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two layer plating method

Jiaxin Li¹

¹South China University of Technology

Jiaxin Li

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We use this protocol and it's
working

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BEFORE STARTING

- 1 Prepare 6 cm diameter petri plates containing solidified LB-agar and dry it in the intercellular incubator for 12 h.
LB medium formulation :

NaCl	10g
TRYPTONE	10g
YEAST EXTRACT	5g
Agar	15g

Take equipping 1L as an example, other volume isometric adjustment

Antibiotic concentration:

Abbreviation	Name	Initial concentration mg/ml	Final concentration mg/ml
Tc	tetracycline	5	50
Gm	gentamicin	50	50

CaCl₂ was added to give a final concentration of 2 mmol/L.

Prepare 0.6% soft agar and store it in a refrigerator at 4°C.

LB medium formulation :

NaCl	10g
TRYPTONE	10g
YEAST EXTRACT	5g
Agar	6g

Take equipping 1L as an example, other volume isometric adjustment.

CaCl₂ was added to give a final concentration of 2 mmol/L.

2 Preparation of SM Buffer

NaCl	5.8g
MgSO ₄ ·7H ₂ O	2g
Tris-HCl	50ml (1M)
H ₂ O	To 1 L

After preparation, sub-Pack (50ml) and store in 4 degree refrigerator.

3 Shake the bacteria in advance, 5 ml each, culture overnight.

- 4 The final concentration of inducer was 0.1% by adding 1 ml bacterial liquid and appropriate amount of inducer into 50 ml centrifuge tube.
- 5 37°C, 220 rpm shaker culture induction for 1 h
- 6 Set the temperature of water bath to 50°C
- 7 Take half of the beaker water, put the soft agar to be melted into the beaker, and heat it in the microwave oven.
- 8 Put the completely melted culture medium into the water bath to keep warm.
- 9 No inducer group: take warm soft agar (10ml/tube) and pour it into the tube containing 100 µl bacterial liquid, shake well and pour it into the plate containing solidified LB-agar.
- 10 Inducer group: add 100 µl 10% Ara and 10 µl 1mol/L IPTG to the tube containing 100 µl bacterial liquid, suck and mix well, pour in warm soft agar, shake and pour it into the plate containing solidified LB-agar.
- 11 Place the poured Petri dish for 30 min to solidify.
- 12 During the waiting process for coagulation, the phages stored at 4°C were removed from the refrigerator and diluted with SM Buffer in a 1.5 ml centrifuge tube.

The amount of addition can be referred to the following figure.

- 13 Add bacteriophage as needed and shake well.
- 14 Take 10 μ l phage spot to the center of each grid of the culture dish, let it dry for 30min, and culture it upside down for 16-20h.