Colony PCR

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1 Works for me

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ABSTRACT

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

MATERIALS TEXT

2×high Taq Master Mix (Enzyme)
Template
F/R primers
ddH_2O
Bio-rad S1000TM Thermo Cycler.

SAFETY WARNINGS

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

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	0.4 µl
Forward Primer (10 µM)	0.4µl
Reverse Primer (10 µM)	0.4 µl
ddH2O	3.8 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.