Last edited by: A. Andrus, 8/2013

Hly* α/β gene PCR typing for *Listeria monocytogenes

Forward Primer **α** : CCT AAG ACG CCA ATC GAA AAG AAA
Reverse Primer **β** : TAG TTC TAC ATC ACC TGA GAC AGA

Master mix:

Master Ingredients	for (1X)
d'H_2O	16.55 µl
10 X PCR Buffer	2.5 µl
MgCl2 (25 mM)	1.5 µl
dNTPs (1mM)	1.25 µl
hly α (20.0 µM)	0.5 µl
hly β (20.0 µM)	0.5 µl
Taq	0.2 µl
	23 ul total

Add 2 µl of template to 23 ul of master mix

Positive Control: any previously typed *L. mono.* lysate
Negative Control: (-) 2 µl of water added to master mix.
Negative Control: (-) 2 µl of (non-*L. mono*) lysate added to master mix.

Thaw your tubes of Stocks. Keep enzymes on ice. Follow the recipe as stated above making sure to mix very well. Keep the mixture on ice. The Taq polymerase should stay in the freezer until it is last added. Make sure the pipettes stay at the desired measurement. Mix gently with the pipette. Allocate 23µl of the master mix into PCR (0.2 mL) tubes.

Ramp up to 90° C and pause to put tubes into machine, then set the PCR machine under the following conditions:

2 min. at	94° C	(1 x)
10 sec.	95° C	----------(40 x)
30 sec.	60° C	
30 sec.	72° C	
10 min. at	72° C	(1 x)
∞	4° C	

The run takes approximately 1.5 hour. Then load 10.0 µl of PCR product with 2 µl of Loading dye (6x) on a 1.5 % gel at approximately 110V for about 1 hour. Stain the gel for 1-10 minutes (depending on frequency of use, the stain can be strong or weak and takes less or more time). De-stain for twice as long (20-60 minutes). Take a picture of the gel and print it immediately.

Reference:

Norton, Dawn M., et al. "Molecular studies on the ecology of Listeria monocytogenes in the smoked fish processing industry." Applied and environmental microbiology 67.1 (2001): 198-205.