InvA colony PCR

Primers:

The primer used here came from:

Kim, J. S., G. G. Lee, J. S. Park, Y. H. Jung, H. S. Kwak, S. B. Kim, Y. S. Nam, and S.-T. Kwon. 2007. A novel multiplex PCR assay for rapid and simultaneous detection of five pathogenic bacteria: *Escherichia coli* O157:H7, *Salmonella*, *Staphylococcus aureus, Listeria monocytogenes*, and *Vibrio parahaemolyticus*. J. Food Prot. 70:1656–1662.

The sequences are (5' => 3'):

invA-F: GAATCCTCAGTTTTTCAACGTTTC
invA-R: TAGCCGTAACAACCAATACAAATG

Master Mix:

PCR Reagents [Concentration]	Vol. (µL) for 1x 50µL rxn	
dH_20	31	
5x Go Taq Flexi buffer	10	
$MgCl_2$ [25mM]	3	
dNTPs [10mM]	1	
primer: invA-F [12.5µM]	2	
primer: invA-R [12.5µM]	2	
Go Taq DNA polymerase	0.25	
TOTAL:	49.25	

Remember:
-Use GoTaq instead of Amplitaq
-refer to Colony PCR Protocol (#8.1.1.1.4) for DNA template preparation -add 1µL (or 1.6µL if using the multichannel pipettor) of dirty lysate to each corresponding master mix tube
-amplicon should be 678 bp

PCR Conditions:

94ºC for 2 min **[1X]**

94ºC for 30 s
60ºC for 30 s 72ºC for 30 s **[20X]**

72°C for 5 min **[1X]**

Troubleshooting:

If colony PCR doesn't work, try making dirty lysates:

- Pick a colony with a toothpick and scrape into PCR tube, add 100µL of dH_2O
- Microwave dirty lysates for 30s to lyse cells