The 5P PCR for O157:H7 Screening method for *E. coli* Serotype O157:H7/STEC fror Foods on the Light Cycler 2.0 Instrument Platform is available here. (**NOTE: Archived Content**)

Archived conventional PCR assays for the confirmation of O157:H7 and the stx. The PCR assay is detailed in LIB 3811 detects only *stx1* and *stx2* (11). When using the LIB 3811, increase annealing temperature to 59°C and reduce extension time to 1 min. The other is 5P multiplex PCR that simultaneously assays for *stx*1, *stx*2, the +93 *uid*A SNP as well as 2 other O157:H7 virulence factors: the enterohemolysin (ehxA) genes and gamma (γ) intimin (*eae*) allele, which is found mostly in O157:H7 and few other serotypes (12). The 5P protocol is described below. For additional details, contact Peter Feng, CFSAN, (240-402-1650).

5P Multiplex PCR for confirmation of O157:H7 isolates

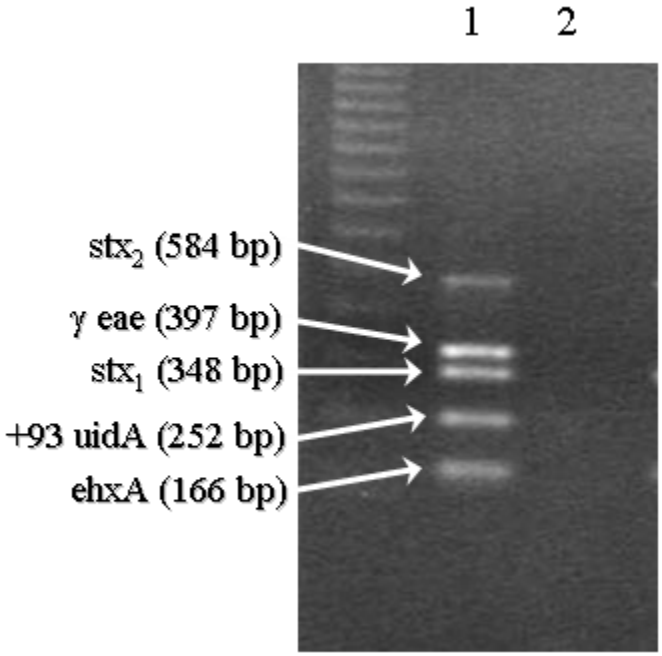
1. The primer sequences and the expected sizes of the amplicons from 5P PCR are shown in Table 7.
2. For positive control, use DNA from an O157:H7 strain, such as EDL933 or any other O157:H7 strain that is known to carry all 5 gene targets.
3. Prepare a 10× primer master mix containing 2000 nM concentrations of each of the 10 primers. Primer master mix may be stored frozen at -20°C. Use 5 µL per 50 µL reaction volume volume to yield a final use concentration of 200 nM for each primer.
4. Use growth from the TSAYE plate in step Q.1 to prepare DNA templates for PCR analysis. Prepare DNA template by resuspending a colony or a small loopful of growth from TSAYE in 100 µl of water. Mix and heat for 5 min in a boiling water bath. Spin in a microcentrifuge to pellet debris. Use 2 µL of supernatant per reaction. Templates may be stored frozen at -20°C.
5. The 50 µL PCR mix contain 1× Taq Polymerase buffer (Qiagen, Valencia, CA), 3 mM MgCl2, 250 µM of dNTP, 2 µL of crude DNA template, 1× primer master mix, 3.75 U of HotStarTaq (Qiagen) and sterile water. The PCR conditions are: 95ºC for 15 min; then 25 cycles, each cycle consisting of: 95ºC for 1 min, 56ºC for 1 min and 72ºC for 1 min and a 72ºC for 5 min final extension. Examine amplicons on agarose gel (1%) electrophoresis in 1× TBE (Tris-borate-EDTA) buffer pH 8.2. Expected results are shown in Fig. 1.7Hill, W.E, K.C. Jinneman, P.A. Trost, J.L Bryant, J. Bond and M.M. Wekell. 1993. Multiplex polymerase chain reaction detection of Shiga-like toxin genes in *Escherichia coli*. FDA LIB 3811

1. The 5P PCR for O157:H7 Screening method for *E. coli* Serotype O157:H7/STEC fror Foods on the Light Cycler 2.0 Instrument Platform is available here. (**NOTE: Archived Content**)
   Archived conventional PCR assays for the confirmation of O157:H7 and the stx. The PCR assay is detailed in LIB 3811 detects only *stx1* and *stx2* (11). When using the LIB 3811, increase annealing temperature to 59°C and reduce extension time to 1 min. The other is 5P multiplex PCR that simultaneously assays for *stx*1, *stx*2, the +93 *uid*A SNP as well as 2 other O157:H7 virulence factors: the enterohemolysin (ehxA) genes and gamma (γ) intimin (eae) allele, which is found mostly in O157:H7 and few other serotypes (12). The 5P protocol is described below. For additional details, contact Peter Feng, CFSAN, (240-402-1650).

1. **Table 7. 5P PCR primer sequences and expected amplicon sizes**

Gene	Primer	Sequence	Amplicon
stx$_1$	LP30	5' - CAGTTAATGTGGTGGCGAAGG - 3'	348 bp
	LP31	5' - CACCAGACAATGTAACCGCTG - 3'	
stx$_2$	LP43	5' - ATCCTATTCCCGGGAGTTTACG - 3'	584 bp
	LP44	5' - GCGTCATCGTATACACAGGAGC - 3'	
+93 uidA	PT-2	5' - GCGAAAACTGTGGAATTGGG - 3'	252 bp
	PT-3	5' - TGATGCTCCATCACTTCCTG - 3'	
γ - eaeA	AE22	5'- ATTACCATCCACACAGACGGT - 3'	397 bp
	AE20-2	5'- ACAGCGTGGTTGGATCAACCT - 3'	
ehxA	MFS1Fb	5'- GTTTATTCTGGGGCAGGCTC - 3'	166 bp
	MFS1R	5'- CTTCACGTCACCATACATAT - 3'	

1. **Figure 7. Agarose gel of 5P PCR amplicons. The samples are: 1. O157:H7 and 2. generic E. coli (negative control).**



NOTE: The 2 conventional PCR assays use *stx* primer sequences that are distinct from those used in the real-time PCR and therefore, confer added verification that the isolate carries *stx* genes.

NOTE: An O157:H7 and O157:NM isolate that carry *stx* are considered pathogenic. However, an O157:NM strain that does not carry *stx* or other EHEC virulence factors is probably non-pathogenic. There are many *E. coli* O157 serotypes that carry other than H7 antigen (ie: H3, H12, H16, H38, H45, etc), and these often do not carry EHEC virulence factors. But, NM variants of these have been isolated (10).

References:

Peter Feng. and S.R. Monday. 2000. Multiplex PCR for detection of trait and virulence factors in enterohemorrhagic Escherichia coli serotypes. *Mol. Cell. Probes*. **14**:333-337.

Hill, W.E, K.C. Jinneman, P.A. Trost, J.L Bryant, J. Bond and M.M. Wekell. 1993. Multiplex Polymerase Chain Reaction Detection of Shiga-like toxin genes in *Escherichia coli*. FDA LIB 3811.