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Colony PCR

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Jiaxin Li¹

¹South China University of Technology



Jiaxin Li

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Protocol status: Working

We use this protocol and it's working

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Keywords: pcr



- 1 Draw grid on clean agar plate (use the appropriate antibiotic). You will use this to inoculate an overnight culture for a positive clone.
- 2 Select colonies to pick. Streak portion of colony to numbered sector and place the remainder in a correspondingly numbered PCR tube with 20 μ L of autoclaved water.
- 3 Heat at 95 °C for 10 minutes.

Note

This can be done in PCR machine

- 4 Use 1 μ L of reaction for colony PCR (T5 Taq Polymerase)

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Component	Volume/ μ L	Final Concentration
2XT5 Super PCR Mix	5 μ L	1x
10 μ M Forward Primer	0.4 μ L	0.4 μ M
10 μ M Reverse Primer	0.4 μ L	0.4 μ M
Template DNA	1 μ L	<1 μ g
ddH ₂ O	Add to 10 μ L	
Total	10 μ L	

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Temperature	time
98°C	03:00
98°C	00:10
T _m -5°C	00:10
72°C	20s/kb



	72°C	02:00
	4°C	∞