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

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

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) causes serious gastrointestinal disease and death in humans. The 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 carriers for STEC. The organism is propagated in the animal gut and shed in the feces, which are a major source of food contamination and human infection (3). Detection of STEC is crucial in intervention strategies.

We have developed an 11-gene multiplex PCR-based method that detects four major *E. coli* virulence genes (*stx1*, *stx2*, *eae*, and *ehxA*) and the seven major serogroups of STEC mentioned above. The throughput level and the interpretation accuracy of this assay were dramatically improved using the QIAxcel capillary electrophoresis system in the workflow.

After PCR, the plate is run on the QIAxcel instrument, which automates the multiple steps of traditional agarose gel separation, including gel casting, PCR product loading, gel electrophoresis, ethidium bromide staining, and gel imaging. It also performs automatic data interpretation. After a run, the positive or negative value of each of the 11 bands from every sample is given and presented in a report.

Materials and methods

Primer design

The *wzx* gene, which encodes for a flippase required for O-polysaccharide export, was used to design 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, were used to design primers for O121. The primers used for virulence genes and O157 were designed ▶

Table 1. Molecular targets and their amplicon sizes used in the 11-gene multiplex PCR.

Target genes	Amplicon size
wzx_{O45}	890 bp
wzx_{O103}	740 bp
stx1	655 bp
$wbqE_{O121}$ $wbqF_{O121}$	587 bp
wzx_{O145}	523 bp
stx2	477 bp
wzx_{O26}	417 bp
eae	375 bp
rfb_{O157}	296 bp
wzx_{O111}	230 bp
ehxA	199 bp

Table 2. Presence (+) or absence (–) of four virulence genes in seven *E. coli* serogroups.

Lane	O-type	*eaeA*	*stx1*	*stx2*	*ehxA*
1	O26	–	+	–	+
2	O26	–	+	+	+
3	O45	–	+	–	+
4	O103	–	+	–	+
5	O103	–	+	+	+
6	O111	+	+	+	+
7	O111	+	+	–	+
8	O121	–	–	+	+
9	O145	+	+	–	+
10	O145	+	+	+	+
11	O157	+	+	+	+

and validated in our previous study (4). The primers amplify targets with distinct amplicon sizes to match all available sequences for the named target genes. The PCR amplicon sizes are given in Table 1.

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 on blood agar plates (BAP, Remel, Lenexa, KS) and 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.

Multiplex PCR

All of the primer stocks were prepared in 1 x TE buffer at concentrations of 100 pM/µl. Equal volumes and concentrations of the 11 primer pairs (22 primers) were mixed, except for the primer pair for O111, which was doubled. 1 µl primer mix was used in a 20 µl PCR reaction resulting in final primer concentrations of 0.42 µM for O111 and 0.21 µM for all the other primers. The reactions also contained 10 µl of a 2X PCR reagent, 1 µl DNA templates and 8 µl nuclease-free water.

A previously described PCR protocol (5) was used. It was 5 min denaturation at 94°C, followed by 25 cycles of 94°C for 30 sec and 67°C for 80 sec.

QIAxcel protocol

After amplification, the PCR products were placed in the QIAxcel system and separated using the QX DNA Screening Kit with the customized AM410 method, which has the following electrophoresis parameters: alignment marker injection at 4 kV for 20 s, sample injection at 5 kV for 10 s, and separation at 4 kV for 410 seconds.

Results and discussion

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

The strains were selected to represent all seven major *E. coli* serogroups and the four virulence genes. All of the samples carried the *ehxA* gene, which is present in most cattle fecal samples. However, the ratios of the presence of the virulence genes in the table do not represent the ratios of these genes in the overall cattle fecal samples. The Shiga toxin genes, especially *stx2*, are present in a much lower ratio in cattle samples, as indicated in the references listed below (4, 5).

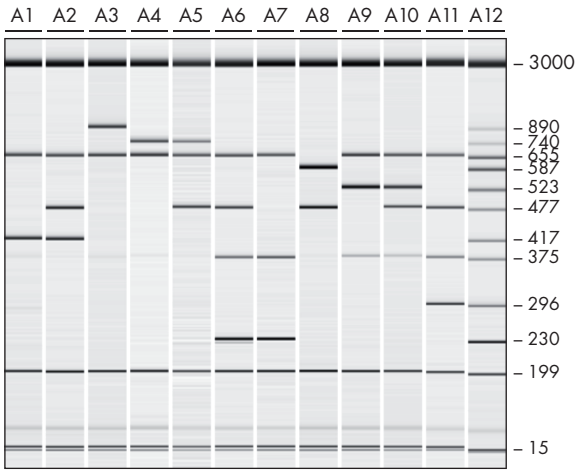

Figure 1. QIAxcel analysis of 11 isolates tested with 11-gene multiplex PCR. The PCR included the major *E. coli* virulence genes, *stx1*, *stx2*, *eae*, and *ehxA*, and *E. coli* O-antigens from the O26, O45, O103, O111, O121, O145, and O157 serogroups. Lane A12 contains a DNA control, which is positive for all 11 genes, and serves as a size marker. Amplicon sizes were verified by sequencing.

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 identification of the genes present in each sample. The peak calling table (not shown) contains the sizes of all 11 amplicons generated by multiplex PCR with the control template DNA.

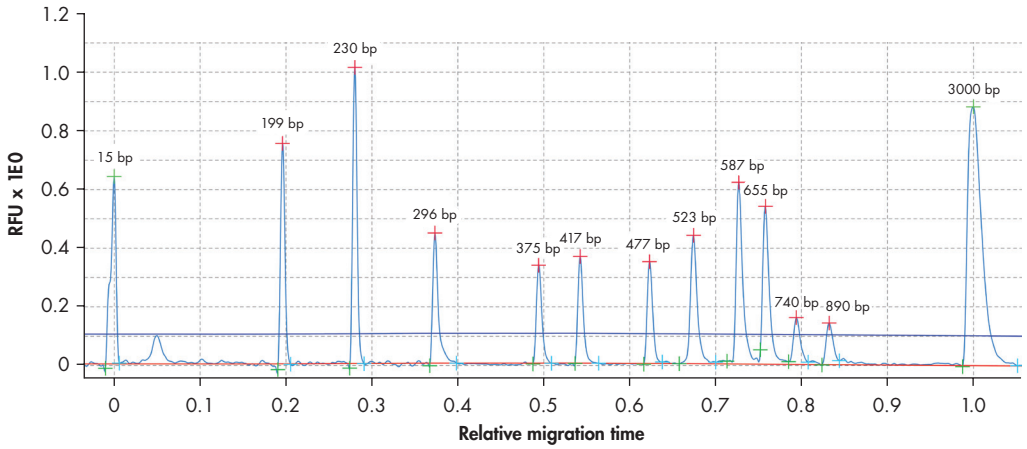

Figure 2. Electropherogram of the control sample. All 11 amplicons used for identification are clearly visible. The amplicon identities and sizes were verified by sequencing

Conclusions

- This 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 system allows detailed, reliable, and rapid analyses of complex samples containing many amplicons within a wide size range.
- Thanks to its fast turnaround and automatic data interpretation, the QIAxcel system is highly suited for post-PCR separation, visualization, and data presentation, especially for multiplex PCR runs in a high-throughput setting.

References

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4. Bai, J., Shi, X., and Nagaraja, T.G. (2010) A multiplex PCR procedure for the detection of six major virulence genes in *Escherichia coli* O157:H7. Journal of Microbiological Methods **82**, 85.
5. Bai, J. et al. (2012) Applicability of a multiplex PCR to detect the seven major Shiga toxin–producing *Escherichia coli* based on genes that code for serogroup-specific O-antigens and major virulence factors in cattle feces. Foodborne Pathogens and Disease **9**, 541.

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 Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
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
QX Alignment Marker 15 bp/3 kb	Alignment marker with 15 bp and 3 kb fragments	929522
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; concentration 100 ng/µl	929559

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High-throughput multiplex PCR detection of shigatoxin-producing *E. coli*

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Rapid screening of multiple genes

We have developed an 11-gene multiplex PCR (mPCR) for the detection and differentiation of 7 major shigatoxin-producing *Escherichia coli* (STEC) serogroups (O26, O45, O103, O111, O121, O145, and O157), and 4 virulence factors (*stx*1, *stx*2, *eae*, and *ehx*A). In combination with the QIAxcel® System, it has been shown to be a powerful approach for concurrent screening of complex sets of genes. It is a unique system when real-time PCR falls short in terms of the number of genes that can be detected.

Gene targets and their amplicon sizes designed in the multiplex PCR.

Target genes	Amplicon size (bp)
wzx_{O45}	890
wzx_{O103}	740
*stx*1	655
$wbqE_{O121}$ $wbqF_{O121}$	587
wzx_{O145}	523
*stx*2	477
wzx_{O26}	417
eae	375
rfb_{O157}	296
wzx_{O111}	230
*ehx*A	199

STEC causes serious gastrointestinal disease and death in humans, especially in children and the elderly. Here, we demonstrate how the method is used to detect STEC from cattle feces, which are of great interest, since they serve as a major source of food and water contamination.

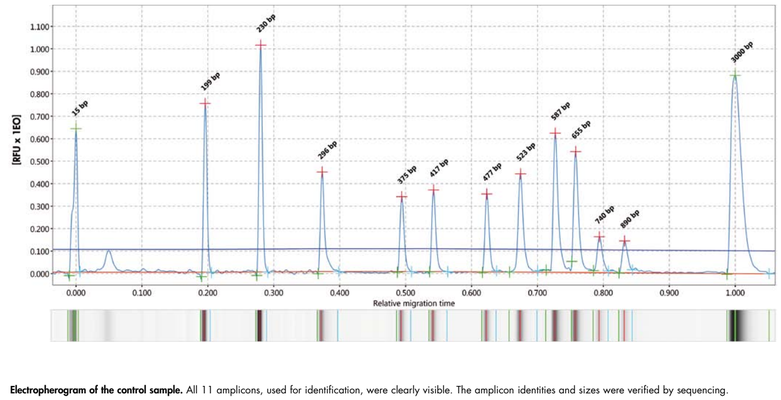
The workflow

Cattle rectal swab samples were collected and shipped to K-State Veterinary Diagnostic Laboratory on ice for overnight delivery. Samples were enriched in EC Broth at 37°C for 14–16 hours. The QIAamp® DNA Stool Mini Kit (QIAGEN) was used for extraction DNA for use in the mPCR amplification. The resulting amplicons were run on the QIAGEN QIAxcel System using capillary electrophoresis, and positive bands were identified using the peak calling function included in the QIAxcel ScreenGel Software.

Detailed multiplex PCR procedure can be found in Bai J, et al. (2012) Foodborne Pathog. Dis. **9**, 541.

Automated identification via peak calling

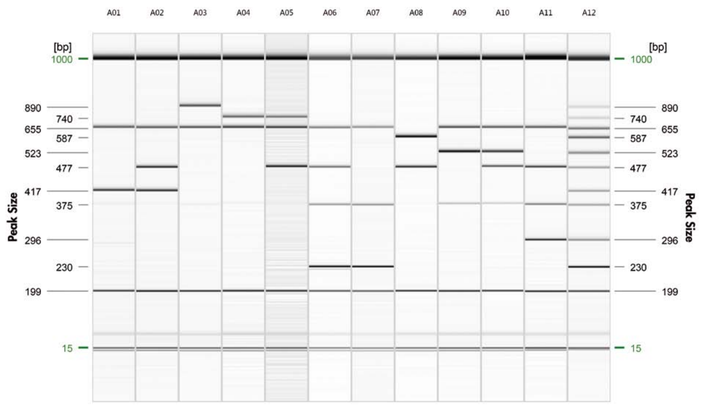
QIAxcel ScreenGel Software automatically identifies DNA fragments from unknown samples with the peak calling function (Peak Calling Table), thereby enabling accurate identification of the genes present in each sample. The peak calling table (not shown) contains sizes of all 11 amplicons generated by the multiplex PCR with the control template DNA.



Electropherogram of the control sample. All 11 amplicons, used for identification, were clearly visible. The amplicon identities and sizes were verified by sequencing.

mPCR products visualized using QIAxcel

As well as using the peak calling function to identify the genes, the separated mPCR products can also be visualized as a gel image, and can be exported for presentation and publication. A positive control, containing template for all amplicons of interest, served as a size marker.



QIAxcel analysis of 11 isolates tested with 11-gene mPCR. The mPCR included the major *E. coli* virulence genes, *stx*1, *stx*2, *eae*, and *ehx*A, and *E. coli* O-antigens from O26, O45, O103, O111, O121, O145, and O157 serogroups. Lane A12 was amplified from a DNA mixture of 7 O-types, and serves as a size marker.

Reliable identification of STEC O-groups and virulence factors

Lane	Q-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	+	+	+	+

Analysis of 11 strains representing 7 *E. coli* serogroups and 4 virulence genes using 11-plex PCR and QIAxcel. –: absent; +: present.

The 11 isolates selected are representative for the 7 major STEC serogroups shown in the previous panel. All 11 strains possessed the *ehx*A gene, 5 had *eae*, 10 had *stx*1, and 6 had *stx*2 genes. The QIAxcel analysis correctly identified the O-antigen genes and the virulence genes affiliated with the STEC strains.

Conclusions

- The method identifies the 7 major STEC serogroups and the 4 main virulence factors in one PCR reaction. This would require multiple reactions using real-time PCR.
- Separating multiplex PCR products with QIAxcel allows detailed, reliable, and fast analyses of a complex PCR result containing many amplicons over a wide size range.
- Use of automation minimizes manual intervention, thus potentially improving inter- and intra-laboratory reproducibility.
- Rapid turnaround and automatic data interpretation make QIAxcel highly suited for post-PCR separation, visualization, and data presentation, especially for multiplex PCR runs in a high-throughput setting.

The applications presented here are for research use only. Not for use in diagnostic procedures.

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