




Oct 11, 2022

Colony PCR for 2022 iGEM

 Forked from [Colony PCR](#)**Team Fudan iGEM¹**¹Fudan University1 *Works for me* Sharedx.doi.org/10.17504/protocols.io.rm7vzb352vx1/v1

2022

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ABSTRACT

Based on manual included in Vazyme Taq product

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PROTOCOL CITATION

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FORK NOTE

FORK FROM

[Forked from Colony PCR, Jiaxin Li](#)

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- 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 

5m

| 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 |

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-GGATTAACTGCTGAATCTGCGCTTC-3

> rev320crtY
5-CCGCGGTATCCATCCACAAG-3
```

| 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.