

Colony PCR

Prepare PCR reactions

	25 μ L system	Final concentration
Taq DNA Polymerase (5 units/ μ l)	0.25 μ L	1.25 unit
5 \times Green Go Taq Buffer (Mg free)	5 μ L	1 \times
dNTP (2.5 mM)	2 μ L	0.2 mM
F primer (10 μ M)	1.25 μ L	0.5 μ M
R primer (10 μ M)	1.25 μ L	0.5 μ M
MgCl ₂ (50 mM)	1.25 μ L	2.5 mM
H ₂ O	13.5 μ L	
DNA template	Pick a single bacterial colony with a sterile toothpick; swirl the toothpick in a PCR tube containing the above components.	
Total	25 μ L	

Cycler setting

	Temperature	Duration	Cycles
Initial heating	95 °C	4 min	1
Denaturation	95 °C	30 sec	20-30
Annealing		30 sec	
Extension	72 °C	1-2 min	
Final extension	72 °C	5 min	1