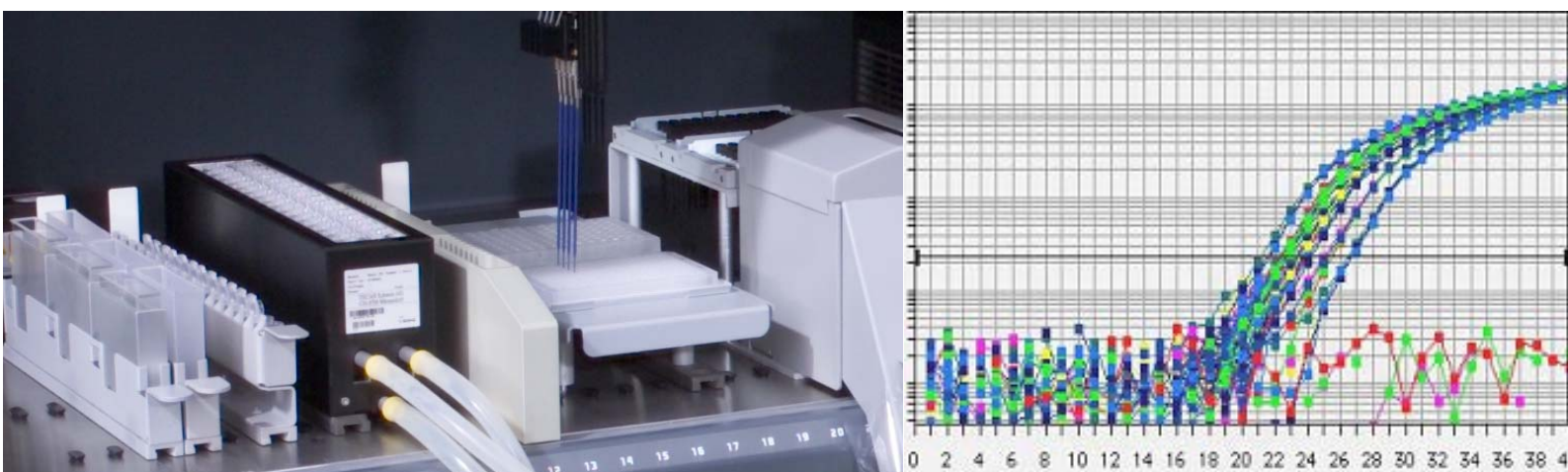


Automated PCR Setup

Automated Real-Time PCR Set-up on a Tecan Freedom PCR Workstation



Introduction

Real-Time chemistry allows for the detection of PCR amplification products during the early phases of the reaction. Measuring the kinetics of the reaction provides a distinct advantage over traditional PCR.

The Tecan Freedom PCR Workstation provides a highly flexible platform for automated set-up of traditional PCR or Real-Time PCR. Using Tecan's disposable tips, precise and accurate set-up even at low volumes can be performed without any risk of cross-contamination.

Moreover, with the integrated robotic manipulator (gripper tool) and appropriate storage modules safe and reliable set-up of multiple batches of 96 samples is possible without any user interaction.

PCR Procedure

Multiplex Real-Time PCR can be used for quantification of downy mildew infestation of grape plants. For this purpose, total DNA from plant (*Vitis vinifera*) and parasite (*Plasmopara viticola*) is extracted from infected leaf punches by traditional extraction methods. Multiplex Real-Time

PCR is performed in an ABI PRISM® 7700 instrument (Applied Biosystems) with specific primers for each species, labeled with different fluorescence labels (FAM™ and VIC™, respectively). Typically 36-40 PCR cycles were performed using AmpliTaq® Gold chemistry (Applied Biosystems). To check for potential cross-contamination, negative controls are placed in every second row on the amplification plate.

Automation

Automation of the Real-Time PCR assay preparation is realized on a Tecan Freedom PCR Workstation. The instrument is equipped with 8 pipetting channels (disposable tips) and specific carriers for precise positioning of Taqman® plates (Applied Biosystems). Pipetting of mastermix (25 µl) and template DNA (5 µl) is performed with either 200-µl or 50-µl disposable tips (approx. 6 minutes for reaction set-up of 96 samples). Gemini™ 4.0 is used for software control of the process.

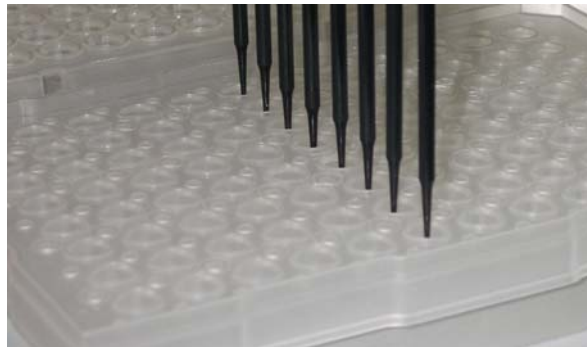


Fig. 1: Assay Set-up

Full automation of the Real-Time PCR set-up on a Tecan Freedom PCR Workstation using 50 µl disposable tips for template pipetting. Multiple plates can be prepared without any user interaction.

To check for potential cross-contamination, a stripe pattern was used for sample (amplification template) and negativ controls (water) distribution into the PCR plate.

Results

Reliable and precise pipetting is demonstrated by consistent low variation of C_t values within replicates ($n=4$). All liquid transfers are performed without any cross-contamination as confirmed by non-appearance of amplification products in negative control wells. Reaction set-up of 96 samples and controls is completed within approximately 10 minutes.

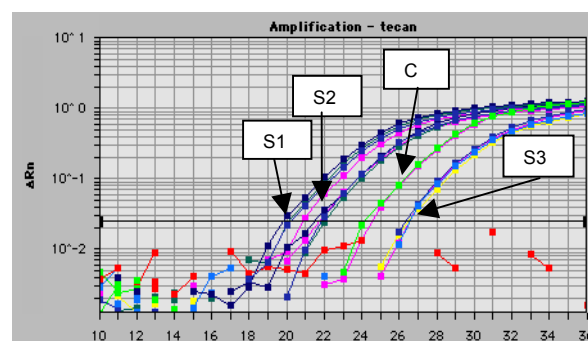


Fig.2: Reliable Real-Time-PCR

Typical Real-Time amplification profile (Taqman[®]; FAM) of *Vitis vinifera* DNA samples (multiples of three samples (S1, S2, S3) and amplification control with standard template (C) are shown).

ΔC_t within replicates is consistently <1.3 . No amplification of negative controls was observed.

Analogous results for *Plasmopara viticola* DNA amplification (data not shown).

Conclusion

The Tecan Freedom PCR Workstation is the ideal instrument for automated Real-Time PCR pipetting:

- high precision pipetting
- no cross-contamination
- deckspace for multiple batches
- flexible access of pipetting tips to various tube and plate types

Complex amplification control patterns are often typical for Real-Time PCR assays. Due to its versatile Liquid Handling Arm (independent z-move-

ment of pipetting tips and flexible tip spacing), the Freedom Sample Preparation Workstation is able to perform any PCR set-up task with high speed and reliability.

Low-volume disposable tips enable precise liquid transfers down to 1 µl.

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