Application Guide

QIAxcel® Advanced

Automated, High-Performance, Versatile Electrophoresis

Sample to Insight

Chapter Contents

Introduction

The QIAxcel Advanced is a high-resolution capillary electrophoresis system designed to provide a versatile solution to the limitations and bottlenecks of slab-gel electrophoresis. The system allows fast and accurate analysis of up to 96 samples and hands-free sample loading and in-built components minimize exposure to hazardous chemicals and error-prone manual steps.

Ready-to-use gel cartridges in combination of pre-programmed methods, allow separation and analysis of both DNA and RNA and caters to a broad range of applications in all field of life sciences such as analysis of single or multiple PCR and restriction fragments including gene editing with CRISPR technology as well as quality control of gDNA, NGS libraries, or RNA samples.

Key benefits of the QIAxcel Advanced include:

- High resolution analysis, down to 3–5 bp for fragments smaller than 0.5 kb, with high-level multiplexing capabilities.
- Quality control of virtually any nucleic acid.
- Flexible throughput with analysis of 12 samples is 3–10 minutes and up to 96 samples per run.
- Cost-effective and environment-friendly reusable and ready-to-use cartridges, bringing unmatched convenience and safety in your electrophoresis assays.
- Digital data output, standardization of experiments and powerful analysis.
- Flexible data reporting and exporting.

We have compiled this collection of application notes to demonstrate the wide range of applications of the QIAxcel Advanced System, in a convenient and easy-to-use format. These application notes are the result of the hard work of scientists across many institutions and disciplines, and we extend our thanks to all the researchers who contributed and shared their results.

For more related information on the QIAxcel Advanced System, please visit **www.qiagen.com/QIAxcel**.

1 gDNA analysis
RNA analysis
NGS library QC

One major factor determining the success of an assay and its reproducibility is the quality of the sample itself. The QIAxcel Advanced addresses this challenge by performing fast and reliable quality control of your nucleic acid samples with only little input material. It measure the concentration of your samples, analyzes their size distribution as well as their integrity so you can assess their potential to achieve quality results. This enables you to standardize the quality of your samples early on and thus make well-informed decision prior to processing them through your workflow.

gDNA extraction from tissues, cells or organisms is a crucial first step for all subsequent DNA analyses. Evaluation of RNA (either for total RNA and/or messenger RNA) quality and quantity is critical for many molecular biology techniques as well as microarray-based research experiments. Accurate quantification of NGS library fragments in a high-throughput automated fashion is becoming significantly important for many research laboratories.

Content

Quality control of genomic DNA using the QIAxcel® system

André Schaller, Christopher Jackson, Division of Human Genetics, Inselspital Bern, Berne, Switzerland

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This application note describes a rapid, reliable, and effective method for quality control of purified genomic DNA using the QIAxcel system. The results are highly reproducible and clearly show whether degradation products are present in a sample.

Introduction

The results of numerous molecular screening and assay methods rely on the quality of the genomic DNA (gDNA) that is used. Effective techniques for purification are crucial to secure gDNA that will give the best results in downstream procedures, but optimized quality control of purified gDNA is equally important. It helps to avoid time and money being wasted, particularly in the case of costly procedures, such as Next Generation Sequencing (1), where gDNA is fragmented and used for library preparation.

The sizes of purified gDNA fragments depend on the purification method. They fall between 20 and 30 kb when spin columns with silica-based membranes are used, and can be up to 100 kb or more when salting-out precipitation is applied. The silica-based method is the most commonly used, and it can be done manually or automated with a sample purification instrument such as the QIAcube®.

Using the QIAxcel system, quality control is possible for all gDNA that has been purified with silica membrane-based methods. It offers a straightforward and effective means for checking the quality of all samples purified with kits that use this approach (e.g., QIAamp® DNA Blood Kit, QIAamp DNA FFPE Tissue kit, QIAamp MinElute Virus Kits).

Materials and Methods

Genomic DNA was isolated from blood samples using QIAGEN's QIAamp DNA Blood Mini Kit, which is based on silica membrane DNA purification. The protocols were automated and run on the QIAcube.

The DNA samples were quantified with a NanoDrop® spectrophotometer. Concentrations of 10–100 ng/µl were chosen for testing. Samples were analyzed on 1% agarose gels in 30 mM TAE buffer for 2.5 h at 80 V. The same samples were analyzed using the QIAxcel system ▷

for capillary electrophoresis with a DNA Screening Cartridge. The AM900 method and QX Alignment Marker 15 bp were used. AM900 is a customized method with the following parameters: alignment marker injection at 4 kV for 20 sec, sample injection at 2 kV for 40 sec, and separation at 3.5 kV for 900 sec.

For degradation analyses, the samples were sonicated with 7, 14, 70 and 105 pulses. Ultra-sound degradation was performed in 500 µl (100 ng/µl) of DNA, sonicated on a Branson Sonifier® 250.

Results

Samples from the dilution series were electrophoresed on a 1% agarose gel (Figure 1A), as well as on the QIAxcel system using a DNA Screening Cartridge (Figure 1B). The results demonstrate that gDNA concentrations between 25 and 100 ng/µl are suitable when using this system.

Various running conditions, such as injection time, separation time and voltage, were tested (data not shown) and optimized. AM900 was identified as the optimal procedure for gDNA quality control with sample injections of 40 sec at 2 KV and a separation time of 900 sec at 3.5 KV.

The results of assessing sonicated gDNA samples with the QIAxcel system revealed that it is very simple to determine the degree of degradation by categorizing the type of peak yielded from each gDNA sample (Figure 2). A major peak with no signals for degradation products before it and a long tailing off after it indicates gDNA of very good quality that is suitable for downstream applications. Electropherograms for partially and highly degraded gDNA have no tailing off after the major peak. The level of degradation can be differentiated based on the signals for the degradation products in the broad peak before the major peak.

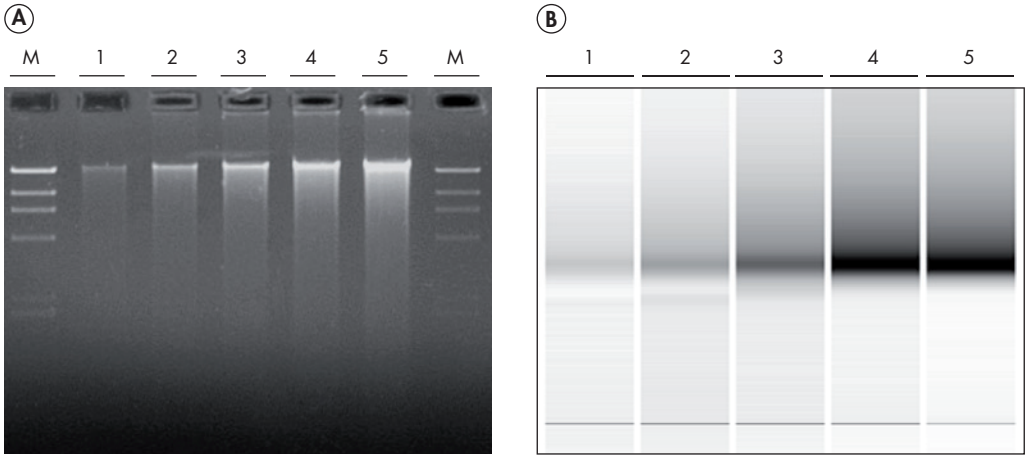

Figure 1. Electrophoresis of samples from a dilution series. A Lanes 1–5: gDNA samples at respective concentrations of 10, 25, 50, 75, and 100 ng/µl run on a 1% agarose gel for 150 min at 80 V. **Lane M:** lambda HindIII size marker. **B** The same samples run using a QIAxcel DNA Screening Cartridge and the AM900 method.

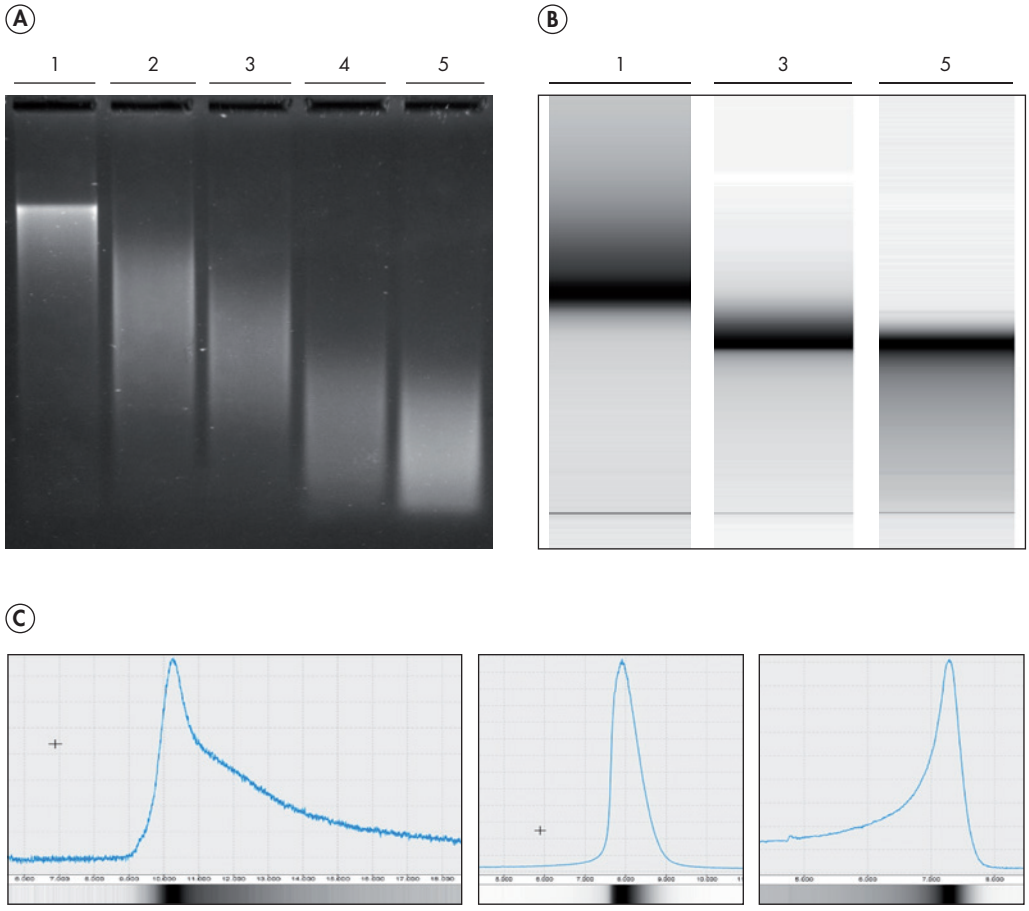

Figure 2. Assessment of DNA degraded by sonication. A Agarose gel photo showing gDNA in various states of degradation. **Lane 1** non-degraded gDNA samples. **Lanes 2 and 3:** gDNA samples partially degraded by sonication with 7 and 14 pulses, respectively. **Lanes 4 and 5:** gDNA samples fully degraded with 70 and 105 pulses, respectively. **B** QIAxcel gel photo showing gDNA samples in three states of degradation. 1, 3, and 5 correspond to lanes 1, 3, and 5 in **A**. **C** Electropherograms and a gel photo of gDNA samples from lanes 1, 3, and 5 in **A**. **Lane 1:** Electropherogram indicates very good quality gDNA (no degradation products before the major peak, long tailing off). **Lane 3:** Partially degraded gDNA (some degradation product signals, no tailing off). **Lane 5:** Highly degraded gDNA (many degradation product signals, no tailing off).

Conclusions

Quality control of genomic DNA purified with silica membrane-based methods can routinely and effectively be performed using the QIAxcel system. The optimal procedure uses the DNA Screening Cartridge and the AM900 method.

The results are highly reproducible, as shown in repeated runs. Using the electropherogram data as well as the results from a gel image allows straightforward verification of the integrity of the purified gDNA. If degradation products are present, they are visible as a broad peak preceding the major gDNA peak.

In order to detect even minute quantities of unwanted degradation products, a long sample injection time of 40 seconds is suitable. It is recommended that gDNA concentrations ranging between 25 and 100 ng/µl are used.

This novel method using the QIAxcel system is optimal for gDNA quality control.

References

1. Shendure, J. and Hanlee, J. (2008) Next Generation Sequencing. Nature Biotechnology **26(10)** 1135.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QX RNA Alignment Marker (1.5 ml)	RNA Alignment marker	929510

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Visit **www.qiagen.com/QIAxcel** and find out how automated gel electrophoresis can benefit your lab.

Quality control of total RNA and cRNA for microarray analysis

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In this application note, the suitability of the QIAxcel® system was assessed for quality control of RNA for microarray analysis. The values obtained for the tested quality control parameters indicate that the QIAxcel system is highly suited for analyzing the quality of total RNA and fragmented or intact cRNA.

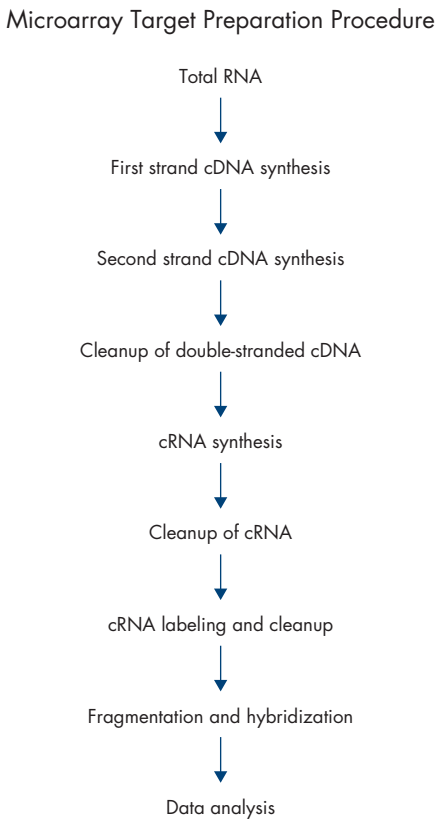

Figure 1. Typical workflow for microarray analysis.

Introduction

Microarray technology is a powerful tool used to determine the expression levels of thousands of genes in a single experiment and, thus, has become increasingly important in biomedical research and life science applications. Preparation of cRNA, which is then hybridized to microarrays, is a multistep procedure (Figure 1) that is prone to random and systematic errors, especially when large numbers of samples are handled. Therefore, it is critical to minimize experimental noise, standardize processing procedures, and include appropriate experimental controls and replicates.

Monitoring the quality of the initial total RNA sample as well as products generated throughout the entire procedure is crucial, since their quality strongly influences the predictive power obtained from microarray data.

Quality control parameters applied to total RNA samples typically include:

- Determination of the A_{260}/A_{280} absorbance ratio to indicate protein contamination
- Determination of the A_{260}/A_{230} absorbance ratio to detect potential chemical contaminants such as guanidinium thiocyanante
- Determination of the 28S/18S rRNA ratio to check the integrity of the total RNA

While double-stranded cDNA is usually not subjected to quality control due to the limited amount of material produced, the size distribution of unfragmented cRNA is usually analyzed. In addition, efficiency of fluorescent labeling of cRNA is also determined. We evaluated the QIAxcel system for quality control of total RNA and cRNA prior to Cy®3/Cy5 labeling.

Materials and methods

Total RNA isolation from *Schizosaccharomyces pombe* (fission yeast) was performed according to standard methods. The protocols described below apply to RNA isolated by different methods and from different organisms.

RNA samples were prepared for capillary electrophoresis according to the protocol in the *QIAxcel RNA Handbook*: A 1 µl aliquot of the RNA eluate was mixed with 1 µl RNA denaturing buffer, heated at 70°C for 2 minutes, and cooled in ice-cold water. Sample volume was adjusted to 10 µl with QX RNA Dilution Buffer, and samples were subsequently analyzed on the QIAxcel system using the QIAxcel RNA QC Kit v2.0 and the CM-RNA method.

cRNA generation and labeling

Synthesis of double-stranded cDNA, amplification to cRNA, and labeling were performed as described (1). After amplification, cRNA was normalized to 600 ng/µl and analyzed using the QIAxcel system. After quality control, the cRNA was labeled with Cy3 or Cy5 dyes and then hybridized to self-spotted or commercial microarrays.

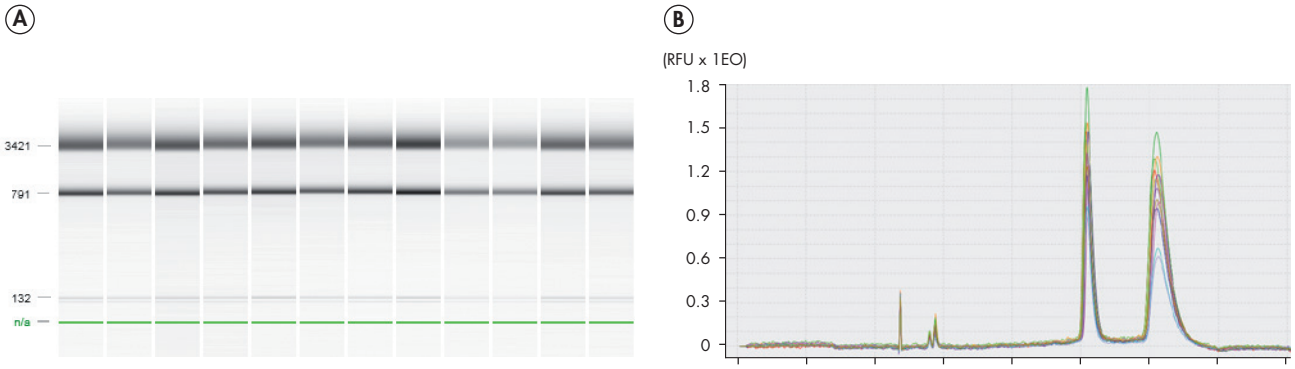

Figure 2. Streamlined RNA analysis using the QIAxcel system. Total RNA purified from *Schizosaccharomyces pombe*. Results presented as **A** a gel image and **B** a superimposed electropherogram view.

Pos.	Plate Id	M	18S	28S	Size 18S	Size 28S	Total RNA Conc.	Ratio
A - 1	100817-TotalRNA	Yes	Yes	Yes	1805.1	3439.9	268.0	1.61
A - 2	100817-TotalRNA	Yes	Yes	Yes	1825.1	3506.2	197.3	1.62
A - 3	100817-TotalRNA	Yes	Yes	Yes	1805.8	3440.6	262.1	1.62
A - 4	100817-TotalRNA	Yes	Yes	Yes	1830.7	3503.7	221.3	1.58
A - 5	100817-TotalRNA	Yes	Yes	Yes	1835.8	3509.0	269.1	1.68
A - 6	100817-TotalRNA	Yes	Yes	Yes	1860.4	3556.8	202.8	1.56
A - 7	100817-TotalRNA	Yes	Yes	Yes	1851.6	3536.3	244.5	1.54
A - 8	100817-TotalRNA	Yes	Yes	Yes	1829.0	3493.8	291.1	1.56
A - 9	100817-TotalRNA	Yes	Yes	Yes	1833.1	3522.1	146.2	1.36
A - 10	100817-TotalRNA	Yes	Yes	Yes	1831.2	3534.1	138.5	1.40
A - 11	100817-TotalRNA	Yes	Yes	Yes	1814.8	3466.0	248.2	1.60
A - 12	100817-TotalRNA	Yes	Yes	Yes	1826.0	3501.6	233.9	1.62

Figure 3. Screenshot showing 28S/18S rRNA peak ratios calculated from samples shown in Figure 2A.

Results

The QIAxcel system is a capillary electrophoresis system that processes samples in batches of 12, for analysis of up to 96 samples without manual intervention. Data can be viewed in both gel image and electropherogram format. Intact RNA is a prerequisite for successful microarray analyses. QIAxcel ScreenGel™ software provides reliable analysis of the size, quantity, and quality of total RNA prior to microarray analysis (Figure 2). The two distinct rRNA peaks and 28S/18Speak ratios of 1.48–1.67 indicate the high quality of the total RNA (Figures 2 and 3).

Analysis of unlabeled cRNA shows expected profiles with a smear of products concentrated in size from 500 bp to 2000 bp. The size distribution should be equal between samples: less intact RNA yields smaller cRNA fragments (Figure 4).

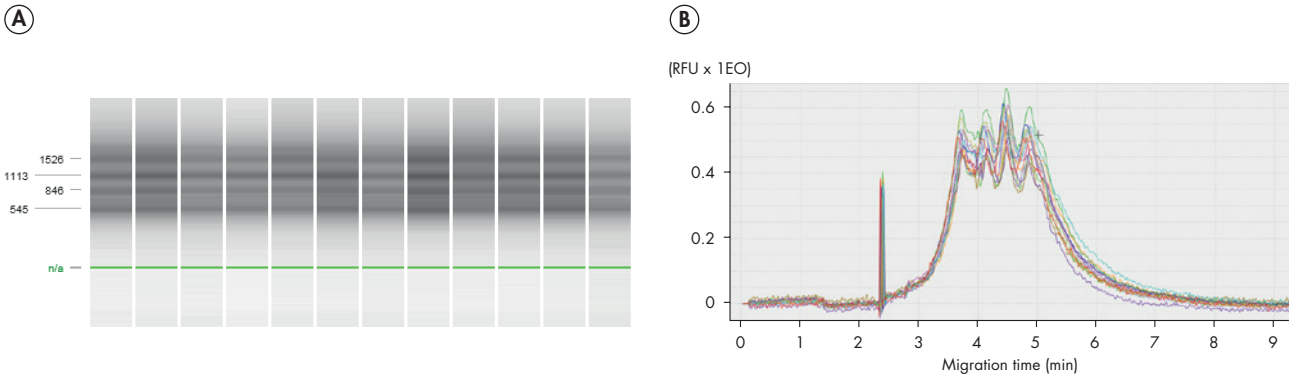

Figure 4. Reliable quality control of unlabeled cRNA generated from yeast total RNA. Results presented as **A** a gel view and **B** a superimposed electropherogram view.

Conclusions

These results demonstrate the high suitability of the QIAxcel system for fast, sensitive analysis of the quality and quantity of total RNA and fragmented or intact cRNA. The 28S/18S rRNA ratio, a measure of RNA integrity, was successfully determined — a crucial factor for meaningful gene expression data. The ease of use, fast processing times, and the ability to analyze up to 96 samples without manual intervention make the QIAxcel system particularly well suited for research laboratories with high-throughput needs.

Reference

1. Lenstra, T.L. et al. (2011) The specificity and topology of chromatin interaction pathways in yeast. Mol. Cell **42** 536.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel Software, and 1-year warranty on parts and labor	9001941
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104

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Identifying rare mutations in Diamond-Blackfan anemia using target enrichment and next-generation sequencing

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Introduction

Diamond-Blackfan anemia (DBA) is a rare congenital stem cell disorder associated with monoallelic inactivating mutations in the ribosomal protein (RP) genes. It leads to bone marrow failure syndrome by causing defects in erythroid progenitor and precursor cell development (1).

Loss of function mutations in 10 of the c. 80 RP genes have been definitively associated with DBA. *RPS19* is mutated in up to 25% of DBA cases, and 13 other RP genes are mutated in a further 25–35%. The molecular basis of the remaining 40–50% of cases is unknown. Since such cases may harbor mutations in one or more of the remaining RP genes and such mutations may occur at very low frequencies, genetic screening using conventional Sanger sequencing on a per-exon/per-gene basis is challenging. Therefore, we developed a methodology based on custom target enrichment technology combined with high-throughput sequencing. To ensure high quality libraries, rapid quality control using the QIAxcel® Advanced System was used at several steps of the library preparation process. We chose the QIAxcel for capillary electrophoresis because it uses ready- to-run gel cartridges and has very short, automated runs that are suitable for high-throughput analysis. Next-generation sequencing was performed using MiSeq®, an Illumina® platform, to screen all 80 RP genes. This proved to be a powerful approach for finding rare mutations in a large set of genes.

Materials and methods

Target enrichment

SureSelect® XP (Agilent®, US) was used for the target enrichment, which employed a custom designed RNA bait hybridization solution to capture the target genes, including the intronic regions and 500 bp of the flanking untranslated region. The regions of interest were collated from the Ribosomal Protein Gene Database (**http://ribosome.med.miyazaki-u.ac.jp**) and uploaded to the Agilent eArray design facility.

Library preparation and sequencing

Library preparation is a complex process with multiple steps. Therefore it is important to assess the sample quality after several steps to ensure the appropriate quality of the final libraries. DNA was purified from peripheral blood leukocytes using QIAamp® DNA Mini Kit on the QIAcube®. A 3 µl sample of gDNA was sheared using a Covaris® e220 sonication platform, and the fragment size was determined via capillary electrophoresis using the QIAxcel Advanced System. Samples were diluted 1 µl to 10 µl with DNA dilution buffer and run on the instrument with the appropriate screening kit. The AM320 method was used in combination with the 15 bp/5 kb alignment marker and a 100 bp – 2.5 kb DNA size marker.

Additional quality control was done after amplification of the ligated libraries and after amplification of the capture libraries. All three steps were analyzed with the QIAxcel DNA Screening Kit using method AM320. The analysis protocol parameters were: baseline filter 160 sec, minimum distance 5 sec, and threshold 10 S/N. The run, reference marker table (RMT), and analysis parameters were defined as a standard protocol that was saved with ScreenGel® software and used for all of the runs.

The capture hybridization was carried out at 65°C for 48 h. Libraries were quantified for pooling using qPCR. Sequencing was performed on an Illumina MiSeq using 150 bp paired-end reads and multiplexed into two runs of 10 samples each. Putative mutations were validated with Sanger sequencing in a CPA-accredited laboratory using an Applied Biosystems® 3500xL Genetic Analyzer.

Results

Per the SureSelect Target Enrichment protocol for Illumina Multiplex sequencing, quality control was performed after shearing of the genomic DNA (Figure 1), amplification of the adapter-ligated libraries (Figure 2), and after amplification of the captured libraries (Figure 3).

The QIAxcel Advanced System allows complete analysis of 12 samples, including reporting, in 8 min. The results are presented as a gel-like image, electropherogram, and result table with different requested parameters (in this case, size and concentration of libraries).

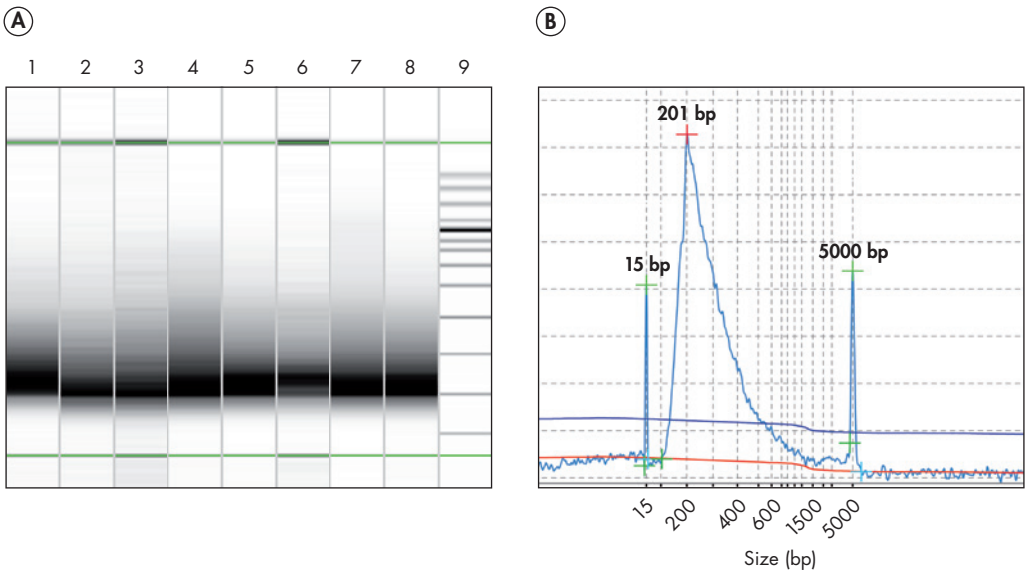

Figure 1. Analysis of sheared genomic DNA. **A** Lanes 1–8: Genomic DNA sheared using a Covaris e220 sonication platform was analyzed using the QIAxcel DNA Screening Kit and method AM320. QX Alignment Marker 15 bp/5 kb (marked in green) was run simultaneously with all of the samples. Lane 9: The QX DNA Size Marker 100 bp – 2.5 kb was used for precise size and concentration assessments. The image shows a smear in the range of 50 – 350 bp. **B** The electropherogram shows the distribution. The medium peak size is 200 bp.

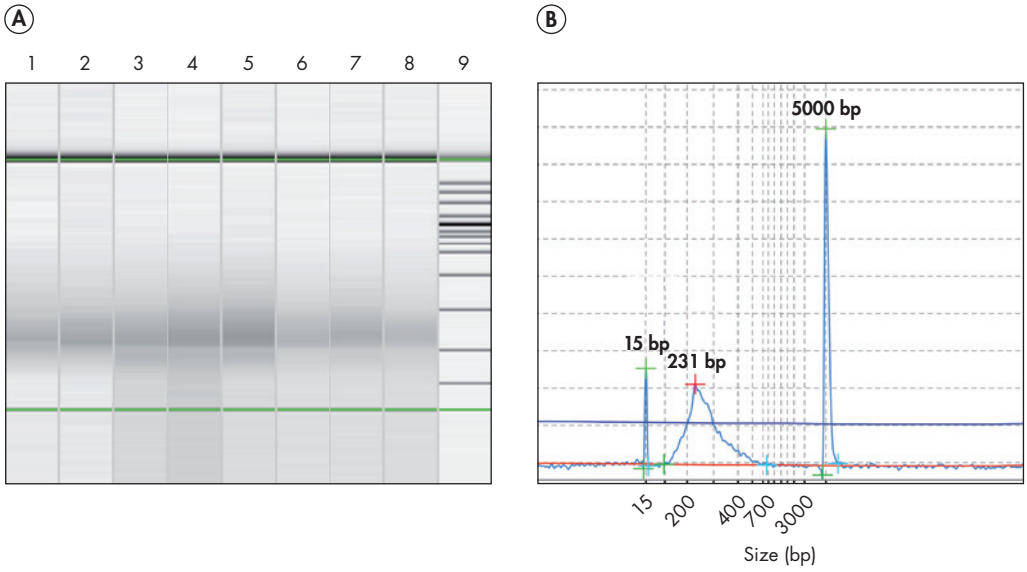

Figure 2. Analysis of amplified, adapter-ligated libraries. **A** Lanes 1–8: The adapter-ligated libraries were analyzed using the QIAxcel DNA Screening Kit and method AM320. Lane 9: QX DNA size marker 100 bp – 2.5 kb. **B** The electropherogram shows a single peak with a median size of 231 bp. The baseline filter is marked with a red line and the threshold with a blue line. As the shortest and longest DNA fragments, the QX Alignment Marker 15 bp/5 kb fragments are visible as sharp peaks at the beginning and the end of the run.

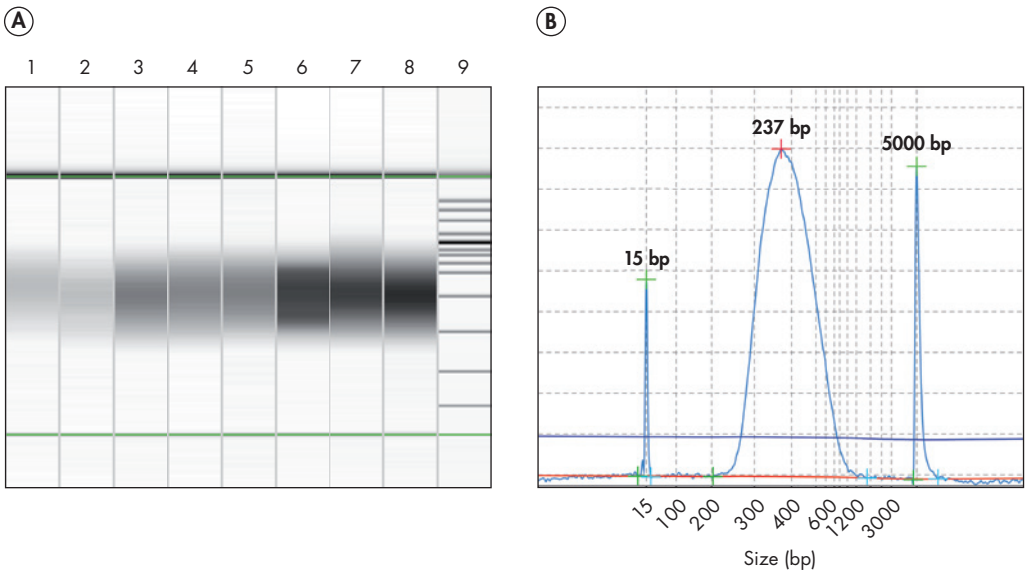

Figure 3. Analysis of amplified capture DNA. **A** Lanes 1–8: The amplified capture DNA was analyzed using the QIAxcel DNA Screening Kit and method AM320. Lane 9: QX DNA size marker 100 bp – 2.5 kb. **B** The electropherogram shows a single peak with a median size of 237 bp.

Using this method, we identified and validated known and novel inactivating mutations in 88% of individuals with DBA. Target enrichment combined with high-throughput sequencing is a robust methodology for the genetic detection of DBA and one that shows considerable improvement over existing methods.

Conclusions

Target-gene enrichment followed by multiplexing and high-throughput sequencing is a powerful approach that allows rare mutations to be found in large sets of genes. Advantages over conventional approaches include the capacity for rapid, accurate, and cost-effective screening of all 80 ribosomal protein genes and the ease of identification of DBA-associated mutations. The method could also prove useful when screening for rare mutations in other genetically complex diseases. The QIAxcel Advanced System allowed reliable and cost-effective library quality control through the whole library preparation procedure. Thus, the sample quality was continuously monitored, securing the good quality of libraries and the high quality of the final sequencing data.

References

1. Gerrard, G., et al. (2013) Target enrichment and high-throughput sequencing of 80 ribosomal protein genes to identify mutations associated with Diamond-Blackfan anaemia. Brit. J. Haematology **162(4)**, 530

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QX Alignment Marker, 15 bp/5 kb (1.5 ml)	Alignment marker with 15 bp and 5 kb fragments	929524
QX DNA Size Marker, 100 bp – 2.5 kb (50 µl)	DNA size marker with fragments of 100, 200, 300, 400, 500, 600, 700, 800, 1000, 1200, 1500, 2000, and 2500 bp, and a concentration of 100 ng/µl	929559

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2 DNA analysis – Basic Research

Nucleic acid gel electrophoresis is a broadly used technique in all fields of basic life science research. The flexibility and versatility of the QIAxcel allows researchers to streamline and accelerate their molecular biology experiments. The sensitivity and resolution of capillary electrophoresis offers an excellent alternative to long or complex slab gel setup.

A wide range of applications in basic research involving microsatellite analysis, mapping mutant genes, linkage analysis, and genotyping transgenes by PCR are all powerful molecular approaches for screening organisms and their genetic profiles. The QIAxcel Advanced provides precise and reliable results to accelerate these analyses and the research projects they are part of.

Content

A. thaliana genotyping with a CAPS marker for a pks3 mutant allele

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The QIAxcel® system was used for genotyping *A. thaliana* with CAPS markers, following mutations in the PKS3 gene (At1g18810), and to identify mutants in various crosses.

Introduction

Arabidopsis thaliana is a small flowering plant that is widely used as a model organism in plant cellular and molecular biology. Its genome sequence is known and is available through the Arabidopsis Information Resource (TAIR), as well as other sources, including seed stocks and collections of genetic and physical markers.

The short life cycle (approximately 6 weeks from germination to seed maturation), enables highly efficient preparation of mutants for in-depth analysis of gene function. Once a mutant of interest has been identified, cleaved amplified polymorphic sequences (CAPS) are used to map the mutation (1). CAPS markers are also used to genotype known mutations.

Previously, no mutations were known for the phytochrome kinase substrate protein 3 (PKS3) gene (At1g18810), a member of a small gene family in A. thaliana (2). The "targeting induced local lesions in genomes" ("tilling") approach was used to identify a mutant in the PKS3 gene, pks3-*7*. Subsequently CAPS markers were used for genotyping, allowing the mutant to be followed in various genetic crosses.

Materials and Methods

Small arabidopsis leaves were homogenized by grinding in an Eppendorf® tube in 500 µl of 200 mM Tris (pH 7.5), 250 mM NaCl, 25 mM EDTA, and 0.5% SDS. After centrifugation, DNA was precipitated by adding equal amounts of isopropanol to the supernatant. After an additional centrifugation at 12,000 x g for 10 minutes, the DNA pellet was washed with 70% ethanol, air dried, and resuspended in 100–200 µl 10mM Tris, 1 mM EDTA (pH 8.0).

PCR amplification was performed with Taq DNA polymerase (proprietary preparation) understandard reaction conditions in a 20 µl volume. PCR amplification using the CF523 (AAACA AGCCG ACATG GAACG) and CF524 (TCGTT ATGTT CTCAA TCTCG) primers yielded a prominent 518 bp fragment.

PCR product (10 µl) was digested in a total volume of 40 µl with 10 U MboI (New England BioLabs) by incubating for 70 minutes at 37°C. The 518 bp wild-type fragment was digested into 3 fragments: 29 bp, 182 bp, and 307 bp. The pks3-7 mutant sequence is missing an MboI restriction site, and digestion of the mutant 518 bp fragment yielded 2 fragments: 211 bp and 307 bp.

Digested samples were analyzed using the QIAxcel capillary electrophoresis system with the QIAxcel DNA Screening Kit and the AM320 method. The QX Alignment Marker 50 bp/500 bp and QX DNA Size Marker 50 bp–800 bp were included in the analysis.

The QIAxcel capillary electrophoresis system processes samples in batches of 12 and allows analysis of up to 96 samples without manual intervention. The results can be displayed as an electropherogram as well as a gel-like image.

Results and Discussion

CAPS analyses were performed to screen the progeny of a backcross of pks3-7 against its isogenic wild-type control. By analyzing the F2 generation for the presence of the pks3-7 mutation using a specific CAPS marker, it was possible to follow the mutant allele through various crosses.

The results of a CAPS analysis using the QIAxcel system are shown in Figure 1. In lanes 2, 4, and 6, DNA fragments from the wild type PKS3 gene are present (29 bp, 182 bp, and 307 bp). The fragments present in lane 3 (211 bp and 307 bp) indicate a homozygote for the pks3-7 mutation. The fragments in lanes 5, 7, and 8 (29 bp, 182 bp, 211 bp, and 307 bp) indicate heterozygosity for the pks3-7 mutation. The sizes of the DNA fragments estimated by BioCalculator Software are given in Table 1.

Although the size differences between the fragments are small, the sharp banding patterns achieved using the QIAxcel system allowed more accurate size estimation than is possible with agarose gel electrophoresis (data not shown).

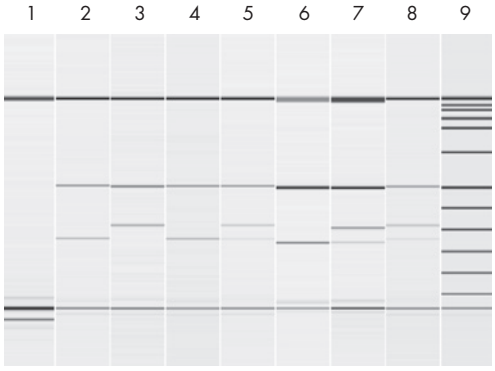

Figure 1. CAPS analysis for the *A. thaliana* PKS3 gene. CAPS of the PKS3 gene were prepared from individual *A. thaliana* plants and resolved on the QIAxcel system using the QIAxcel DNA Screening Kit.
1: negative control;
2, 4, 6: wild type;
3: homozygous pks3-7 mutant;
5, 7, 8: heterozygote pks3-7 mutant;
9: QX DNA Size Marker 50 bp/800 bp.

Table 1. BioCalculator analysis of the gel image in Figure 1

Lane	Estimated fragment size (bp)										
1	41										
2	180	306									
3	210	303									
4	179	304									
5	179	210	304								
6	29	170	300								
7	34	171	204	299							
8	178	210	303								
9	50	100	150	200	250	300	400	500	600	700	800

Conclusions

- The sharp banding patterns achieved with the QIAxcel capillary electrophoresis system simplified and accelerated the routine sizing of wild type and mutant DNA fragments. Due to the accurate sizing of DNA fragments compared to conventional agarose gel electrophoresis (data not shown), the QIAxcel system enabled unambiguous size estimation in significantly shorter time.
- Up to 96 samples can be analyzed in a single run without manual intervention using the QIAxcel system. In addition, the QIAxcel system provides more information from CAPS analyses than traditional methods, saving time and effort. Controlled running conditions and automated data acquisition ensure data safety, reliability, and reproducibility.
- QIAxcel capillary electrophoresis uses only minute quantities of DNA for electrokinetic injection, allowing the samples to be used for downstream procedures, such as sequencing or cloning.

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Detection of alternative Tra2β regulated splicing

Andrew Best, Sushma Grellscheid, and David J. Elliott, Institute of Genetic Medicine, Newcastle University, UK

This application note describes how the QIAxcel® system was used to successfully determine the splicing pattern of exonic sequences targeted by Tra2β protein isoforms.

Introduction

Tra2β (*Sfrs10*) is an evolutionarily conserved splicing protein that is crucial for mouse embryogenesis (1), but its biological role is not fully understood. It has a modular structure with domains rich in arginine and serine (RS1 and RS2) and a central RNA recognition motif (RRM) that binds to target RNA sequences (2, 3). Furthermore, at least 3 isoforms of Tra2β have been identified. Tra2β is known to splice the *Nasp* histone chaperone gene, which monitors DNA double strand breaks (4). An evolutionarily conserved cassette exon (annotated *Nasp*-T) may play a crucial role in developmental processes. Tra2β splices *Nasp* via a number of binding sites, but the exact role of these interactions is not known.

Because of the high levels of splicing inclusion observed for the wild type *Nasp*-T exon at endogenous cellular concentrations of Tra2β, we tested a mutated exon ("M3+M4"), which is less efficiently spliced, to find out whether the Tra2β binding sites are necessary for splicing activation. We also investigated the need for the Tra2β RRM and RS1 domains in these interactions (5).

The QIAxcel system provides rapid, sensitive, and reproducible analyses of Tra2β regulated splicing. This system may also prove advantageous for studying the role of other splicing proteins and their target sequences.

Materials and Methods

HEK 293 cells were cotransfected with a mutated *Nasp*-T construct (M3+M4) and one of 3 Tra2β–GFP constructs encoding full length Tra2β, Tra2βΔRRM, or Tra2βΔRS1. Control cells were cotransfected with the *Nasp*-T construct (M3+M4) and GFP only. ▷

The extracted pre-mRNAs were subjected to RT-PCR and subsequently analyzed using the QIAxcel system. Samples of low DNA concentration were analyzed using Method OL400. Samples were injected at 8 kV for 20 s, and separation was performed at 6 kV for 400 s. Alignment marker, with fragments of 15 bp and 3 kb, was injected at 4 kV and 20 s and run simultaneously with the samples. QX DNA size marker, with fragments ranging from 50–800 bp, was used for size and concentration estimation.

High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) was performed as previously described (6) using an antibody specific to Tra2β (7).

Results and Discussion

As expected, analyses on the QIAxcel system demonstrated very high splicing activation by full length Tra2β, but also significant percentage splicing inclusion (PSI) activation by the Tra2βΔRRM–GFP protein (Figure 1). These results indicate that for some exons, Tra2β can act as a coactivator as well as a splicing activator.

Interestingly, Tra2βΔRS1 seems to behave as a potent splicing repressor. This indicates that the endogenous Tra2βΔRS1 isoform acts a splicing repressor and/or that the RS1 domain plays a central role in splicing activation.

Conclusions

- The QIAxcel system is a valuable tool for revealing exon splicing patterns.
- Analyses of the splicing patterns using the QIAxcel system were both qualitative and quantitative.
- The QIAxcel system can help to identify the roles of other splicing proteins as activators, coactivators, or repressors.

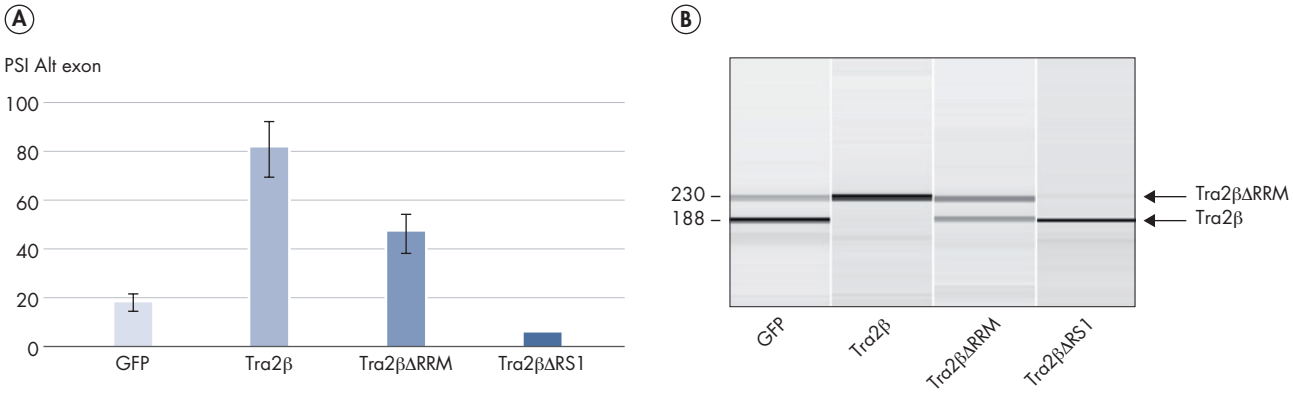

Figure 1. Splicing of *Nasp*-T M3+M4 (mutant) exon. A. Percentage splicing inclusion (PSI) of a panel of exons identified through HITS-CLIP in response to GFP and Tra2β-GFP fusion proteins. Data represent at least 3 biological replicates, and the error bars are shown as standard errors. **B.** Representative image from RT-PCR analysis on the QIAxcel system. Probability (***p***) values were calculated using an independent two-sample T-test between the PSI levels for cells cotransfected with GFP and each of the different Tra2β-GFP constructs ($p \leq 0.05$, $p \leq 0.01$) (adapted from reference 5).

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QIAxcel® system — linkage analysis of zebrafish mutants

Takashi Akanuma, Okazaki Institute for Integrative Biosciences, National Institutes of Natural Sciences, Okazaki, Japan

In this application note, we describe the transfer of methods based on agarose gel electrophoresis for linkage analysis of zebrafish mutants to the QIAxcel system. The simple sequence length polymorphisms (SSLP) marker 'a' was analyzed. Using the QIAxcel system, we were able to resolve size differences of DNA fragment s down to a few base pairs, a resolution that was not attainable using conventional agarose gel electrophoresis. The QIAxcel system provides significant advantages in determination of genotype for gene linkage studies.

Introduction

To understand the roles genes play in development, there is a need to identify as many of the genes involved in development as possible. Identification can be problematic, since vertebrates are not as amenable to genetic analysis as invertebrates. However, the zebrafish is an excellent genetic system for the study of vertebrate development and disease.

Materials and methods

Linkage studies of zebrafish mutants were performed by using the SSLP marker 'a'.

Genomic DNA was isolated from a zebrafish recessive mutant X heterozygote, wild-type control, TL/India heterozygous mutant, plus normal individuals and homozygous mutants obtained by crossing 2 TL/India heterozygous mutants.

PCR products were separated by electrophoresis on a 3% agarose gel and by electrophoresis using the QIAxcel system together with the QIAxcel DNA High Resolution Kit, and the OM700 method. Genotypes were determined based on the sizes of the amplified products.

Results

PCR products of SSLP marker 'a' for the TL line resolve at 242 bp (Figures 1 and 2; lane 1) and for the India line at 233 bp (Figures 1 and 2; lane 2). In the 3% agarose gel, separation of PCR products derived from the TL line and India line was incomplete (Figure 1; lane 3), and as a result, it was very difficult to assess the genotypes in the F_2 population (Figure 1; lanes 4–9).

By comparison, using the QIAxcel system, PCR products derived from the TL line and India line were clearly separated into 2 bands (Figure 2; lane 3), enabling the simple determination of genotypes in the F_2 population (Figure 2; lanes 4–9).

Based on these results, it is clear that the gene responsible for mutant X is linked to SSLP marker 'a'.These results demonstrated that the QIAxcel capillary electrophoresis system resolves DNA fragments that are very close in size much more clearly than conventional agarose gel electrophoresis.

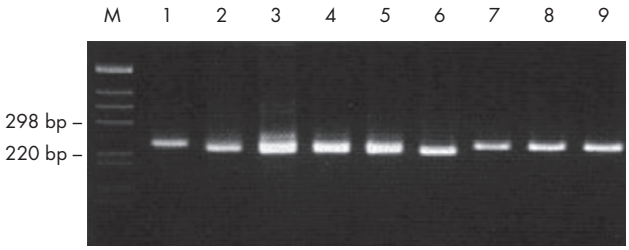

Figure 1. Results using 3% agarose gel. M: Marker; **Lane 1**: TL line (P_0); **Lane 2**: India line (P_0); **Lane 3**: Individual (F_1) obtained from TL x India cross; **Lanes 4, 5, 6**: Normal individual (F_2) of brood; **Lanes 7, 8, 9**: Homozygous mutant (F_2) of brood.

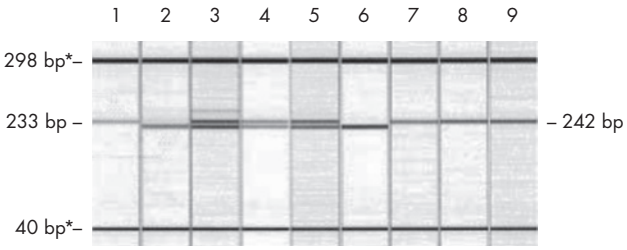

Figure 2. Results using the QIAxcel system with the QIAxcel DNA High Resolution Kit. Lane 1: TL line (P_0); **Lane 2**: India line (P_0); **Lane 3**: Individual (F_1) obtained from TL x India cross; **Lanes 4, 5, 6**: Normal individual (F_2) of brood; **Lanes 7, 8, 9**: Homozygous mutant (F_2) of brood. * Alignment marker.

Conclusions

These results demonstrated that the QIAxcel system can resolve the size difference of DNA fragments down to just a few base pairs, a resolution that was not achievable using conventional agarose gel electrophoresis. The QIAxcel system provides significant advantages in determination of genotype for gene linkage studies.

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QIAxcel® system — mapping mutant gene loci in *Arabidopsis thaliana*

Nobuyoshi Mochizuki, Kyoto University, Graduate School of Science, Division of Biological Science, Kyoto, Japan

In this application note, we describe the assessment of simple sequence length polymorphism (SSLP) and cleaved amplified polymorphisms (CAPS) markers in the mapping of mutant gene loci and the homo/hetero examination of known mutant gene loci using the mutated CAPS or derived CAPS (dCAPS) markers. Both applications utilize the QIAxcel system and the QIAxcel DNA High Resolution Kit, with the OM500 method.

The QIAxcel system demonstrated superior separation capability, enabling the resolution of markers differing by only 4 base pairs in size. It is predicted that the QIAxcel system will be a powerful tool for increasing the speed of mapping and genotyping in the future.

Introduction

There are several methods used in the identification of gene loci responsible for the mutation in *Arabidopsis thaliana*. Of these, mapping of the F_2 populations from crosses between different ecotypes has been a highly effective technique. However, as the entire genomic sequence became available, the increasing numbers of DNA markers made gene cloning much easier and faster using mapping and chromosomal walking. In addition, these DNA markers have often been used to detect polymorphisms of *Arabidopsis thaliana*. For polymorphism studies, we have routinely performed the following experiment types:

- Assessment of SSLP and CAPS markers in the mapping of mutant gene loci
- Homo/hetero examination of known mutant gene loci using the mutated CAPS or dCAPS markers
- Homo/hetero examination of T-DNA insertion in T-DNA insertion mutations

Design of PCR primers for the SSLP and dCAPS markers was extremely challenging, due to the sequence limitation, and the resulting PCR products were small (less than 200 bp). Consequently, the size differences among PCR products or restriction-enzyme–digested fragments are often just a few base pairs. Until recently, polyacrylamide gels or high concentration (2–4%) agarose gels were used to resolve such small size differences between DNA fragments. The introduction of the QIAxcel system has enabled the generation of rapid, reproducible results for these types of analysis.

Materials and methods

Assessment of Col and Ler using the SSLP marker NGA707

The sizes of SSLP marker NGA707 for two ecotypes of Col and Ler are 132 bp and 128 bp, respectively, meaning a difference in size of only 4 bp. Genomic DNA samples from Col, Ler, and a hybrid of Col and Ler cross were extracted using a miniprep system and PCR was performed on a 10 µl scale. PCR products were analyzed using the QIAxcel system together with the QIAxcel DNA High Resolution Kit and the OM500 method.

Hetero/homozygote examination of mutants using dCAPS markers

When base sequence polymorphisms, derived from the mutations of substitution, insertion, or deletion do not generate or modify a restriction enzyme recognition sequence, the PCR primers can be designed to introduce a new restriction enzyme recognition sequence to enable the easy assessment of polymorphisms (dCAPS method). The *gun5-1* mutation is shown as an example (Figure 1 shows the sequence) and has a base substitution (from G to A). PCR products amplified using specially designed, mismatching primers will include the *Eco*T14 I recognition site in the wild type, but not in the *gun5-1* mutant (Figure 1).

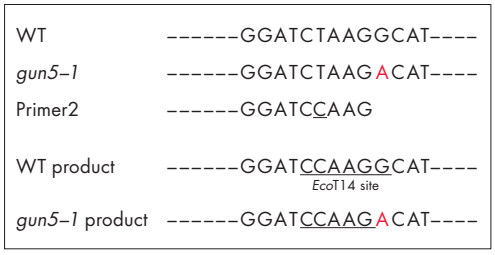

Figure 1. Sequence of the *gun5-1* mutant.

Results and Discussion

Assessment of Col and Ler using the SSLP marker NGA707

Figure 2 shows the analysis of the PCR products using the QIAxcel system in conjunction with the QIAxcel DNA High Resolution Kit. In the hybrid (H) sample, the two DNA bands were easily distinguished and the identification of hybrid was clear with no ambiguity. Presence of additional 2 heteroduplex bands confirmed heterozygosity.

Hetero/homozygote examination of mutants using dCAPS markers

The predicted sizes of *Eco*T14 I digested fragments from wild-type and mutant are shown in Figure 3. The *Eco*T14 I digested samples were analyzed on the QIAxcel system after a 5- to 10-fold dilution with water (Figure 4). The gel view of the QIAxcel BioCalculator Software enables easy assessment of wild-type, heterozygote, and mutant — with no ambiguity.

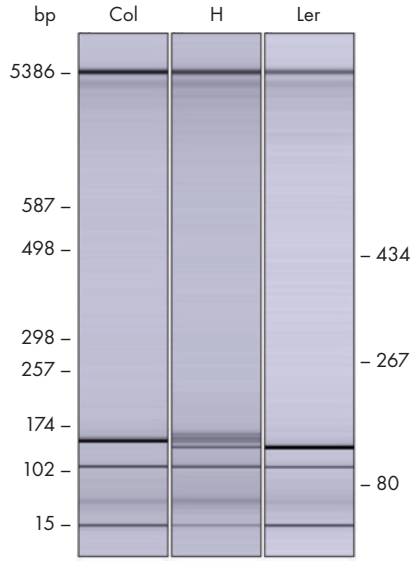

Figure 2. Assessment of ecotypes based on the NGA707 marker.

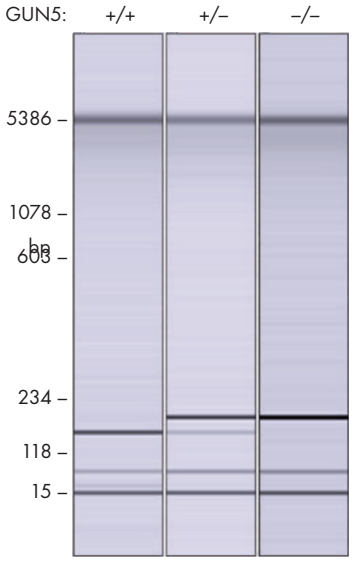

Figure 4. Examination of mutations based on dCAPS.

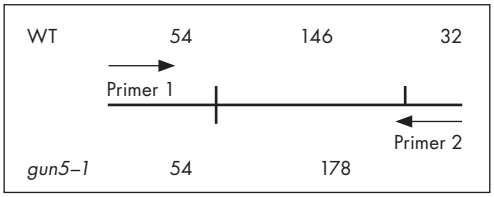

Figure 3. Predicted sizes of restriction digested wild-type and mutant fragments.

Conclusions

Although many DNA markers are recorded in the *Arabidopsis thaliana* database, markers with differences of only several bases pairs may be difficult to analyze. We anticipate that the use of the QIAxcel system, which demonstrates superior separation capability and simplicity, will enable the effective use of these markers and further increase the speed of mapping and genotyping.

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Rapid and effective genotyping of Cre transgenic mice

Norman Moullan

Laboratory of Integrative Systems Physiology (LISP), École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Sizes of Cre gene-specific DNA fragments from the Cre gene were unambiguously identified using the QIAxcel® system. The accuracy of the system allowed rapid and reliable identification of Cre transgenic mice.

Introduction

The Cre-lox system, which is not naturally present in the mouse genome, is a molecular tool for genome manipulation and has been successfully used to generate mouse mutants (1, 2). Initially, transgenic mouse lines are produced: one expressing the Cre recombinase and one carrying 2 *loxP* sites (34 bp sequences). Upon crossing Cre and loxP strains, the Cre recombinase cuts specifically at the *loxP* sites in tissues where Cre transgene is expressed (3, 4). Subsequent recombination of *loxP* sequences leads to rearrangements of the genome. Depending on the orientation of the *loxP* sites, deletions, inversions, or chromosomal translocations are generated. By targeting Cre recombinase to tissues of interest, conditional knockout mutants can be generated (2).

To test the successful insertion of the Cre transgene into the genome of transgenic mice, PCR-amplified Cre gene sequences were identified using QIAxcel system.

Materials and methods

Rapid genomic DNA extraction from mouse ear tissue was performed by incubating samples in 0.1N NaOH at 70–95°C for up to 10 minutes. Samples were placed on ice for 5 minutes and 30 µl 170 mM Tris·HCl (pH 8.0) was added. Samples were vortexed and centrifuged for 4 minutes at maximum speed, and 3 µl of the supernatant was used for PCR amplification.

Primers specific for the Cre transgene (sense: 5′- GAACC TGATG GACAT GTTCA GG -3′; anti-sense: 5′- AGTGC GTTCG AACGC TAGAG CCTGT -3′) were used to amplify a 320 bp fragment. Primers specific for the myogenin gene (sense 5′- TTACG TCCAT CGTGG ACAGC -3′ and anti-sense 5′- TGGGC TGGGT GTTAG CCTTA -3′) were included in the reaction to amplify a 250 bp control fragment. ▷

Standard amplification reactions (25 µl) were prepared using 0.4 µM of each primer. After initial denaturation at 95°C for 5 minutes, reactions were subjected to 35 cycles of 95°C, 62°C, and 72°C for 30 seconds each. Reactions were incubated for a final elongation at 72°C for 5 minutes.

Samples were analyzed using the QIAxcel system with the QIAxcel DNA Screening Kit and the AL320 method. The QX Alignment Marker 15 bp/1 kb and the QX DNA Size Marker 50–800 bp were included in the analysis.

Results and Discussion

The QIAxcel system was used to detect the presence of the Cre transgene in putative transgenic mice. Figure 1 shows the results of genotyping in which the Cre-specific DNA fragment (320 bp, present in lanes 2, 6, 11, and 12) as well as the control fragment from the myogenin gene (250 bp, present in all lanes) were unambiguously identified.

The QIAxcel capillary electrophoresis system processes samples in batches of 12 and allows analysis of up to 96 samples without manual intervention. Results can be displayed as a gel-like image as well as an electropherogram. Using the BioCalculator Software, the presence of specific DNA fragments as well as their sizes were accurately determined.

Cre transgenic mice will be used in breeding for knockout functional analysis.

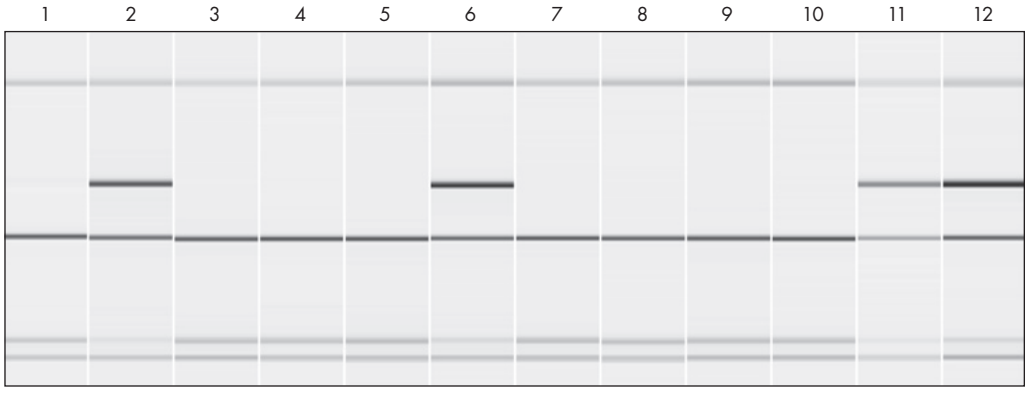

Figure 1. Detection of the Cre transgene in mice. DNA was isolated from mouse ear tissue and subjected to PCR amplification using primers for the Cre transgene as well as for a myogenin control. PCR products were analyzed on the QIAxcel system using the QIAxcel DNA Screening Kit. The 250 bp control fragment amplified from the myogenin gene is present in all lanes, indicating that amplification reactions were successful. The 320 bp fragment present in lanes 2, 6, 11, and 12 indicates the presence of the Cre transgene in these mice.

Conclusions

- The fragments amplified from the Cre transgene and from the myogenin control (320 bp and 250 bp, respectively) were easily distinguished using the QIAxcel system, allowing reliable identification of transgenic mice. The sizing accuracy and sensitivity obtained using the QIAxcel system is superior to conventional methods, such as agarose gel electrophoresis (data not shown).
- Due to automated electrophoresis analysis as well as automated data acquisition, the QIAxcel system ensures safe and reliable results.
- Because the QIAxcel system enables short running times and analysis of up to 96 samples in a single run, it is ideally suited for medium- to high-throughput mouse genotyping, significantly saving time.
- QIAxcel analysis results are fully reproducible due to controlled running conditions and automated data acquisition.
- Since QIAxcel capillary electrophoresis uses only minute quantities of DNA for electrokinetic injection, the samples are retained for downstream procedures, such as sequencing.

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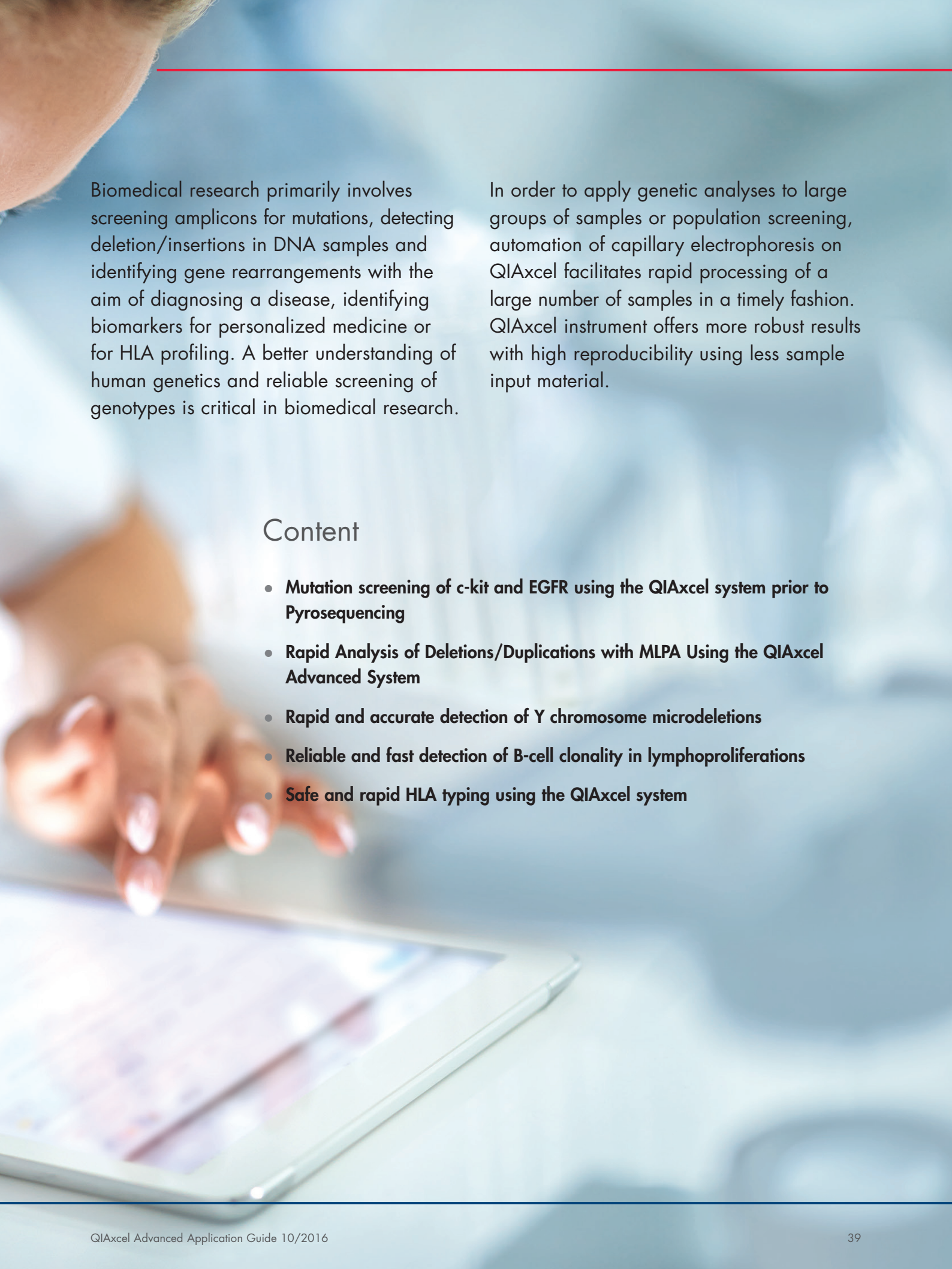
3 DNA analysis – Biomedical Research



Biomedical research primarily involves screening amplicons for mutations, detecting deletion/insertions in DNA samples and identifying gene rearrangements with the aim of diagnosing a disease, identifying biomarkers for personalized medicine or for HLA profiling. A better understanding of human genetics and reliable screening of genotypes is critical in biomedical research.

In order to apply genetic analyses to large groups of samples or population screening, automation of capillary electrophoresis on QIAxcel facilitates rapid processing of a large number of samples in a timely fashion. QIAxcel instrument offers more robust results with high reproducibility using less sample input material.

Content

Mutation screening of c-kit and EGFR using the QIAxcel® system prior to Pyrosequencing®

Mehran Ghaderi, Department of Pathology and Cytology, Karolinska University Hospital Solna, Stockholm, Sweden

The QIAxcel Advanced System was successfully used to separate PCR amplicons and detect mutations in exons of *c-kit* and *EGFR* genes found in gastrointestinal stromal tumors (GIST) and non-small cell lung cancers. It reduced analysis time and provided high-quality, reproducible determination of deletions and insertions of key genes. This study shows that the QIAxcel system can be used to increase the efficiency of Pyrosequencing applications.

Introduction

Screening amplicons for mutations prior to sequencing is a critical step, but it can be tedious and time consuming. It is therefore important to develop rapid and reliable detection techniques that yield results of sufficiently high quality for downstream Sanger sequencing and Pyrosequencing applications. In this study, a method for detecting activating mutations of *c-kit* and *EGFR* genes using the QIAxcel Advanced System was assessed. It proved highly suitable for this purpose.

Activating mutations of *c-kit* and *EGFR* genes can be detected in DNA samples from gastrointestinal stromal tumors (GIST) and non-small cell lung cancers (1–3). Some of the most common mutations are the *EGFR* exon 19 deletion and exon 20 insertion, and the duplication of the *c-kit* gene within exon 9 or deletion within exon 11. The assessed QIAxcel-based technique readily detected the mutations in *EGFR* exon 19 and *c-kit* exon 11.

Materials and methods

DNA isolation and purification

Genomic DNA was isolated from formalin-fixed paraffin embedded (FFPE) samples with representative tumor cellularity. Sections with a thickness of 10 µm were taken and deparaffinized with xylene. DNA was extracted using the QIAamp® DNA FFPE Tissue Kit. The genomic DNA concentration was measured with a NanoDrop® spectrophotometer (ThermoFisher® Scientific Inc.). ▷

* The *therascreen* EGFR Pyro Kit is not available in the US and Canada.

Real-time PCR

Amplification was performed using the *therascreen*® EGFR Pyro Kit* on a real-time PCR system. As per the manufacturer's protocol, a 25 µl PCR mix was prepared containing 30 ng of template DNA and 8 µM of primers. The initial denaturation step was at 95°C for 15 min, followed by 42 amplification cycles of denaturation at 95°C for 20 s, annealing at 53°C for 30 s, and elongation at 72°C for 20 s. The final elongation step was at 72°C for 10 min.

Electrophoresis and DNA size estimation

After amplification, the PCR products were separated using the QIAxcel Advanced, the QIAxcel DNA High Resolution Kit, and the 0M800 method. Amplicon size determination was done with the QX Alignment Marker 15 bp/600 bp and QX DNA Size Marker 25–500 bp. Data were analyzed and visualized using the QIAxcel ScreenGel® software. Pyrosequencing was then performed.

Results

PCR fragments were amplified from *EGFR* exon 19 and *c-kit* exon 11 using samples with known deletions compared to the wild type (Figure 1 and 2). Analyses using the QIAxcel Advanced System showed high accuracy in identifying wild-type and mutated DNA fragments based on the size estimation. Human *EGFR* exon 19 has an amplicon size of about 250 bp. When there were deletions, extra bands could be seen in the gel images (Figure 1). Human *c-kit* exon 11 has an amplicon size of about 220 bp. Mutations were found at various sizes (Figure 2). All of the samples with deletion mutations were detected and the corresponding deletion size was correctly scored, allowing for the exclusion of wild-type samples from subsequent Pyrosequencing.

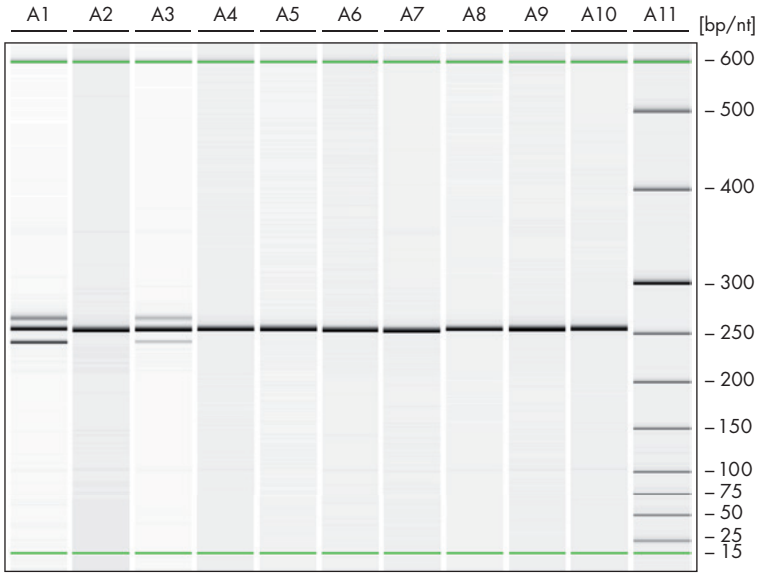


Figure 1. Detection of deletion mutations in *EGFR* exon 19. Human *EGFR* exon 19 has an amplicon size of 250 bp. Lanes A1 and A3 are from samples with *EGFR* exon 19 deletions and have extra bands, while the remaining lanes are from wild-type *EGFR*.

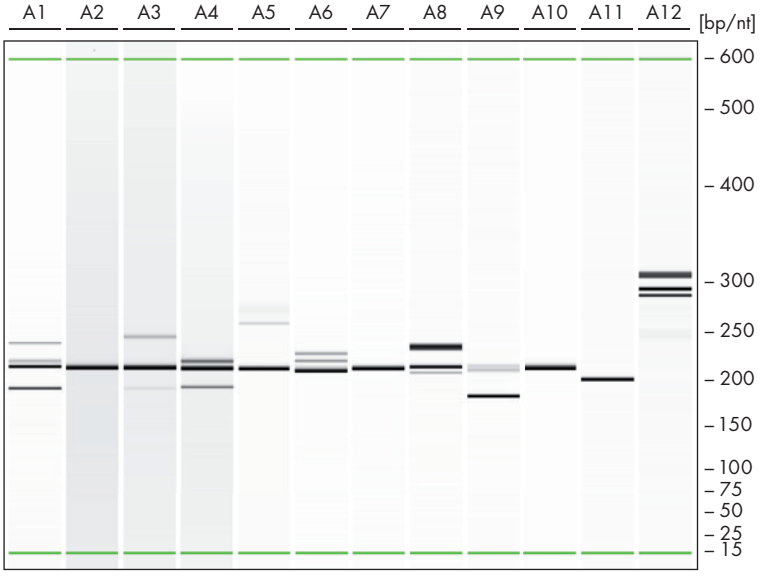


Figure 2. Deletion and duplication mutations in *c-kit* exon 11. Human *c-kit* exon 11 has an amplicon size of about 220 bp. **Lane A11**: sample with an exon 11 homozygous deletion. **Lanes A2, A7, and A10**: the wild-type *c-kit* exon 11 allele. **Lane A12**: sample with an exon 9 duplication (p.A502 Y503dup).

Conclusions

- The QIAxcel system proved to be highly suitable for screening amplicons prior to Pyrosequencing using *EGFR* and *c-kit* as the model systems. All of the deletion mutants were detected and the corresponding deletion size was correctly scored, allowing the exclusion of wild-type samples from the downstream sequencing step.
- The described QIAxcel-based screening method yields robust and reproducible results of sufficiently high quality for more efficient downstream Sanger sequencing and Pyrosequencing applications.
- This method using the QIAxcel system can be applied to increase the speed and reduce the costs of deletion/insertion studies.

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3. Lopes, L.F. and Bacchi, C.E. (2007) *EGFR* and gastrointestinal stromal tumor: an immunohistochemical and FISH study of 82 cases. Mod. Pathol. **20**, 990.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device, including computer and QIAxcel ScreenGel Software, 1-year warranty on parts and labor	9001941
QIAamp DNA FFPE Tissue Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
therascreen EGFR Pyro Kit (24)*	For 24 reactions: Sequencing Primers, PCR Primers, Unmethylated Control DNA, PyroMark PCR Master Mix, CoralLoad Concentrate, and therascreen Buffers and Reagents	971480
QX Alignment Marker 15 bp/600 bp	Alignment marker with 15 bp and 600 bp fragments	929530
QX DNA Size Marker 25–500 bp (50 µl) v2.	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500 bp; concentration 100 ng/µl	929560

* The *therascreen* EGFR Pyro Kit is not available in the US and Canada.

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Discover more about the QIAxcel Advanced System at **www.qiagen.com/QIAxcel**.

Rapid Analysis of Deletions/Duplications with MLPA® Using the QIAxcel® Advanced System

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Introduction

Adenomatous polyposis syndromes are characterized by polyps in the colorectum. When untreated, these pre-malignant polyps almost always become malignant and lead to colorectal cancer (1). Age of onset for colon cancer is around 39 years, though individuals may develop multiple benign colon polyps in their teenage years. Three monogenic inherited forms have been molecularly diagnosed and distinguished:

- Familial adenomatous polyposis (FAP)
- *MUTYH*-associated polyposis (MAP)
- Proofreading-associated polyposis (PPAP)

FAP is the autosomal dominant form caused by mutations or deletions/duplications in the adenomatouspolyposis coli (*APC*) gene located on chromosome 5q22. Hundreds of different variations in the *APC* gene have been described. The detection rate for a pathogenic APC mutation in index patients with classical FAP is 80–90%. Attenuated FAP (AFAP) is a second form of FAP with a milder course of disease. The detection rate for *APC* and *MUTYH* mutations in AFAP for index patients is around 20–30%. For clinical diagnosis of a classical or attenuated FAP, persons must have at least 10 synchronous adenomatous polyps (2,3).

Molecular genetic analysis frequently uses either capillary (Sanger) or next-generation sequencing of all exons of the genes involved in the development of FAP, AFAP and PPAP. In classical FAP, genomic rearrangements are caused by large deletions in <10%–15% and rarely by large duplications (3,4). To detect these events in the *APC* and *MUTYH* genes, a Multiplex Ligation-dependent Probe Amplification (MLPA®) can be conducted with subsequent fragment analysis on a capillary (Sanger) sequencer, which is a costly and time-consuming method.

In our pilot-study we used a faster, more efficient alternative for the subsequent MLPA analysis using the QIAxcel Advanced System. The proposed protocol enabled detection of exonic deletions/duplications with the same resolution and accuracy as using a sequencer. The efficiency of the MLPA analysis using the QIAxcel Advanced System is more cost effective, compared to other detection systems in use.

Materials and methods

This pilot study was based on two different groups of clinical samples:

- 12 FAP patients and 5 healthy controls
- 9 HNPCC (hereditary non-polyposis colorectal cancer, Lynch syndrome) patients and 5 healthy controls

MLPA

The MLPA was performed using the SALSA® MLPA P043 *APC* probemix for the FAP samples and the SALSA MLPA P003 MLH1/MSH2 probemix (MRC-Holland®) for the HNPCC samples following the manufacturer's manual.

Amplicon visualization

The separation and visualization of MLPA-generated amplification products was performed using both a sequencer and QIAxcel Advanced System (QIAGEN) for capillary electrophoresis, following the manufacturer's instructions for the sequencer and a modified protocol for the QIAxcel Advanced System (QIAGEN). Samples were separated using the QX DNA High Resolution Kit with a customized 0M1100_2kV_AM10s_S5kV method, with the following electrophoresis parameters: alignment marker injection at 4 kV for 10 s, sample injection at 5 kV for 10 s and separation at 2 kV for 1100 s. Alignment marker 15 bp/600 bp was run simultaneously with the samples and the size was estimated in comparison with the QX DNA size marker 25 bp–500 bp.

Data analysis

Analysis of the data generated by the sequencer was performed with Coffalyser® software (MRC-Holland), while QIAxcel ScreenGel® 1.2 software (QIAGEN) was used for data analyses of the results from the QIAxcel Advanced System.

Note: The layouts of all graphics in this Application Note are slightly changed from originals to more clearly visualize the results. No modifications were conducted that influenced the results themselves and the authors will provide original graphics/data on request.

Results

Electropherograms

Electropherograms from the Coffalyser software analyses showed peak patterns comparable to the corresponding electropherograms from QIAxcel ScreenGel 1.2 analyses (Figures 1–3). The electropherograms represent typical samples from the *APC* MLPA analyses.

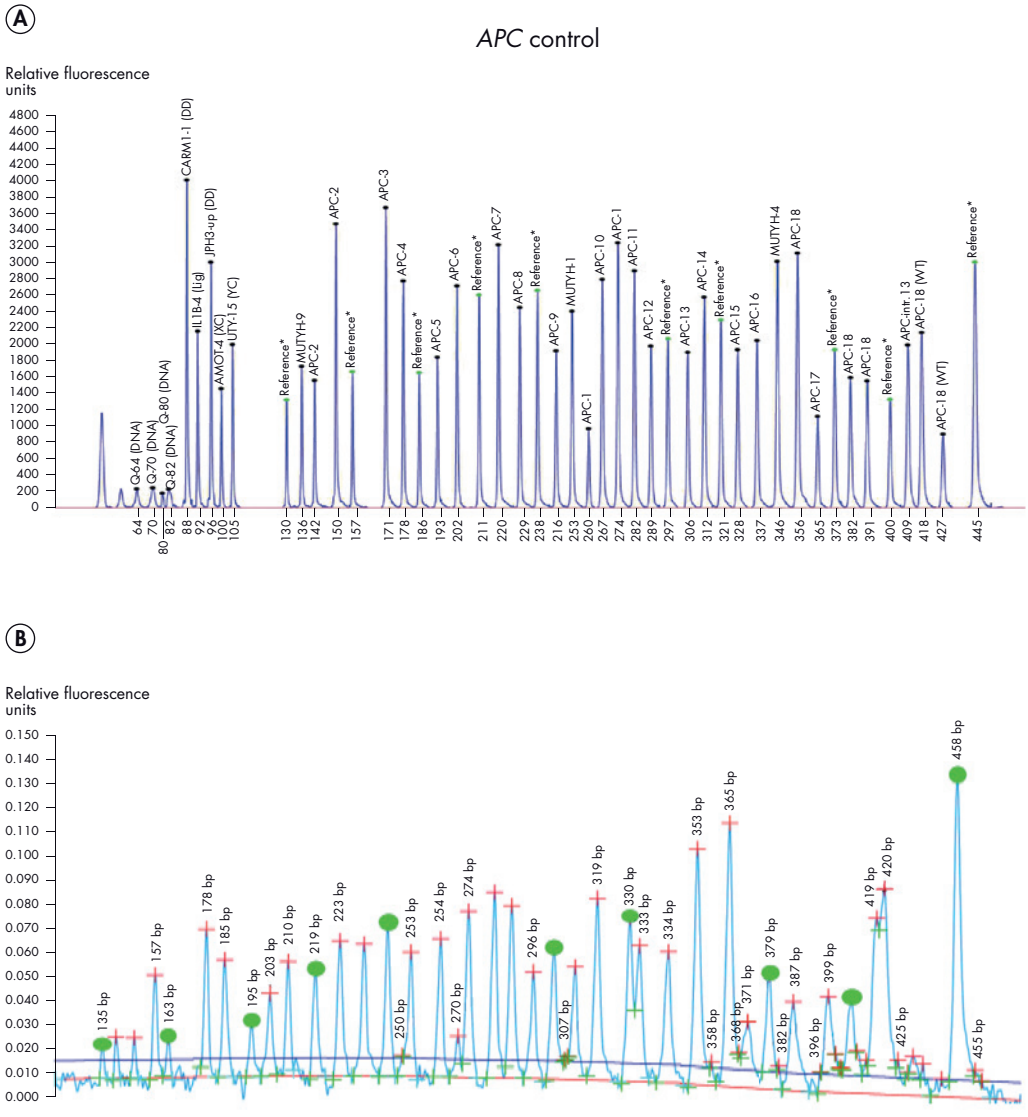

Figure 1. Comparable pattern of peaks for *APC* control using an ABI PRISM® 3100 Genetic Analyzer and QIAxcel Advanced System. A Electropherogram from Coffalyser software analysis. **B** Electropherogram from the QIAxcel Screen Gel 1.2 software analysis. Green circles show reference peaks.

The peak height ratio between deleted (Figure 2) or duplicated (Figure 3) exon probes with their corresponding reference probes showed changes in the specific target region.

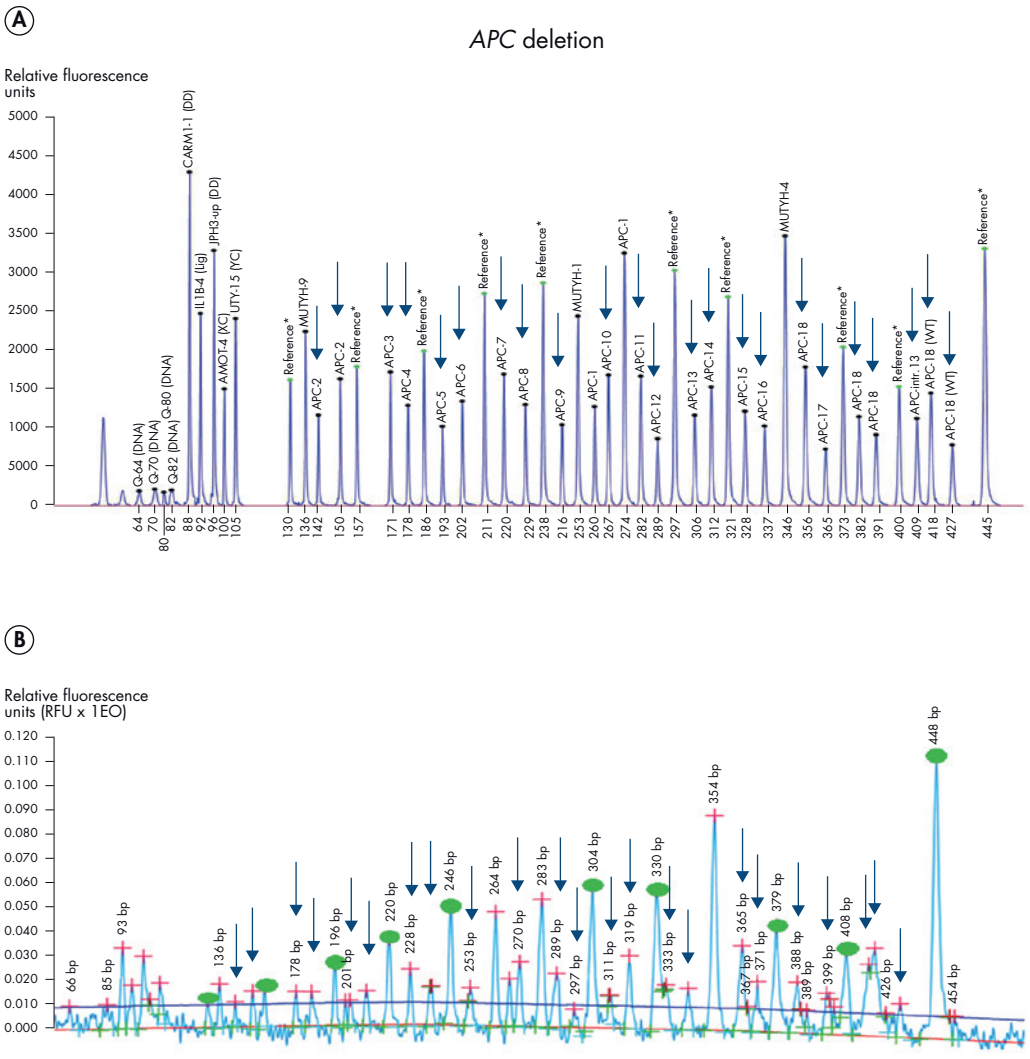

Figure 2. Comparable pattern of peaks for *APC* deletion using sequencing and QIAxcel Advanced System amplicon visualization techniques. A Electropherogram from Coffalyser software analysis of sequencing. **B** Electropherogram from the QIAxcel Screen Gel 1.2 software analysis of QIAxcel Advanced System capillary electrophoresis. Green circles show reference peaks. Black arrows indicate *APC* peaks reduced compared with the corresponding *APC* control electropherograms in Figure 1. Arrows are similarly placed between electropherograms A and B.

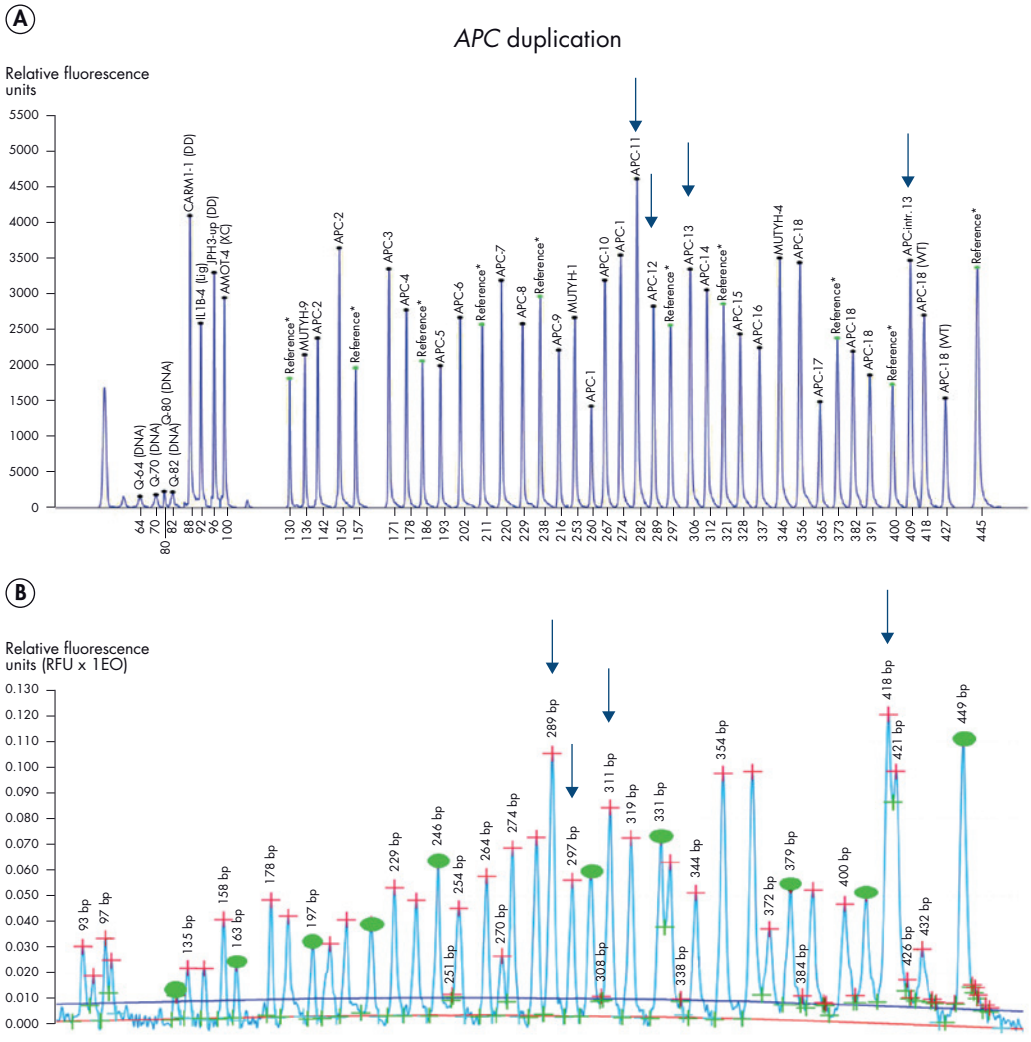

Figure 3. Comparable pattern of peaks for *APC* duplication using ABI PRISM 3100 Genetic Analyzer and QIAxcel Advanced System. A Electropherogram from Coffalyser software analysis. **B** Electropherogram from the QIAxcel ScreenGel 1.2 software analysis. Green circles show reference peaks. Black arrows indicate *APC* peaks increased compared with the corresponding *APC* control electropherograms in Figure 1. Arrows are similarly placed between electropherograms **A** and **B**.

Ratio charts

Coffalyser ratio charts are an additional overview of MLPA results and were available for the results from the sequencer only (Figure 4). Complementary to electropherograms, the ratio charts re-interpret the same exonic information.

The displayed confidence interval enables assessment of the success of the MLPA run. Mutation state is indicated by the ratio. Ratios of around 0.5 suggest a heterozygous deletion of a specific allele (Figure 4B, Exons 2–18). Duplication of specific alleles are shown with a ratio of around 1.5 (Figure 4C, Exons 11–13).

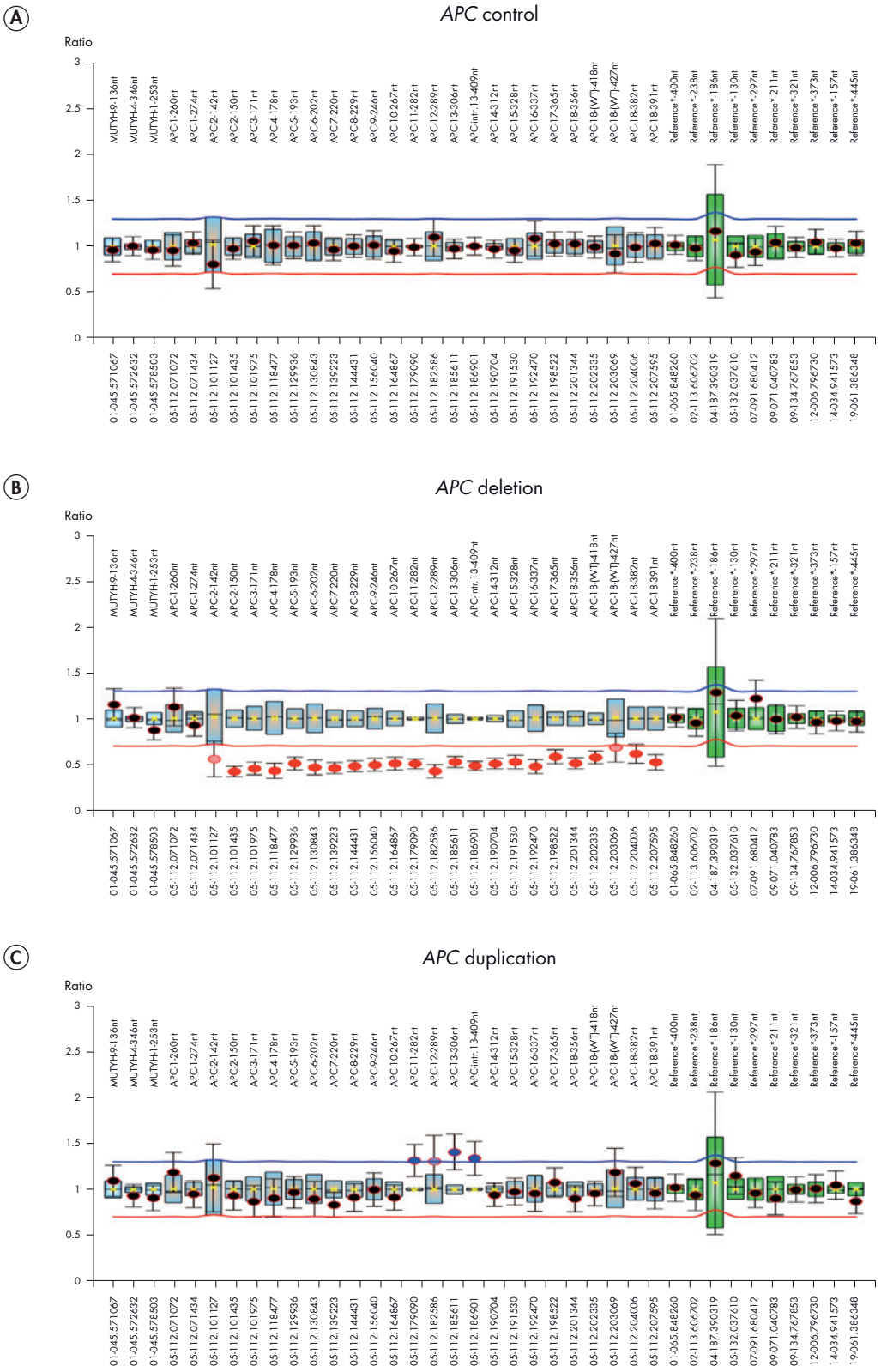

Figure 4. Ratio chart interpretations of the data from *APC* samples by the Coffalyser software. **A** *APC* control data indicating a ratio of 1, meaning no deletion/duplication at any of the specific genomic regions. **B** Heterozygous deletion of specific *APC* alleles indicated by a ratio tending towards 0.5 (ratio of 0 would indicate homozygous deletion). **C** Duplication of specific *APC* alleles indicated by a ratio tending towards 1.5. Mean ± 95% confidence interval of ratio of signal intensity between sample and reference. Green/blue boxes indicate the 95% confidence range of reference.

Discussion

This pilot study showed detection of exonic deletions/duplications in both FAP and HNPCC samples using the QIAxcel Advanced System with comparable resolution and accuracy when using a sequencer.

Probemix sets (MRC-Holland) for MLPA analysis have specific sizes (in bp) for the different probes in each kit, which are either reference probes or target exonic regions. This specific peak pattern is needed to differentiate the different peaks in the analysis and the pattern of peaks is specific for each analysis. We observed a deviation of 1–9 bp in our generated electropherogram peak pattern for *APC* with the QIAxcel ScreenGel software version 1.2 compared with the fragment size analysis using the capillary sequencer.

Corrections for the size deviation are normally done at two points: within the sequencer software by applying the correct binning and then subsequently within the Coffalyser software by choosing the correct kit. The Coffalyser software is currently incompatible with the QIAxcel file format, since the Coffalyser requires .fsa file format (the output file format from the sequencer) to perform the analysis. This necessitated QIAxcel results to be analyzed manually. Nevertheless, all events in the pilot samples identified by ABI PRISM 3100 Genetic Analyzer and Coffalyser analysis were still identifiable with the QIAxcel ScreenGel analysis software after using the QIAxcel Advanced System for separation, detection and analysis.

To further develop the MLPA analysis with QIAxcel Advanced System, we are currently trying to convert the output format to be compatible with the Coffalyser.

Conclusions

- The quality of the MLPA analysis when using the QIAxcel Advanced system is comparable with the data provided by a sequencer, both in terms of resolution and accuracy.
- The QIAxcel Advanced system provides a faster alternative to current sequencer-based methods and is more cost-effective.
- QIAxcel ScreenGel software can be used for MLPA analysis if the analysis is performed manually. Achieving software compatibility between QIAxcel ScreenGel and Coffalyser software would offer an even more attractive approach to MLPA analysis.

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Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel ScreenGel Software	Ten licenses for use of QIAxcel ScreenGel Software on additional computers	9021165
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QX Alignment Marker 15 bp/600 bp (1.5 ml)	Alignment marker with 15 bp and 600 bp fragments	929530
QX DNA Size Marker 25-500bp (50 ul) v2.0	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500bp; concentration 100 ng/ul	929560

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Rapid and accurate detection of Y chromosome microdeletions

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[2] QIAGEN Instruments AG, Hombrechtikon, Switzerland

Y chromosome microdeletions and rearrangements that are relatively common causes of aberrant sperm profiles were detected using the QIAxcel® system. Samples were analyzed directly without prior manipulation, and the type of deletion was detected by automatic fragment-size determination. Use of the QIAxcel system significantly accelerated research on male infertility.

Introduction

The 3 azoospermia factor (AZF) regions of the Y chromosome accommodate genes required for spermatogenesis. The most distal region, AZFc, is one of the most genetically dynamic regions in the human genome (1, 2).

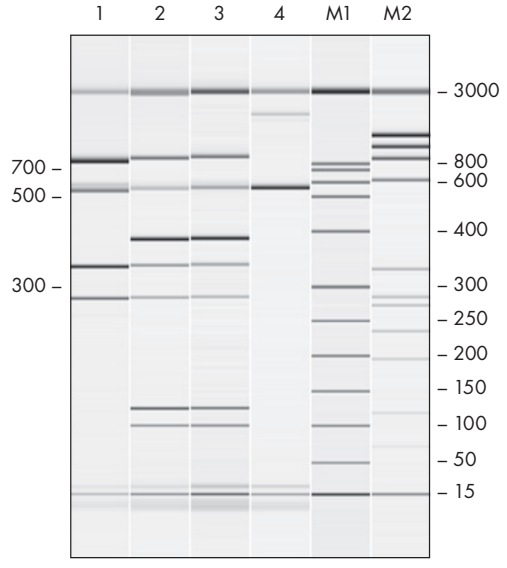
Some AZF rearrangements are responsible for marked spermatogenic defects. For example, men with deletions in AZF regions exhibit drastically reduced sperm concentrations (<1 million sperm/ml compared to normal levels of >20 million sperm/ml) (3).

A rapid and reproducible method for determining AZF deletions would support effective research on the mechanisms of Y chromosome microdeletions. To develop an optimal screening strategy for male subfertility, a large database of microdeletion data was constructed and analyzed (4, 5). Most of the published Y chromosome microdeletions (85.6%) can be detected by PCR using a set of 6 genetic markers: sY84, sY127, sY152, RBM1, sY147 and sY254-DAZ as well as for the ZFY/ZFX gene as an internal control for the multiplex PCR reaction (5).

The QIAxcel system.

Materials and methods

After routine PCR amplification of Y-chromosome-specific, sequence-tagged site (STS) markers as described in (5), samples were placed directly into the QIAxcel system and analyzed using the QIAxcel DNA High Resolution Kit and the QX Alignment Marker 15 bp/3 kb. The analysis included the QX DNA Size Marker 50–800 bp and the QX DNA Size Marker FX174/*Hae*III. Analysis was performed with the OM500 method at 5 kV voltage and a 500 second separation time. The alignment marker was injected at 4 kV for 20 seconds and samples at 5 kV for 10 seconds.



Results

Results of separation of specific amplicons from the AZF regions of the Y chromosome are presented in Figure 1. In lane 3 all fragments were present: sY147 (100 bp), sY152 (125 bp), sY127 (274 bp), sY84 (326 bp), sY254-DAZ (350 bp), ZFY/ZFX (495 bp) and RBM1 (800 bp).

Using the QIAxcel DNA High Resolution Kit, 12 samples can be analyzed in less than 10 minutes. Results can be displayed as a gel-like image as well as an electropherogram.

The analysis of PCR products was simplified by the sharp banding pattern achieved with the QIAxcel system. The automated data acquisition enhanced analysis reliability and eliminated error in sample identification.

No manipulation of the PCR samples was required before analysis on the QIAxcel system, saving time and minimizing human error.

Since the QIAxcel capillary electrophoresis system uses only minute amounts of DNA through electrokinetic injection, the samples are retained for downstream procedures, such as sequencing.

Figure 1. Identification of Y chromosome microdeletions. PCR was prepared with primers for detecting Y chromosome microdeletions and analyzed directly on the QIAxcel system using the QIAxcel DNA High Resolution Kit. **1**: male exhibiting deletions sY152, sY147, and sY254-DAZ; **2**: unknown sample exhibiting no deletions; **3**: male control; **4**: female control; **M1**: QX DNA Size Marker 50–800 bp; **M2**: QX DNA Size Marker FX174/*Hae*III.

Conclusions

- The QIAxcel capillary electrophoresis system is highly suited for reproducibly detecting Y chromosome microdeletions and rearrangements in a fast and reliable manner.
- Using the QIAxcel system with the QIAxcel DNA High Resolution Kit, Y chromosome microdeletions were detected by direct analysis of PCR products: prior manipulation of the samples was not necessary.
- Results of this research, described in (4), facilitated the construction of a minimal set of markers to be used for detecting AZF microdeletions. The use of these markers will assist the research on the effect of AZF mutations on male fertility.

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Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device, including computer, and QIAxcel ScreenGel Software; 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Fast Analysis Kit (3000)	QIAxcel DNA Fast Analysis Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX DNA Size Marker 50 bp – 1.5 kb, QX Alignment Marker 15 bp/3 kb, 12-Tube Strips	929008
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104
QX DNA Size Marker FX174/*Hae*III (50 µl)	DNA size marker with fragments of 72, 118, 194, 234, 271, 281, 310, 603, 872, 1078, and 1353 bp; concentration 100 ng/µl	929551
QX DNA Size Marker 50–800 bp (50 µl)	DNA size marker with fragments of 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, and 800 bp; concentration 100 ng/µl	929556
QX Alignment Marker 15 bp/3 kb (1.5 ml)	Alignment marker with 15 bp and 3 kb fragments	929522

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Reliable and fast detection of B-cell clonality in lymphoproliferations

Jean Benhattar

Institute of Pathology, Centre Hospitalier Universitaire Vaudois (CHUV) and University of Lausanne, Lausanne, Switzerland

DNA fragments amplified using multiplex PCR that was developed to detect immunoglobulin and T-cell receptor gene rearrangements were resolved using the QIAxcel® system. Accurate size determination enabled identification of the type of B-cell clonality in lymphoproliferation.

Introduction

Lymphoma is a cancer of the lymphatic or the immune system (1). The World Health Organization (WHO) lists 43 different forms of lymphoma, including well-known Hodgkin's and several non-Hodgkin lymphomas (2). Research on lymphoid malignancies relies on histomorphological, cytomorphological, and immunohistological methods and evaluations.

The vast majority of lymphomas (>98%) exhibit rearrangements in immunoglobulin (Ig) and/or T-cell receptor (TCR) genes. Because of the clonal origin, all cells of a lymphoma exhibit identical gene rearrangements, and, therefore, monoclonality in lymphoid tissue suggests a malignant disease. Polyclonality, in contrast, indicates normal B-cell maturation (3). Thus, clonal gene rearrangement assessments are of great importance for understanding lymphoid malignancies.

In the BIOMED-2 European collaborative project, multiplex PCR assays were developed and standardized to study such rearrangements. By detecting rearrangements in the in IGH and IGK genes using multiplex PCR, it is possible to identify virtually all B-cell proliferations of clonal origin (3).

Here, we describe the use of the QIAxcel system for fast and reliable capillary electrophoresis analysis to identify the clonality of B-cells in lymphoproliferative tissues.

Materials and methods

Detection of B-cell clonality was performed using BIOMED-2 primer sets for IGH (IGH-FR3 and IGH-FR2) and IGK (IGK-A and IGK-B) gene rearrangements (3). DNA was extracted from archival formalin-fixed, paraffin-embedded tissue sections.

PCR amplification (40 cycles) was performed in duplicate with 10–100 ng of extracted DNA in a total volume of 20 µl. PCR products were denatured at high temperature (5 minutes at 95°C) before being subjected to rapid renaturation at low temperature (1 hour at 4°C) to enhance the formation of multiple heteroduplexes. Homoduplexes exhibit the same migration rate during electrophoretic separation, whereas, heteroduplexes exhibit different migration rates, allowing monoclonal lymphoproliferations to be distinguished from polyclonal lymphoproliferations. ▷

PCR products were analyzed using the QIAxcel system with the QIAxcel DNA Screening Kit and the QX Alignment Marker 15 bp/450 bp. Electrophoretic separation was performed using 2 kV separation voltage and 700 second separation time. Alignment marker and samples were injected with an injection time of 35 seconds at 2 kV and 3 kV, respectively.

Results and discussion

Results of the analysis of 5 samples (S1–S5) are shown in Figure 1. The presence and size of fragments was accurately determined using BioCalculator Software.

B-cell monoclonality was observed for the S1, S4, and S5 samples. B-cell oligoclonality was detected for the S3 sample. For sample S5a and S5b, taken at different times during the course of the disease, the same monoclonal rearrangements were observed for the IGH and IGK genes. (Table 1).

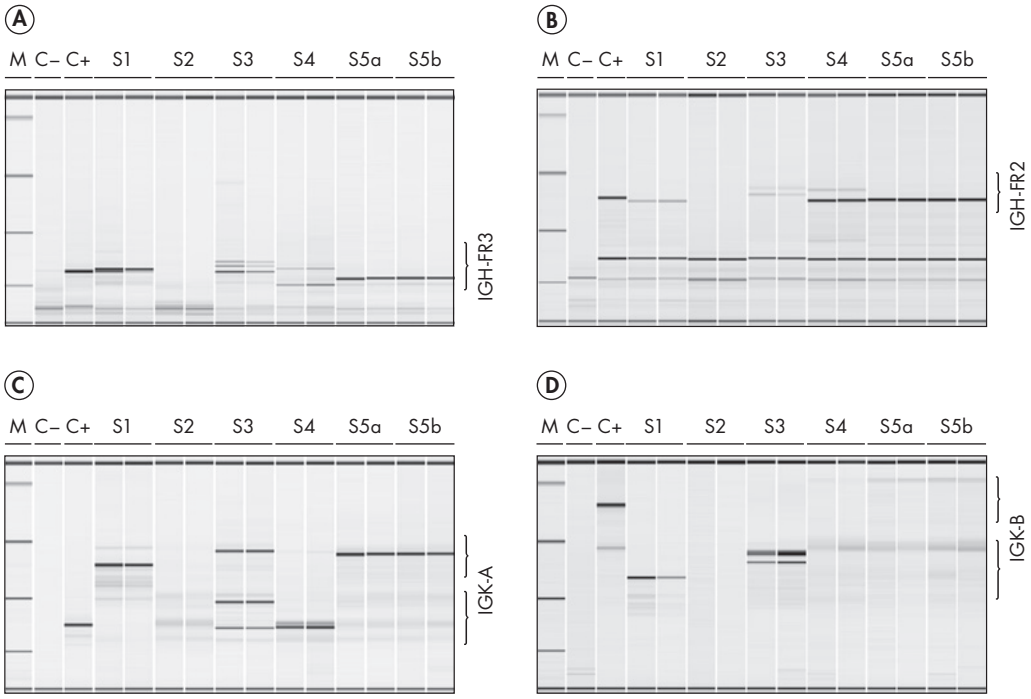

Figure 1. Identification of gene rearrangements in proliferating B-cells. DNA from lymphoid tissues was amplified using primers developed in the BIOMED-2 study and analyzed using the QIAxcel system with the QIAxcel DNA Screening Kit. **M**: size marker; **C–**: negative control; **C+**: positive control. **S1–S5b**: duplicate samples of multiplex PCR products. Primers were chosen to detect rearrangements in FR3 (**A**) and FR2 (**B**) VD FR regions of the IGH gene as well as the IGK-A (**C**) and IGK-B (**D**) genes.

Table 1. Observed IGH and IGK gene rearrangements

Gene	S1	S2	S3	S4	S5a	S5b
IGH-FR3	+	–	+ *	+	+ †	+ †
IGH-FR2	+	–	+	+	+ †	+ †
IGK-A	+	–	+ *	+	+ †	+ †
IGK-B	+	–	+ *	–	–	–

Monoclonal gene rearrangement present (+) or absent (–).

* Oligoclonality.

† The same monoclonal gene rearrangements were observed in both S5 samples.

Conclusions

- The QIAxcel capillary electrophoresis system is an optimal tool to resolve amplification products from multiplex PCR samples. Since even very small differences in fragment size could be resolved, it was possible to unambiguously detect immunoglobulin gene rearrangements. This allowed us to determine whether a lymphoid tissue was of monoclonal, oligoclonal, or polyclonal origin.
- Fast and accurate detection of gene rearrangement in B-cells facilitates the understanding of the development of malignancies during maturation of B-cells.
- The QIAxcel system processes samples in batches of 12 and allows analysis of up to 96 samples without manual intervention. Results can be displayed as a gel-like image as well as an electropherogram. The size and concentration data can be exported as a Microsoft® Excel® table.
- QIAxcel capillary electrophoresis uses electrokinetic injection of minute quantities of DNA, allowing the samples to be retained for downstream procedures, such as sequencing.

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QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Fast Analysis Kit (3000)	QIAxcel DNA Fast Analysis Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX DNA Size Marker 50 bp – 1.5 kb, QX Alignment Marker 15 bp/3 kb, 12-Tube Strips	929008
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104

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Visit **www.qiagen.com/clonality-analysis** and find out how automated gel electrophoresis can benefit your lab!

Safe and rapid HLA typing using the QIAxcel® system

Jean-Luc Sambucy

Merck Serono SA, Geneva, Switzerland

Using the QIAxcel system and data exchange with HLA genotyping software, PCR products from different HLA loci can be analyzed and typed in a few minutes. This method is simple and reliable and significantly reduces hands-on time compared to traditional methods in clinical research.

Introduction

Human leukocyte antigens (HLA) are proteins that are presenton each cell surface and allow the immune system to recognize "self" from "foreign" (1). Each individual human exhibits approximately 8 HLA types. Since each type comprises hundreds of subtypes, the chance that 2 unrelated individuals will have identical HLA patterns is very low (2).

Although HLA typing is primarily used to match organ donors and recipients, it is also often used in biomedical research because HLA profiles are associated with a number of diseases.

For example, the presence of HLA-DR4 and HLA-DRB1 increases the risk of rheumatoid arthritis 7-fold (3). A correlation between HLA type and the resistance or susceptibility to certain infectious diseases has also been observed (4). Epstein-Barr virus requires HLA Class II-Dr (in addition to CD21) to penetrate cells (5). Other diseases associated with HLA types include autoimmune diseases, post-infectious arthritis, type I diabetes, autoimmune hepatitis, and several others (see Table 1).

Rapid and routine HLA typing of individual humans and populations will facilitate understanding of the role of HLA types with respect to disease.

Table 1. HLA types correlated with disease*

Disease	HLA loci	Reference
Autoimmune diseases	HLA-B27	6
Rheumatoid arthritis	HLA-DR4, HLA-DRB1, HLA-DRB1*0404	3
Post-infectious arthritis	HLA-B27	7
Type I diabetes	HLA-DR3, HLA-DR4, HLA-DQ	8
Autoimmune hepatitis	HLA-DRB1*1301, HLA-DR3	9
Infectious diseases	HLA-DR2	10
Malaria	HLA-B53	11
Cancer	HLA-G	12
Breast cancer	HLA DQB*03032, HLA DRB1*11	13
Epstein-Barr virus infection	HLA Class II	5

* This list is not complete and includes only prominent examples of HLA genotypes that correlate with a disease or are suspected to play a role in a disease.

Materials and methods

PCR products produced using the A-B-DR Combi Tray Kit (Olerup SSP AB) were analyzed using the QIAxcel system with the QIAxcel DNA Fast Analysis Kit and the DM150 method. QX Alignment Marker 15 bp/3 kb and QX DNA Size Marker 50 bp – 1.5 kb were included in the analysis. Electrophoretic separation was performed using 10 kV voltage and 150 s separation time. Samples were injected with an injection time of 10 s at 10 kV.

HLA types were determined after analysis using BioCalculator software and HLA genotyping software.

Results

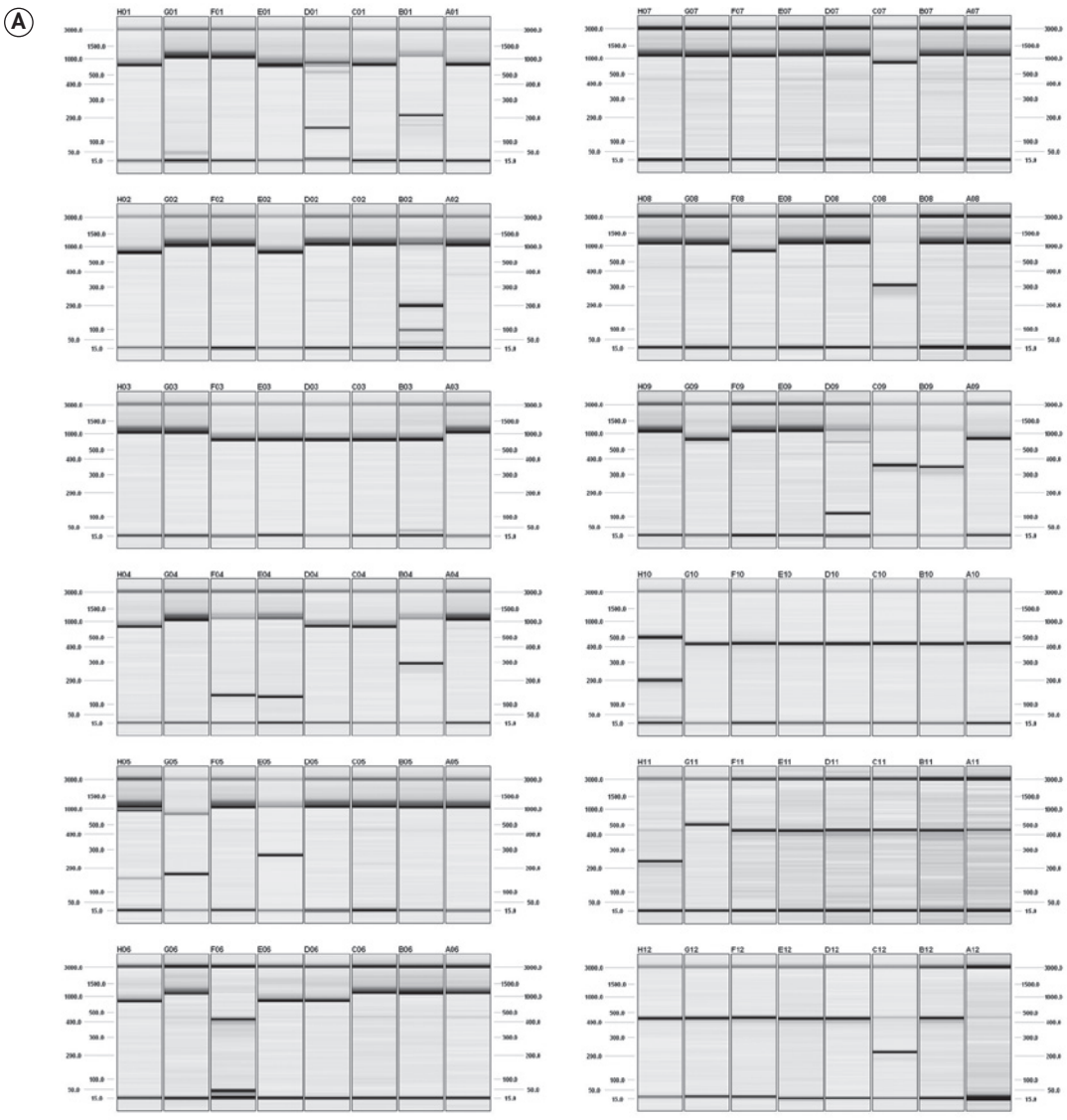
Three different types of HLA typing kits are available — sequence-specific oligonucleotides (SSO), sequencing-based typing (SBT), and sequence-specific primers (SSP) — and these are used in combination with different detection systems. For gel electrophoresis, sequence-specific primer kits are used. These kits are primarily dedicated to analysis of the A, B, C, DR, DQ, and DP loci of polymorphic HLA genes.

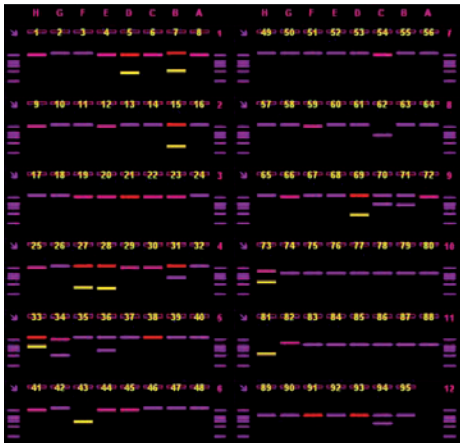
Figure 1 presents the results of analysis of 96 samples for one person. Separation of 12 samples was completed on the QIAxcel in 3 minutes using the QIAxcel DNA Fast Analysis Kit. Ready-to-run gel cartridges allow 96 samples to be processed automatically without manual intervention, thus reducing handling errors. The QIAxcel system minimizes hands-on time significantly compared to traditional methods. Electrophoresis and data acquisition are fully automated. Since results obtained using the QIAxcel system are compatible with HLA genotyping software, data can be imported and exported easily, and HLA genotypes are obtained within a few minutes.

Since the QIAxcel capillary electrophoresis system uses only minute amounts of DNA through electrokinetic injection, the samples are retained for downstream procedures.

Conclusions

- The combination of the QIAxcel system and QIAxcel DNA Fast Analysis Kit is an optimal solution for fast PCR checking, enabling rapid, reproducible, and standardized analysis of multiple HLA loci. The QIAxcel system is, therefore, highly suited for HLA genotyping and examining the role of HLA genes in multiple diseases in clinical research.
- BioCalculator software provides automated data generation and data acquisition, using formats that allow data exchange with other analysis software.
- The QIAxcel system is a powerful and versatile tool for researching and determining HLA types. Analysis of PCR products using the QIAxcel system facilitates a better understanding of the role of highly polymorphic HLA genes in multiple diseases in clinical research.





Allele combinations			summarised results
common	alleles	serological	equivalents
DRB1*01	DRB1*07:01:01:01	0 DR1	DR7
DRB4		0	
rare	combinations	serological	equivalents
DRB1*01	DRB1*07	0 DR1, –	DR7, Null, –

Figure 1. Routine automated HLA genotyping. HLA loci were amplified from 96 samples using the A-B-DR Combi Tray (Olerup SSP AB). **A.** PCR products were analyzed using the QIAxcel system. **B.** Analysis results were exported from BioCalculator software for further analysis using HLA genotyping software. **C.** HLA genotypes were obtained within a few minutes.

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4 DNA analysis – Microbiology

Bacteria, fungi and viruses show a great diversity of genotypes. There are vast numbers of biological assays designed to fingerprint this diversity, the prevalence of microbial communities or perform genotyping of strains of interest. A wide range of applications of the QIAxcel Advanced System in microbiology research includes, but are not limited to PCR-ribotyping, plant pathogen detection, pathogen strain typing, identification and analysis of bacterial DNA fingerprints, etc.

Thanks to significantly shorter run times, high resolution and multiplexing capacities, the QIAxcel Advanced is an instrument of choice to automate electrophoresis assays for microbiology applications and holds the potential to reduce costs, without compromising on sizing accuracy or sensitivity of detection.

Content

A Novel Approach for Identification of 16 Respiratory Viral Targets

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Introduction

Viral respiratory tract infections are associated with various types of virus, including adenovirus, coronavirus, human rhinovirus, influenza A, influenza B, metapneumovirus, parainfluenza and respiratory syncytial virus. To efficiently identify all these viruses requires the establishment of an accurate and reliable method that is rapid, sensitive and affordable.

Current non-molecular methods, such as viral culture or immunofluorescence (direct fluorescence antibody, DFA), are insufficiently sensitive, time consuming and labor intensive (1,2). Consequently, molecular methods are used and a well-accepted approach is an automated electrophoresis system to identify differences in amplicon size. Recently, multiplex RT-PCR assays have been developed specifically to detect respiratory viruses, but the limited availability of required equipment renders them difficult to standardize.

The method described herein uses a two-tube multiplex reverse-transcription PCR assay followed by amplicon-size separation using the QIAxcel® Advanced System (3). It is fast, highly automated, and based on reliable, affordable and readily available equipment and reagents. As such, this method may make an important contribution to routine virus identification.

Materials and Methods

Samples were collected and total RNA/DNA was extracted using the QIAamp® Viral RNA Mini Kit. A two-tube multiplex reverse-transcription PCR assay (two-tube assay) was used to detect 16 respiratory viruses based on their amplicon size differences. ▷

Test A was set up for simultaneous detection of nine respiratory viruses:

- Influenza A virus (FluA)
- Influenza B virus (FluB)
- Seasonal influenza A virus subtypes H1N1 (sH1N1)
- Parainfluenza virus type 1 (PIV1)
- Human rhinovirus (HRV)
- Coronavirus subtypes OC43 (CoV OC43)
- Coronavirus subtypes 229E (CoV 229E)
- Coronavirus subtypes HKU1 (CoV HKU1)
- Adenovirus (Adv)

In Test B, seven respiratory viruses were detected:

- Parainfluenza virus type 2 (PIV2)
- Parainfluenza virus type 3 (PIV3)
- Respiratory syncytial virus A (RSVA)
- Respiratory syncytial virus B (RSVB)
- Coronavirus subtypes NL63 (CoV NL63)
- Human metapneumovirus (HMPV)
- Human bocavirus (HBoV)

The QIAGEN® OneStep RT-PCR Kit was used for the amplification and PCR was performed as previously described (3). All PCR tubes (or 96-well reaction plate) can be loaded directly onto the QIAxcel Advanced electrophoresis system without post-PCR operation. For interpretation during analysis, the QIAxcel DNA High Resolution Kit in combination with the QX DNA Size Marker 25-500 bp and the QX Alignment Marker 15 bp/600 bp were used.

Results and Discussion

Test A allowed identification of nine respiratory viral targets (Figure 1A), while test B enabled identification of another seven respiratory viral targets (Figure 1B). The results show discrete band patterns for each of the virus type/subtype-specific amplicons. The QIAxcel DNA High Resolution Kit is capable of resolving amplicons that differ by as few as 5 bp.

It is estimated that, with this assay, 100 samples can be tested within 1 working day. Of that, a maximum of 1 hour is hands-on time. This estimate is based on 30 minutes to prepare the PCR mixture, 3 hours to complete the RT-PCR process, and time for detection using the QIAxcel Advanced System (15 minutes for 12 tests).

The fast and high resolving capacity provided by this experimental setup increases the potential to assess these 16 respiratory virus types/subtypes in future routine surveillance analyses.

Conclusions

- The two-tube assay in combination with the QIAxcel Advanced System and the QIAxcel DNA High Resolution Kit provided a reliable method for respiratory virus identification.
- The assay had sensitivity and efficiency suitable for consideration for routine surveillance.

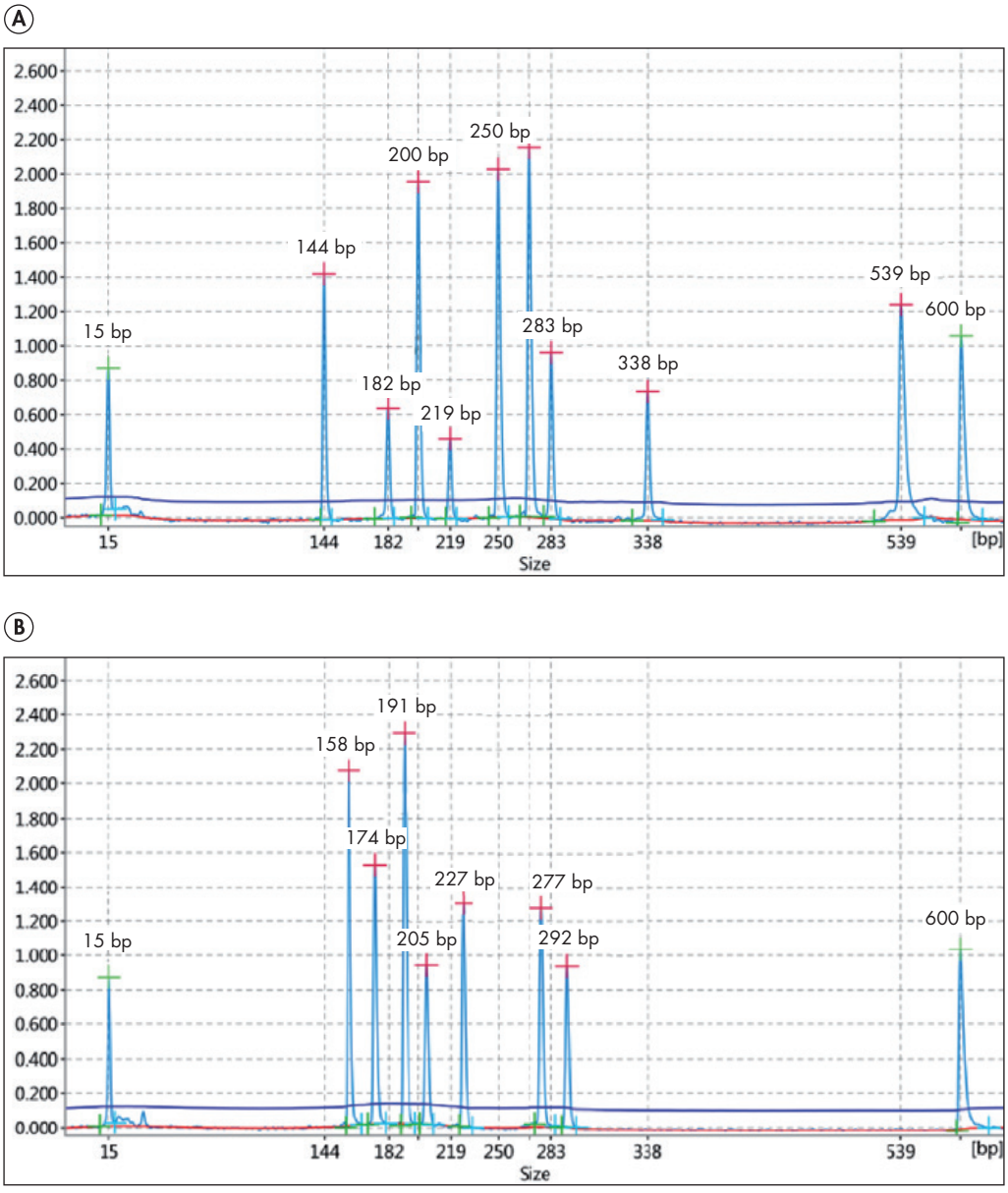

Figure 1. Discrete band identification of 16 respiratory targets with QIAxcel analysis. A Respiratory virus Test A. **B** Respiratory virus Test B. Test peaks are indicated by a red cross; peaks from QX Alignment Marker 15 bp/600 bp are indicated by a green cross.

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QIAGEN OneStep RT-PCR Kit (25)	For 25 x 50 µl reactions: QIAGEN OneStep RT-PCR Enzyme Mix (1 x 50 µl), 5x QIAGEN OneStep RT-PCR Buffer (1 x 250 µl), dNTP Mix (1 x 50 µl, 10 mM each), 5x Q-Solution (1 x 400 µl), RNase-Free Water (1 x 1.9 ml)	210210
Buffer ATL (4 x 50 ml)	4 x 50 ml lysis buffer for use in purification of nucleic acids using QIAsymphony DSP Virus/Pathogen Kits and the QIAsymphony DSP DNA Mini Kit	939016
QIAsymphony® SP	QIAsymphony sample prep module: includes 1-year warranty on parts and labor	9001297
QIAsymphony DSP DNA Mini Kit (192)	For 192 preps of 200 µl each: Includes 2 reagent cartridges and enzyme racks and accessories.	937236
TopTaq® Master Mix Kit	For 200 x 50 µl reactions: 2x TopTaq Master Mix containing 250 units of TopTaq Polymerase in total, 10x CoralLoad Concentrate, and RNase-Free Water	200403
QX Alignment Marker 15 bp/600 bp (1.5 ml)	Alignment marker with 15 bp and 600 bp fragments	929530
QX DNA Size Marker 25-500 bp (50 µl) v2.0	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500 bp; concentration 100 ng/µl	929560

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Advantages of the QIAxcel® system for bacterial genotyping

Steven Mutschall,[1] Susan Ross,[2] Cody Buchanan,[1] and Eduardo N. Taboada[1].

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

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

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 µl reaction volume, with ▷

each reaction containing 5–20 ng of template DNA, 1x PCR buffer, 1.0 mM $MgCl_2$, 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.

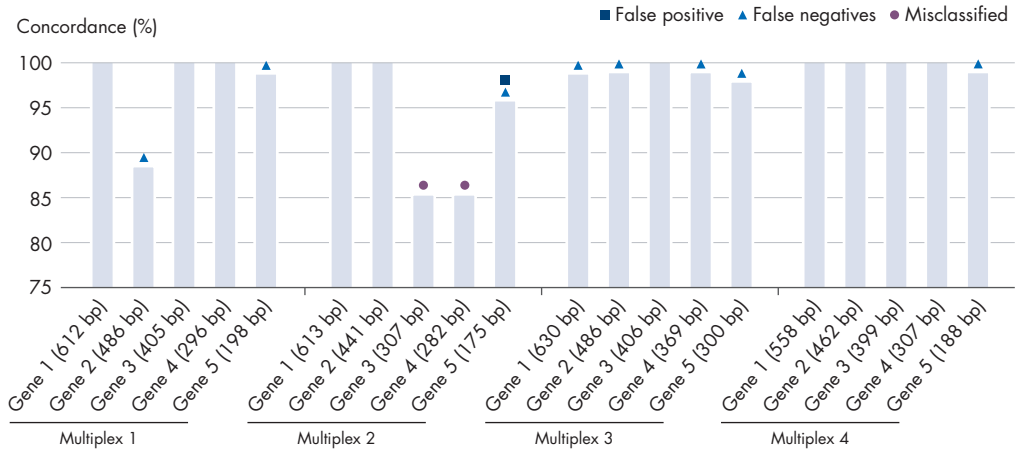

Figure 1. Percent concordance of band calls from agarose vs. QIAxcel data (n=96). A total of 50 mismatches were observed for a global concordance of 97.4% (1870 concordant datapoints in 1920 observations, i.e., 96 samples x 20 genes). Differences were primarily found in 3 of the 20 genes of the assay,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

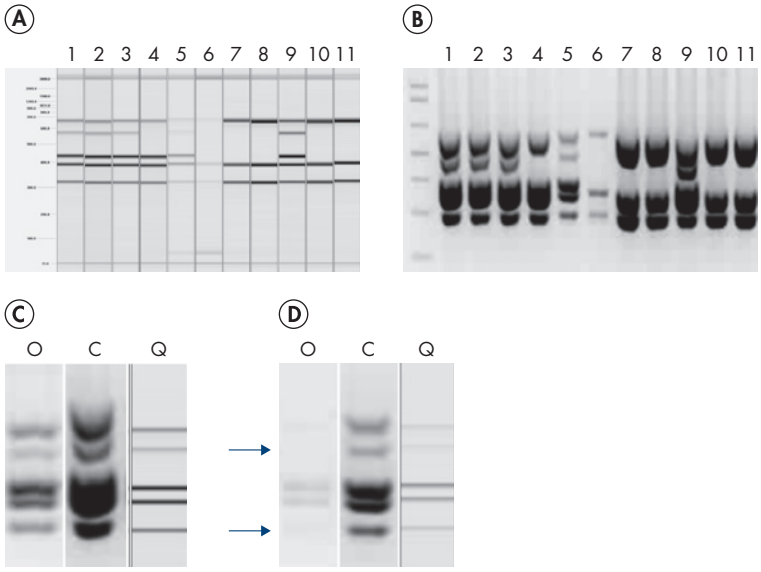

Figure 2. False negative error calls due to weak amplicons when analyzing samples by agarose gel electrophoresis. Samples were analyzed on either **A.** the QIAxcel at the original concentration or **B.** by agarose gel electrophoresis with five-fold concentration. Samples 3 **C.** and 5 **D.** were 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.

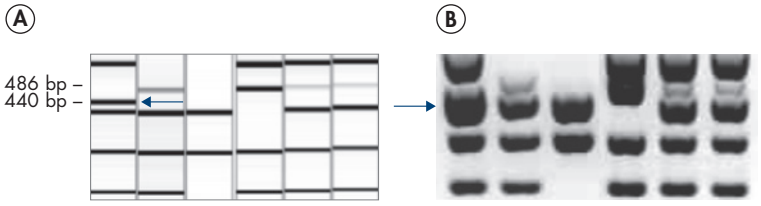

Figure 3. False positive error calls due to poor resolution when analyzing samples by agarose gel electrophoresis. Samples were analyzed either on **A.** the QIAxcel or **B.** by agarose gel electrophoresis. The arrows represent the bands of approximately 440 bp that was originally scored as positive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

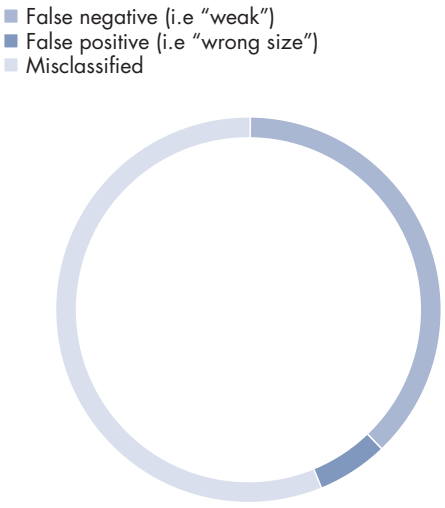

Figure 5. Classification of mismatches observed in the dataset after analysis by agarose gel electrophoresis or on the QIAxcel. Each of the 50 mismatches was examined to determine the nature of the mismatch. False negatives (38%, 19/50) were weak amplicons that were below the detection limit for agarose 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 maintainance 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.

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Automated MIRU-VNTR genotyping of *Mycobacterium tuberculosis* strains using the QIAxcel® Advanced Systems

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Introduction

MIRU-VNTR (mycobacterial interspersed repetitive units–variable number of tandem repeats) genotyping is commonly applied in studies of *Mycobacterium tuberculosis* strains. The protocol based on mini-satellite allele numbering developed by Supply et al. (1) is widely used.

The conventional method for MIRU-VNTR genotyping requires PCR amplification of each targeted locus, followed by detection using high-resolution agarose gel electrophoresis. The allele calling is done manually based on the gel analysis data. Poor gel quality or human error can lead to significant rates of false interpretation. An alternative method is needed to simplify the procedure and increase the reliability of the data assessment to help with the implementation of *M. tuberculosis* genotyping in high-burden countries.

The QIAxcel Advanced System is a high-throughput capillary electrophoresis system with automated data interpretation features. The aim of this study was to assess the instrument's performance in terms of ease of *M. tuberculosis* genotyping and reliability of MIRU-VNTR pattern generation. Sizing accuracy, reproducibility, repeatability, and automated allele calling analysis were studied using a MIRU-24 genotyping panel of the most prevalent strains.

Materials and Methods

Amplification

All of the samples were amplified via PCR using the QIAGEN HotStarTaq® Master Mix Kit, as previously described (2). Two controls were included in each run: one known sample (H37Rv or H37Ra) and one PCR blank (molecular-grade pure water).

Conventional method for electrophoresis and allele calling

Electrophoresis was run on NuSieve® 3:1 3% Agarose 100 mL Gel for 5 h at 120 V in the presence of the intercalating agent and using several size markers per gel. Allele calling was manually calculated using theoretical tables (2) and reported before submission to a free online genotyping database.

QIAxcel-based method for electrophoresis and allele calling

PCR products were analyzed using a QIAxcel DNA High Resolution Kit on the QIAxcel Advanced System (protocol OM1700). The QIAxcel Alignment Marker 15 bp/3 kb was run simultaneously with all of the samples to frame or delineate DNA fragments with two clearly visible peaks. The QIAxcel DNA Size Marker 100 bp – 2.5 bp ▷

was used to estimate the size of the PCR amplicons. Sizing was done automatically using the QIAxcel ScreenGel® software.

Allele calling was performed automatically using a peak calling table that defines the size of the alleles according to theoretical tables (2) and a tolerance in % for each locus to exclude any overlapping data. Then the results were merged into an Excel® spreadsheet and submitted to the free online genotyping database.

Reference sizing method

Sanger sequencing was used as a reference method to determine the amplicon sizes.

Table 1. Summary of MIRU-VNTR analyzed in this study

	Alias/Locus	Minimum size (bp)	Maximum size (bp)	Allele Distribution												
				0	1	2	3–3s	4	5	6	7	8	9	10	15	Mixture
15-locus MIRU-VNTR panel (n = 82)	MIRU 04/580	250	757		2	63	3	7	2	3	2					
	MIRU 10/960	590	1262			8	48	17	2	3	2		1			1
	MIRU 16/1644	565	821	1	4	19	57									1
	MIRU 26/2996	335	649		4	8	10	6	40	2	11					1
	MIRU 31/3192	492	816		1	10	24	3	40	3						1
	MIRU 40/802	398	692		8	9	42	10	12							1
	Mtub 04/424	602	920		1	25	12	38	6							
	ETR C/577	270	445			3	14	63	2							
	Mtub 21/1955	164	1053		1	16	14	8	42						1	
	QUB-11b/2163b	140	626		6	12	6	11	2	38	5	1				1
	ETR A/2165	274	732		1	6	17	50		2	5					1
	Mtub 30/2401	248	533	1	7	22		52								
	Mtub 39/3690	387	783			7	53	8	9	2	1	1	1			
	QUB-26/4052	385	1317			1	6	4	7	17	11	29	4	1		2
	QUB-4156/4156	560	849	4	8	56	8	6								
24-locus MIRU-VNTR (n = 66)	MIRU 02/154	456	513		3	62										1
	MIRU 20/2059	508	605		2	64										
	MIRU 23/2531	306	472				1		59	5						1
	MIRU 24/2687	439	505		59	7										
	MIRU 27/3007	644	665				65									1
	MIRU 39/4348	584	700		1	17	47									1
	Mtub 29/2347	451	579			1	7	58								
	ETR B/2461	403	647		1	57	1	6	1							
	Mtub 35/3171	386	492		1		64									1

Results and Discussion

MIRU-VNTR loci

The 15-locus panel for epidemiological studies and the 24-locus panel for phylogenetic resolution were used as the standard sets for identification of the main mycobacterial genotypes (Table 1). Other locus panels can be used when addressing specific lineages, such as Beijing strains.

VNTR allele-calling accuracy

The overall concordance on allele calling observed between the two methods is 1803/1824 (98.8%). For the size range of 140–900 bp, it is 99.9%. We observed 21 discrepancies: 19 on locus VNTR4052 (alleles 8 to 10), 1 on locus VNTR3690 (allele 8), and 1 on locus VNTR1955 (allele 15). Sanger sequencing confirmed all the allele sizes obtained by conventional gel electrophoresis. The QIAxcel data showed overestimated sizes for alleles above 900 bp. Optimization of the sizing algorithm for OM1700 is in progress. An in-house allelic ladder can act as an alternative internal size marker for accurate allele calling.

Influence of DNA concentration on sizing

It has been proved that conventional gel electrophoresis is negatively affected by high DNA concentrations. Therefore, we investigated the influence of DNA concentration on the resolution and size estimation for the two methods, and compared the results (Figure 1). Dilution series of the same PCR products were used to estimate the impact of DNA concentration on sizing accuracy.

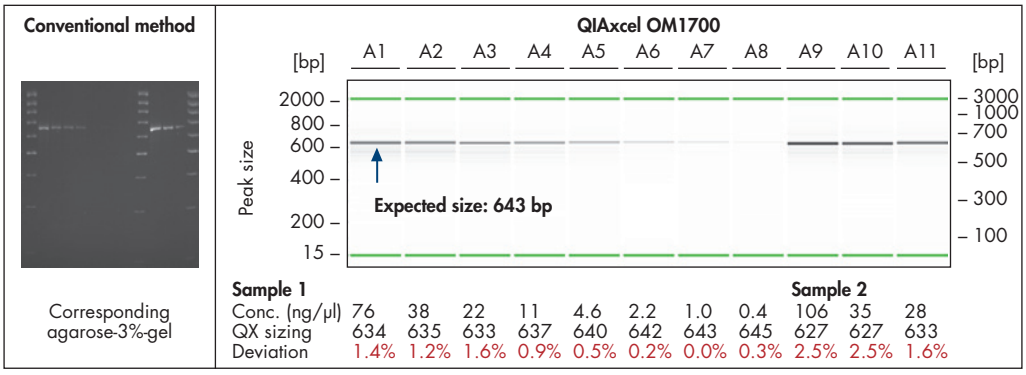

Figure 1. QIAxcel Advanced performs accurate DNA fragment sizing. Gel electrophoresis and fragment sizing of DNA fragments at various concentrations were performed using conventional gel electrophoresis and the QIAxcel Advanced instrument. Locus MIRU10 has repeats of 55 bp. The 643-bp fragment represents 3 alleles.

The results show no difference in allele calling at concentrations between 0.4 and 106 ng/µl. The QIAxcel Advanced System enables detection of DNA at concentrations as low as 0.4 ng/µl. The limit of detection using the conventional method is 2.2 ng/µl. Thus, PCR DNA quantification and/or sample dilution are not required before the QIAxcel run under these conditions.

Reproducibility and repeatability

A number of variables (operator, different alignment marker and cartridge batches, and cartridges of different ages) were tested using a ladder of previously sequenced amplicons of known sizes in order to cover the broad size range (100, 206, 382, 348, 562, 639, 681, 1065 bp). Tests were done with two cartridges. The maximum deviation for the estimated sizes ranged between 1.9 bp (100-bp amplicon) and 6.5 bp (1065-bp amplicon). The results indicate that the automated analysis with the QIAxcel protocol is repeatable (0.4 and 0.8% at the beginning and end of kit shelf life, respectively) and reproducible (0.4 and 1.2%) for MIRU-VNTR genotyping.

Conclusions

- The precision and accuracy of sample size estimation of Mycobacterium tuberculosis DNA with the QIAxcel Advanced method is compatible with that of conventional methods, and the repeatability and reproducibility are better.
- The QIAxcel Advanced method is significantly faster and less expensive.
- Implementing QIAxcel Advanced-based MIRU-VNTR genotyping of M. tuberculosis in the field would benefit epidemiological studies on tuberculosis worldwide.

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Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel Alignment Marker 15 bp/3 kb (1.5 ml)	Alignment marker with 15 bp and 3 kb fragments	929522
QIAxcel DNA Size Marker 100 bp – 2.5 bp (50 µl)	DNA size marker with fragments of varying size from 100 to 2500 bp; concentration 100 ng/µl	929559

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ERIC-PCR fingerprinting of indigenous *Sinorhizobium meliloti* strains

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[2] QIAGEN Instruments AG, Hombrechtikon, Switzerland

The QIAxcel® system was successfully used to identify and analyze DNA fingerprints of *Sinorhizobium* strains. Analysis using the QIAxcel system involved significantly shorter handling and running times compared to conventional methods, providing an effective, reproducible, and time-saving method for determining genetic diversity in bacteria.

Introduction

Upon infection with rhizobial bacteria, nitrogen-fixing nodules are formed in the roots of legumes. It is a common agricultural practice to inoculate leguminous seeds with nitrogen fixing bacteria such as *Sinorhizobium meliloti* to enhance root nodulation and, subsequently, nitrogen uptake of the plant. Individual strains of *S. meliloti*, however, vary in their symbiotic effectiveness (1). Commercially available inoculants often fail to establish nodules when indigenous rhizobial populations are already present (2). The selection of highly competitive strains is essential for effective inoculation (3, 4).

Indigenous *S. meliloti* strains from different field sites in Croatia were analyzed (4). DNA fingerprints of the enterobacterial repetitive intergenic consensus (ERIC) sequences (5) were established to assess the genetic diversity of the isolates, as well as to establish their relationship to natural populations. The results of the study provided information about nodulation and symbiotic efficiency of individual *S. meliloti* strains (4).

The QIAxcel System.

Materials and Methods

Amplification reactions (25 µl) were prepared with 20 mM Tris·HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 200 mM each dNTP, 2.5 µl primer, 40 ng genomic DNA, and 1.5 units *Taq* DNA polymerase (Life Technologies). Primers used to fingerprint repetitive ERIC sequences, ERIC 1R and ERIC 2, are described in (6).

Samples were analyzed using the QIAxcel system together with the QIAxcel DNA High Resolution Kit and the 0M700 method with additional 120 second separation time. The QX Alignment Marker 15 bp/3 kb, the QX DNA Size Marker FX174/*Hae*III, and bacteriophage lambda DNA digested with *Eco47*I (*Ava*II) (Fermentas) were included in the run.

Results

The QIAxcel capillary electrophoresis system processes samples in batches of 12 and allows analysis of up to 96 samples without manual intervention. Results can be displayed as a gel-like image as well as an electropherogram. PCR-amplified ERIC sequences of individual *S. meliloti* strains were resolved, exhibiting well separated, sharp bands in the range of 100–2000 bp. A representative gel image is shown in Figure 1. Results were compared with those from 6% poly (NAT) gel (Elchrom Scientific AG) analysis (data not shown). The binary call function of the BioCalculator Software was used to determine the presence or absence of specific fragments in the samples. DNA sizes were precisely and reproducibly calculated. The results were used in a later study to prepare a dendrogram displaying the relatedness of the isolated strains.

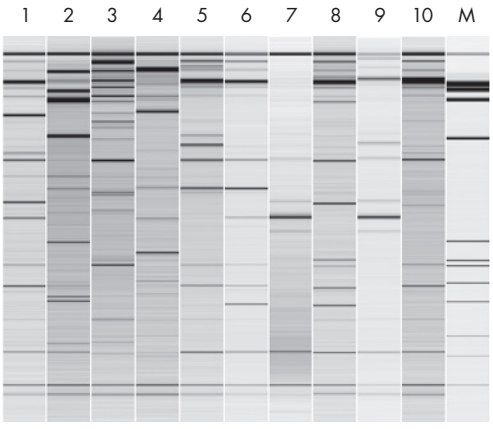

Figure 1. Detection of genetic diversity for *Sinorhizobium meliloti*. PCR amplified ERIC sequences of individual *S. meliloti* strains were analyzed using the QIAxcel DNA High Resolution Kit. **1–9**: Strains isolated from the nodules of alfalfa (*Medicago sativa* L.); **10**: reference strain 2011; **M**: QX DNA Size Marker FX174/*Hae*III.

Conclusions

- ERIC PCR fragments were separated and unambiguously identified using the QIAxcel system and BioCalculator Software. Subsequent comparison of the ERIC PCR patterns led to the precise determination of the genetic relationship of bacterial strains.
- The QIAxcel DNA High Resolution Kit resolves ERIC PCR fragments more effectively than agarose or polyacrylamide gels, providing greater sizing accuracy and improved sensitivity. The results are fully reproducible due to controlled running conditions and automated data acquisition. Since up to 96 samples can be analyzed in a single run, the QIAxcel system yields more information from DNA fingerprints while saving time.
- Since the QIAxcel capillary electrophoresis uses only minute quantities of DNA through electrokinetic injection, the samples are retained for downstream procedures, such as sequencing or cloning.

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Ordering Information

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QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104

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

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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).

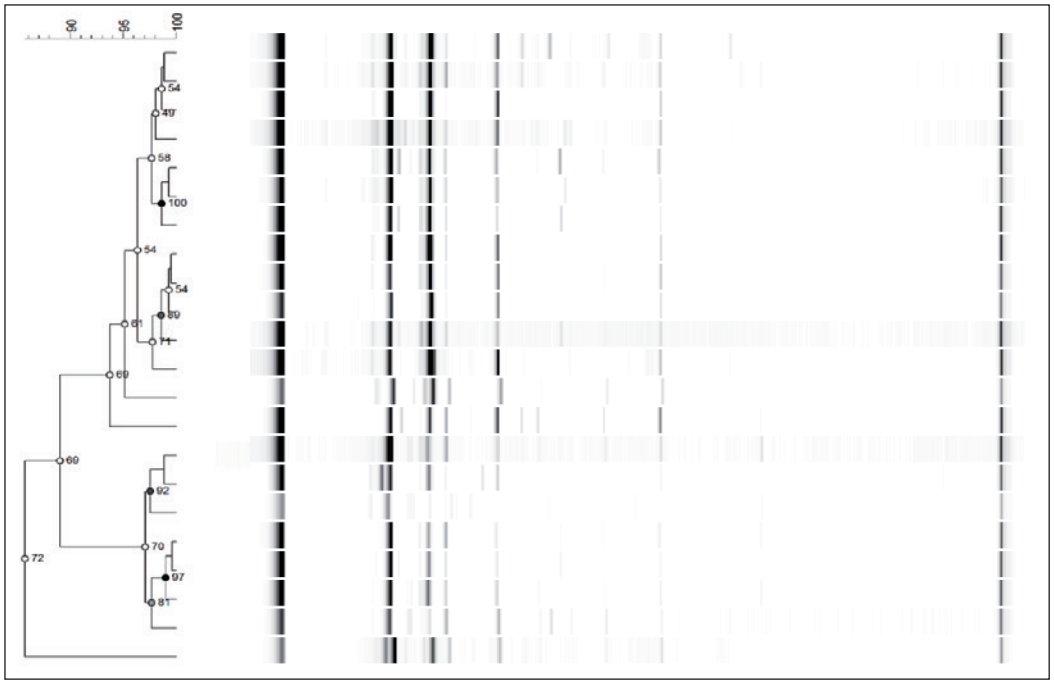
The QIAxcel Advanced System ScreenGel® software is fully compatible with BioNumerics software. Using the QIAxcel plugin, raw data files from the QIAxcel software can be imported as densitometric curve files (with the file extensions .xml and .csv) or peak table files (with the file extensions .xdr). 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 $MgCl_2$ 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.



Year of isolation	Strain
– 1983	1
– 1962	2
– 1998	3
– 1996	4
– 1945	5
– 2006	6
– 1982	7
– 1984	8
– 1993	9
– 2007	10
– 2000	11
– 1990	12
– 1968	13
– 1967	14
– 1967	15
– ?	16
– 2012	17
– 2003	18
– 1978	19
– 1967	20
– 2008	21
– ?	22

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.

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.

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 high-throughput 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.

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Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QX Alignment Marker 50 bp/5 kb (1.5 ml)	Alignment marker with 50 bp and 5 kb fragments	929529

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QIAxcel® System — *Clostridium difficile* ribotype determination

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Abstract

In this application note, we describe the transfer of methods based on agarose gel electrophoresis for ribotype detection and genotype characterization of *Clostridium difficile* to the QIAxcel system. Using the QIAxcel system, we were able to detect the fragment pattern characteristic of the 027 ribotype *C. difficile* strain in both gel and electropherogram views. The system also proved highly suitable for PCR-based detection of the *tcdA* enterotoxin and *tcdB* cytotoxin genes as well as the *tcdC* deletion. These loci together with the *tcdR* and *tcdE* genes form a chromosomal pathogenicity locus (PaLoc) (1, 2). The *cdtA/B* binary toxin gene was also detected.

Introduction

Clostridium difficile is the major cause of nosocomial diarrhoea and antimicrobial-associated colitis. Millions of infections a year cause diarrhoea, sometimes with abdominal pain and vomiting. Enteritis caused by *C. difficile* infection has become an increasing problem in the past few years. Since 2002, severe outbreaks have been reported with increased mortality for both elderly and young patients.

Antibiotics, especially those with a broad-spectrum activity, cause disruption of normal intestinal flora and can lead to overgrowth of *C. difficile*, which flourishes under these conditions. It is suspected that use of these antibiotics drives the formation of hypervirulent strains. Identified hypervirulent strains are of the 027 ribotype and express 3 toxins: enterotoxin TcdA, cytotoxin TcdB, and binary toxin CdtA/B. Furthermore, a deletion within the regulatory *tcdC* gene, normally restricting production of the TcdA and TcdB toxins, is characteristic for these strains.

We evaluated use of the QIAxcel, a capillary electrophoresis system, for ribotyping *C. difficile* strains and analysis of toxin genes. In comparison to conventional agarose gel based methods, this system provides significantly shorter run times and fewer manual handling steps, freeing up time for more demanding lab work and reducing manual error rates.

Materials and methods

Nucleic acid purification and PCR ribotyping

C. difficile isolates were subcultured on CCF (cycloserin, cephalosporin, fructose) selective plates and incubated anaerobically for 24–48 hours. After harvesting, genomic DNA was isolated using a Chelex 100-based method (3) and PCR ribotyping was performed as described previously (4). After PCR amplification, samples were concentrated by heating at 75°C for 55 minutes, the volume was adjusted to 10 µl with QX DNA Dilution Buffer, and analysis was performed on the QIAxcel system using the "OM500" method and QX Alignment Marker 15 bp/1 kb.

PaLoc analysis

The 027 sample, which is positive for ribotyping, was used for detection of the *tcdA, tcdB*, and *cdtA/B* genes and the *tcdC* deletion. Gene sequences were amplified using primers which are described elsewhere (1, 5, 6) and amplicons were analyzed on the QIAxcel system using the "0M500" method and QX Alignment Marker 15 bp/1 kb. DNA izing was performed using the QX DNA Size Marker 50 bp/800 bp.

Results and discussion

C. difficile PCR ribotyping patterns are based on size variations in the 16S–23S intergenic spacer regions of the bacterial rRNA (*rrn*) operon. Traditionally, analysis of these variations is performed using agarose gel electrophoresis. While this analysis method is easy to use and relatively cheap, it also requires long run times as well as significant manual effort to pour and prepare gels and often provides poor resolution. Recently, the use of methods based on capillary electrophoresis has been described to discriminate between different *C. difficile* strains and to analyze infection clusters (7–9).

The QIAxcel system is a capillary electrophoresis system that processes sample in batches of 12 and allows analysis of up to 96 samples without manual intervention. The system displays data as both a gel-like image and electropherogram. The QIAxcel system was used for analysis of *C. difficile* reference strains, and the typical ribotype patterns can be observed in the gel view (Figure 1A and 1B). Comparison of the 027 sample with classical agarose gel electrophoresis reveals a comparable fragment pattern (Figure 1C).

The QIAxcel system also proved to be highly suitable for PCR-based detection of the *tcdA, tcdB*, and *cdtA/B* genes (Figure 2A). The 18 bp deletion of the *tcdC* gene was accurately detected by the QIAxcel BioCalculator Software (Figure 2B).

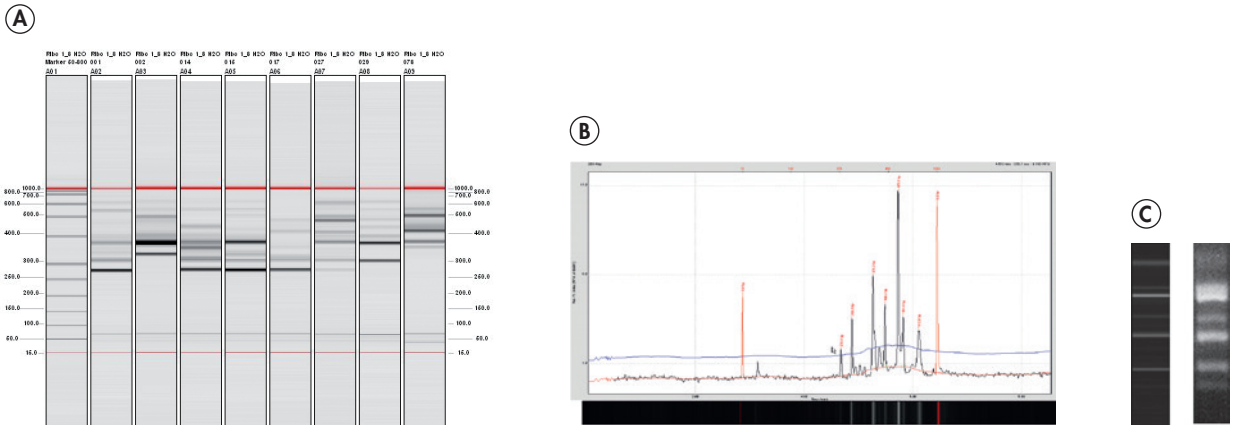

Figure 1. *C. difficile* ribotyping. A. Gel and **B.** electropherogram views of the 027 ribotype pattern obtained with the QIAxcel system. Alignment markers are indicated in red. **C.** Comparison of the 027 ribotype pattern obtained with the QIAxcel method (left) and with the traditional agarose gel method (right).

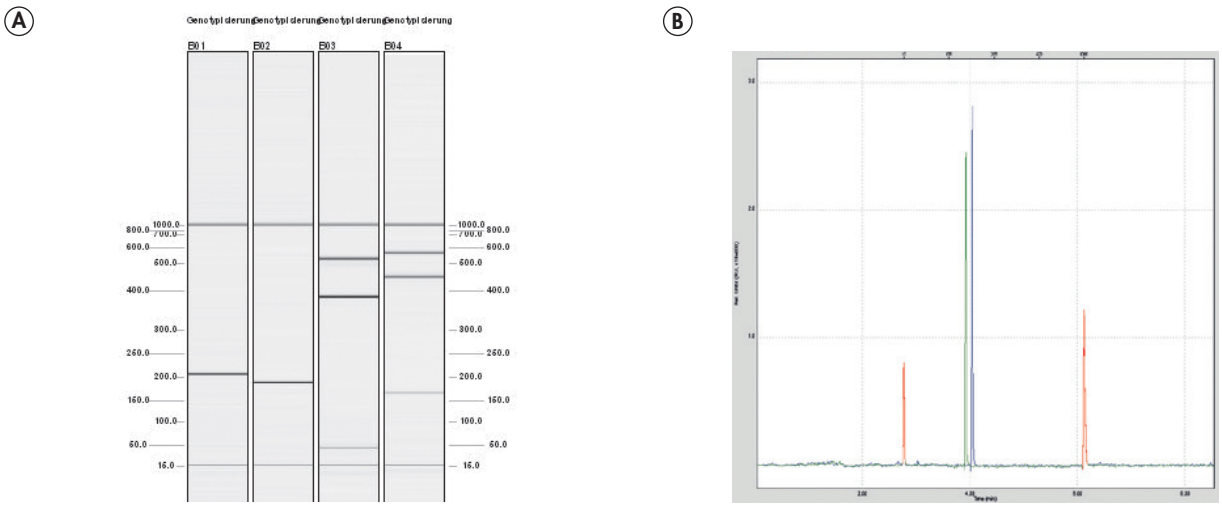

Figure 2. *C. difficile* genotyping. A. Partial genotyping of a *C. difficile* 027 ribotype sample. **B01**: *tcdC* control; **B02**: *tcdC* with deletion; **B03**: *cdtA/B*; **B04**: *tcdA/B*. **B.** Overlay of electropherogram views of B01 (206.8 bp, blue) and B02 (188.4 bp, green) lanes from part **C.** demonstrating the 18 bp *tcdC* deletion. Peaks for the upper and lower alignment marker are indicated in red.

Conclusions

The QIAxcel system proved to be suitable for *C. difficile* ribotyping applications and toxin-gene detection. Due to significantly shorter run times in comparison to conventional methods, the system has the potential to reduce the cost of PCR ribotyping by drastically reducing the hands-on time. Standardized automated processing facilitates inter-laboratory data exchange without the need for cumbersome standardization of equipment, reagents, and operating procedures.

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QIAxcel DNALarge Fragment Kit (600)	QIAxcel DNA Large Fragment Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929006
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5 DNA analysis – Applied Testing

Thanks to its high-throughput capacities and low cost per sample without compromising on the performance, the QIAxcel Advanced is a solution of choice for genetic testing and screening of large pools of samples, be it food or veterinary samples. The potential to perform complex multiplexing within one electrophoresis run makes DNA analysis more cost efficient, thus improving your laboratory throughput and reducing time-to-result.

Overcoming the limitations of conventional gel electrophoresis for food, veterinary and GMO testing by addressing size heterogeneity, QIAxcel Advanced enables screening, identification and quantification of many more genetic targets in less time than one can imagine. This faster and more efficient process for DNA analysis can increase the amount of information available to scientists making informed decisions about how to advance their research in order to enhance food or crop characteristics.

Content

Authentication of Basmati rice using SSR-PCR and QIAxcel® Advanced

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Introduction

Basmati is one of the most popular types of rice in the world, representing approximately 40% of the dry rice market. Being twice as expensive as non-Basmati rice, it is also often mixed with other types of rice. Approximately 46% of sold Basmati rice is adulterated. The term Basmati is a customer generic name for 15 varieties of rice produced exclusively in India and Pakistan. Nine of the 15 varieties are eligible for zero import duty in the European Union (Regulation (EC) 1549/2004). The 6 other varieties are approved only in India and Pakistan. These two countries also define which varieties can bear the name "Basmati" (Table 1).

Import of Basmati rice into the European Union must comply with several regulations (EC No 1234/2007, 1785/2003 and 972/2006) and requires an import certificate (1). The rice must be analyzed before and sometimes after import. Therefore, analyses that enable identification and quantification of Basmati rice are of great importance. The price of the rice depends on the variety as well as on several physical properties, including the proportion of green grains and broken grains. Various physical methods have been developed for identification based on examining grain dimension, amylose content, grain elongation through ▷

Table 1. Varieties of Basmati rice (reference: the Basmati Code)

Eligible for zero import duty under Regulation (EC) 1549/2004	
Basmati 217	Ranbir Basmati (IET 11348)
Basmati 370	Super Basmati
Basmati 386	Taraori Basmati (HBC-19, Kamal Local)
Kernel Basmati (Basmati Pakistan)	Type – 3 (Dehradun)
Pusa Basmati (IET 10364)	
Other varieties approved in India and Pakistan	
Basmati 198	Kasturi (IET 8580)
Basmati 385	Mahi Suganda
Haryana Basmati	Punjab Basmati (Buani Basmati)
(HKR 228/IET 10367)	

cooking and aroma, but these are time-consuming or unreliable. Microsatellites or simple sequence repeats (SSR) are molecular markers used for identification (2–4). Generally, interpretation of SSR data can be difficult, especially for an admixture of more than 3 individuals. In addition, fragment sizing requires a high degree of accuracy. Consequently, this method can be very time and resource-consuming, especially when performing simplex analyses using conventional gel electrophoresis. The QIAxcel Advanced capillary electrophoresis system can replace conventional gel electrophoresis with an automated process that minimizes manual intervention and errors. Analysis is fast (96 samples in 1 h 30 min) and inexpensive. Additionally, the QIAxcel ScreenGel® software estimates the sizes and areas of the fragments analyzed, rendering complex analysis software unnecessary because interpretation can be done directly with ScreenGel and the Excel® spreadsheets it produces. Using the QIAxcel DNA High Resolution DNA Kit makes an accuracy of 3–5 bp possible, which is crucial for correct sizing of SSRs. We have developed an SSR-PCR protocol for routine analysis of Basmati rice using the QIAxcel Advanced System, where all Basmati as well as other types of rice can be identified and quantified as DNA percentage of the Basmati variety.

Materials and methods

Grains from a range of Basmati and non-Basmati rice samples were selected and homogenized. Homogenized samples were mixed with lysis buffer (Qiagen ATL), heated to 65°C, and treated mechanically by agitation at 1400 rpm. DNA was purified with the QIAsymphony® DSP DNA Mini Kit. DNA amplification by duplex PCR was performed with a set of 8 SSR markers (RM1 + RM72, RM44 + RM55, RM202 + RM241 and RM171 + RM348) using the QIAGEN Multiplex PCR Kit. For some Basmati rice, 2 additional SSR markers were used for more accurate discrimination (data not shown). Size range of the PCR products was 80–400 bp (Figure 1). Capillary electrophoresis was carried out using the QIAxcel DNA High Resolution Kit, QX Alignment Marker 15 bp/600 bp, and QX DNA Size Marker 25–500 bp v2.0. QIAxcel ScreenGel software was used to estimate fragment size and percentage of target rice in the analyzed samples. The type of rice was identified using data from the Excel spreadsheets produced by the software.

Results and discussion

Identification

Analysis of different types of Basmati rice at 8 loci demonstrated that the rice varieties could be identified with high discriminatory power. Results showed characteristic, stable and reproducible DNA fragment profiles for each rice variety (Figure 1). Reproducibility and stability were assessed by repeating the analysis 12 times with a Taraori Basmati rice sample (Figure 2).

Quantification

When non-Basmati rice was detected in a sample, the quantity of Basmati rice DNA was presented as a percentage (Figure 3). This method is based on the comparison of the allele profiles of a known pure-grain sample and the unknown, tested sample. If a new peak was detected above a given threshold level, all possible profiles of all loci were identified. The area under the peak of every marker was calculated as a percentage. The average of all values was considered the quantity of Basmati rice DNA in the tested sample (Figure 4). The overall uncertainty of the analysis was 5.2%. The uncertainty when over 95% of the mixture was Basmati rice was 1.9%, which complies with EU regulations. The quantification had an accuracy of 0.2% and a dispersion of 2.0% based on 133 measurements made on 37 different mixtures of Basmati and non-Basmati varieties.

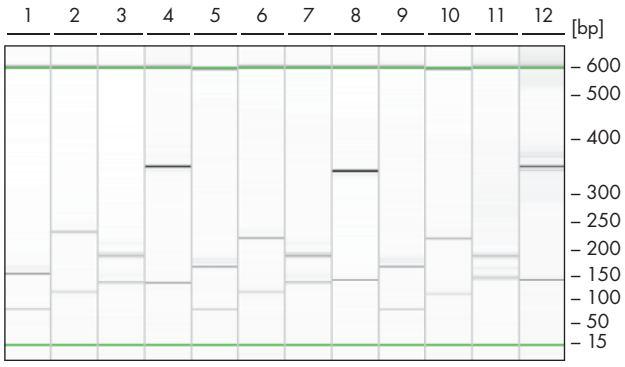


Figure 1. Reproducible and stable profiles of 3 Basmati rice varieties. **Lanes 1–4**: Pusa. **Lanes 5–8**: Taraori. **Lanes 9–12**: Super Basmati. The SSR markers were RM1 + RM72, RM44 + RM55, RM202 + RM241 and RM171 + RM348.

Figure 2. SSR profiles for Basmati rice are reproducible. The Taraori variety was analyzed 12 times with the SSR markers RM171 + RM348 and yielded the same fragment profile.

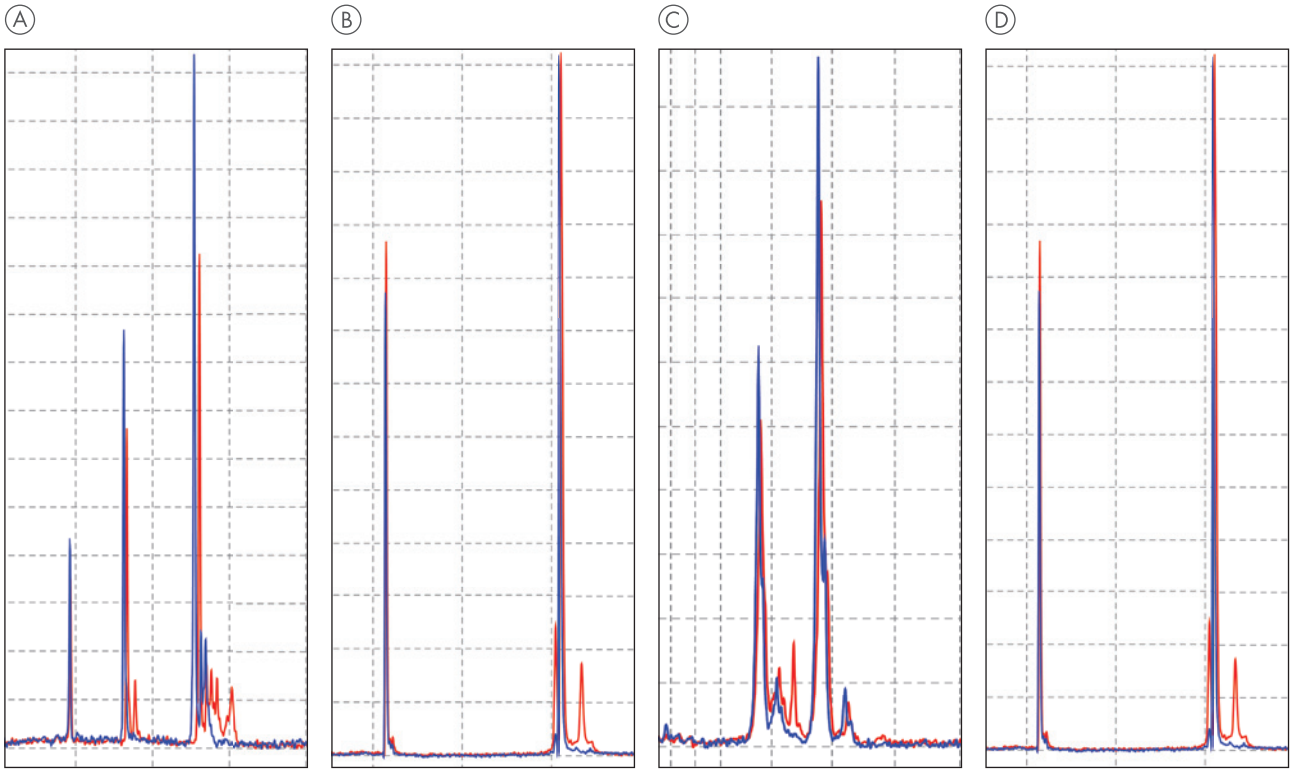
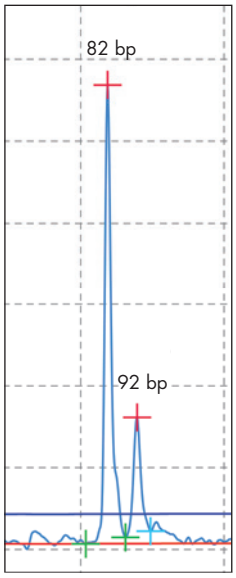


Figure 3. Sensitive detection of non-Basmati rice in a sample. Comparison of pure Taraori grain (blue peak) and a sample containing 75% Taraori and 25% adulterant (red peak). **A**: RM1 + RM72, **B**: RM44 + RM55, **C**: RM202 + RM241, **D**: RM171 + RM348.



#	Size (bp)	NA	NA %
1	15	0.0018099	n/a
2	82	0.0026379	75.86
3	92	0.0008393	24.10
4	600	0.0020555	n/a

Figure 4. Quantification of the RM1 marker in a sample of 75% Taraori Basmati and 25% non-Basmati rice. The 82 bp peak comes from Basmati and the 92 bp peak from the non-Basmati rice. The normalized area percentage (NA %) of each peak represents the quantity of Basmati rice and adulterant, respectively.

Analysis limitations

The identification of rice varieties was based on a database. A sample that generates a profile that is not present in the database cannot be identified. The 8 markers in this study were not sufficient to unambiguously identify all 15 Basmati rice varieties. In ambiguous cases, we used 2 additional markers to identify the variety (data not shown). When a sample consisted of 3 or more rice varieties, the allele patterns became complex (Figure 5), making complete sample identification and quantification difficult or at times, impossible. However, in most cases, identification and quantification could be done by independently analyzing grains that differ in physical characteristics (grain profiles). This method is more time-consuming and expensive, with an uncertainty between 6.5% for samples with >95% Basmati rice content and 22.2% for samples with lower Basmati rice content (average 14.1%).

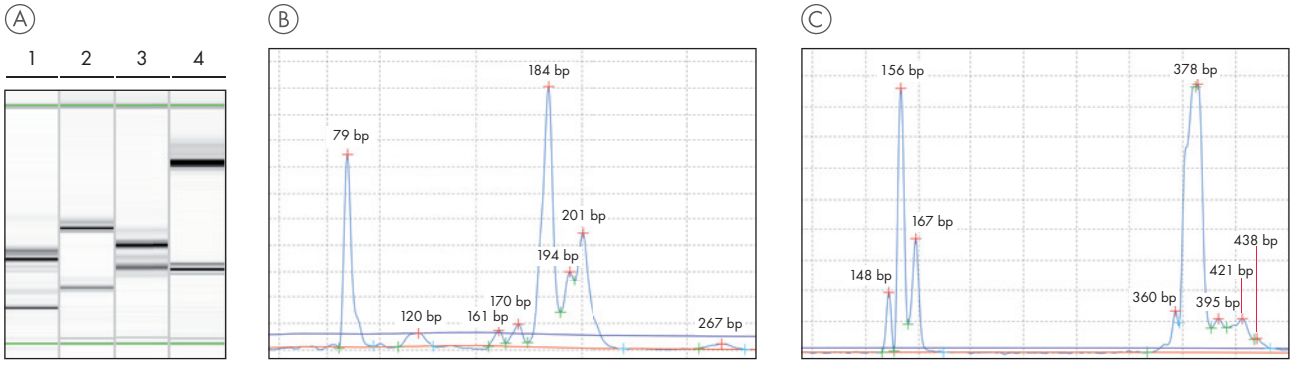


Figure 5. Analysis of a sample containing more than 3 rice varieties. A. **Lane 1**: RM1 + RM72. **Lane 2**: RM44 + RM55. **Lane 3**: RM202 + RM241. **Lane 4**: RM171 + RM348. **B**. Electropherogram of the RM1 and RM72 markers. **C**. Electropherogram of the RM171 and RM348 markers. With such complex allele patterns, identification requires additional analysis of physically different grains present in the sample.

Conclusions

- The SSR-PCR protocol for the QIAxcel Advanced System combined with a SSR marker allele database for rice proved to be accurate, reliable and fast, making this experimental setup a good candidate for routine Basmati rice authentication analysis.
- Identification and quantification of Basmati rice and its adulterants was successfully performed using the QIAxcel High Resolution Kit, which discriminates SSR fragment sizes with an accuracy of 3–5 bp.
- The method is best suited for samples containing 1 or 2 different rice varieties. Analysis of samples with 3 or more different rice types is more complex and requires additional testing.
- The method detects adulterant contents as low as 0.1%, with quantification of up to 99.0% Basmati rice. The overall uncertainty is 5.2%, and only 1.9% for mixtures with a Basmati rice proportion higher than 95%.

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QIAsymphony DSP DNA Mini Kit (192)	For 192 preps of 200 µl each. Includes 2 reagent cartridges and enzyme racks and accessories	937236
QIAsymphony SP	QIAsymphony sample prep module: includes 1-year warranty on parts and labor	9001297
QIAGEN Multiplex PCR Kit (100)	For 100 x 50 µl multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 mM $MgCl_2$, 3 x 0.85 ml), 5x Q-solution (1 x 2.0 ml), RNase-free water (2 x 1.7 ml)	206143
QX Alignment Marker 15 bp/600 bp (1.5 ml)	Alignment marker with 15 bp and 600 bp fragments	929530
QX DNA Size Marker 25–500 bp (50 µl) v2.0	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400 and 500 bp; concentration 100 ng/µl	929560

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See how you can benefit from automated gel electrophoresis at **www.qiagen.com/QIAxcel**.

Detection of genetically modified plants using the QIAxcel® System

BeeNa Lee, QIAGEN, Irvine, CA

The QIAxcel System was successfully used to detect DNA derived from genetically modified organisms (GMOs) at a level suitable for GMO testing according to EU standards.

Introduction

According to current EU regulations that went into effect in 2004, food and feed that contain at least 0.9% content from GMOs, or that were derived from GMOs, must be labeled as such. Each GMO that is present must be specifically declared using the system of unique identifiers published by the EU. For products for which compliance can not be conclusively demonstrated by analysis (such as for highly refined materials), the non-GMO origin of the material must be documented. Enforcement of the regulation depends upon the availability of accurate methods for detection and tracing of GMO materials. This report describes the validation of a reliable and cost-effective system for GMO detection and analysis which facilitates compliance with EU guidelines.

The following performance characteristics were determined for GMO material mixed with non-modified plant material:

- DNA concentration
- Fragmentation state of DNA
- Specificity

The QIAxcel System. The fully automated size separation and quantification capability of the QIAxcel System provides unmatched resolution, speed, and throughput.

Sensitive GMO detection

To determine whether the limit of detection (LOD) of the QIAxcel System is sufficient for GMO testing, template DNA of GMO origin (35S promoter) was mixed with genomic DNA from non-modified plants. Amplification of the GMO band was detected using the QIAxcel System (Figure 1) and quantification was performed to determine the sensitivity of detection (Figure 2 and Table 1).

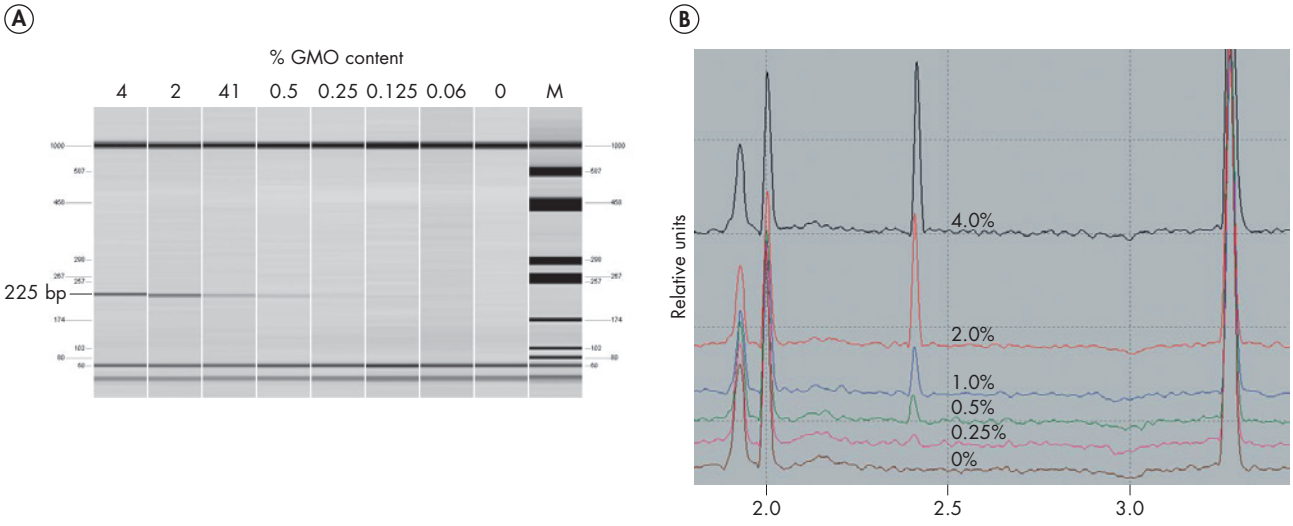

Figure 1. Amplification of target DNA mixed with genomic DNA from non-modified plants. Genomic DNA (supplied by University of Tennesee) was purified from young dogwood leaves using the DNeasy® Plant Mini Kit and quantified with a TD-360 fluorometer (Turner Designs, Sunnyvale, USA). The integrity of the genomic DNA was examined by visual inspection on a 1% agarose gel. Control 35S DNA template (provided by GeneScan, Freiberg, Germany) was mixed with the plant genomic DNA to the final concentrations listed in Table 1. Reactions were prepared with 50 ng/µl of the genomic DNA mixtures and proprietary primers (provided by Biotools, Madrid, Spain). PCR was performed on an Mastercycler® Gradient (Eppendorf, Hamburg, Germany): after an initial denaturation step (94°C for 3 minutes), 40 PCR cycles (denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 40 seconds) were followed by 1 step of 72°C for 3 minutes. PCR products were analyzed in the automatic multi-capillary electrophoresis QIAxcel System using the QIAxcel DNA Screening Kit. Non-diluted PCR products were placed in the instrument sample tray. The DNA samples were automatically injected into the capillary channel and subjected to electrophoresis according to the standard protocol. Separation was performed by the AM320 method using 10 seconds injection time and 320 seconds separation time. **A.** The expected 225 bp band amplified from the 35S promoter is clearly visible in the gel image. **M**: The molecular weight and concentration of the amplicons were determined based on the standard pUC18/HaeIII DNA size marker (Sigma, St. Louis, USA). **B.** BioCalculator software labeled the integrated peak area automatically. The added 35S target DNA was detected at a level of 0.25% in the electropherogram.

Table 1. Detection of GMO content

Template copies	GMO content (%)	Detected
2000	4	Yes
1000	2	Yes
500	1	Yes
250	0.5	Yes
125	0.25	Yes
62	0.125	No
31	0.06	No
No template control	0.0	No

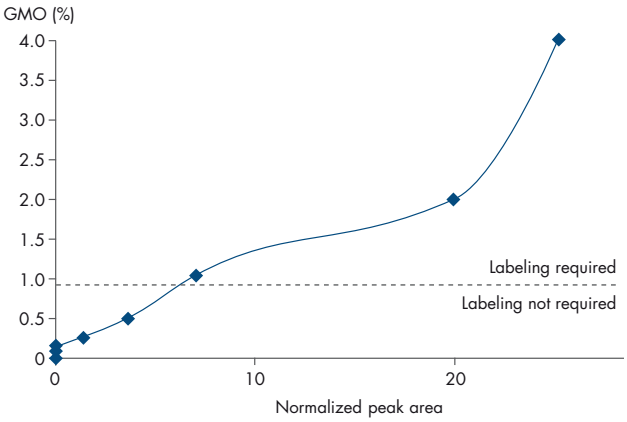

Figure 2. LOD of the QIAxcel System. The LOD was calculated for the quantitative normalized area (peak area) of the amplified 35S product at a defined number of copies per reaction. According to the EU GMO regulation, the limit of detection for a GMO marker must be below 0.9%. Food containing over 0.9% GMO content must be labeled.

Conclusions

- This system detected as few as 125 copies of GMO-origin target mixed with 50 ng genomic DNA from non-modified plants, corresponding to 0.25% GMO content (see Table 1). This result indicates that the sensitivity of the system allows detection of GMO content well below the cutoff limit for labeling (0.9%).
- The QIAxcel System includes a consumable multi-channel cartridge that can be used to inject and analyze multiple DNA samples simultaneously: up to 12 samples can be analyzed in less than 7 minutes and 96 samples in a multiwell plate in less than 50 minutes. This throughput is sufficient to meet the needs of many GMO-testing facilities.

QIAxcel Kits. Precast, reusable gel cartridges allow up to 200 runs of 12 samples to be performed.

Reference

Liu M.S. and Amirkhanian V.D. (2003) DNA fragment analysis by an affordable multiple-channel capillary electrophoresis system. Electrophoresis **24**, 93.0.

Ordering Information

Product	Contents	Cat. no.
QIAxcel System	Capillary electrophoresis device, including computer, and BioCalculator Analysis software; 1-year warranty on parts and labor	9001421
Warranty PLUS 2 Basic, QIAxcel	3-year warranty, 5-working day response time, all labor, travel, and repair parts	9241202
Gel cartridge kits		
QIAxcel DNA High Resolution Kit (1200)*	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)*	QIAxcel DNA Screening Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Large Fragment Kit (600)*	QIAxcel DNA Large Fragment Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929006
Software		
BioCalculator Software†	Separate license for use of BioCalculator software on an additional computer	9018391
Purification of plant genomic DNA		
DNeasy Plant Mini Kit (50)	50 DNeasy Mini Spin Columns, 50 QIAshredder Mini Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69104
DNeasy Plant Mini Kit (250)	250 DNeasy Mini Spin Columns, 250 QIAshredder Mini Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69106

* QX DNA Size Markers and QX Alignment Markers are not provided with QIAxcel DNA Kits and must be ordered separately. For information about markers suitable for your application, visit **www.qiagen.com/QIAxcel**.

† The software key is for analysis of results only. It does not provide any instrument control functions.

The QIAxcel instrument is intended to be used only in combination with QIAxcel Kits for applications described in the respective QIAxcel Kit handbooks. QIAxcel Kits are intended for research only. Not for use in diagnostic procedures.

The DNeasy Plant Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention or treatment of a disease.

Discover more about how the QIAxcel system can speed up and increase sensitivity for GMO detection at **www.qiagen.com/goto/QIAxcel**.

Fish species identification using PCR-RFLP and the QIAxcel® Advanced

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Introduction

Fishing and aquaculture play a considerable economic role in the European Union (1), accounting for 24% of the worldwide fish market (52.2 billion € for 12.3 million tons in 2011). In the EU, aquaculture is regulated through the definition of feed allowed in aquaculture production, including clear restrictions regarding the use of PAP (processed animal proteins), by-products from healthy animals developed to feed other animals (2). Intra-species feeding or cannibalism is prohibited, which means that feeding salmon with Salmonidae species, for example, is forbidden. These regulations are crucial in controlling fish feed composition and ensuring a supply of correctly labelled, high-quality fish to consumers.

Despite a long history of regulations to maintain a healthy fish industry, fraudulent practices still exist. One of the most common is the mislabeling of fish species. An initial study in the US between 2010 and 2012 showed that 33% of fish products were mislabeled. The study covered 1200 seafood products originating from 674 retailers and revealed fraud involving mainly red snapper and tuna (3). A similar study in France, based on 371 samples, showed that 3.5% of fish were mislabeled. Fraud involved mainly tuna and cod (4, 5). These studies attest to the importance of controlling all stages of fish distribution.

Various techniques are used for fish species identification. The most commonly used is PCR-RFLP (restriction fragment length polymorphism), but other methods, including qPCR, sequencing, and species-specific PCR, are also used (Table 1).

PCR-RFLP is a well-documented, easily mastered and inexpensive technique. However, RFLP can be time-consuming and requires special equipment, which makes it a poor candidate for standardized workflows. Furthermore, mutagenic and hazardous products, such as ethidium bromide, are often used in the visualization of DNA sequences. Results from gel electrophoresis can also be complex and interpretation may require specific software, although misidentification can be minimized by maintaining a database of possible profiles. To overcome these problems, we tested the QIAxcel native capillary electrophoresis system as an alternative to conventional gel electrophoresis. QIAxcel Advanced has numerous advantages: analysis is fast (96 samples in 1 h 30 m), inexpensive, and does not require handling ethidium bromide. Additionally, the QIAxcel ScreenGel® software enables semi-automated interpretation. The software calculates size of the analyzed fragments, which can then be interpreted with the ScreenGel software and the Excel® spreadsheets it produces. ▷

Table 1. Most common methods used for identification of fish species

Analytical method	Advantages	Inconveniences	References
PCR-RFLP	• Enables admixture analysis • Reproducible, sensitive and specific results • Enables analysis of processed samples	• Point mutations can induce false positives or negatives • Identification relies on a profiles database • Without automation, analysis is time-consuming	6, 7
qPCR	• Produces quantitative results • Enables analysis of processed samples • Detects trace amounts • Reproducible, sensitive and specific results	• Detects only prespecified species • Expensive when examining several fish species (requires 1 analysis per species)	8
PCR sequencing (Sanger Method)	• No prior knowledge required (internet database) • Delivers high-quality information • Reproducible and specific results	• Does not enable admixture analysis • High analysis costs	9
PCR sequencing (NGS)	• Enables admixture analysis • Produces quantitative results • No prior knowledge required (internet database) • Delivers high-quality information • Reproducible and specific results	• Time-intensive (1 week for results) • High analysis costs	10, 11
Species-specific PCR	• Simple method • Enables analysis of processed samples • Reproducible, sensitive and specific results	• Detects only prespecified species • Expensive when examining several fish species (requires 1 analysis per species)	12

Materials and methods

The analysis of 8 samples, from sample grinding to identification, was performed in less than 6 h. Samples were ground for homogenization and then lysed via chemical (Buffer ATL), thermal (up to 65°C) or mechanical (1400 rpm agitation) treatment for up to 1 h. DNA was then extracted and purified using the QIAsymphony® DSP DNA Mini Kit on the QIAsymphony. Cytochrome B was amplified with TopTaq Master Mix to yield a fragment of 470 bp. The fragment was digested with a panel of suitable enzymes (AluI, HaeIII, HinfI, DdeI, and TaqI) and the resulting fragments were separated on the QIAxcel Advanced using the QIAxcel DNA High Resolution Kit, the OM500 method, QX Alignment Marker 15 bp/600 bp and QX DNA Size Marker 25–500 bp. Fragment sizes were estimated with the QIAxcel ScreenGel software and results were interpreted from the resulting Excel spreadsheets by comparison to a database of possible profiles. The database contained matching internal and published data. The internal data were validated by analyzing fresh fish with all enzymes at 3 different times, and the published data were validated by testing fish samples with our method.

Results and discussion

This validation study examined PCR-RFLP for routine identification of fish species, generating results within 8 h. Several fish species, fish mixtures and processed samples were used to validate the method. Our assessment took into consideration that the validation may be limited by either polymorphisms or point mutations causing false negatives and false positives. We also accounted for low-quality food samples, given that overly degraded DNA, even from barely processed food, cannot be analyzed. Table 2 lists the 34 fish species (salmons, tuna, trout, hake) that could be identified with this method based on our database.

Figure 1 presents an analysis of DNA from crude fish, both a fish mixture and individual fish species. The HinfI digest (top right) can be used as an example to illustrate the principle of the RFLP approach. The fish mixture (lane 1) revealed 5 bands corresponding to different fish species comprising the mixture. These bands appear also in lanes 2–4 containing DNA from the individual fish species. The bands at 55 and 221 bp were also visible in lane 2 for *Scomber scombrus*.

Table 2. List of fish species that could be identified.

Scientific species names			
Anguilla anguilla	*Merluccius merluccius*	*Scomber scombrus*	*Boops boops*
Microstomus kitt	*Scophthalmus rhombus*	*Coryphaenoides rupestris*	*Mulus surmuletus*
Sparus aurata	*Cynoglossus senegalensis*	*Oncorhynchus gorbuscha*	*Stizostedion luciperca; Sander lucioperca*
Dicentrarchus labrax	*Oncorhynchus keta*	*Theragra chalcogramma*	*Epinephelus sp.*
Oncorhynchus kisutch	*Thunnus albacares*	*Gadus morhua*	*Oncorhynchus mykiss or Salmo gaidneri*
Thunnus thynnus	*Glyptocephalus cynoglossus*	*Perca fluviatilis*	*Trachurus trachurus*
Lates niloticus	*Pleuronectes platessa*	*Xiphias gladius*	*Lophius sp.*
Pollachius virens	*Zeus faber*	*Melanogrammus aeglefinus*	*Salmo salar*
Merlanguis merlanguis	*Sardina pilchardus*		

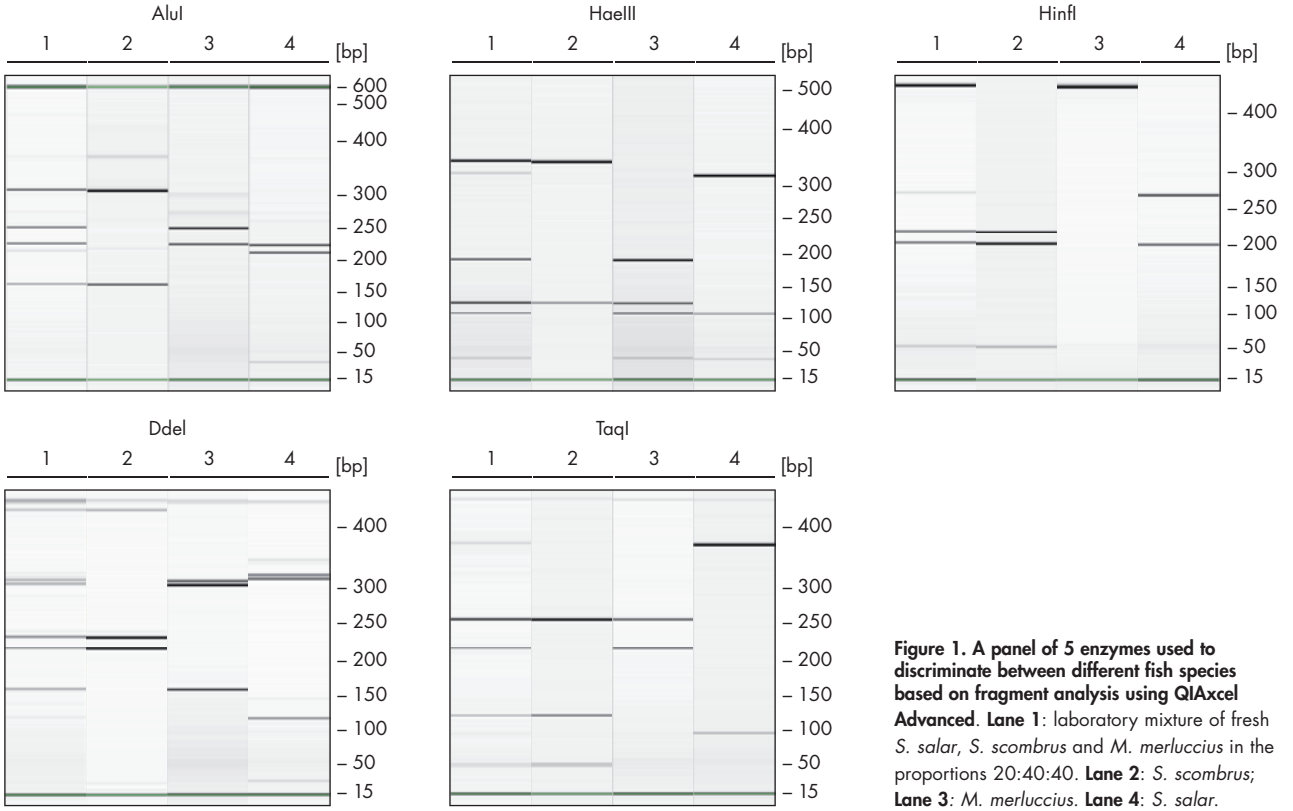

Figure 1. A panel of 5 enzymes used to discriminate between different fish species based on fragment analysis using QIAxcel Advanced. **Lane 1**: laboratory mixture of fresh *S. salar*, *S. scombrus* and *M. merluccius* in the proportions 20:40:40. **Lane 2**: *S. scombrus*; **Lane 3**: *M. merluccius*. **Lane 4**: *S. salar*.

The band at 204 bp was also present in lane 2 for *S. scombrus*, and in lane 4 for *Salmo salar*. The band at 273 bp appeared in lane 4 for *S. salar*, and finally, the band at 466 bp was visible in lane 3 for *Merluccius merluccius*.

Table 3 compares sizes of the observed HinfI restriction fragments with the theoretically expected fragments. The deviation average between theoretical and observed data was 4 bp. Experiments with the other enzymes generated

similar results. To assess utility of the method for processed commercial foodsamples, salmon spinach lasagna was digested with 4 enzymes, of which 1 enzyme was added for result confirmation (data not shown). Three different salmon species were identified in the lasagna, *S. salar*, *Oncorhynchus gorbuscha* and *O. keta* (Figure 2). The AluI digest was uninformative as it gave rise to the same band pattern for all 3 species, however, fragments from HaeIII, HinfI and DdeI digestion enabled discrimination of *S. salar* from *O. keta* and *O. gorbusha*. For example, the HaeIII restriction profile consisted of 4 bands, each coming from the band patterns of different salmons. The band at 41 bp was a fragment common to all three species, the bands at 109 bp and 309 bp came from *S. salar*, and the band at 419 bp came from *O. keta/O. gorbusha*. The two *Oncorhynchus* species were then identified using a fifth enzyme (data not shown).

The PCR-RFLP method proved successful in fish species identification. Each fish species had a unique profile when using the set of enzymes described above. Correlation between theoretical and observed data was good for both fresh crude and frozen fish (data no shown). Moreover, the method enabled discrimination of mixtures containing up to 3 species. During the validation, we observed that some fish, like *Theragra chalcogramma*, had a polymorphic profile or point mutations. Such samples may give rise to false negatives or false positives, which must be taken into consideration for interpretation. The method also works well for several processed foods, such as salmon spinach lasagna, crab sticks or salmon parmentier, as long as the extracted DNA is not overly degraded. Only severly processed foods (e.g., canned rillettes) contain excessively degraded DNA that cannot be analyzed.

Table 3. Comparison of theoretical and observed band sizes arising from HinfI restriction digests

Fish species	Theoretical band sizes (bp)	Observed band sizes (bp)	Differences
S. scombrus	56	55	–1
	201	204	+3
	214	221	+7
M. merluccius	464	466	+2
S. salar	198	204	+6
	266	273	+7

Conclusions

- The tested fish species generate unique digestion profiles and can be readily identified based on comparison to known profiles in a database. Up to 3 different species can be identified in mixtures.
- Commercial samples of varying processing degree can be analyzed, as long as the DNA has not been overly degraded.
- The QIAxcel Advanced facilitates the identification of fish species based on PCR-RFLP and provides results in less than 8 h.
- Using the QIAxcel Advanced, the method is inexpensive and reliable, making it a good candidate for routine use in fish species identification.

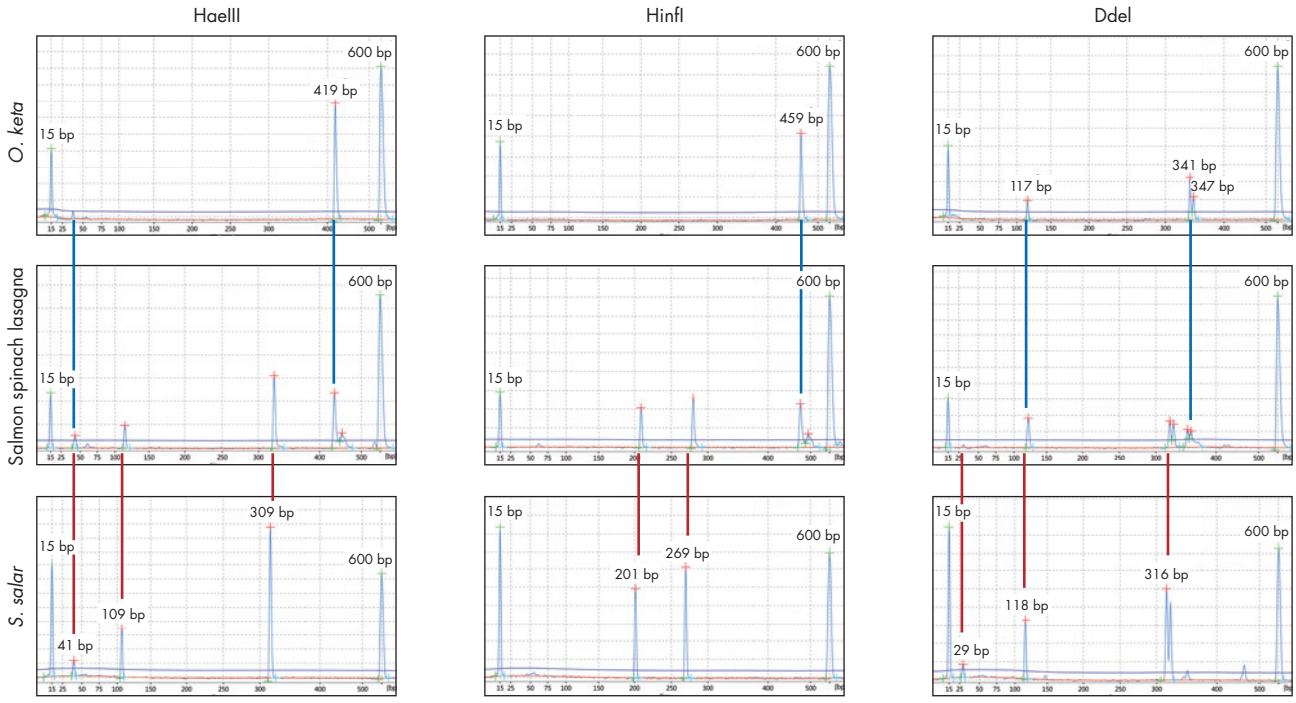

Figure 2. Analysis of a commercial food (salmon spinach lasagna) by fragment analysis using QIAxcel Advanced. *S. salar*, *O. gorbuscha* and *O. keta* were identified. HaeIII, HinfI and DdeI digestion enabled discrimination between *S. salar* and the 2 *Onchorhynchus* species. *O. gorbuscha* and *O. keta* were later identified using another enzyme (data not shown). Blue lines indicate bands in the lasagna sample corresponding to *O. keta*. Red lines indicate bands in the lasagna sample corresponding to *S. salar*.

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Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced Instrument	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High-Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
Buffer ATL (4 x 50 ml)	Lysis buffer use in purification of nucleic acids using QIAsymphony DSP Virus/Pathogen kits	939016
QIAsymphony DSP DNA Mini Kit (192)	For 192 preps of 200 µl each. Includes 2 reagent cartridges and enzyme racks and accessories	937236
QIAsymphony SP	QIAsymphony sample prep module: includes 1-year warranty on parts and labor	9001297
TopTaq Master Mix Kit (250)	For 200 x 50 µl reactions: 2x TopTaq Master Mix containing 250 units of TopTaq DNA Polymerase in total, 10x CoralLoad Concentrate and RNase-Free Water	200403
QX Alignment Marker 15 bp/600 bp (1.5 ml)	Alignment marker with 15 bp and 600 bp fragments	929530
QX DNA Size Marker 25–500 bp (50 µl) v2.0	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400 and 500 bp; concentration 100 ng/µl	929560

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

See how you can benefit from automated gel electrophoresis at **www.qiagen.com/QIAxcel**.

High-throughput detection of Shiga toxin-producing *Escherichia coli* using multiplex PCR and the QIAxcel® Advanced system

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Using the QIAxcel Advanced system after a multiplex PCR allowed us to perform efficient and reliable high-throughput screening of cattle feces for the presence of four major *E. coli* virulence genes and the seven major Shiga toxin-producing serogroups that give rise to infection in humans.

Introduction

Shiga toxin-producing *E. coli* (STEC) causes serious gastrointestinal disease and death in humans. The seven major *E. coli* serogroups that give rise to infections in humans are O26, O45, O103, O111, O121, O145 and O157 (1, 2). Cattle are considered one of the major asymptomatic STEC carriers, and the bacteria propagate in their gut and are shed in the feces, which is a major source of food contamination and human infection (3). Early detection of STEC is crucial for prevention and intervention strategies.

Nowadays most tests for STEC in veterinary laboratories are performed using real-time PCR, but this method is limited by the number of targets it can detect. Most real-time cyclers are limited to five channels, and in addition, developing multiplex qPCR using more than three channels is technically challenging. Detecting more targets by real-time PCR would require multiple reactions and result in significant cost increases.

We have developed an 11-gene multiplex PCR assay that detects four major *E. coli* virulence genes (*stx*1, *stx*2, *eae*, *ehx*A) along with the seven major STEC serogroups mentioned above. The level of throughput and the interpretation accuracy of this assay have been dramatically improved by using the QIAxcel Advanced, a fast capillary electrophoresis system with high resolution and reproducibility. ▷

After PCR amplification, samples in either strip tubes or 96-well plates can be analyzed directly using the QIAxcel Advanced instrument without further manipulation. Multiple steps of traditional agarose gel separation, including gel casting, PCR product loading, gel electrophoresis, ethidium bromide staining and gel imaging are not necessary when QIAxcel is used. The QIAxcel Advanced system can also perform automated data interpretation by recognizing predefined electrophoretic patterns. After a run, the positive or negative value for each of the eleven possible amplicons in every sample is presented in a report.

Materials and methods

Primer design

The *wzx* gene, which encodes a flippase required for O-polysaccharide export, was used to design specific primers for serogroups O26, O45, O103, O111 and O145. The *wbqE* gene, which encodes a putative glycosyl transferase, and *wbqF*, which encodes a putative acetyl transferase (6), were used to design primers for the detection of serogroup O121. The primers for O157 were designed from the *rfb*E gene, and primers used for virulence genes were designed and validated in our previous study (4). The 11 pairs of primers were specifically designed to match the majority of available target sequences and to amplify different amplicon sizes that could be easily separated on a gel or on the QIAxcel Advanced system (Table 1).

Table 1. The molecular targets and their amplicon sizes used in the 11-gene multiplex PCR. Specificity and amplicon sizes were confirmed by sequencing.

Target gene	Amplicon size (bp)	Target gene	Amplicon size (bp)
wzx_{O45}	890	wzx_{O26}	417
wzx_{O103}	740	*eae*	375
*stx*1	655	$rfbE_{O157}$	296
$wbqE_{O121}$ $wbqF_{O121}$	587	wzx_{O111}	230
wzx_{O145}	523	*ehxA*	168
*stx*2	477		

DNA templates

Field isolates obtained from cattle fecal samples were stored in CryoCare beads (Key Scientific Products, Stamford, TX) at –80°C. Single colonies were streaked onto blood agar plates (BAP, Remel, Lenexa, KS) and were incubated overnight at 37°C. One or two colonies of each strain were suspended in 1 ml of distilled water and boiled for 10 min. After a short centrifugation, 1 µl of the supernatant was used as the DNA template (approximate amount ranging from 1–100 ng DNA per 25 µl reaction). For cattle fecal sample preparation, approximately 1 g of feces was added to a tube of 9 mL Escherichia coli broth (Difco, ThermoFisher), and cultured at 40°C for 6 hr. One milliliter of the enrichment was transferred to a 1.5 ml tube and centrifuged. The supernatant was

discarded and the pellet was used for DNA extraction with QIAamp DNA Stool Mini Kit. The MagAttract 96 *cador* Pathogen Kit can be used for high-throughput DNA extractions. One microliter of extracted DNA was used for each PCR reaction (data not shown).

Multiplex PCR

The primer mix was prepared in 1x TE buffer, and each primer had a final concentration of 0.11 µM. The PCR reaction mix (Table 2) was prepared by mixing 12.5 µl of 2x QIAGEN Multiplex PCR Master Mix, 2.0 µl of 25 mM $MgCl_2$, 8.5 µl RNase-free water and 1 µl template DNA (1–100 ng per 25 µl reaction; 10 ng DNA template is recommended).

A modified PCR protocol described by Bai et al (4, 5) was used in this study. A 15 min denaturation step at 95° C was followed by a 35 cycles of amplification at 95° C for 20 s, 63° C for 30 s and 72° C for 90 s, with a final extension at 68° C for 10 min (Table 3).

Table 2. Preparation of reaction mix.

Component	Volume/reaction	Final concentration
RNase-free water	8.5 µl	
2x QIAGEN Multiplex PCR Master Mix	12.5 µl	1x
E. coli 11-plex Primer Mix	1.0 µl	0.11 µM
25 mM $MgCl_2$	2.0 µl	5 mM*
Total volume	**24.0 µl**	

* Final concentration includes $MgCl_2$ in the QIAGEN Multiplex PCR Master Mix

Table 3. PCR cycling conditions.

Initial activation step	**15 min**	**95°C**
3-step cycling: Denaturation	20 s	95°C
Annealing	30 s	63°C
Extension	90 s	72°C
Number of cycles	35	
Final extension	10 min	68°C
Hold		8°C

The following thermal cyclers have been tested and are compatible with this procedure and the described cycling conditions: TP600 thermal cycler (TaKaRa Bio Inc.), Eppendorf® Mastercycler® ep Gradient, Arktik Thermal cycler, Labnet PCR thermal cycler, Bio-Rad® C1000, Bio-Rad T 100 Thermal Cycler, MJ Research PTC-200, GeneAmp® PCR System 9700.

QIAxcel Advanced protocol

After amplification, PCR products were directly placed into the QIAxcel Advanced capillary electrophoresis system and separated using the QIAxcel DNA Screening Kit with the AM700 method, which includes the following electrophoresis parameters: alignment marker injection at ▷

4 kV for 10 s, sample injection at 2 kV for 10 s and separation at 3.5 kV for 700 seconds. The QX Alignment Marker 15 bp/3 kb was run simultaneously with the samples, and the sizes were estimated by comparison with a control sample containing all eleven possible amplicons (Table 1).

Following analysis with the QIAxcel ScreenGel® software, the operating and analysis software for the QIAxcel Advanced system, the results are displayed along with comprehensive reports including gel images, electropherograms and the result table. It is possible to customize the report according to different laboratory requirements.

Results and discussion

The analysis showed that the samples tested were positive for various combinations of O groups and virulence genes, as shown in Table 4 and Figures 1 and 2.

Table 4. Result table showing the presence (+) or absence (–) of four virulence genes in seven *E. coli* serogroups.

Lane	O-type	*eaeA*	*stx*1	*stx*2	*ehxA*
1	O26	–	+	–	+
2	O26	–	+	+	+
3	O45	–	+	–	+
4	O103	–	+	–	+
5	O103	–	+	+	+
6	O111	+	+	+	+
7	O111	+	+	–	+
8	O121	–	–	+	+
9	O145	+	+	–	+
10	O145	+	+	+	+
11	O157	+	+	+	+

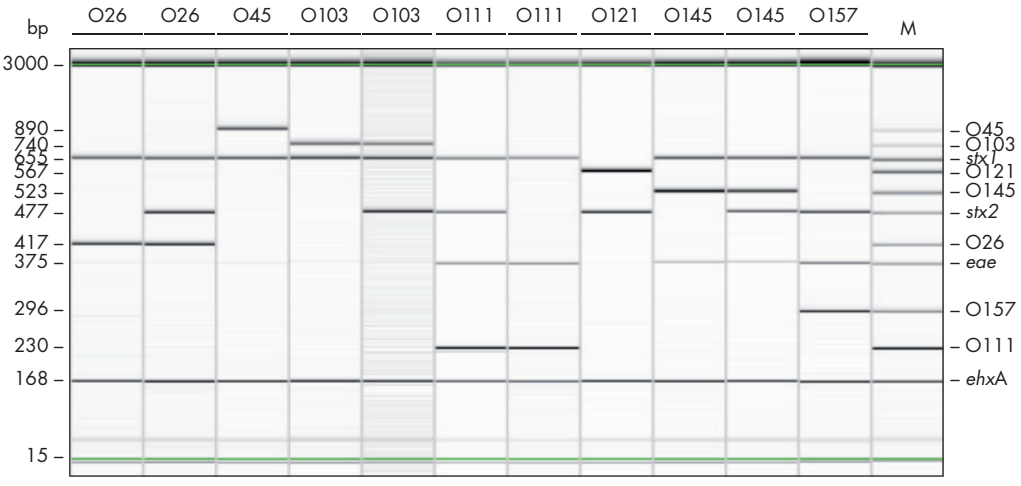

Figure 1. QIAxcel Advanced analysis of 11 isolates tested with 11-gene multiplex PCR. The PCR included the major *E. coli* virulence genes *stx*1, *stx*2, *eae* and *ehx*A, along with *E. coli* O-antigens from O26, O45, O103, O111, O121, O145 and O157 serogroups. Lane A12 contains a DNA ladder, which includes all 11 possible amplicons sizes and serves as a size marker. Amplicon sizes were verified by sequencing.

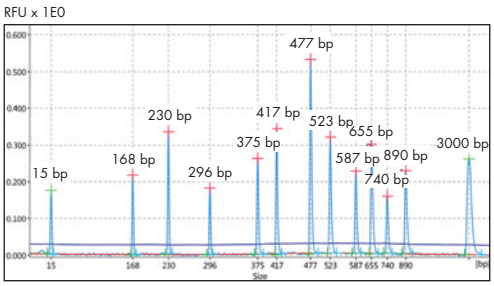

Figure 2. Electropherogram of the control sample from lane 12. All 11 amplicons used for identification are clearly visible and well separated. Peaks at 15 bp and 3000 bp correspond to alignment markers.

The strains were selected to represent all seven major *E. coli* serogroups and the four virulence genes. All of the samples carried the *ehx*A gene, which is present in most cattle fecal samples. However, the assay is only qualitative, and the ratios of the presence of the virulence genes in the table do not relate to STEC relative abundance or gene copy numbers within the cattle fecal samples. The Shiga toxin genes, especially *stx*2, are present in a much lower ratio in cattle samples, as described in previous publications (4, 5).

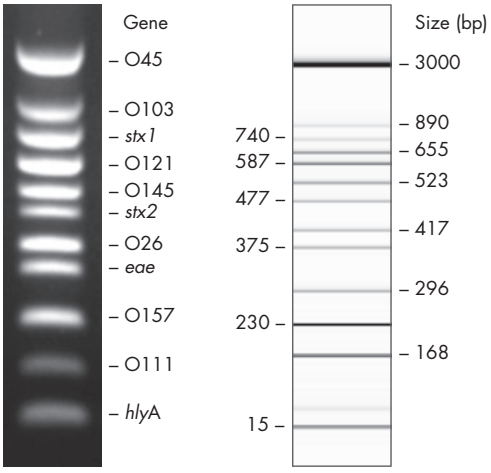

Figure 3. Comparison of the analysis results obtained with agarose gel electrophoresis and the QIAxcel Advanced. Sample containing all 11 amplicons was used as a positive control (5).

QIAxcel Advanced is a high sensitivity detection platform. When dealing with samples such as cattle feces, concentration of an *E. coli* strain can vary significantly. With the QIAxcel ScreenGel software, weak peaks can be observed in samples containing low concentrations of *E. coli* strains and may be difficult to interpret (Figure 4). After analyzing several thousands of field samples, we have established the best level of confidence in data analysis by using a 5% cutoff in the QIAxcel threshold setting. This correlates best with manual interpretations.

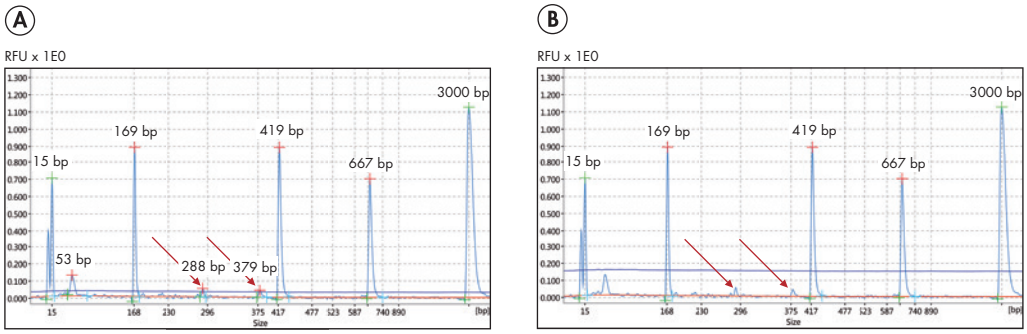

Figure 4. A **The threshold is used during peak detection.** Signals that exceed the threshold value (dark blue line) are detected as peaks. **B** If non-relevant peaks are identified, the threshold can be increased (e.g., to 5 %) to exclude the non-relevant or unspecific data from the analysis.

Automated sample loading, processing and analysis prevent human errors that can occur with manual sample handling, thus ensuring reliability and reproducibility of the measurements. Since no hazardous compounds are handled manually, this analysis method affords both convenience and safety. Using the recommended method, up to 96 samples per run can be analyzed unattended in about 90 minutes, or 12 samples in as little as 13 minutes. Digital data collection and management of experiments ensure traceability and standardized results.

Automated identification via peak calling

QIAxcel ScreenGel software automatically identifies DNA fragments from unknown samples using its peak calling function (peak calling table). This enables accurate detection and identification of the amplicons present in each sample. The peak calling table contains the sizes of all 11 possible amplicons generated by multiplex PCR and the tolerance in % for automated peak detection (Table 5).

Table 5. Peak calling instructions for 11-multiplex PCR.

Peak Calling Instruction *E.coli* 11-multiplex PCR					
Name	**Position**	**Tolerance**	**Name**	**Position**	**Tolerance**
ehxA	168 bp	18.000 %	wzx_{O145}	523 bp	4.400 %
wzx_{O111}	230 bp	13.500 %	$wbqE_{O121}$ $wbqF_{O121}$	587 bp	5.500 %
rfb_{O157}	296 bp	11.100 %	*stx*1	655 bp	5.200 %
eae	375 bp	5.600 %	wzx_{O103}	740 bp	5.700 %
wzx_{O26}	417 bp	5.000 %	wzx_{O45}	890 bp	8.400 %
*stx*2	477 bp	4.800 %			

The QIAxcel ScreenGel software compares the electrophoretic pattern obtained for each sample with the peak calling table and reports detected peaks in a Peak Calling Result Table that summarizes the results of the experiment (Table 6). This automated primary analysis of the sample makes results interpretation faster and more reliable for screening of a large number of samples.

Table 6. Based on the data presented in Figure 1and the peak calling instructions, a peak calling result table was generated. The table summarizes the experiment by reporting for each sample presence or absence of amplification products and their size estimates.

Peak Calling Result Table E.coli 11-multiplex PCR

Plate |R0|E0

Pos	Sample Info	*ehxA*	wzx_{O111}	rfb_{O157}	*eae*	wzx_{O26}	*stx*2	wzx_{O145}	wbq_{EO121} wbq_{FO121}	*stx*1	wzx_{O103}	wzx_{O45}
A1	*E.coli*-A-01	169	n/a	n/a	n/a	419	n/a	n/a	n/a	667	n/a	n/a
A2	*E.coli*-A-02	168	n/a	n/a	n/a	418	479	n/a	n/a	664	n/a	n/a
A3	*E.coli*-A-03	169	n/a	n/a	n/a	n/a	n/a	n/a	n/a	666	n/a	947
A4	*E.coli*-A-04	169	n/a	n/a	n/a	n/a	n/a	n/a	n/a	667	759	n/a
A5	*E.coli*-A-05	169	n/a	n/a	n/a	n/a	481	n/a	n/a	666	757	n/a
A6	*E.coli*-A-06	169	231	n/a	376	n/a	479	n/a	n/a	664	n/a	n/a
A7	*E.coli*-A-07	169	231	n/a	376	n/a	n/a	n/a	n/a	666	n/a	n/a
A8	*E.coli*-A-08	169	n/a	n/a	n/a	n/a	479	n/a	595	n/a	n/a	n/a
A9	*E.coli*-A-09	169	n/a	n/a	380	n/a	n/a	529	n/a	667	n/a	n/a
A10	*E.coli*-A-010	169	n/a	n/a	380	n/a	482	529	n/a	667	n/a	n/a
A11	*E.coli*-A-011	168	n/a	297	378	n/a	480	n/a	n/a	667	n/a	n/a

Conclusions

- The method presented here can identify the seven major *E. coli* serogroups and the four main virulence factors in one reaction.
- Separating multiplex PCR products with the QIAxcel Advanced system allows detailed, reliable and rapid analyses of complex samples containing many amplicons within a wide size range.
- Due to its fast turnaround time and automatic data interpretation, the QIAxcel Advanced system is highly suited for post-PCR separation, visualization and data presentation, especially for multiplex PCR runs in a high-throughput setting.

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Ordering Information

Product	Contents	Cat. no.
E. coli 11-plex primer mix (200)	Mixture of 11 pairs of primers for 200 reactions	WBQX-001-200
E. coli 11-plex primer mix (1000)	Mixture of 11 pairs of primers for 1000 reactions	WBQX-001-1000
QIAGEN Multiplex PCR Kit (100)	For 100 x 50 µl multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 mM $MgCl_2$, 3 x 0.85 ml), 5x Q-Solution (1 x 2.0 ml), RNase-Free Water (2 x 1.7 ml)	206143
QIAGEN Multiplex PCR Kit (1000)	For 1000 x 50 µl multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 mM $MgCl_2$, 1 x 25 ml), 5x Q-Solution (1 x 10 ml), RNase-Free Water (1 x 20 ml)	206145
QIAxcel Advanced system	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QX Alignment Marker 15 bp/3 kb	Alignment marker with 15 bp and 3 kb fragments	929552
QX Nitrogen Cylinder (6)	6 QIAxcel Nitrogen Cylinders	929705
QIAamp DNA Stool Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, InhibitEX® tablets, Buffers, Collection Tubes (2 ml)	51504

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Identifying allergenic nut species using the QIAxcel® system

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Introduction

The global food industry faces numerous challenges in terms of providing confidence in the authenticity of foods. There is a commercial interest in ensuring that foods are correctly labeled for the presence and levels of specific ingredients, including the occurrence of GMOs and incidence of allergens, and in preventing the fraudulent replacement of expensive food ingredients with inferior ones.

For the benefit of consumers who are allergic to certain food ingredients, current EU regulations require labeling disclosure of the presence of any of 14 major allergens if used as ingredients in prepacked foods (Directive 2003/89/EC, as amended). PCR assays have been developed to detect and identify DNA originating from allergenic nut materials in processed foods. They consisted of a suite of singleplex and multiplex assays to detect DNA from almond, hazelnut, macadamia nut, and peanut. The assays have been validated by LGC (UK) and Premier Analytical Services (UK) with regards to sensitivity and specificity.

This study demonstrates the successful use of the QIAxcel system for the analysis of PCR products as part of an allergen detection workflow. The QIAxcel system provided rapid, reliable, and inexpensive identification of four nut species.

Materials and Methods

Samples from a panel of 17 food products (Table 1) were analyzed using singleplex and multiplex PCR assays developed for the nut species almond, hazelnut, macadamia nut, and peanut. The PCR products were analyzed using the QIAxcel system in combination with the QIAxcel DNA High Resolution Kit.

End-point PCR was conducted for each nut species using 200 µM primers and 50 ng template DNA in a final volume of 25 µl. The PCR products were in the size range 75 to 134 bp. Short fragment sizes were chosen to ensure that the targets remained intact during food processing. The DNA concentration of the samples tested was in the range 2–5 ng/µl.

Samples were analyzed using a QIAxcel DNA High Resolution Cartridge. The method designated OM500 was chosen as the optimal one for the nut species under analysis. It has the following parameters: QX Alignment Marker injection at 4 kV and 20 s, sample injection at 5 kV for 10 s and separation at 5 kV for 500 s. The sample injection time was increased to 40 s to ensure identification of all of the amplicons present in the sample. The analysis was performed with a suspend integration time of 2.53 min. QX Alignment Marker 15 bp/400 bp and QX DNA Size Marker 25–500 bp were run simultaneously. Two fragments from the QX DNA Size Marker were excluded from the analysis since they were longer than the 400 bp fragment in the QX Alignment Marker.

Results and Discussion

The amplicons from the singleplex and multiplex PCR assays were analyzed using the QIAxcel system and QIAxcel DNA High Resolution Kit (Figure 1). An overview of the assays and the fragments detected is presented in Table 1. All of the nuts present on the ingredients list of the samples were accurately identified. An electropherogram of sample 16 is also presented to illustrate the successful application of the newly developed PCR method (Figure 2). The limit of detection for all of the nut species based on singleplex assays using 100% nut material was less than 20 pg of nut DNA.

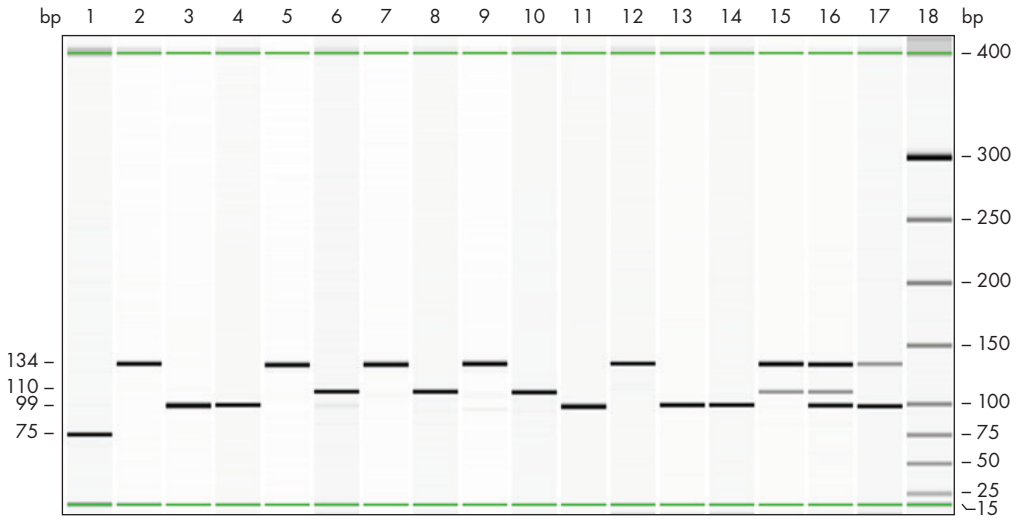

Figure 1. Successful use of the QIAxcel system for DNA fragment analysis. DNA fragments from macadamia, peanut, hazelnut, and almond are clearly visible. **Lane 1**: Macadamia (75 bp); **lanes 3, 4, 11, 13, 14, 16, and 17**: peanut (99 bp); **lanes 6, 8, 10, 15 and 16**: hazelnut (110 bp); **lanes 2, 5, 7, 9, 12, 15, 16, and 17**: almond (134 bp); **lane 18**: QX DNA Size Marker 25–500 bp.

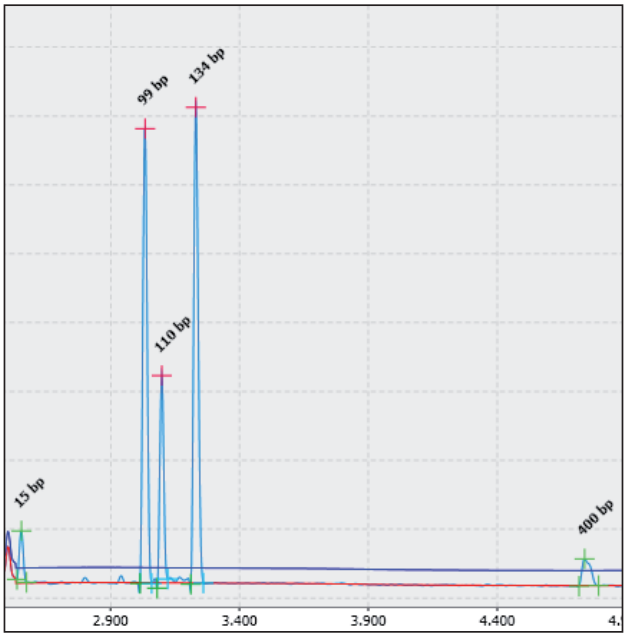

Figure 2. Electropherogram displaying the result from the multiplex PCR, sample 16 (cereal bar). DNA fragments from peanut (99 bp), hazelnut (110 bp), and almond (134 bp) were detected. The ingredients listed in the cereal bar included almonds (15%), peanuts (15%), and hazelnuts (9%).

Table 1. Foods tested for their nut contents with singleplex and multiplex assays using the QIAxcel system

Food sample	Lane	Nuts present on ingredient list	Assay	Fragment(s) detected (bp)
Macadamia cookies	1	Macadamia	Macadamia	75
Marzipan	2	Almond	Almond	134
Peanut butter	3	Peanut	Peanut	99
Brownies	4	May contain nuts	Peanut	99
Pistachio baklawa	5	Bakery (Pistachio)	Almond	133
Pistachio baklawa	6	Bakery (Pistachio)	Hazelnut	110
Dark chocolate	7	Almond, hazelnut	Almond	133
Dark chocolate	8	Almond, hazelnut	Hazelnut	110
Cereal bars	9	Almond, peanut, hazelnut	Almond	134
Cereal bars	10	Almond, peanut, hazelnut	Hazelnut	110
Cereal bars	11	Hazelnut	Peanut	98
Marzipan	12	Almond	Multiplex	134
Peanut butter	13	Peanut	Multiplex	99
Brownies	14	May contain nuts	Multiplex	99
Dark chocolate	15	Almond and hazelnut	Multiplex	110/134
Cereal bars	16	Almond, peanut, hazelnut	Multiplex	99/110/134
Pistachio baklawa	17	Bakery (Pistachio)	Multiplex	98/134

Detecting the singleplex and multiplex nut assays and analyzing the data using the QIAxcel ScreenGel® software demonstrated that the QIAxcel system can be used to detect DNA of specific nut species in the foods tested. The specific assays yielded clear results with strong bands corresponding to the targeted fragment sizes.

The results of the analysis can also be presented in other formats.

1. A table with up to 16 user-defined parameters, including size, concentration, and percentage.
2. Peak calling tables with information about the presence or absence, size, and concentration of the targeted amplicons in the analyzed samples

Conclusions

The QIAxcel system can be successfully used to identify PCR products from the DNA of the specific nut varieties almond, hazelnut, macadamia nut, and peanut. Thanks to the fully automated procedure, the results are highly reproducible and reliable. Safety and reproducibility is ensured by defining the process profile, which cannot be changed by a routine user. The process profile defines the electrophoresis parameters, analysis, and reporting of the data.

This method used with the QIAxcel DNA High Resolution Kit and QIAxcel system proved to be an excellent tool for identifying nut DNA. These results demonstrate the successful application of the PCR method across a range of food samples and also the high sizing accuracy and reproducibility of results yielded by the QIAxcel electrophoresis system.

Acknowledgements

The authors gratefully acknowledge funding from the UK National Measurement Office under the Government Chemist Programme 2011-2014 for the development of the PCR assays described in this article. We would like to thank Victoria Kyle for her help with the PCR assays and Waitrose for providing food materials for the validation of the approaches.

References

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Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device: includes computer, software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QX Alignment Marker 15 bp/400 bp	Alignment marker with 15 bp and 400 kb fragments	929521
QX DNA Size Marker 25–500 bp (50 µl) v2.0	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500 bp; concentration 100 ng/µl	929563

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Identifying meat species using RFLP-PCR and the QIAxcel® Advanced system

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Food producers are obliged to monitor the manufacturing process for compliance with regulations on the quality and originof food products. Technology for rapid and accurate meat species authentication is crucial to the meat industry. This paperlooks at a sensitive method using restriction fragment length polymorphism PCR and the QIAxcel Advanced system.

Introduction

The requirement to put labels on food products providing consumers with information about the quality and origin of ingredients first appeared in the European Union in 1992. Directives on the legal protection of geographical indications and designations of origin for agricultural products and foodstuffs followed a few years later (1).

To protect consumers' interests, it was necessary to develop effective methods to authenticate the species composition of various food products, including meat. The prices for high quality meat are higher, so fraud does occur. Species identification requirements are also connected with the prohibition of sale of meat from certain protected animal species. Furthermore, certain cultures have religious restrictions on the meat composition of food. Finally, allergy sufferers need to know that they are getting meat that is safe for their consumption.

Fortunately, it is now possible to identify individual food species using molecular biology techniques, some of which allow unequivocal species identification in both raw and processed food.

Protein-based methods are not sensitive enough and cannot be used with processed meat because soluble muscle proteins are destroyed by processing. Therefore, most methods use PCR-based DNA amplification. PCR is characterized by high specificity and a relatively short analysis time.

The most commonly used PCR methods for meat identification are:

1. **PCR with species-specific primers** that are designed based on cytochrome b mitochondrial DNA (2) has been used in multiplex PCR for the qualitative identification of 6 meat species: cattle, swine, chicken, sheep, goat, and horse (3). Multiplex PCR has also been used for the distinct and specific detection of chicken, turkey, duck, goose, pheasant, quail, and guinea fowl in raw meat and processed meat products (4). These experimental setups enabled authentication for correct food labeling and for compliance with ingredient composition covering the range of all common domestic poultry species available on the EU market. ▷

2. **RFLP-PCR** allows the identification of various mammal, bird, and fish species (5). Wolf et al. developed a method that allows recognition of 25 animal species. The DNA fragment obtained by amplifying a specific region of the mitochondrial genome (tRNAGlu/cytochrome b) is treated with 11 different restriction endonucleases.

3. **Real-time PCR** allows quantitative contamination assessment, including the identification of meats of different, even closely related, animal species. It is very efficient in detecting traces of specific animal DNA, even if the DNA has been degraded during a meat preparation process. As such, it serves an excellent screening tool for high-throughput routine testing where the target is known.

RFLP-PCR satisfies crucial aspects such as specificity, sensitivity, flexibility, and efficiency. Previous studies demonstrated that RFLP-PCR (5, 6, 7, 8) is successful in identifying meat species.

Sample analysis using slab gel electrophoresis is unsuitable for routine work because the method is time-consuming, requires more manual handling, and uses hazardous products such as ethidium bromide. The results are difficult to interpret and may require specific software.

Native capillary electrophoresis with the QIAxcel Advanced system overcomes these issues. The automated procedure is fast and inexpensive. The QIAxcel ScreenGel® Software analyzes the electrophoresis data and provides the sizes and concentrations, so no other software is needed.

The purpose of this study was to optimize a procedure using RFLP-PCR in conjunction with the QIAxcel Advanced system for use in rapid (results in less than 8 h) and accurate routine analyses. The method involves amplification of a 359-bp product that is common to all vertebrates, followed by one or more enzymatic digestions. The proportion of individual meats could range from 1 to 99%. By applying four enzymes, it was possible to distinguish meat from 15 different animals. In addition, 6 animals served as contamination markers.

Criteria for optimization

We defined four criteria for optimizing the meat authentication method: sensitivity, flexibility, speed, and simplicity.

The limit of detection (LOD) should range between < 0.5% and 1%. Several different pure animal samples were tested and a sensitivity of 0.01% was validated for beef, pork and chicken. The meat was tested either as a water dilution or in a maize mixture.

The method should be suitable for a broad range of sample material to give maximum flexibility of application. It should take less than one working day to receive results as longer delays lead to higher costs for food producers. Finally, since the method is intended for routine analyses, it should be easy to implement and perform.

If RFLP-PCR is intended for species identification, the reference samples must be analyzed using the same procedure and QIAxcel DNA kit as the unknown samples. The disadvantage of this technique is the possibility of incomplete digestion. In such cases, other methods, such as real-time PCR and/or sequencing, should be used for confirmation.

Materials and methods

Meat homogenization was followed by lysis with chemical (QIAGEN ATL lysis buffer), thermal (up to 65°C), and mechanical (1400 rpm agitation) treatment for up to one hour (internal method). DNA purification was performed on a QIAsymphony® SP using the QIAsymphony DSP DNA mini kit. PCR was performed as previously described (7, 8) using TopTaq Master Mix (QIAGEN) and the primersCYT b1 and CYT b2. This yielded a product of 359 bp that is common to all vertebrates. The PCR product was digested with different enzymes as previously described (7), and the digests were analyzed with the QIAxcel system, which provided information about the size and concentration of the products. The results were further interpreted using a programmed Excel sheet.

The sample analysis was performed according to method OM500 for the QIAxcel Advanced system, using the QIAxcel High Resolution Kit, QX Alignment Marker 15 bp/600 bp and QX DNA Size Marker 25 – 500 bp.

Results and discussion

We used the PCR-RFLP approach where the amplified mitochondrial DNA region encoding cytochrome b (7) undergoes enzyme digestion for species identification. This was achieved using four restriction enzymes: AluI, Hae III, Hinf I, and RsaI. The meat was tested raw and after processing methods, such as cooking, freezing, reheating, smoking, dehydration, and sterilization. The results proved that the PCR-RFLP method can successfully identify and authenticate the species of meat used in commercial products subjected to various processing.

The method was first tested on a range of reference samples containing either a single meat or mixed meat species. Initially, the samples were analyzed with real-time PCR and the results were compared to data obtained using RFLP-PCR to validate the procedure and establish whether detection using the QIAxcel system was comparable to detection using real-time PCR. A number of pure and mixed animal products were used as references (Figure 1). The number of enzymes needed for the analyses was species-specific. For example, AluI is sufficient to detect cattle DNA but not that of the other species. In our study, a maximum of four enzymes was needed to identify all of the animals tested.

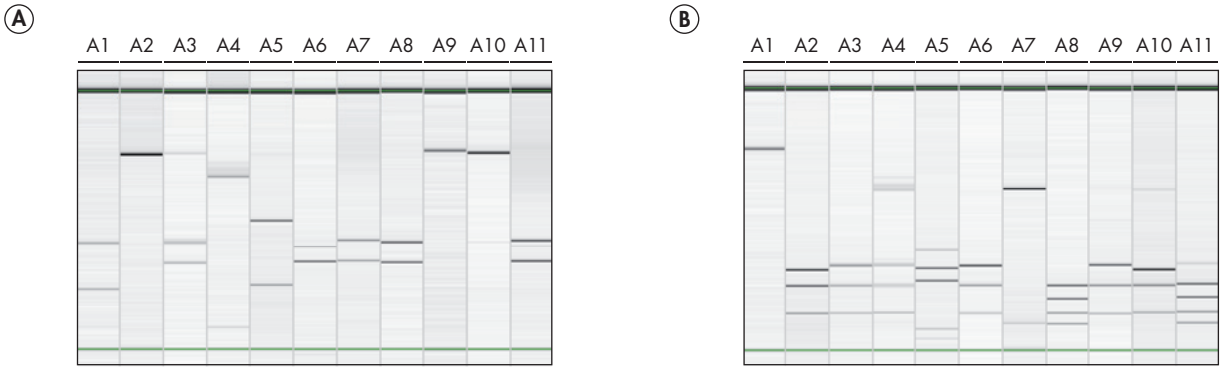

Figure 1. Successful identification of meat species using the QIAxcel Advanced System. The analysis of the 359-bp PCR fragment digested with **A.** HinfI enzyme and **B.** HaeIII enzyme. The 359-bp fragment was amplified from nine reference samples. The starting material was fresh meat. **Lane 1**: Cattle; **Lane 2**: Pig; **Lane 3**: Sheep; **Lane 4**: Deer; **Lane 5**: Rabbit; **Lane 6**: Chicken; **Lane 7**: Duck; **Lane 8**: Turkey; **Lane 9**: Goose. Only two mixed samples were prepared in the laboratory: **Lane 10**: Beef and pork and **Lane 11**: Chicken and turkey.

Table 1. Fragment sizes after digestion of 359-bp mitochondrial cytb fragment with restriction enzymes: Data are presented for nine animal species. Fragment sizes are given in base pairs.

	Restriction enzyme			
Animal species	**HaeIII**	**AluI**	**HinfI**	**RsaI**
Cattle	74, 285	190, 169	44, 198, 117	359
Pig	74, 132, 153	115, 244	359	359
Sheep	74, 126, 159	359	161, 198	359
Deer	74, 285, 126, 159	359	44, 315	359
Rabbit	44, 132, 30	359	233, 126, 153	359
Chicken	74, 21, 159	359	105, 161, 188, 10	149, 210
Duck	55, 286, 18	359	161, 198	359
Turkey	74, 285	359	161, 198	149, 109, 101
Goose	74, 126, 159	130, 229	359	154, 205

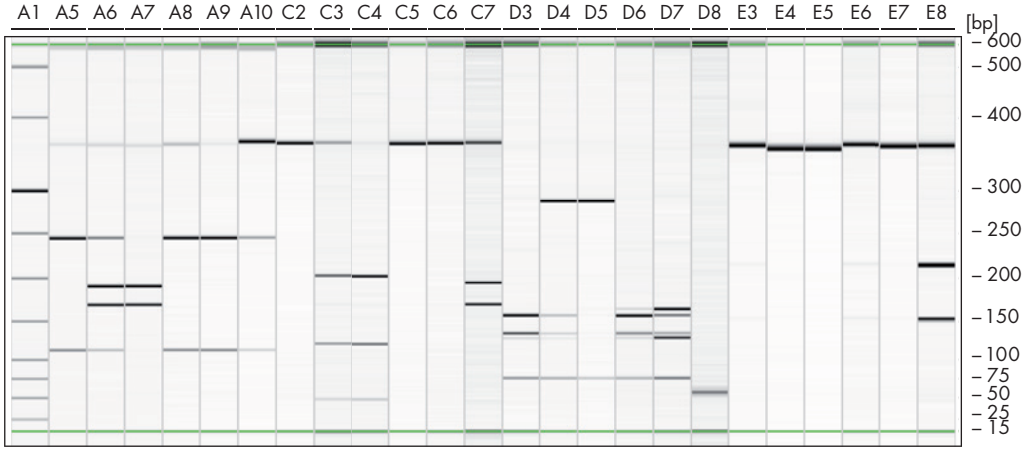

Figure 2. QIAxcel analysis of six commercial food samples. The samples were as follows — **FF1**: pork pâté (100% pig); **FF2**: hamburger meat (100% beef); **FF3**: Parmentier beef hash (100% beef); **FF4**: pork terrine (100% pig); **FF5**: pork liver mousse (100% pig); **FF6**: poultry liver terrine of (75% pig and 25% poultry). These samples were digested by the four studied enzymes, as follows — **Lanes A5–A10**: AluI; **Lanes C2–C7**: HinfI; **Lanes D3–D8**: HaeIII; **Lanes E3–E8**: RsaI. **Lane A1**: DNA Size Marker 25 – 500 bp.

Table 2. Summary of the analysis of the six samples, based on the data from Figure 2.

Sample	**Expected result**	**Observed result**	**Compliance**
FF1 (Pork pâté)	Pig	Pig	Yes
FF2 (Hamburger meat)	Beef (cattle)	Beef + Pig	No
FF3 (Parmentier beef hash)	Beef	Beef + Pig (traces)	No
FF4 (Pork terrine)	Pig	Pig	Yes
FF5 (Pork liver mousse)	Pig	Pig	Yes
FF6 (Poultry liver terrine)	Pig + Poultry	Pig + Chicken	Yes

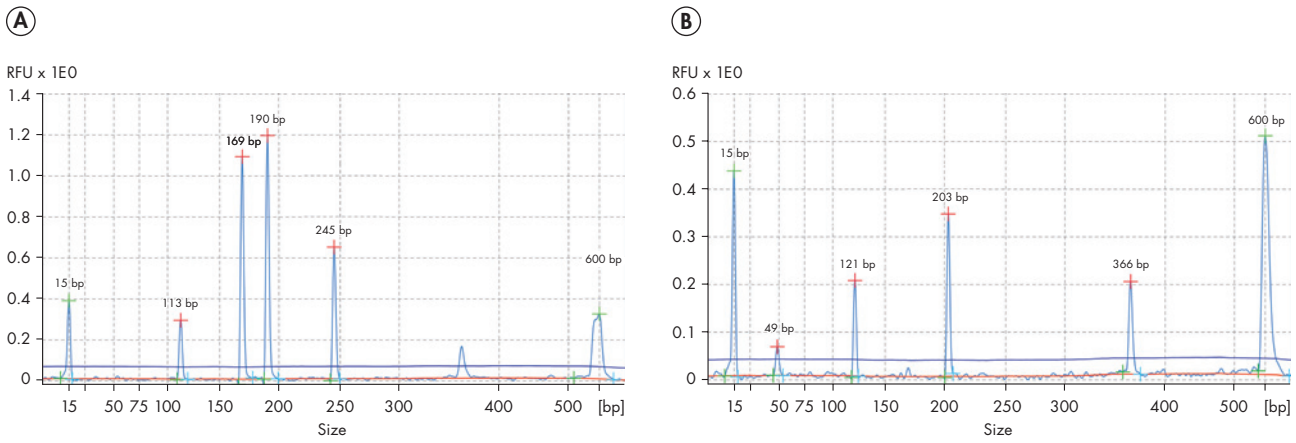

Figure 3. An electropherogram view of the data for the analyzed samples. A. Sample FF2 digested with AluI (corresponding to Lane A6 in Figure 2). **B.** Sample FF2 digested with HinfI (corresponding to Lane C3 in Figure 2).

In this study, four out of six samples were found to be correctly labeled with the RFLP-PCR yielding the expected results. However, we found that samples FF2 and FF3 were not properly labeled: they contained various amounts of pork despite being labeled as pure beef.

All of the commercial samples analyzed had undergone different production processes. The results indicated that the enzyme digestions were incomplete in some samples (lanes A5 to A9, Figure 2). Therefore, the samples were additionally analyzed with real-time PCR (results not shown), which confirmed that the digestion with AluI was incomplete for those samples.

Conclusion

- The QIAxcel Advanced System facilitates analysis of PCR-RFLP samples for meat species identification, especially for large-scale analyses.
- Using this native capillary electrophoresis system significantly reduces analysis time for the authentication of meat samples and minimizes the potential for procedural errors that would influence the accuracy of analysis.
- The method also eliminates exposure to hazardous chemicals and is easy to handle, making it an excellent routine method for the control of meat and meat products.

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QIAsymphony SP	QIAsymphony sample prep module	9001297
QIAsymphony DSP DNA Mini Kit (192)	For 192 preps of 200 ul each: Includes 2 reagent cartridges and enzyme racks and accessories.	937236
TopTaq Master Mix Kit	For 200 x 50 ul reactions: 2x TopTaq Master Mix containing 250 units of TopTaq Polymerase in total, 10x CoralLoad Concentrate, and RNase-Free Water	200403
QX Alignment Marker 15 bp/600 bp (1.5 ml)	Alignment marker with 15 bp and 600 bp fragments	929530
QX DNA Size Marker 25–500 bp (50 ul) v2.0	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500 bp; concentration 100 ng/ul	929560

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Marker-assisted selection (MAS) of wheat lines for udon noodle production

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The QIAxcel® system was used for analysis of PCR products generated for marker-assisted selection in wheat. The high-throughput capacity of the system allowed large numbers of plants to be quickly and reliably analyzed, making it highly suitable for plant breeding applications.

Introduction

The development of wheat varieties begins with a cross between 2 varieties or lines. The plants grown from the seeds of the cross are considered to be the first generation (F_1); the suitability and productivity of the subsequent generations in multiple regions are examined from the F_8 generation onwards for several years. When a variety is found to be superior, it becomes certified and can be grown on a farm. Since a generation requires approximately one year in the field, the development of a variety traditionally requires approximately 10 years.

The QIAxcel system.

Due to recent progress in genomic research, this breeding approach is undergoing a major change. Rather than selecting traits based on phenotypes, the genes or gene regions that control the traits can be used to design markers, and selection is based on studies of these markers using a method called marker-assisted selection (MAS). The use of DNA markers in MAS enables the selection of plants with the targeted traits by analyzing results from F_2 individuals. Thus, MAS is a breakthrough technology that changes the process of variety development from the traditional field-based format to a laboratory-based format. As a representative example, we present here a DNA marker selection system for the viscoelasticity trait in udon noodles. Viscoelasticity of udon noodles improves as the amylose content of the starch decreases. Amylose synthesis in seed starch is controlled by the *Wx* gene family. In wheat, amylose synthesis is influenced by genes from the A, B, and D genomes (*Wx*-A1, *Wx*-B1, and *Wx*-D1 genes). ▷

When no *Wx* gene products are present, the wheat line is referred to as "waxy wheat" which is not suitable for producing udon noodles. When only 1 or 2 gene products are missing (partially waxy wheat), the amylose content of the wheat is reduced and the wheat is more suitable for making udon noodles.

Selection of a partial waxy line based on the actual amylose content is problematic because measuring amylose content is difficult and imprecise and measurements must be obtained from several generations of plants. Therefore, we developed the DNA markers listed in Table 1 for PCR analysis to select partial waxy wheat plants (1). An effective method for multiple selections requires a high-throughput system. Our laboratory used a simple DNA extraction method in conjunction with the QIAxcel system to efficiently select lines from many breeding sites throughout the country.

Materials and methods

Genomic DNA from Chinese Spring (CS, wild type), Mochi Otome (MO, mutations in all 3 *Wx* genes), and a heterozygous plant (H) was prepared for the detection of mutations. PCR amplification was performed using primers for the detection of mutations in *Wx* genes (Table 1), and the amplified products were analyzed on the QIAxcel system with the QIAxcel DNA Screening Kit and the AM420 method.

Table 1. Sizes of fragments that are specific PCR markers for mutants of *Wx* alleles

		Amplification product (bp)	
Gene	**Primer**	**Wild type**	**Mutant**
***Wx*-A**	AFC	389	370
	AR2	(408, 410)	(408, 410)
***Wx*-B**	BDFL	425	none
	BRD	(455, 497)	(455, 497)
***Wx*-D**	BDFL	2307	1731
	DRSL	—	—

Fragments amplified from other *Wx* genes are indicated in parentheses.

Results and Discussion

Representative results are shown in Figure 1. Clearly distinguishable separation was obtained for the PCR-amplified fragments, as seen with the 370 bp and 389 bp fragments in Figure 1A. The PCR products for *Wx*-A1 and *Wx*-D1 alleles (Figures 1A and 1C, respectively) are co-dominant, and the assessment of heterozygosity using the gel image generated by the QIAxcel system is easy and straightforward. BDFL and BRD primers (*Wx*-B gene) amplify a 425 bp fragment that is present in the wild type but not the mutant (Figure 1B). In the electropherograms, the height of the *Wx*-B peak from the heterozygote (Figure 1D) is half the height of the peak from the homozygous wild type (Figure 1E), clearly demonstrating differences in gene dosage. The throughput capacity of the QIAxcel system allowed simultaneous analysis of up to 96 samples to be performed (Figure 2).

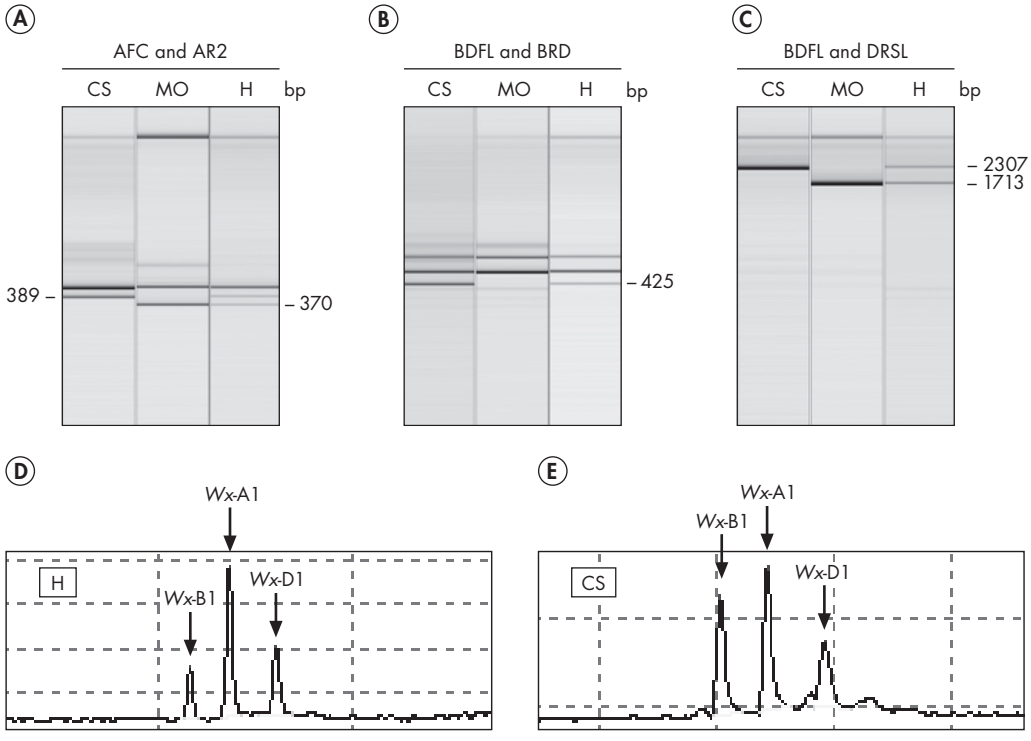

Figure 1. *Wx* gene analysis in a segregating F_3 generation of wheat. Genomic DNA was isolated from wild type (CS), Mochi- Otome (MO), and heterozygous (H) plants and amplified using primers for the **A.** *Wx*-A1, **B.** *Wx*-B1, and **C.** *Wx*-D1 genes. PCR products were analyzed on the QIAxcel system using the QIAxcel DNA Screening Kit. The sizes of expected fragments are listed in Table 1. Electropherogram views of fragments amplified from **D.** heterozygous and **E.** wild type plants using BDFL and BDR primers.

Figure 2. Analysis of Wx-A1 gene types in a segregating F_3 generation of wheat. **W:** wild type; **N:** mutant; **H:** heterozygote.

Conclusions

- The high-throughput capacity of the QIAxcel system provides an efficient way to handle large numbers of plants from breeding programs and is highly suitable for the wheat breeding industry.
- Selection of wild type, mutant, and heterozygous plants is feasible and simple using the QIAxcel system.

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Species determination for meat using PCR-RFLP analysis on the QIAxcel® system

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A strategy using PCR followed by restriction fragment length polymorphism analysis (PCR-RFLP) is used to identify meat from various animal species in food. Analysis results from the QIAxcel system and from agarose gel electrophoresis were compared for 14 exotic and game species. Although both techniques were suitable for species differentiation, the use of the QIAxcel was less time consuming and provided the advantage of electronic documentation.

Introduction

Today, meats from exotic and game animals from all around the world provide an attractive alternative for people looking for unforgettable sensory experiences. Falsification of specialty meat is very common due to the tremendous profit gained by selling less costly meat labeled as meat from a more expensive species. A method that is sensitive enough to detect the small but relevant difference between meat from specific species is essential for official food control authorities to verify claims made about specialty meat. Protein-based methods for species identification, such as isoelectric focusing (IEF) or immunological methods, are not adequate because the soluble muscle proteins in processed meat products (heated or marinated) are rapidly and efficiently degraded. Nucleic acid-based analytical methods for the differentiation and identification of animal species in food have been commonly used in the last 20 years (1, 2).

In this study, PCR amplification of the cytochrome b (cytb) gene followed by restriction fragment length polymorphism analysis (PCR-RFLP) was used to differentiate 14 different exotic or game species. Traditionally, gel electrophoresis has been used to detect PCR-RFLP DNA fragments. However, this method is laborious, time consuming, and hazardous due to the use of ethidium bromide or similar intercalating dyes that are mutagenic and dangerous for human health. In addition, gel data can not be used directly for publication or archiving. As an alternative method for discriminating animal species in processed food and meat, we evaluated the QIAxcel capillary electrophoresis system, a computer-controlled system that provides electronic documentation.

Materials and Methods

Samples were collected and total RNA/DNA was extracted using the QIAamp® Viral RNA Mini Kit. A two-tube multiplex reverse-transcription PCR assay (two-tube assay) was used to detect 16 respiratory viruses based on their amplicon size differences.

Nucleic acid purification

DNA extraction was performed using the Wizard Plus Miniprep® DNA Purification System (Promega) according to the *Swiss Food Manual* (4). DNA was extracted from 200 mg ground meat from bison, chamois, crocodile, duck, emu, kangaroo, kudu, ostrich, quail, rabbit, red deer, roe deer, springbok, and water buffalo and was eluted in 50 µl of elution buffer according to the manual.

PCR-RFLP analysis

Primers to conserved regions of the vertebrate mitochondrial cytb gene were used to amplify a 359 bp fragment (1, 3, 5). PCR amplification of 25 µl reactions, as well as restriction analysis, was performed as described in (5). PCR-RFLP products were separated on 3% agarose gels in TAE buffer, and the BenchTop 100 bp DNA Ladder (Promega) was included in the analysis. In addition, analysis was performed on the QIAxcel system using the QIAxcel DNA High Resolution Kit with the OM700 method. The QX Alignment Marker 15 bp/1 kb was included in the analysis.

Results

Using a set of endonucleases, all species could be identified with both conventional agarose gel electrophoresis and the QIAxcel capillary electrophoresis system. Using the QIAxcel system, 24 samples were analyzed in approximately 30 minutes. Analysis using agarose gel electrophoresis, which involves more steps for handling and documentation, required at least three times as long.

Representative analysis results are shown in Figures 1 and 2. Fragments shorter than 100 bp were not visible on the agarose gel. A 35 bp fragment from samples 11 and 12 (Figure 1) and fragments between 50 bp and 80 bp from samples 11, 12, 14, and 15 (Figure 2) were detected using the QIAxcel system but not with agarose gel electrophoresis.

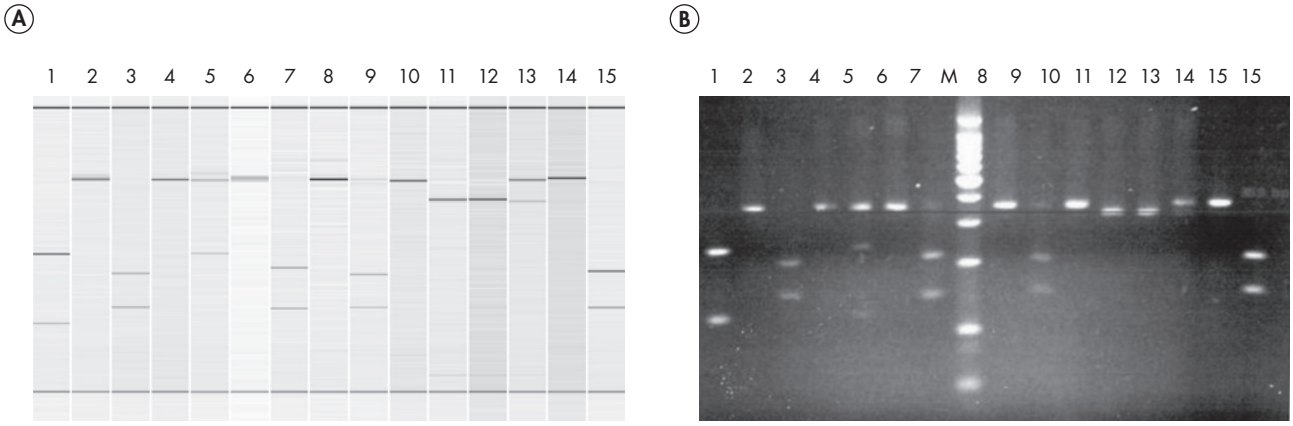

Figure 1. PCR-RFLP analysis (*RsaI*) of meat from several exotic and game species. DNA isolated from crocodile (1), kangaroo (2), ostrich (3), duck (4), red deer (5), roe deer (6), kudu (7), springbok (8), quail (9), rabbit (10), bison (11, 12); water buffalo (13), chamois (14), and emu (15) was amplified by PCR, digested with *Rsa*I and analyzed using **A.** the QIAxcel system with the QIAxcel DNA High Resolution Kit or **B.** conventional agarose gel electrophoresis. **M**: 100 bp DNA Ladder.

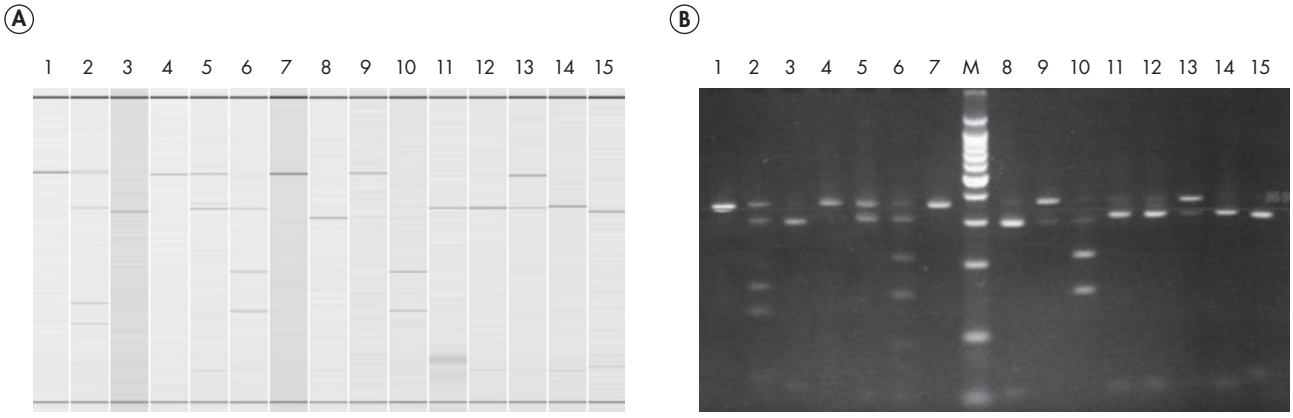

Figure 2. PCR-RFLP analysis (*Tru*9 I) of meat from several exotic and game species. DNA isolated from crocodile (1), kangaroo (2), ostrich (3), duck (4), red deer (5), roe deer (6), kudu (7), springbok (8), quail (9), rabbit (10), bison (11, 12); water buffalo (13), chamois (14), and emu (15) was amplified by PCR, digested with *Tru*9 I and analyzed using **A.** the QIAxcel system with the QIAxcel DNA High Resolution Kit or **B.** traditional agarose gel electrophoresis. **M**: 100 bp DNA Ladder.

Conclusions

- The QIAxcel capillary electrophoresis system as well as conventional agarose gel electrophoresis provided suitable differentiation of relevant animal species. However, compared to agarose gel electrophoresis, QIAxcel analysis enables significantly shorter running time, eliminates sample preparation and exposure to mutagenic reagents, and requires fewer analysis and handling steps, freeing time for more demanding laboratory work and reducing manual errors.
- Fragments shorter than 100 bp were detected with the QIAxcel system but not with agarose gels, greatly reducing the practical value of the agarose gel system.
- QIAxcel documentation of results, presented as a gel-like image, an electropherogram, or in table format, is automatic and reliable. The QIAxcel system provides more detailed information than conventional agarose gel electrophoresis.

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