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Colony PCR

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1 Works for me dx.doi.org/10.17504/protocols.io.bnihmcb6

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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 µL of autoclaved water.

3 Heat at 95 °C for 10 minutes.

This can be done in PCR machine

4 Use 1 µl of reaction for colony PCR (T5 Taq Polymerase)

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Component	Volume/ul	Final Concentration

2XT5 Super PCR Mix	5ul	1x
10uM Forward Primer	0.4ul	0.4uM
10uM Reverse Primer	0.4ul	0.4uM
Template DNA	1ul	<1ug
ddH2O	Add to 10ul	
Total	10ul	

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Temperature	time
98°C	03:00
98°C	00:10
Tm-5°C	00:10
72°C	20s/kb
72°C	02:00
4°C	∞

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