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Full plate & spot test plaque assays + PFU/mL calc. - anaerobic/slow growing bacteria

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FOOD Micro UCPH

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Protocol for plaque assays - either a full plate plaque assay that are more laborious but with high accuracy, or a spot test plaque assay that are for higher throughput but with less accuracy.

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Materials needed: Broth, agar plates, soft agar, bacterial culture, phage culture, anaerobic 1M CaCl2, anaerobic 1x SM buffer, centrifuge tubes/falcon tubes, anaerobic sachet, anaerobic chamber, 1000 µL and 200 µL filter tips, incubation jar, heating block/bath.

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Initial preparation of bacterial culture

1 Inside anaerobic chamber, spread bacterial culture on an agar plate and incubate under anaerobic conditions (e.g. an incubation jar with an anaerobic sachet) at required temperature until clear colonies appear. All handling during this protocol of the bacteria should be inside the anaerobic chamber, unless otherwise stated.

2 Inoculate with a single colony of the bacteria to prepared anerobic media, incubate at required temperature, and wait until exponential phase has been reached. Preferably an OD600 between 0.2 – 0.5 - however, obtaining a proper bacterial lawn to observe clear plaques may be challeging with some anaerobic bacteria, and the additional steps below are therefore to increase the efficiency of the assay.

Full plate plaque assay

3 Prepare a 10-fold dilution of phage culture in SM buffer

4 Clearly mark on each agar plate the chosen phage dilution.

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5 Per. 10 mL bacterial culture (exponential phase) divide this into two Falcon tubes inside anaerobic chamber, tighten the screw cap, and bring out for centrifugation (5000 x g for 10 min). Thus, you may need 4-5 mL bacterial culture in exponential phase per agar plate.

6 Suspend bacterial pellet in 100 µl 40 mM CaCl2 (anaerobic)

7 Add 100µL of the 10-fold dilution series made of the phage lysate. Wait 10 min. for potential phage adsorption.

8 Do not vortex, mix by pipetting and inversion

9 Transfer this suspension into a tube with 3-4 ml soft agar cooled down to 50°C in a heating block.Immediately after pour on an agar plate. Let it solidify for 15-20 min.

10 Incubate plates at required temperature and at anaerobic conditions (e.g. incubation jar with anaerobic sachet)

11 Count the plaques and choose the plate with 30-300 plaques to calculate PFU/mL

12 (PFU / 0.1 mL) * (1/dilution) = PFU/mL

Spot test plaque assay

13 Prepare a 10-fold dilution of phage culture in SM buffer

14 Clearly mark on each plate the area allocated for each dilution.

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15 Per. 10 mL bacterial culture (exponential phase) divide this into two Falcon tubes inside anaerobic chamber, tighten the screw cap, and bring out for centrifugation (5000 x g for 10 min). Thus, you may need 4-5 mL bacterial culture in exponential phase per agar plate.

16 Suspend bacterial pellet in 100 µl 40 mM CaCl2 (anaerobic)

17 Transfer this suspension into a tube with 3-4 ml soft agar cooled down to 50°C in a heating block. Immediately after pour on an agar plate. Let it solidify for 15-20 min.

18 Then carefully spot 10 µL phage solution at each of the allocated areas and wait 1-2 hours for the droplet to dry inside the chamber. The lid can be slightly opened to speed up the drying.

19 Incubate plates at required temperature and at anaerobic conditions (e.g. incubation jar with anaerobic sachet)

20 Count the plaques lysis and choose the plate with 10-50 plaques to calculate PFU/mL

21 (PFU / 0.01 mL) * (1/dilution) = PFU/mL

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