Oct 05, 2025

Colony PCR

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gpm5×8gzp/v1

igemnthuth[1]
[1]NTHU_igem

iGEM NTHU

igemnthuth

DOI: https://dx.doi.org/10.17504/protocols.io.dm6gpm5x8gzp/v1

Protocol Citation: igemnthuth 2025. Colony PCR. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gpm5x8gzp/v1

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

Created: October 03, 2025

Last Modified: October 05, 2025

Protocol Integer ID: 228961

Keywords: colony pcr, target dna fragment, colonies by pcr, pcr, dna, transformed colony, colony

Abstract

Rapidly screen transformed colonies by PCR to determine whether they successfully contain the target DNA fragment.

Materials

(I) Colony Source

- Single colony grown 16–18 hr on an LB agar plate containing the appropriate antibiotic

(II) PCR Reagents

- Forward and reverse primers
- 10× Taq polymerase buffer
- ddH_2O
- Taq DNA polymerase
- 10 mM dNTPs

(III) Instruments / Equipment

- Thermal cycler (PCR machine)
- PCR tubes (0.2 mL)
- Microcentrifuge tubes
- Micropipettes (pipetman)
- Microcentrifuge
- Vortex mixer (or shaker)

Colony Picking and Template Preparation

1 Select colonies from the agar plate. Inoculate a portion of each colony into LB medium containing the appropriate antibiotic and incubate at 37 °C. Place the remaining portion of the colony into a correspondingly numbered PCR tube containing 25 µL of reaction mix (see below), and pipette to fully resuspend the bacteria.

PCR Reaction Setup (20 µL per reaction)

2 Prepare the PCR reaction mix before starting Step 1 and keep the mix on ic

10 mM dNTPs	0.5 µl
Forward primer (10 µM)	0.5 µl
Reverse primer (10 µM)	0.5 µl
10X Standard Taq Reaction Buffer	2.5 µl
Taq DNA Polymerase	0.125 µl
ddH_2O	to 25 µl
total	**25**

3 Gently mix the reaction components, then briefly centrifuge the tubes to collect the contents at the bottom.

4 Place the PCR tubes into the thermal cycler and run the following program:

Step	Temperature	Time
Initial Denaturation	95°C	30 seconds
25-30 Cycles	95°C 45-68°C* 68°C	15-30 seconds 15-60 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	