PIP Strip/Array Protocol

1) Cut the membrane (Amersham Hybond-C Extra – RPN203E) into the appropriate size and notch one corner for orientation. *Tip: Use the 12.5ul Matrix Multi-channel pipette to gauge the size to cut. In general use (m+1) and (n+1) tip distances for the lengths of the sides where m and n are the number of PIPs being spotted and the number of dilutions. For example: To make a strip with 5 dilutions of 2PIPs, measure 6 by 3 tip distances as the membrane size.*
2) Make the buffers you will use in glass tubes or vials. Scintilation vials are cheap and do the trick nicely. Be sure to pipette the components up and down until all the bubbles are gone from the tip to ensure accurate pipetting. This will be important for all of the following steps as well. Chilling the components and the buffers on ice speeds this up by decreasing the vapor pressure. Below are the recipes for the required buffers. If you are making a lot of strips, you may want to make more Spot Buffer. These buffers must be made fresh shortly before making strips.
    a. Resuspension Buffer - 250ul Chloroform + 500ul MeOH + 200ul Water
    b. Spot Buffer – 250ul Chloroform + 500ul MeOH + 200ul 50mM HCl + 2ul Ponceau S (Fluka 09276)
3) Setup the cold room for blot making. Lay out all of the blots on foil and number them. Chill a polypropylene 96well plate and label eppendorf tubes for each of the lipid species you will dilute. All future steps will be conducted in the cold room. *For example: To make PI4P strips, label one tube PI and another PI4P.*
4) Resuspend the first lipid in the correct amount of Resuspension Buffer to make a 1mM stock solution. Work in the glass vial provided by Echelon and be sure to wash the walls of the vial well to ensure that all of the lipid is resuspended. See the chart below for proper volumes. *For example: Wash the walls of a 0.1mg tube of PI4P with 104.5ul Resuspension Buffer.*

Lipid	Amount	ul Resuspion Buffer for 1mM
PI	0.1 mg	109.6 ul
PI3P	0.1 mg	104.5 ul
PI4P	0.1 mg	104.5 ul
PI5P	0.1 mg	104.5 ul
PI(3,4)P2	0.1 mg	92.5 ul
PI(3,5)P2	0.1 mg	92.5 ul
PI(4,5)P2	0.1 mg	92.5 ul
PIP3	0.1 mg	83.0 ul

5) Dilute the lipid as appropriate in the labeled eppendorf tube. Use the Spot Buffer as a diluent. *For example: To make 200pmol spots, add 72ul Spot Buffer to the eppendorf tube and then add 18ul of the resuspended lipid at 1mM. 1ul of this solution will contain 200pmol of lipid.*

6) Transfer the desired amount of this solution to the microplate. Be sure to transfer at least 10-15ul more than the absolute minimum needed to make your blots. You will use 1ul of this solution for each blot.
7) Add additional Spot Buffer to the eppendorf tube with the lipid to serially dilute your sample and continue to transfer to the microplate until you have made all appropriate dilutions. *For example: To make 3-fold dilutions from the sample discussed in step 5, you will transfer 60ul of this solution to the microplate in step 6 and then add 60ul of fresh Spot Buffer to the eppendorf. Continue transferring out and 60ul of solution and adding 60ul of Spot Buffer until you have generated the proper number of samples to spot your membranes.*
8) Using the Matrix multi-channel pipette, aspirate all of the samples for this lipid and begin spotting the membranes. Be sure to draw up and purge the tip at least three times before spotting to eliminate air bubbles. Spot 1ul of each solution on each membrane. Even though the tip will take up 12.5ul you should only spot 10 blots before returning to the plate for additional sample. Also, wait for the pipette to beep before removing the tips from the membrane. This will ensure accurate dispensing.
9) Repeat steps 4-8 for all additional lipids until you have completed your strip or array.
10) Dry the blots at room temperature for 1 hour in a dark place. I use an empty drawer.
11) Store the blots in foil at 4C until ready to use.