



MULTIPLE IDENTICAL HIGH DENSITY MACRO ARRAYS WITH MULTI-PRINT™

1. From a 30 cm roll of nylon membrane (VP 503) cut a piece 22 cm long and place the membrane on a blotting paper such as VP 521V (22 cm by 32 cm).
2. Remove protective cover from the low tack tape on 4 MULTI-PRINT™ locator bases.
3. Place 4 MULTI-PRINTS (VP 382, VP 382B or VP 382D) on the membrane and press to attach.
4. Mark the 4 corners of each membrane with a pencil for orientation purposes.
5. Select a 96, 384 or 1536 well source plate "a". Select a 96, 384 or 1536 MULTI-BLOT™ Replicator and prepare it as described in Technical Note 10.
6. Place a LIBRARY COPIER™ (VP 381, VP 381C, VP 381D, VP 381F, VP 381M or VP 381N) over a 96, 384 or 1536 well source plate "a" with the single alignment hole side of the device closest to the last row of the plate. Slide the LIBRARY COPIER™ to make sure the plate is seated within the device and therefore registered.
7. Hold a sterile 96, 384 or 1536 MULTI-BLOT™ Replicator at a 45° angle to the source plate LIBRARY COPIER™ and 20° angle to the left alignment hole. Place the right guide pin into the right alignment hole. Then slowly decrease the 20° angle and place the left guide pin into the left alignment hole. Then rotate the Replicator forward until guide pins line up vertically and slide down the alignment holes and the Replicator pins drop into the wells (see diagram).
8. Hold the LIBRARY COPIER™ in one hand and mix contents of wells by raising and lowering the Replicator 3X through the meniscus with the other hand. The speed at which the pins are removed from the wells on the final withdrawal will affect the size of the hanging drops and the amount of liquid on the sides of the pin. Removing the pins quickly from the source plate produces large, hanging drops on the tips of the pins and more liquid on the sides. We recommend removing the pins on the final withdrawal at a slow even speed each time (~.5 cm/sec). This action produces very uniform transfers from plate to plate and reduces the amount of liquid hanging on the tip and sides of the pins. Performing this operation with the LIBRARY COPIER keeps the pins in the middle of the well and prevents hanging drops from being accidentally touched off.
9. Lift the Replicator from the source plate and holding it at a 45° angle to the first MULTI-PRINT™ and at a 20° angle to the left alignment holes, place the right guide pin into the "A" position of the right alignment holes. Then slowly decrease the 20° angle and place the left guide pin into the "A" position of the left alignment holes.

10. Rotate the Replicator forward until the Replicator pins rest on the surface of the membrane making the first stamp of blots.
11. Remove the Replicator and place it back in the same source plate.
12. Repeat steps 7 through 10 until all 4 membranes have been blotted at alignment hole position "A."
13. Sterilize the Replicator by dipping in tip lid box (VP 421) containing 10% bleach solution, blot on lint free blotting paper (VP 522) and then dip and blot twice in sterile distilled water in tip lid boxes then dip in isopropanol reservoir, flame and place in new source plate "b."
14. Repeat steps 7 through 10 on all the membranes at registration alignment hole position "B."
15. Repeat step 14 for remaining source plates.
16. Remove MULTI-PRINTS from membranes and hybridize, assay or culture. Replace protective covers on tape of MULTI-PRINT™. Membranes can be cultured on nutrient agar in Nunc, Inc. Bioassay Polystyrene Dishes (VP 411) or on Pyrex Baking Dishes (VP 412).

The VP 430 reading mat can be used after assay to determine the plate # and well position of spots on the membrane

Four alignment hole pattern for 384 pin replicator

left set
 A B
 ● ●
 ● ●
 C D

right set
 A B
 ● ●
 ● ●
 C D

Nine alignment hole pattern for 96 pin replicator

left set
 A B C
 ● ● ●
 D E F
 ● ● ●
 G H I
 ● ● ●

right set
 A B C
 ● ● ●
 D E F
 ● ● ●
 G H I
 ● ● ●