Plaque assay protocol for Cellulophaga

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Abstract

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Before start

What you need before you start:

1. Zobell plates
2. Top agar – 3.5 ml per plate
    a. MSM
    b. 6g LMP agarose/liter
3. Your host growing somewhere in exponential phase – 0.3 ml per plate
4. Phages – these could be from:
    a. A lysate
    b. A plaque picked into buffer
    c. An environmental sample
    d. A sample from an experiment (e.g., a one-step)
5. A 35 °C water bath

Protocol

Step 1.
First, microwave the top agar to melt it

NOTES
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Make sure the cap is loose.

Step 2.
Let your plates warm to room temperature and label them

Step 3.
Make whatever dilutions of your phages you plan to plate

Step 4.
Aliquot the agar into tubes (either 15ml or 50ml, depending on how much top agar and cell culture you need) and place in 35 °C water bath

NOTES
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Before you use the agar, make sure the tube feels the same temperature as the water bath. If the

agar is still too hot, you'll kill your host. You'll need to wait about 5–15 minutes for it to cool down enough. If the agar is too cool, it might start getting chunky. If your agar is starting to solidify, do NOT use it. Make up a new tube if your big bottle is still hot or re-microwave it.

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If you're doing more than one plate with the same host, you can aliquot enough agar for however many plates you're comfortable infecting at once (up to 12). Be sure to leave room in your tubes to add host.

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Use 3.5 ml per plate.

Step 5.

Pipet 100 µl of your phage sample onto the plate

NOTES

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If you made up agar aliquots for more than one plate, add phage to each plate now.

Step 6.

Remove the agar tube from the water bath

Step 7.

Add 0.3 ml of host per plate

ANNOTATIONS

Bonnie Poulos 08 Mar 2016

Multiply the number of plates you will be plating by 0.3 and this will be the total volume of host to add to the tube of agar.

Step 8.

Pipet up and down or gently invert to mix

NOTES

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Try not to introduce a lot of bubbles (i.e., do not shake or vortex the tube).

Step 9.

Add 3.5 ml of agar/host mixture to each plate

NOTES

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If there is only one plate worth of agar and host in the tube, you can pour it onto the plate. Otherwise, pipet 3.5 ml onto each plate.

Step 10.

Swirl each plate to spread the agar as you go

NOTES

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Make sure it covers the whole plate.

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If you want your plates in stacks, stack them as you go. They cannot be moved after plating for a least 30 minutes.

Step 11.

Leave the plates upside-down in the dark (e.g., in a drawer or box) overnight

DURATION

18:00:00

Step 12.
Count or pick plaques the next day

NOTES
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If you’re calculating PFU, you will probably need to leave them out and count them for one to two more days.