

InvA colony PCR

Master Mix:

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

Remember:

- Use GoTaq instead of Amplitaq
- Make dirty lysates in 100 μL of dH₂O by picking a colony with a toothpick and scraping into PCR tube
- Microwave dirty lysates for 30s to lyse cells
- add 1 μL (or 1.6 μL if using the multichannel pipettor) of dirty lysate to each corresponding master mix tube
- amplicon should be 678 bp

PCR Conditions:

94°C for 2 min **[1X]**

94°C for 30 s
60°C for 30 s **[20X]**
72°C for 30 s

72°C for 5 min **[1X]**