

# Detection of Shiga toxin-producing *E. coli* using multiplex colony-direct PCR with KOD XL DNA Polymerase

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Certain strains of *E. coli* are known to produce a family of related toxins, referred to as Shiga toxin 1 (Stx1, encoded by *stx1*) and Shiga toxin 2 (Stx2, encoded by *stx2*). Shiga toxin-producing *E. coli* (STEC), represented by serotype O157:H7, has a strong infectious capacity and pathogenicity. In recent years, this bacterium has been affecting an increasing number of victims, resulting in life-threatening illness such as hemorrhagic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura (1). The morbidity and mortality associated with STEC disease highlight the threat these organisms pose to public health. For this reason, there is an increasing demand for fast and efficient methods for the detection of virulent strains of STEC in fecal

samples and in meat and dairy products.

PCR is generally considered the most sensitive means for determining if a food or fecal sample contains STEC. A multiplex PCR method developed by Paton and Paton (2) enables simultaneous determination of *stx1*, *stx2*, and correlated genes that encode accessory STEC virulence factors, such as *eaeA* and *hlyA*, in crude DNA extracts from primary fecal cultures.

In this study, we developed a rapid typing system for STEC that improves upon the original multiplex PCR assay. With our method, a bacterial colony from a food or fecal culture was used directly as the template. In addition, four target genes were examined for the presence of the IS1203v insertion sequence discovered in *stx2* genes (3, 4) with IS1203v-specific primers. To reduce the time needed for the PCR, *Taq* DNA polymerase was replaced with the faster KOD XL DNA Polymerase\*. Multiplex CD-PCR analysis for eleven STEC strains and one control K-12 strain isolated at Kanagawa Prefecture, Japan, between 1996 and 1999 was performed (Figure 1). Table 1 identifies the final concentrations of the PCR reaction components. PCR reactions were performed using the following conditions: initial denaturation at 94° for 5 minutes, 30 cycles

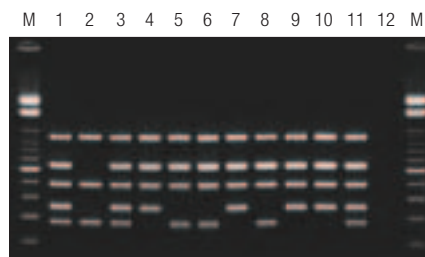
of 98° for 15 seconds, 60° for 5 seconds, and 74° for 30 seconds. After the PCR, one-tenth of the reaction solution was analyzed by agarose gel electrophoresis (2% TAE gel, Figure 1). The results of the multiplex-PCR showed a clear amplification for each target: 180 bp for *stx1*, 255 bp for *stx2*, 384 bp for *eaeA*, 534 bp for *hlyA*, and 910 bp for IS1203v, in 11 STEC strains (Figure 1). Amplification of the target genes from the control K-12 strain was negative. The results clearly demonstrated that this system is effective for STEC typing.

By using KOD XL DNA Polymerase, PCR was completed in less than 1.5 hours, a significant time savings compared to the nearly 3.5 hours of the original method, and a great advantage when multiple specimens require quick processing.

**Table 1. STEC PCR reaction setup<sup>1</sup>**

Component	Concentration
PCR buffer for KOD XL	1X
dNTP mix	0.2 mM
<i>stx1</i> primers ( <i>stx1</i> -F + <i>stx1</i> -R)	0.2 μM each
<i>stx2</i> primers ( <i>stx2</i> -F + <i>stx2</i> -R)	0.2 μM each
<i>eaeA</i> primers ( <i>eaeA</i> -F + <i>eaeA</i> -R)	0.2 μM each
<i>hlyA</i> primers ( <i>hlyA</i> -F + <i>hlyA</i> -R)	0.2 μM each
IS1203v primers (1203v-F + 1203v-R)	0.1 μM each
Bacterial colony	approximately 10 <sup>4</sup> cfu
KOD XL DNA Polymerase	2.5 U

<sup>1</sup> When using KOD XL DNA Polymerase, set up the PCR reaction on ice.



Lane(s)	Sample
M	Markers (100-bp ladder)
1–11	STEC strains
12	K-12 strain

**Figure 1. STEC identification by CD-PCR**

Eleven STEC strains and one K-12 strain were used for CD-PCR. Reaction products were analyzed by agarose gel electrophoresis and stained with ethidium bromide.

## REFERENCES

1. Karmali, M. A. (1989) *Clin. Microbiol. Rev.* 2, 15–38.
2. Paton, A. W. and Paton, J. C. (1998) *J. Clin. Microbiol.* 36, 598–602.
3. M., Nishiya, Y., Kawamura, Y., and Shinagawa, K. (1999) *J. Biosci. Bioeng.* 87, 93–96.
4. Okitsu, T., Suzuki, R., and Yamai, S. (2001) *Upload* 63 (Toyobo Co., Ltd., Japan newsletter).

Product	Size	Cat. No.
KOD XL DNA Polymerase	250 U	71087-3
[includes KOD XL DNA Polymerase (2.5 U/μl), 10X PCR Buffer for KOD XL DNA Polymerase, dNTP Mix (2 mM each)]	5 × 250 U	71087-4

\* Manufactured by Toyobo and distributed by EMD Biosciences Inc. Novagen Brand. Not available through Novagen in Japan.