Protocol From: **SOP-Testing cheese samples for presence of STEC_Al09292015.doc**

PCR product size:

Gene fragment:	Amplicon size:
Stx1 (Slt I)	210 bp
Stx2 (Slt II)	484 bp

Making Lysates (from *E. coli* 6-gene multiplex PCR)

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

Running the Multiplex Touch-down PCR for Stx1 and Stx 2

Multiplex PCR master-mix for sample:

Component	Volume (V)	V x (No. of samples+controls+1)
dH_20	28,75 µL	
5x GoTaq Flexi Buffer	10,0 µL	
$MgCl_2$ (25mM)	5,0 µL	
dNTPs (10mM)	1,0 µL	
slt I-F (10uM)	1,0 µL	
slt I-R (10uM)	1,0 µL	
slt II-F (10uM)	1,0 µL	
slt II-R (10uM)	1,0 µL	
GoTaq DNA polymerase	0,25 µL	
Total volume	**49 µL**	

1. Transfer 1 µL of lysate to the first tube of master mix. Continue doing so with the rest of the sample lysates, putting 1 µL of sample lysate in each tube.
2. Perform a short spin-down step is on mini bench-top centrifuge in order to collect entire mixture on the bottom without any air bubbles.
3. The tubes are placed in to PCR cycler and the touch-down PCR reaction performed under following conditions.

Touch-down PCR cycling conditions:

Temperature:	**Time:**	**Number of cycles:**
94°C	2 min	1x
94°C	30s	20x
59°C (TD -0.50°C per cycle)	1 min	
72°C	1 min	
94°C	30s	20x
49°C	1 min	
72°C	1 min	
72°C	7 min	1x

Separating PCR products on agarose gel

After the PCR reaction is finished the PCR products are separated on 1.5% agarose gel.

How to prepare 1.5% agarose gel:

1. Weigh 1.5g of agarose in to 250 mL Erlenmeyer flask and add 100 mL of 0.5x TBE Buffer.
2. Place a folded kimwipe in to the neck of the flask to partial close the opening and prevent excessive evaporation during heating.
3. Heat the suspension for approximately 2 min, using microwave oven. Heat for a minute and swirl flask every 20 s.
4. During the second minute of heating swirl every 7 s.
5. The final gel should be clear without any undissolved particles.
6. After heating swirl the flask one last time and let it sit for 2-3 min, use this time to set up the gel tray.

   NOTE: When preparing gel for separation of PCR products from Multiplex Touch-down PCR for *Stx1* and *Stx2*, each gel is prepared to have only one/top row for loading. To separate these PCR products you need entire length of the gel, half of the gel is not enough.