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# Colony PCR

Forked from Colony PCR and electrophrosis

An.Huang[1]

[1]XJTLU



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An.Huang

DISCLAIMER



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.

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FORK NOTE

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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



## 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.



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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



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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 |
|---|---|---|
| $dH_2O$ | 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 |
| *Taq* polymerase | 0.3 µl | µl |
| *Total* | *15* µl | 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** $ddH_2O$ 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**



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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**



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