

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

Master mix:

Master Ingredients	for (1X)
d'H ₂ O	16.55 μ l
10 X PCR Buffer	2.5 μ l
MgCl ₂ (25 mM)	1.5 μ l
dNTPs (1mM)	1.25 μ l
<i>hly α</i> (20.0 μ M)	0.5 μ l
<i>hly β</i> (20.0 μ M)	0.5 μ l
<i>Taq</i>	<u>0.2 μl</u>
	23 ul total

Add 2 μ l of template
to 23 ul of master
mix

Positive Control: any previously typed lysate

Negative Control: (-) 2 μ l of water added to master mix.

Negative Control: (-) 2 μ l of 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 one 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 23 μ l 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 μ l of PCR product with 2 μ l of Loading dye (6x) on a 1.5 % gel at approximately 110V for about 1 hour. Stain the gel for 10 minutes if the stain is freshly made (depending on frequency of use, the stain can be strong or weak and takes less or more time). De-stain for twice as long (at least 20 minutes). Take a picture of the gel and print it immediately.