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Colony PCR

Forked from a private protocol

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We use this protocol and it's working

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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.

Materials

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

Troubleshooting

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.

Note

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

6 Prepare several sterilized 1.5ml microcentrifuge tubes.

Note

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, +, -".

Note

"+" 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_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

Note

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.

Note

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

Protocol

NAME

Plasmid transduction using competent cell

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Preview

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