Plating Prochlorococcus and Synechococcus strains in top agarose for plaque assays

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Abstract

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Guidelines

(using GIBCO LM agarose = now called Invitrogen Life Technologies LMP agarose, 1-800-955-6288, cat# 1-5517-014 for 50 g, 1-5517-022 for 100 g)
Note: new catalog number is16520-050.

Protocol

Base Plates

Step 1.
Prepare base plates using 0.4% GIBCO LM agarose (0.4g agarose / 100 ml)

NOTES
VERVE Team 08 Jul 2015
(using GIBCO LM agarose = now called Invitrogen Life Technologies LMP agarose, 1-800-955-6288, cat# 1-5517-014 for 50 g, 1-5517-022 for 100 g)
VERVE Team 08 Jul 2015
Note: new catalog number is 16520-050 for LMP agarose.

Base Plates

Step 2.
Make up plates using filtered, autoclaved 75% Sargasso Sea Water (SSW)

Base Plates

Step 3.
Add appropriate agarose, then microwave to a boil

NOTES
VERVE Team 17 Jun 2015
NO agarose floaties

Base Plates

Step 4.
Add appropriate nutrients to bring levels up to media of choice (SN, Pro99?)

Base Plates

Step 5.
Vortex / shake to mix nutrients thoroughly and asceptically pour plates in hood

Base Plates

Step 6.
Allow plates to sit overnight to solidify before adding top agarose

DURATION
18:00:00

NOTES
VERVE Team 17 Jun 2015
Synechococcus base plates can be used 1-3 days after being made, whereas Prochlorococcus base plates should be used the next day
VERVE Team 17 Jun 2015
Some strains (MED4, NATL2A, WH 8102, WH 7803) can go without base plates but may not stay pigmented as long as those plates with base plates, whereas other strains may do better without base plates (SS120 and MIT 9312?)

Dilutions

Step 7.
Prepare your cyanophage dilutions ahead of plating cells in top agarose by using media for dilutions in 5 ml Falcon tubes

NOTES
VERVE Team 08 Jul 2015
I like to have the cyanophage dilution volume total 300 µl.
VERVE Team 22 Jul 2015
I prepare cyanophage dilutions with media in 5 ml Falcon tubes to plate the cyanophage by simply adding 2.7 ml of the cell-agarose mixture to the cyanophage dilution that can be vortexed right in the tube and pour plated immediately.

Top Agarose

Step 8.
Prepare top agarose as 0.5% GIBCO Low-Melt agarose

REAGENTS
UltraPure™ Low Melting Point Agarose 16520-100 by Contributed by users

NOTES
VERVE Team 22 Jul 2015
1 part cells : 4 parts agarose yields ~0.4% agarose final concentration.

Top Agarose

Step 9.
Use filtered, autoclaved 75% SSW to make up plates.

Top Agarose

Step 10.
Add appropriate agarose (0.5 g / 100 ml SSW) and microwave as above

Top Agarose

Step 11.
Allow to cool in a pre-heated water bath to 29°C (solidifies 24-28°C)

Top Agarose

Step 12.

Asceptically add filter sterilized nutrients to bring level of top agarose nutrients to desired media of choice

Top Agarose

Step 13.
Add appropriate volume of cells to top agarose, vortex to thoroughly mix and plate immediately

NOTES

VERVE Team 22 Jul 2015

I add 10 ml of a dense stock of exponentially growing cells (~ 10^8 cells ml^-1) to 40 ml of top agarose in an orange capped 50 ml centrifuge tube.

Step 14.
Inoculate a liquid culture using the same dilution chosen for the plates as an indicator of when the plates might turn green (within a few days of each other)

Step 15.
Incubate all plates overnight at 2-5 uE light without parafilming the plates to allow the top agarose to solidify completely

DURATION

18:00:00

Step 16.
After the o/n incubation parafilm the plates (humidity control) and place at the appropriate light levels

NOTES

VERVE Team 22 Jul 2015

Due to the harshness of the plating procedures, the light levels for plating might need be slightly reduced (~ to 33% less) to those used for liquid cultures. Also, marine cyanobacterial cells will not likely grow if there is any liquid in the agarose (we are plating at the limits of where this agarose can solidify, so if the humidity is wrong I may not get solid top agarose.