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Colony PCR

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ABSTRACT

Protocol for a PCR from a colony

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1 Dissolve one colony in **20 µL** of nuclease-free water and heat at 80°C for 10 minutes.

Prepare the following 50 uL reaction in a 0.2mL PCR tube on ice:

2

Component	uL Reaction
Magnesium chloride (MgCl2) 25 mM	4 µL
5X Colorless GoTaq Buffer	5 µL
PCR Nucleotide Mix 10 mM	0.5 µL
Forward primer 10 uM	1 µL
Reverse primer 10 uM	1 µL
GoTaq DNA polymerase (5u/uL)	0.2 µL
DNA Colony	10 µL
Nuclease-free water	3.3 µL
TOTAL	25 µL

3 Gently mix the reaction and spin down in the microcentrifuge

4 Cycling conditions for a routine PCR:

A	B	C	D
1 Cycle	Initial activation	2 minutes	94ºC
35 Cycles	Denaturation	30 seconds	94ºC
	Alignment	30 seconds	50ºC
	Extension	2 minutes	72ºC
1 Cycle			72ºC
Maintenance		∞	4ºC

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