Dot Blot Preparation

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Abstract

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Guidelines

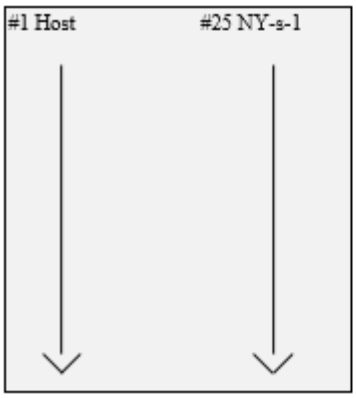
Materials:

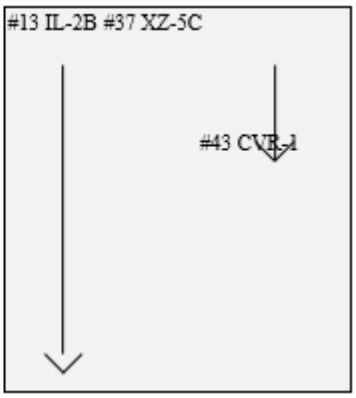
1.) host and viral DNAs
2.) 1X TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA)
3.) 1 M NaOH
4.) 1 M Acetic acid
5.) nylon membrane, cut to 12.3 X16 cm (2 sheets)
6.) Whatman 3MM filter paper, cut 12.3X16 cm (2 sheets)
7.) SSC solutions: 1X, 5X, 20X
20X SSC (1000 mL):

- 175.3 g NaCl
- 88.2 g sodium citrate
- adjust pH to 7.0 with NaOH

8.) 96-well assay plates
9.) hybridot dot blot apparatus and pump

Note: Load the membranes so that when scanned, the second blot of the set may be placed under the first, i.e., load as the following diagram shows:





Protocol

DNA dilutions

Step 1.
Prepare DNAs in 1.2 µg/48.6 µl concentrations.

DNA dilutions

Step 2.
Distribute 97.2 µl of each to 96-well plates in the same pattern as the 1.0 µg samples will be loaded onto the membrane.

DNA dilutions

Step 3.
Make 3 serial 1/2 dilutions with each DNA sample (48.6 µl into 48.6 µl 1XTE).

DNA dilutions

Step 4.
Discard 48.6 µl from the final dilution.

DNA dilutions

Step 5.
Add 5.4 µl of 1 M NaOH to each sample.

DNA dilutions

Step 6.
Cover plates with parafilm.

DNA dilutions

Step 7.
Incubate at 37°C, 30 min.

DURATION
00:30:00

DNA dilutions

Step 8.
Add 6.0 µl of 1 M acetic acid to each sample.

DNA dilutions

Step 9.
Chill on ice.

Membrane preparation

Step 10.
Wet the membrane in 1X SSC ≥60 min.

DURATION
01:00:00

Membrane preparation

Step 11.
On the hybridot manifold, place a piece of Whatman 3MM filter paper and wet with 20X SSC.

Membrane preparation

Step 12.
Roll air pockets out.

Membrane preparation

Step 13.

Mount the membrane on top of the 3MM paper (centered).

Membrane preparation

Step 14.
Roll air pockets out.

Membrane preparation

Step 15.
Wash the membrane with 5X SSC (ca. 25 ml) with vacuum filtration.

Membrane preparation

Step 16.
Screw the top of the hybridot manifold in place, then shut off the vacuum pump.

Membrane preparation

Step 17.
Add 200 µl of 5X SSC to each well.

Membrane preparation

Step 18.
Add DNAs (50 µl each) to the wells.

Membrane preparation

Step 19.
Turn on the vacuum pump and draw samples onto the membrane.

Membrane preparation

Step 20.
Wash each well with 400 µl of 5X SSC (2X).

Membrane preparation

Step 21.
Remove membrane and crosslink under UV light (200 mJ).

Step 22.
Store the membranes wrapped in plastic at 4°C.

NOTES

Irina Agarkova 05 Apr 2016

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