

Application Note

Advantages of the QIAxcel[®] system for bacterial genotyping

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The QIAxcel system was successfully used together with the QIAxcel DNA Screening Kit for high-throughput genotyping of bacteria. The QIAxcel system enabled greater sizing accuracy and more sensitive detection than conventional agarose gel electrophoresis.

Introduction

Comparative genomic studies have demonstrated extensive intraspecies genomic variability in some bacterial species and have led to identification of “accessory” genes that are present in some but not all strains of *C. jejuni* and verotoxigenic *E. coli* (1–3).

Comparative genomic fingerprinting (CGF) is a novel method of comparative genomics-based bacterial characterization based on the concept that differential carriage of these accessory genes can be used to generate unique genomic fingerprints for genotyping purposes. A CGF assay for the analysis of *E. coli* was recently developed in our laboratory and shows great promise as a high-throughput comparative genomics-based method for genotyping that yields epidemiologically relevant information (4). We recently developed a CGF method for *C. jejuni* based on assessing the conservation status of 20 accessory genes. These 20 genes are targeted by a series of four 5-plex PCRs designed based on data from multiple sequenced genomes. Target genes were selected to represent whole-genome genetic diversity by targeting hyper-variable regions previously identified (1). The genes selected were either present or absent on different genome strains and displayed little sequence variation when present. The latter enabled



The QIAxcel system enables fully automated analysis of up to 96 samples per run.

PCR primers to be easily designed in SNP-free regions.

Although the CGF method has a favorable throughput when compared to standard methods for *C. jejuni* genotyping, we sought to adapt the assay to the QIAxcel system to increase our throughput and facilitate data analysis. As part of this process, we performed extensive cross-validation to compare conventional agarose gel results to those obtained using the QIAxcel instrument.

Materials and Methods

CGF assay PCR

Each gene in the assay is represented by a signature amplicon, with each 5-plex PCR producing a unique 5-band fingerprint, and this presence/absence profile of the 20 genes is used to produce the comparative genomic fingerprint. PCR was carried out in a 50 μ l reaction volume, with \triangleright

each reaction containing 5–20 ng of template DNA, 1x PCR buffer, 1.0 mM MgCl₂, 0.2 μM of each dNTP, 0.5 μM of each primer, and 1 U of QIAGEN *Taq* DNA polymerase. Amplification was performed on a GeneAmp® PCR System 9700 thermal cycler with an initial denaturation step at 94°C for 5 min, followed by 34 amplification cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s. A final elongation step at 72°C was carried out for 5 min before storing the sample at 4°C for short-term storage, or at –20°C for long-term storage.

CGF assay analysis by agarose gel electrophoresis

Multiplex PCRs were analyzed by electrophoresis in 2.5% agarose gels containing 1 μg/ml ethidium bromide. Band sizes were estimated using a 1 kb molecular size marker. Gels were run at 10 V/cm until the Bromophenol Blue loading dye reached a distance of 8 cm from the loading well. Gels were visualized using UV light and photographed using a Syngene transilluminator.

CGF assay analysis using the QIAxcel platform

Multiplex PCRs were analyzed using the QIAxcel system using the AM320 method with an injection time of 20 s. The

15 bp–3000 bp alignment marker was used as internal standard marker and band sizes were determined using the QX 100 bp to 3 kb DNA size marker. Data were visualized using BioCalculator Software (version 3).

Analysis of concordance

Direct comparison of agarose gel electrophoresis with capillary electrophoresis was performed by analyzing the same four sets of 5-plex PCRs for 96 samples using both methods. The presence/absence of each gene was scored and global concordance was assessed by calculating the number of matches as a proportion of the total number of data points (i.e., 96 samples x 20 genes).

Results and discussion

The overall concordance between the QIAxcel and agarose gel electrophoresis was 97.4% (1870/1920). A large proportion of all mismatches (39/50) was in three of the 20 genes assayed (Figure 1); the concordance in the remaining genes was 99.3%. Every single mismatch could be traced to problems with the agarose gel data.

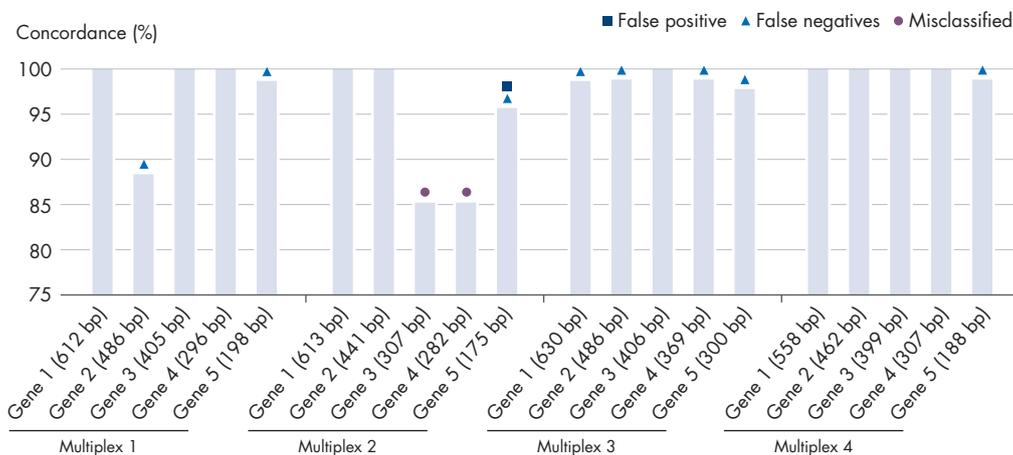


Figure 1. Percent concordance of band calls from agarose vs. QIAxcel data (n=96). A total of 50 mismatches were observed for a global concordance of 97.4% (1870 concordant datapoints in 1920 observations, i.e., 96 samples x 20 genes). Differences were primarily found in 3 of the 20 genes of the assay, which accounted for 39 of the mismatches observed. The concordance of the remaining 17 genes was 99.3% (1621 concordant data points in 1932 observations, i.e., 96 samples x 17 genes).

The increased sensitivity of the QIAxcel compared to agarose gels revealed erroneous calls in agarose gels arising from weak amplicons that could not be easily detected (Figure 2). The weak amplicons detected by QIAxcel in the original samples could only be detected by conventional gel electrophoresis after the original sample was concentrated five-fold. Although this resulted in enhanced detection of weak bands, it also led to problems visualizing other samples due to overloading. In contrast, we had no problems visualizing either the original samples or the concentrated samples with the QIAxcel instrument.

The sizing accuracy and resolution of the QIAxcel compared to agarose gels revealed erroneous calls in agarose gels arising from band sizing errors (Figures 3 and 4). Slight gel anomalies may lead to erroneous calls when amplicons are sufficiently similar in size unless extreme precaution is taken to maintain reproducibility and quality control during electrophoresis and during subsequent analysis of the gel image data. This is a major challenge for high-throughput environments and represents the source of all of the band size-related errors observed in our dataset (Figure 5).

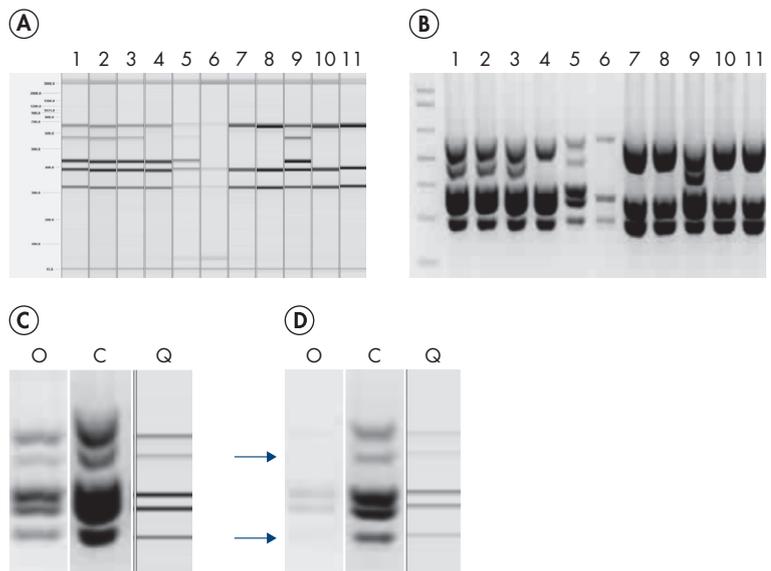


Figure 2. False negative error calls due to weak amplicons when analyzing samples by agarose gel electrophoresis. Samples were analyzed on either **A.** the QIAxcel at the original concentration or **B.** by agarose gel electrophoresis with five-fold concentration. Samples **3 C.** and **5 D.** were analyzed by agarose gel electrophoresis at the original concentration (**O**), with five-fold concentration (**C**), and at the original concentration on the QIAxcel (**Q**). The arrows represent bands originally scored as negative.

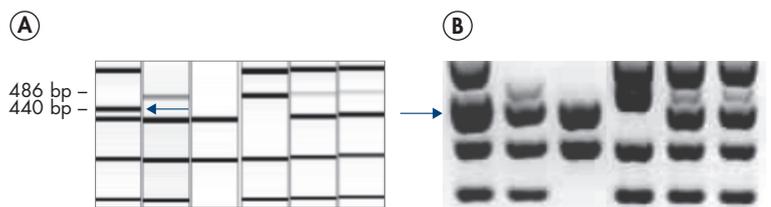


Figure 3. False positive error calls due to poor resolution when analyzing samples by agarose gel electrophoresis. Samples were analyzed either **A.** the QIAxcel or **B.** by agarose gel electrophoresis. The arrows represent the bands of approximately 440 bp that was originally scored as positive due to its proximity to the band of expected size (486 bp).

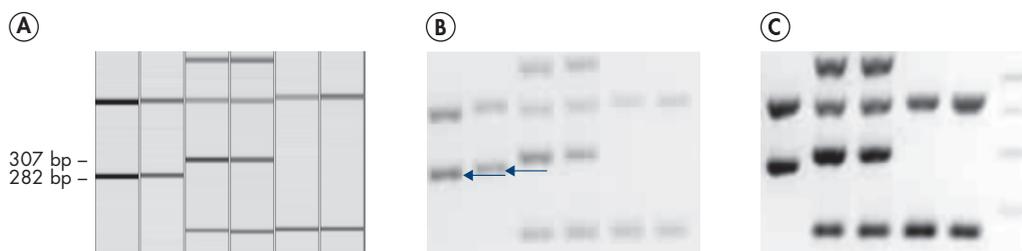


Figure 4. Misclassification of error calls due to poor resolution coupled with gel anomaly when analyzing samples by gel electrophoresis. Samples were analyzed either **A.** the QIAxcel or **B.** and **C.** by agarose gel electrophoresis. The arrows in **B.** represent the bands that were misclassified as the 307 bp band due to a gel artifact. These samples were reanalyzed by agarose gel electrophoresis in **C.**

- False negative (i.e. "weak")
- False positive (i.e. "wrong size")
- Misclassified

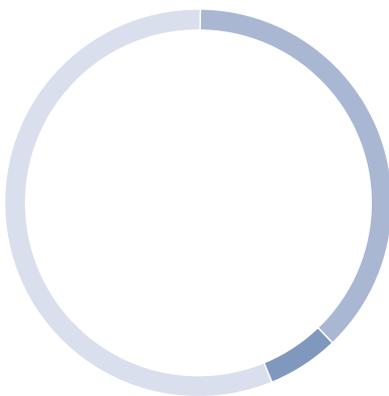


Figure 5. Classification of mismatches observed in the dataset after analysis by agarose gel electrophoresis or on the QIAxcel. Each of the 50 mismatches was examined to determine the nature of the mismatch. False negatives (38%, 19/50) were weak amplicons that were below the detection limit for agarose gel electrophoresis but could be detected on the QIAxcel (see Figure 1). False positives (6%, 3/50) were amplicons of incorrect size and called positives after agarose gel electrophoresis due to sufficient similarity to the expected amplicon size (see Figure 2). Misclassified (56%, 28/50) were amplicons misclassified after agarose gel electrophoresis because they were of sufficiently similar size to the expected bands, e.g., 282 bp compared with 307 bp (see Figure 3).

Three of the 20 genes assayed were found to be a source of problems in the manual agarose gel-based assay, with one gene prone to false negatives and two genes prone to misclassification. Despite the high concordance between the platforms, fully a third of the samples analyzed (32/96 samples) were affected by problems with these genes and had at least 1 erroneous band call on agarose gel electrophoresis. This would affect our ability to detect strains with 100% matching fingerprints, a key aspect of molecular epidemiological investigations. The data obtained from the QIAxcel was instrumental in identifying these genes so that PCR primers could be re-designed to mitigate these adverse effects.

Conclusions

Although the QIAxcel displayed extremely high concordance with conventional agarose gel electrophoresis, greater sizing accuracy and greater sensitivity of detection allowed the QIAxcel data to outperform the agarose gel data.

The QIAxcel system enables easy maintenance of high quality control over multiple electrophoresis runs and displays a wider dynamic range than conventional gel electrophoresis. As a result, bands both in weak samples and in highly concentrated samples are accurately detected, making the QIAxcel an extremely robust, high-throughput platform for these types of genotyping applications.

References

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