Colony PCR

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

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ABSTRACT

This PCR method can be used to screen for inserted target genes or DNA sequencing analysis.

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MATERIALS TEXT

2×high Taq Master Mix (Enzyme)
Template
F/R primers
ddH2O
Bio-rad S1000TM Thermo Cycler.

SAFETY WARNINGS

Please wear gloves for the experiment, don't try to touch the lid after PCR program initiation.

ABSTRACT

This PCR method can be used to screen for inserted target genes or DNA sequencing analysis.

BEFORE STARTING

Synthesize primers in advance.

1 Pick colonies as the template for colony PCR. The number picked for each plate depends on the difference between the positive and negative controls.

2×high Taq Master Mix (Enzyme)	5 µl
Template	1 µl
Forward Primer (10 µM)	0.4µl
Reverse Primer (10 µM)	0.4 µl
ddH2O	3.2 µl

Fill the rest with water.

2 Test digest performed and products analysed using agarose gel electrophoresis to confirm if correct construct was present.

3 Use colony PCR to enlarge colony numbers. Then only the positive clones were mini-prepped.

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