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Protocol status: Working

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.
- Put the selected colonies into different 15mL centrifuge tubes, each with 45 mL LB broth.
- Incubate in an orbital shaker at 171 rpm, 37°C overnight.
- Pipette \perp 30 μ L culture from each tube to different agar plates. Spread the culture evenly on the plate.

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

Add \perp 30 μ L 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 5 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 \$\mathbb{\mathbb{I}}\$ 100 °C for \$\infty\$ 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	<i>15</i> µl	μl

Note

Keep all PCR reagents on ice.

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

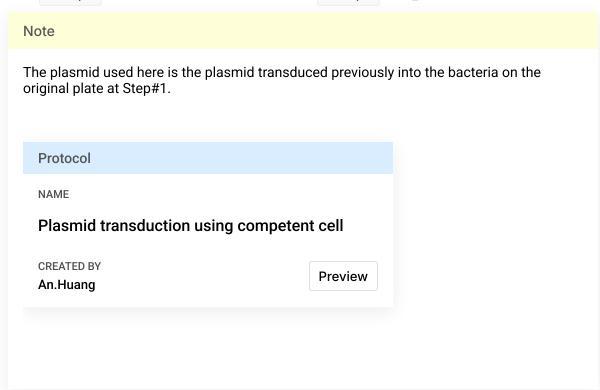
This means if you have X samples, you need to prepare X+3 reactions for X samples, one positive control, one negative control and another portion in case of pipette inaccuracies.

- 11 Label X+2 0.2mL PCR tubes as "1, 2, 3, ..., X, +, -"
- 12 Pipette 4 15 µL from the Master Mix into all X+2 0.2 mL PCR tubes.



Pipette Δ 5 μ L from the colony lysate from tube $30 \pm 30 \pm 30 \pm 30$ "1, 2, 3, ..., X" into corresponding 0.2 mL PCR tubes.

Add \perp 5 μ L plasmid into "+" PCR tube. Add \perp 5 μ L ddH₂O into "-" PCR tube.



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

```
1 cycle of $\mathbb{s} \text{ 95 °C 2 min}$

30 cycles of $\mathbb{s} \text{ 94 °C 30 sec}$

$\mathbb{s} \text{ 50 °C 30 sec}$

$\mathbb{s} \text{ 72 °C 2 min}$

Final extension $\mathbb{s} \text{ 72 °C 10 min}$
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When the programme is finished, store the tubes at 4 °C