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two layer plating method

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Protocol status: Working
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
	MgSO4·7H2O	2g
	Tris-HCl	50ml（1M）
	H2O	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.

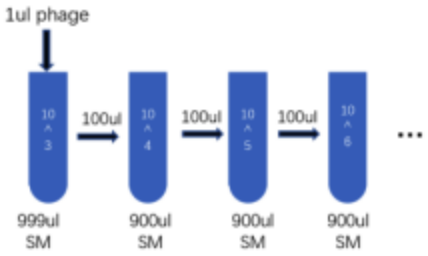
8 Put the completely melted culture medium into the water bath to keep warm.

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 µl 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.