

Nov 21, 2023

Colony PCR (E. coli)

DOI

<https://dx.doi.org/10.17504/protocols.io.bp2l69ekrlqe/v1>

a.koh¹

¹MRC LMS



Alan Koh

MRC LMS

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bp2l69ekrlqe/v1>

Protocol Citation: a.koh 2023. Colony PCR (E. coli). protocols.io <https://dx.doi.org/10.17504/protocols.io.bp2l69ekrlqe/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: March 16, 2023

Last Modified: November 21, 2023



Protocol Integer ID: 78924

Keywords: colony pcr, amplified pcr product, easy pcr, pcr, cloning insert

Abstract

Fast and easy PCR to check for cloning inserts. This protocol is not suitable for downstream application of the amplified PCR products.



1 **Set up 1x PCR buffer mix**

5X Green or Colorless GoTaq® Flexi Buffer: 10µl (1x)
PCR Nucleotide Mix, 10mM each: 1µl (0.2mM each dNTP)
upstream primer: 2µl (1.0µM)
downstream primer: 2µl (1.0µM)
GoTaq® G2 Flexi DNA Polymerase (5u/µl): 0.25µl (1.25u)
MgCl₂: 2ul (1 mM)
Nuclease-Free Water to: 50µl

Note: Can reduce the final volume to 25µl to save reagents.

- 2 Using a sterile toothpick or 200µl pipette tips, gently touch a single bacterial colony and dip it into the PCR buffer mix.

3 **PCR reaction**

Initial denaturation: 95°C, 2 mins
*Denaturation: 95°C, 1 min
*Annealing: 55°C, 30 sec
*Extension: 73°C, 1 min for every 1kb of amplification
Final extension: 73°C, 5 mins
Storage: 12°C or room temperature depending on when the products will be analysed
*20 to 30 cycles (Generally 20 cycles is more than enough to amplify)

Note: Increasing the annealing temperature will increase the specificity of the PCR reaction.

Important: Run gel electrophoresis immediately as PCR products will normally be degraded due to the presence of DNase from the lysed E. coli. PCR products not recommended for long term storage.