

InvA colony PCR

Master Mix:

PCR Reagents [Concentration]	Vol. (μ L) for 1x 50 μ L rxn	
dH ₂ O	31	
5x Go Taq Flexi buffer	10	
MgCl ₂ [25mM]	3	
dNTPs [10mM]	1	
primer: invA-F [12.5 μ M]	2	
primer: invA-R [12.5 μ M]	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 1 μ L (or 1.6 μ L 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]**

Troubleshooting:

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

- Pick a colony with a toothpick and scrape into PCR tube, add 100 μ L of dH₂O
- Microwave dirty lysates for 30s to lyse cells