Slot blotting DNA [from genomic DNA isolation or squish prep+]
Danna Eickbush

1 Solutions/ Buffers needed:
   a. 0.4 M Tris, pH 7.5
   b. 20X SSC
   c. 2X Denaturing Solution (1X is 0.25 N NaOH, 0.5 M NaCl)
   d. Loading Solution (0.1X SSC, 0.125 N NaOH)

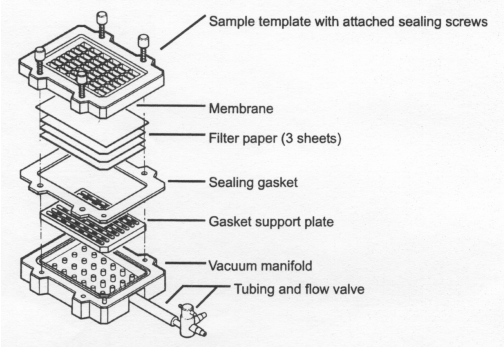
2 Cut nylon membrane to appropriate size and soak in 0.4 M Tris, pH 7.5 until needed.

3 Label 48 tubes (or appropriate number), pipet 100 ul Loading Solution in each tube, and put tubes in the minus 20^0C freezer while preparing DNA.

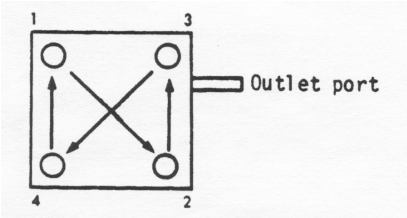
4 Prep DNA
   a. For each sample, mix DNA and H_2O for a final volume of 50 ul
   b. Put 50 ul 2X Denaturing solution into each tube
   c. Vortex each sample 1-2 seconds, centrifuge tubes briefly
   d. Incubate for 10 minutes at room temperature.

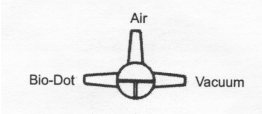
5 Quick cool the denatured DNA by pipetting each 100 ul sample into a tube with 100 ul of cold Loading Solution (from freezer). Leave tubes on ice.

6 Prepare slot blotter, use prewet filter paper (0.4 M Tris, pH 7.5), remove air bubbles between filter paper and membrane:

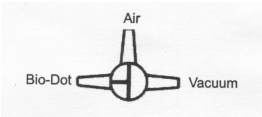


Tighten screws in order shown below:

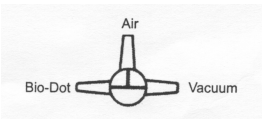




Attach vacuum to the port, turn on vacuum, and with manifold exposed to vacuum (flow valve position1) tighten screws again.



Adjust so manifold is exposed to air (flow valve position 2). Add 200 ul of 0.4 M, Tris pH 7.5 to each slot to rehydrate membrane.



Adjust so manifold is exposed to vacuum and air (flow valve position 3) & gently remove the buffer from the wells. Use finger over the air portion to control vacuum. As soon as buffer solution drains from all the wells, adjust flow valve to position 2 & turn off vacuum.

7 Load samples into wells.
   a. Gently remove sample solution with flow valve in position 3
   b. As soon as sample buffer drains from all the wells, adjust flow valve to position 2 & turn off vacuum.

8 Wash wells by adding 200 ul of Loading Solution.
   a. Remove solution with flow valve in position 1
   b. Leave vacuum on, loosen the screws, remove sample template.
   c. Turn off the vacuum and remove the membrane.

9 Rinse membrane in 2X SSC for 2 minutes

10 Cross link the DNA to the membrane while it is still damp (Stratalinker in Gorbunova/Seluanov lab).

2X Denaturing Solution (0.5 N NaOH, 1 M NaCl):
2 g NaOH
5.8 g NaCl
H_2O to 100 ml

Loading Solution:
0.5 g NaOH
0.5 ml 20X SSC
H_2O to 100 ml