




Oct 06, 2022

Colony PCR

Forked from [Colony PCR and electrophoresis](#)An.Huang¹¹XJTLU

1 Works for me

 Sharedx.doi.org/10.17504/protocols.io.bp2l69bnklqe/v1 An.Huang

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

ABSTRACT

Colony PCR using the whole organism of bacteria instead of purified DNA template. This simplifies PCR procedure. This protocol helps conduct a simple colony PCR procedure.

DOI

dx.doi.org/10.17504/protocols.io.bp2l69bnklqe/v1

PROTOCOL CITATION

An.Huang 2022. Colony PCR. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bp2l69bnklqe/v1>



FORK NOTE

FORK FROM

Forked from [Colony PCR and electrophoresis](#), Weizhuo.Chen

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

CREATED

Oct 06, 2022

LAST MODIFIED

Oct 06, 2022

PROTOCOL INTEGER ID

70896

MATERIALS TEXT

Your LB agar plate with colonies of transformed E. coli, TE solution, PCR buffer, dNTP stock solution, Forward Primer
Reverse Primer, Taq DNA polymerase


DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to [protocols.io](#) is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with [protocols.io](#), can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Preparation for experiment

2d

- 1 Pick several colonies of bacteria from the plate using pipette tips.
- 2 Put the selected colonies into different 15mL centrifuge tubes, each with  5 mL LB broth.

- 3 Incubate in an orbital shaker at 🌀 **171 rpm, 37°C** overnight.
- 4 Pipette 📄 **30 µL** culture from each tube to different agar plates. Spread the culture evenly on the plate.
- 5 Incubate the plates in a biochemical incubator at 🌡 **37 °C** overnight.

These steps help obtain adequate (and genetically pure) colonies for testing and research in the future.

- 6 Prepare several sterilized 1.5ml microcentrifuge tubes.

If you have X samples to test, prepare X+2 tubes at least. You may prepare more in case you make mistakes.

Colony PCR

5m

- 7 Add 📄 **30 µL** TE buffer to X+2 1.5ml microcentrifuge tubes each. Label the tubes as "1, 2, 3, ..., X, +, -".

"+" tube means the positive control group and "-" tube means the negative control group.

- 8 Pick one colony from each plate ➡ **go to step #5** using a sterilized pipette tip and put the colonies into different 1.5ml microcentrifuge tubes numbered "1, 2, 3, ..., X"

- 9 Place the tubes in a heating block, heating at 🌡 **100 °C** for ⌚ **00:05:00** .

5m

10 Prepare Master Mix for colony PCR. The recipe for the Master Mix is as follows:

| Item | For one reaction | For Y reactions (prepare one more in case pipette inaccuracies) Y+1 |
|-----------------------|------------------|------------------------------------------------------------------------------|
| dH ₂ O | 11.5 µl | µl |
| 10X PCR buffer | 2 µl | µl |
| dNTP mix | 0.4 µl | µl |
| forward primer | 0.4 µl | µl |
| reverse prime | 0.4 µl | µl |
| <i>Taq</i> polymerase | 0.3 µl | µl |
| <i>Total</i> | <i>15 µl</i> | µl |

Keep all PCR reagents on ice.

If you have Y reactions, prepare Master Mix for Y+1 reactions.

This means if you have X samples, you need to prepare X+3 reactions for X samples, one positive control, one negative control and another portion in case of pipette inaccuracies.

11 Label X+2 0.2mL PCR tubes as "1, 2, 3, ..., X, +, -"

12 Pipette **15 µL** from the Master Mix into all X+2 0.2 mL PCR tubes.

13 Pipette **5 µL** from the colony lysate from tube **go to step #9** "1, 2, 3, ..., X" into corresponding 0.2 mL PCR tubes.

Add **5 µL** plasmid into "+" PCR tube. Add **5 µL** ddH₂O into "-" PCR tube.

The plasmid used here is the plasmid transduced previously into the bacteria on the original plate at Step#1.



Plasmid transduction using competent cell
by An.Huang

14 Place the X+2 PCR tubes in Thermocycler. PCR procedure will be set as the following programme:

1 cycle of ⌚ **95 °C 2 min**

30 cycles of ⌚ **94 °C 30 sec**

⌚ **50 °C 30 sec**

⌚ **72 °C 2 min**

Final extension ⌚ **72 °C 10 min**

15 When the programme is finished, store the tubes at ⌚ **4 °C**