

HIGH DENSITY COLONY INOCULATION ON OMNI TRAYS

1. Prepare 1.5% nutrient agar plates on a flat level surface. Pour 35 ml of nutrient agar into either Nunc Omni Trays or Omni Tray lids. **DO NOT MOVE THE TRAY UNTIL IT HAS SOLIDIFIED.** This will give you a very flat surface and all the pins will touch the agar surface. Invert the plates as soon as they solidify to minimize condensation on the agar surface. After the plates reach room temperature use immediately or store by refrigerating inverted in plastic bags or Tupperware®. If condensation has collected on the agar surface simply incubate at 30°C until excess fluid has evaporated. The agar surface can be inoculated directly or for hybridization studies by placing a transfer membrane ([VP 504 or 506](#)) on the agar surface. Use care not to trap air bubbles under the membrane. Air bubbles can be removed using a sterile glass rod with a 90° bend. An alternative transfer membrane support is Wicking Paper (VP 521) soaked in a nutrient broth. The wicking paper has the advantage of producing a "cleaner transfer membrane" which has a lower nonspecific background binding in hybridization assays.
2. Place a LIBRARY COPIER™ (VP 381N) over a 96 or 384 well source plate with the set of single alignment holes closest to the "H" or "P" row on bottom of the plate ([see COLONY COPIER™ application diagram](#)). Slide the LIBRARY COPIER™ to make sure plate is seated within the device and therefore registered.
3. Place a COLONY COPIER™ (VP 380N) over a Nunc Omni Tray or lid. Slide the COLONY COPIER™ to make sure the Omni Tray or lid is seated within the device and therefore registered. Position the COLONY COPIER™ with the two sets of four alignment holes closest to the side of the Omni Tray or lid with the two cut corners ([see diagram](#)).
4. Hold a sterile 96 or 384 FP series 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).
5. 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 to re-suspend settled microorganisms. 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.

6. Position the Replicator over the COLONY COPIER™. Hold the Replicator at a 45° angle to the COLONY COPIER™ and at a 20° angle to the left set of 4 alignment holes. Place the right guide pin into the right "A" alignment hole. Then slowly decrease the 20° angle and place the left guide pin into the left "A" alignment hole ([see diagram](#)). Then rotate the Replicator forward until guide pins line up vertically and slide down the alignment holes and the Replicator pins rest on the surface of the membrane or agar. Gently tap the Replicator - The pins won't penetrate the membrane or the agar surface unless great force is applied.
7. Holding the COLONY COPIER™ down, remove the Replicator. Sterilize it by dipping in 10% bleach, blotting on lint free paper (VP 522) rinsing twice in sterile distilled water, blotting and dipping in isopropanol followed by flaming. Then repeat step 4 in a new source plate "B" and repeat steps 5 and 6 in the same Omni Tray using alignment hole "B."
8. Remove and sterilize the Replicator and repeat step 4 in a new source plates as necessary.

The LIBRARY COPIER™ and the COLONY COPIER™ may be sterilized by wiping with alcohol or bleach.

Nalge Nunc Omni Trays ([VP 413](#)), Nalge Nunc 384 well plates ([VP 414](#)), Nalge Nunc 96 well plates ([VP 416](#)), precut Nytran Nylon Transfer Membranes ([VP 504](#)), precut Optitran nitrocellulose transfer Membranes ([VP 506](#)) and precut Wicking Paper ([VP 521](#)) may be purchased from V & P Scientific, Inc.

Four alignment hole pattern for 384 pin replicator

left set
 A B
 ● ●
 ● ●
 C D

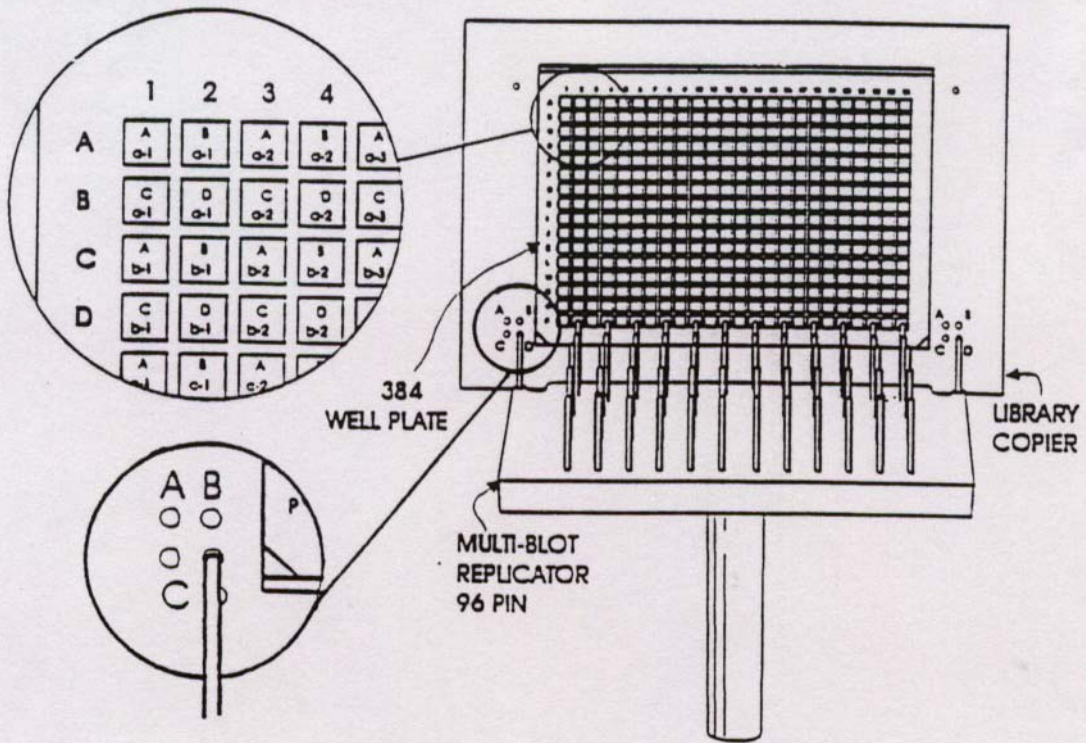
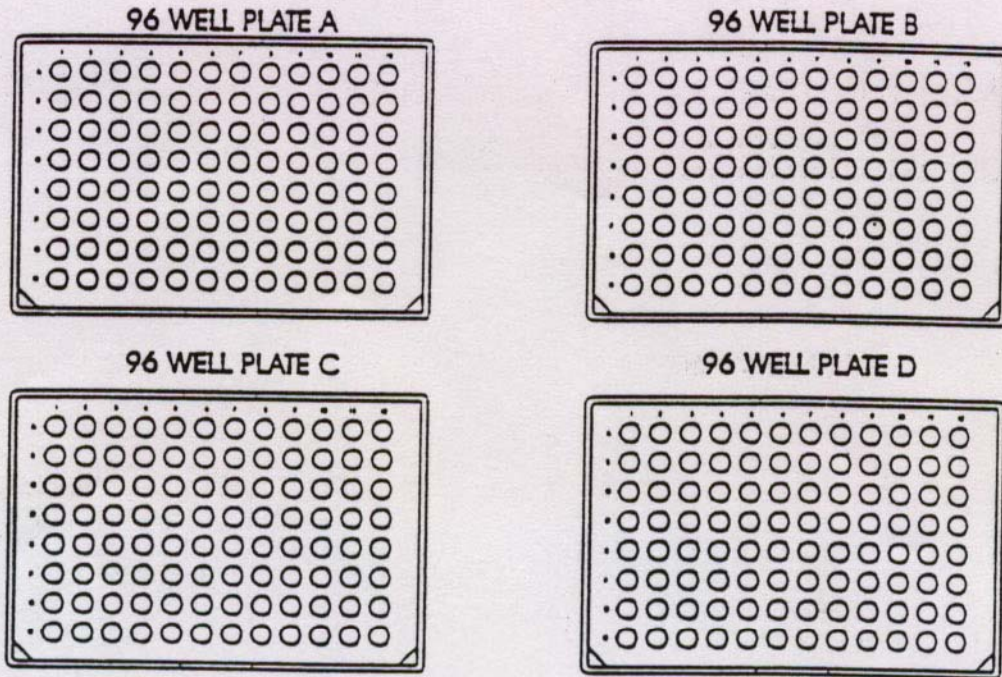
right set
 A B
 ● ●
 ● ●
 C D

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
 ● ● ●

CONDENSING FOUR 96 WELL PLATES INTO ONE 384 WELL PLATE



COLONY COPIER APPLICATION

