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Colony PCR

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Protocol status: Working

We use this protocol and it's working



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Abstract

Protocol for a PCR from a colony



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

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

	Component	μL Reaction
	Magnesium chloride (MgCl ₂) 25 mM	4 μL
	5X Colorless GoTaq Buffer	5 μL
	PCR Nucleotide Mix 10 mM	0.5 μL
	Forward primer 10 μM	1 μL
	Reverse primer 10 μM	1 μL
	GoTaq DNA polymerase (5u/ μL)	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	94°C



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