ISSR-PCR fingerprinting of plant pathogen strains using the QIAxcel® system and BioNumerics®

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A reliable method for genotypic fingerprinting of plant pathogen strains would be an important tool for tracking sources of infections and determining effective control strategies. This application note describes a reliable method for this purpose using the QIAxcel Advanced System in combination with BioNumerics software. The plant pathogen model used was bacterial canker of tomato (*Clavibacter michiganensis* subsp. *michiganensis*).

Introduction

The causal organism of bacterial canker in tomato plants is the seed-borne pathogen *Clavibacter michiganensis* subsp. *michiganensis* (1). It is a quarantine organism under the European Union Plant Health legislation (2). It poses serious economic risks in tomato cultivation, as epidemics can result from the spread of contaminated seed and transmission of the pathogen to seedlings and plants (3).

In this study, ISSR sequences of individual *C. michiganensis* subsp. *michiganensis* strains were amplified using ISSR-PCR and analyzed using the QIAxcel Advanced System. ISSR stands for Inter Simple Sequence Repeat and refers to the genome regions between microsatellite loci. It is a rapid and inexpensive genotyping technique with a wide range of applications, including the characterization of genetic relatedness among the organisms of a population (4). ISSR profiling is as reproducible as AFLP, but more cost effective (5).

The QIAxcel Advanced System ScreenGel® software is fully compatible with BioNumerics software. Using the QIAxcel plugin, raw data files from the QIAxcel software can be imported as densitometric curve files (with the file extensions .xml and .csv) or peak table files (with the file extensions .xdr). BioNumerics Plug-in v1.0 makes it possible to link any sample information (e.g., sample names) provided in ScreenGel (v1.0.1.0) to entries in a BioNumerics database. After ISSR fingerprints have been imported, they can be reliably normalized. Automated peak-calling algorithms allow rapid assignment of bands. Optionally, the software can exclude interfering primer dimers by discarding all peaks below or above a certain peak height.

Since the BioNumerics platform allows the storage of genomic and phenotypic biological data in one program, it also enables the combination of fingerprint patterns obtained using ISSR-PCR ▶

with results from other band-based techniques (e.g., RFLP and PFGE sequencing data and binary and numerical character arrays). With these possibilities in mind, the combination of the QIAxcel Advanced System with BioNumerics was assessed as a method for pinpointing sources of pathogenic plant infections, such as bacterial canker of tomato.

Materials and methods

The amplification reaction mixtures (25 µl) consisted of 1× PCR buffer (containing 100 mM Tris-HCl, 15 mM MgCl2 and 500 mM KCl at pH 8.3), 0.2 mM each of dNTPs, 0.5 µM primer (5'-TGCCGCCGCCGCC-3'), 0.5 U AmpliTaq DNA polymerase and 50–60 ng template DNA. DNA extracts were prepared according to Pitcher's protocol (6) which was adapted for Gram-positive bacteria with an additional lysozyme step (5 mg lysozyme in 150 µl TE buffer per sample). The PCR program consisted of initial denaturation at 94°C for 5 min followed by 36 cycles (94°C for 5 min, 64°C for 45 s, 72°C for 2 min) and a final extension at 72°C for 10 min. Samples were analyzed with the QIAxcel Advanced System and QIAxcel DNA High Resolution Kit using method OM1200 with an additional 120-second separation time. QX Alignment Marker 50 bp/5 kb was included in the run. QIAxcel ScreenGel software v1.0.1.0 was used to run the samples and export the raw .xml or .csv data. The QIAxcel plugin v1.00 was used to import the raw fingerprint files to BioNumerics version 6.6, using an OD range of 20,000 points and a normalized track resolution of 2500 points as the fingerprint conversion setting.

Figure 1. Dendrogram and gel image showing the relatedness of *Clavibacter michiganensis* subsp. *michiganensis* strains. The primer in all cases was ISSR5 and the annealing temperature was 64°C.

Results

PCR-amplified ISSR sequences of individual *C. michiganensis* subsp. *michiganensis* strains were resolved, showing well separated, sharp bands in the range of 500–3000 bp. Figure 1 is a dendrogram displaying the relatedness of the isolated strains.

Conclusions

■ The QIAxcel Advanced System gave high-resolution electropherograms, with high bootstrap values, objective band scoring, and minimized background variation. It enables highthroughput analyses of samples in the 96-well plate format.

■ The analysis data stored in the QIAxcel ScreenGel software can easily be transferred to BioNumerics via the QIAxcel plugin. This enables rapid, reproducible, inexpensive genotyping. Furthermore, it contains integrated analysis and visualization tools for data mining, clustering, identification and statistical analyses. This functionality enables decisive conclusions to be reached.

■ The combination of the QIAxcel Advanced System and BioNumerics should prove a useful approach in isolating sources of infectious plant diseases, based on the results of this study using *Clavibacter michiganesis* subsp. *michiganesis* as a model.

References
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Ordering Information

BioNumerics configuration	Description
Fingerprint data module	Module to store and process fingerprint data
Tree & Network Inference module	Module to cluster data and create dendrograms
QIAxcel plugin	Specialized module to handle QIAxcel data in an intuitive and fast way

Visit www.applied-maths.com/bionumerics/modules for more information

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