

Oct 11, 2022

Colony PCR for 2022 iGEM



Forked from [Colony PCR](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.rm7vzb352vx1/v1>

igem¹

¹Fudan University

2022



Team Fudan iGEM

Fudan University

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.rm7vzb352vx1/v1>

Protocol Citation: igem 2022. Colony PCR for 2022 iGEM. protocols.io
<https://dx.doi.org/10.17504/protocols.io.rm7vzb352vx1/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: October 10, 2022

Last Modified: October 11, 2022

Protocol Integer ID: 71102

Keywords: colony pcr, pcr, igem, manual

Abstract

Based on manual included in Vazyme Taq product



- 1 Select colonies on the agar plate to pick. Culture portion of the colony in LB media with antibiotic and grow in 37 degree. Put the remainder of the colony into a correspondingly numbered PCR tube with 20 μ L of reaction mix (as below), and pipetting to complete resuspend the bacteria.

- 2

A	B
Component	Volume/ μ L
10x Taq Buffer	2 μ L
10 μ M Forward Primer	0.5 μ L
10 μ M Reverse Primer	0.5 μ L
Template DNA (bacteria colony)	added later
dNTP 10 mM mix	0.5 μ L
Taq DNA polymerase	0.5 μ L
distilled water	16 μ L
Total	20 μ L

5m



Prepare PCR reaction mix before step 1, and place the mix on ice.

Primers used for screening crt genes insertion are:



```
> 5-crtB
5-ATGAATAATCCGTCGTTACTCAATCATGC-3

> 5-crtE
5-ATGACGGTCTGCGCAAAAAAAC-3

> 5-crtI
5-ATGAAACCAACTACGGTAATTGGTGC-3

> 5-crtY
5-ATGCAACCGCATTATGATCTGATTC-3

> rev320crtB
5-CCTTCCAGATGATCAAACGCGTAAG-3

> rev320crtE
5-ATGAGAATGAATGGTAGGGCGTC-3

> rev320crtI
5-GGATTAAACTGCTGAATCTGCGCTTC-3

> rev320crtY
5-CCGCGGTATCCATCCACAAG-3
```

3

10m

	A	B
	Temperature	Time
	94°C	05:00
	94°C	00:30
	60°C	00:30
	72°C	03:30, cycle 3-4-5 for 25 times
	72°C	05:00
	16°C	∞

The PCR program above is for screening crt genes insertion during 2022 iGEM season.



- 4 Agarose gel electrophoresis of PCR products. One lane on the gel is loaded with 6 uL D2000 DNA ladder. Run the gel at 100 V for 20-30 minutes, and examine the gel under UV.
- 5 The correct bacterial clones are sent for Sanger sequencing. Once verified, these clones would be used for further experiments.