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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

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

## Materials

2×high Taq Master Mix (Enzyme)

Template


F/R primers

ddH<sub>2</sub>O

Bio-rad S1000<sup>TM</sup> Thermo Cycler.

## Troubleshooting

## Safety warnings

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

## Before start

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)	0.4 µl
	Reverse Primer (10 µM)	0.4 µl
	ddH <sub>2</sub> O	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.