QIAxcel® System and Multiplex PCR for STEC Virulence Gene Detection and Serogroup Differentiation

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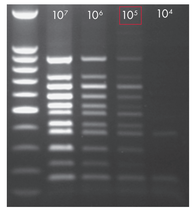
Sensitive, Efficient and Rapid Screening of 11 Genes

We have developed an 11-gene multiplex PCR (mPCR) for the detection and differentiation of 7 major Shiga toxin- producing *Escherichia coli* (STEC) serogroups (O26, O45, O103, O111, O121, O145, and O157), and 4 virulence factors (*stx1, stx2, eae,* and *ehxA*).

STEC are commonly associated with more severe forms of human infections. The bacteria often reside and are propagated in the gastrointestinal tract in cattle. The organisms are subsequently shed in feces and serve as a major source of food and water contamination, causing disease and death in humans, especially in children and the elderly.

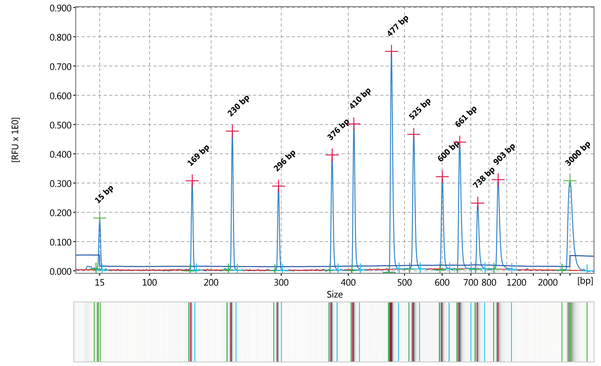
Here, we demonstrate how the multiplex PCR, in combination with the QIAxcel System, provides a powerful approach for concurrent screening of complex sets of genes. It provides a valuable system when real-time PCR falls short in terms of multiplexing capacity.



Sensitivity of the 11-gene PCR on a spiked cattle fecal sample.

Using Peak Calling for Gene Identification

A total of 185 enriched cattle fecal swab pools (5 per pool) were amplified by the 11-gene mPCR assay. PCR products were run directly on the QIAxcel without further manipulation. Amplicons corresponding to each of the 11 genes were manually identified and the results were compared with data generated automatically by the peak calling function of the QIAxcel ScreenGel software.



Electropherogram of the control sample. All 11 amplicons used for identification were clearly visible and well separated.

mPCR Products Visualized Using QIAxcel

In addition to the peak calling function to identify genes, the separated mPCR products can also be visualized as a gel image and can be exported for presentation and publication.

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 Analysis of STEC-O Groups and Virulence Factors

The 11 selected samples, representing the 7 major STEC serogroups, and 2 additional O104 samples were further grouped into particular *E. coli* O-groups based on the band sizes. The 4 virulence gene profiles for each sample are also shown in the table below.

Lane	O-type	*eae*A	*stx*1	*stx*2	*ehx*A
1	O26	–	+	–	+
2	O26	–	+	+	+
3	O45	–	+	–	+
4	O103	–	+	–	+
5	O103	–	+	+	+
6	O111	+	+	+	+
7	O111	+	+	–	+
8	O121	–	–	+	+
9	O145	+	+	–	+
10	O145	+	+	+	+
11	O157	+	+	+	+
12	O104	+	+	–	+
13	O104	+	–	–	+

Analysis of 13 strains representing 8 *E. coli* serogroups and 4 virulence genes using 12-plex PCR and QIAxcel. –: absent; +: present.

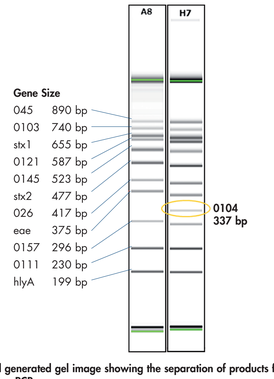
Future Potential: 12-Gene mPCR

In 2011, a food-borne *E. coli* outbreak killed over 50 people, mainly in Germany. The source of contamination was sprouts of fenugreek seeds. The causal agent was identified as *E. coli* O104:H4, and a full genome sequencing indicated that it is a hybrid strain of enteroaggregative and Shiga toxigenic strains. Using a 12-gene PCR, the O104:H4 strain could readily be detected.

The 12-gene mPCR was simply set up by adding an O104 antigen primer pair to the 11-gene mPCR. The results indicated that all 12 genes can be detected in a single PCR reaction, and are separated well on QIAxcel.

Preliminary screening on cattle fecal samples indicated that the prevalence of O104 *E. coli* strains extended up to 40%. Further analysis using a different mPCR showed that none of the US cattle O104 strains resembled the characteristics of the German outbreak O104 strain.

To avoid the false alarm the O104 test may generate from the US cattle feces, the O104 primers were removed from the mPCR. However, the 12-gene PCR illustrated the feasibility of adding additional genes into our 11-gene PCR should other STEC serogroups become more prevalent in causing human disease in the future.



QIAxcel generated gel image showing the separation of products from a 12-gene mPCR.

Conclusions

- Multiplex PCR in combination with the QIAxcel System is a powerful approach for concurrent screening of complex sets of genes in one PCR reaction. It provides a useful method to screen a set of genes when real-time PCR has insufficient multiplexing capabilities. Sets of 11 or more genes can be analyzed simultaneously.
- The method identifies the 7 major STEC serogroups and the 4 main virulence factors in one PCR reaction.
- Automation minimizes manual intervention. Therefore, the protocol may minimize human error and improve inter- and intra-laboratory reproducibility.
- Due to its fast separation and automated data interpretation, the QIAxcel System is also highly suited for other post-PCR separation, visualization, and data presentation, especially for highly multiplexed PCR assays in high-throughput settings.

References

1. Bai, J., et al. (2010) A multiplex PCR procedure for the detection of six major virulence genes in *Escherichia coli* O157:H7. J Microbiol. Methods. **82**, 85.
2. 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 Pathog. Dis. **9**, 541.
3. Dargatz, D.A., et al. (2013) Prevalence of Escherichia coli O-types and Shiga toxin genes in fecal samples from feedlot cattle. Foodborne Pathog. Dis. **10**, 392.

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