***E. coli* 6-gene multiplex PCR**

1. Master Mix preparation for conventional PCR (Hu, Zhang et al. 1999).

PCR Reagents [Concentration]	Primer sequence 5' –3'	Vol (ul) for 1X 50 ul rxn	
dH2O	- - -	24.39	
5X Go Taq Flexi buff	- - -	10.0	
$MgCl_2$ [25mM]	- - -	5.0	
dNTPs [10mM]	- - -	1.0	
EC hly-F [10µM]	CCCTggCAgACCTTTgATg	1.0	
EC hly-R [10µM]	CCgTgTCTTTTCTgATACTCA	1.0	
flicH7-F [10µM]	gCgCTgTCgAgTTCTATCgAgC	0.3	
flicH7-R [10µM]	CAACggTgACTTATCgCCATTCC	0.3	
int-F [10µM]	gACTgTCgATgCATCAggCAAAg	0.38	
int-R [10µM]	TTggAgTATTAACATTAACCCCAgg	0.38	
rfb-F [10µM]	gTgTCCATTTATACggACATCCATg	0.5	
rfb-R [10µM]	CCTATAACgTCATgCCAATATTgCC	0.5	
slt I-F [10µM]	TgTAACTggAAAggTggAgTATAC	1.0	
slt I-R [10µM]	gCTATTCTgAgTCAACgAAAAATAAC	1.0	
slt II-F [10µM]	gTTTTTCTTCggTATCCTATTCCg	1.0	
slt II-R [10µM]	gATgCATCTCTggTCATTgTATTAC	1.0	
Go Taq DNA polym.	- - -	0.25	
	TOTAL	49	

Using a sterile toothpick, select a single colony per isolate and scrape into a sterile PCR tube. Add 95ul of sterile dH20 and microwave *E. coli* for 30 seconds. Add 1ul of "dirty lysate" to PCR tube containing 49ul of aliquoted master mix.

+ control FSL F6-699 is *E. coli* O157:H7 (stx 1 & 2)
+ control FSL F6-704 is *E. Coli* O26:H11 (stx 1)

2. Amplification conditions.

94°C for 2 min **[1X]**

94°C for 30 s
59°C for 1 min (TD -0.50°C per cycle) **[20X]**
72°C for 1 min

94°C for 30 s
49°C for 1 min **[20X]**
72°C for 1 min

72°C for 7 min **[1X]**

3. **Analysis of PCR products.** PCR products (5 µl) should be run on a 2% agarose gel at 110V for 55 minutes and visualized by ethidium bromide staining.

4. **Interpretation of results:**

Gene fragment (primers)	Amplicon size	Target present in
hly	774 bp	O157:H7 & Non-O157 STEC
H7 (*fliC* primers)	625 bp	O157:H7
slt II	484 bp	O157:H7 & Non-O157 STEC
intimin (int primers)	368 bp	O157:H7
O157 (*rfb*)	292 bp	O157:H7
slt I	210 bp	O157:H7 & Non-O157 STEC

Any samples in which amplification of one or both genes encoding a Shiga-like toxin occurs will be further characterized with a second PCR. The secondary PCR utilizes primers designed for the conserved region of the eaeA gene (541 bp) present within both *E. coli* O157 and non-O157 STEC. Once the secondary PCR is completed, the presence or absence of the eaeA, H7 and fliC amplicons in the first PCR determines if you have a non-O157 STEC.

References:

Hu, Y., Q. Zhang, et al. (1999). "Rapid and sensitive detection of Escherichia coli O157:H7 in bovine faeces by a multiplex PCR." J Appl Microbiol **87**(6): 867-76.