Sep 20, 2019

Colony PCR

DOI
dx.doi.org/10.17504/protocols.io.7h2hj8e

宏亮 董[1]

[1]Northeast Forest University

2019 iGEM NEFU_China
Tech. support email: **shengyiyanwork@gmail.com**

宏亮 董

DOI: https://dx.doi.org/10.17504/protocols.io.7h2hj8e

Protocol Citation: 宏亮 董 2019. Colony PCR. **protocols.io https://dx.doi.org/10.17504/protocols.io.7h2hj8e**

Protocol status: Working
We use this protocol and it's working

Created: September 20, 2019

Last Modified: September 20, 2019

Protocol Integer ID: 27930

Keywords: Colony PCR, pcr method, pcr, sequencing analysis, inserted target gene, target gene, dna, gene

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_2O
Bio-rad S1000TM 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)	0.4µl
	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.