Plaque Assay Protocol for Pseudoalteromonas

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Abstract

Citation: Natalie Solonenko Plaque Assay Protocol for Pseudoalteromonas. **protocols.io** dx.doi.org/10.17504/protocols.io.de33gm
Published: 02 Feb 2016

Before start

What you need before you start:
1. 20% nutrient Zobell plates
2. Top agar – 3.5 ml per plate
    a. 100% nutrient Zobell
    b. 6g agar/liter
3. Your host growing somewhere in exponential phase – 0.4 ml per plate
4. Phages – this could be:
    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 40°C water bath

Protocol

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

Step 2.
While the autoclave is running:
a. Let your plates warm to room temperature and label them
b. Make whatever dilutions of your phages you plan to plate

Step 3.
Aliquot the agar into tubes and place in 40°C water bath

NOTES
VERVE Team 15 Jul 2015
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-autoclave it If it will take you more than 30 minutes to do all your infections, you're doing too many at a time – your agar will start to solidify

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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 doing at once (up to 12) Be sure to leave room in your tubes to add host.
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Use 35 ml per plate.

Step 4.
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 5.
Remove the agar tube from the water bath and add the host to it

NOTES
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Pipet up and down or gently invert to mix. Try not to introduce a lot of bubbles (i.e., do not shake or vortex the tube).
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Use 0.4 ml of host per plate.

Step 6.
Add 3.5 ml of the agar/host mixture

NOTES
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If you want your plates in stacks, stack them as you go. They cannot be moved after plating for at least an hour.
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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 7.
Swirl each plate to spread the agar as you go

NOTES
VERVE Team 24 Aug 2015
Make sure it covers the whole plate.

Step 8.
Leave the plates on the bench overnight

DURATION
18:00:00

Step 9.
Count or pick plaques the next day

NOTES
VERVE Team 15 Jul 2015
If you’re calculating PFU, you will probably need to leave them out and count them for one more day.