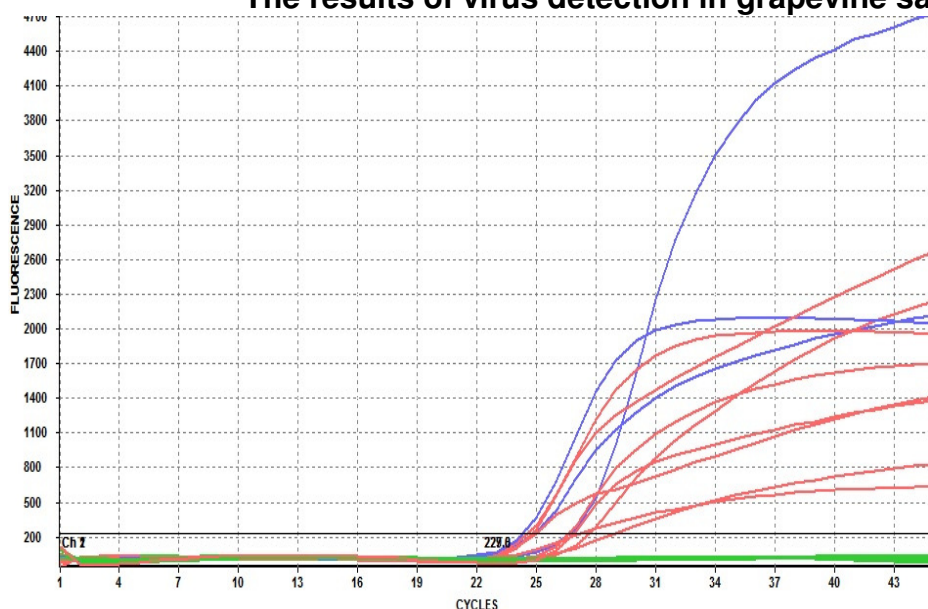
- rapid determination of pathogens: the time of analysis is 45 minutes (DNA) and 60 minutes (RNA)
- reduced consumption of reagents and samples: the volume of x2 PCR reagents master mix is 0.6 μL per microreactor, the volume of sample is 1.2 μL per analysis

Analysis Flow-Chart

1 Extract DNA and/or RNA from grapevine samples

2 Mix extracted DNA/RNA samples with PCR reagent mixes and add them into the microchip reactors

The results of virus detection in grapevine samples



Results of real-time RT-PCR of grapevine sample with GFkV and GFLV pathogens (**blue curves**). Also shown positive control samples C+ (**red curves**) and negative control samples C- (**green curves**) for all six pathogens and internal control sample IC.

3 Insert the microchip into the AriaDNA[®] analyzer and run the analysis via the software on a PC

4 Obtain real-time PCR results and print report in 45-60 minutes

The information and specifications in this publication are subject to change without notice.

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