V&P SCIENTIFIC, INC.
Innovators in Liquid Handling, Arraying and Mixing

TECHNICAL NOTE 31

TIPS ON CARE AND USE OF FLOATING PIN REPLICATORS

Disinfecting:

The Replicators can be sterilized by hot air oven, treating in 10% bleach or isopropyl alcohol.

The pins can be cleaned between source plates by dipping briefly in a 10% bleach solution, followed by a series of two sterile dH2O baths then a 99% isopropanol bath (all in tip lid boxes). Between baths, remove the liquid from the pins by blotting on a Lint-Free Blotting Paper (VP 522). This blotting step is very important to reduce carry over. It is important that the pins be dry before going into the next source plate. The pins can be air-dried or dried using a portable hair drier.

Hydrogen peroxide may also be used to disinfect the pins as long as it is rinsed off with distilled water. The % of hydrogen peroxide necessary will vary between applications.

It is important that the liquid in the baths not get into the pin slide holes in the float plate as that will interfere with the pins floating freely. We recommend only filling the baths with just enough liquid to cover the "high water mark" of the liquid in the microplates. Thus it is best not to "float" the pins when cleaning them.

Care:

Before each day's use we recommend that the pins be cleaned with VP 110 Pin Cleaning Solution, which is designed to clean and condition the stainless steel pins. If the pins should become coated with organic material they can be mechanically cleaned with the VP 425 brush and Ivory dish detergent. For heavy duty cleaning we recommend using ultrasonic bath with MICRO 90®, from Cole Parmer®, at a 1/100 dilution in the ultrasonic bath. When using an ultrasonic bath, hold the Replicator in the bath without letting the pins touch the bottom of the reservoir (the vibrating bottom surface of the sonicator reservoir may damage the pin tips). The Cole Parmer Catalog #P-08857-02 Ultrasonic Cleaner is ideal for cleaning the Replicator pins. It is not necessary to clean the Replicator in an ultrasonic cleaner if the pins are cleaned with bleach and brushed with detergent after each day's use.

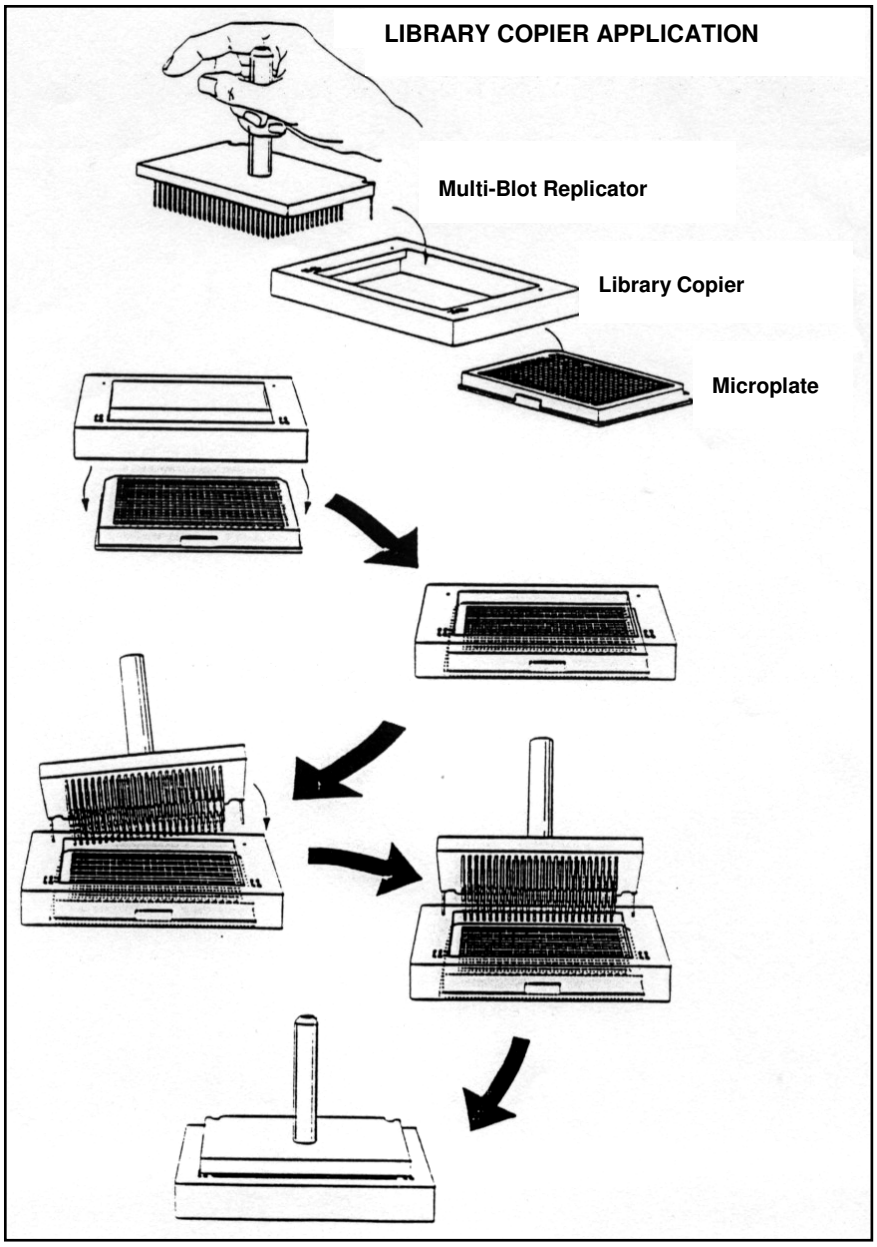
Use:

1. Place a LIBRARY COPIER™ (VP 381, VP 381C, VP 381D, VP 381F, VP 381M or VP 381N) over a 48, 96, 384 or 1536 well source plate 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.

2. Place a second LIBRARY COPIER™ (VP 381, VP 381C, VP 381D, VP 381F, VP 381M or VP 381N) over a 48, 96, 384 or 1536 well reception plate 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 centered and seated within the device and therefore registered.

3. Hold a sterile 96, 384 or 1536 Floating 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. Slowly decrease the 20° angle and place the left guide pin into the left alignment hole. 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).



4. Hold the LIBRARY COPIER™ in one hand and mix contents of wells by raising and lowering the Replicator three times 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 (~0.5 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 on the sides of the well.

5. To deliver to another microplate with liquid in the wells, place the Replicator in the LIBRARY COPIER™ as described for the source plate, dip and raise the pins three times through the recipient plate's meniscus. Blot the pins on Lint-Free Blotting Paper to reduce carry over if the pin tool is put back into the source plate or to the wash bath.

6. Repeat steps 3-5 for each replicate plate as needed.

7. To deliver to a membrane, have a soft absorbing pad under the membrane (VP 522, VP 521, or VP 521V) and press gently on the replicator. We recommend using one of our MULTI-PRINT Registration systems (VP 382, VP 382B, VP 382D or the FU-MEEI registration system) to prevent the pins from making skid marks on the membrane.

8. Repeat steps 3, 4 and 7 for each replicate blot as needed.

Test your Replicator using dye (5% red food coloring) in 10 mM Tris, pH 8.0 with 0.005% Sarcosyl or Tween 20 as wetting agents in water.

Note: If you are having problems with varying volumes of liquid on the tips, clean the pins with the VP 110 Pin Cleaning Solution. Cleaning with the V&P Pin Cleaning Solution will reduce the surface tension on the pin and this will solve 99% of your pin loading problems. Also, you can add 0.005% Sarcosyl, Tween 20, protein or carrier DNA to lower the liquid surface tension.

WARNING

- Do not soak in bleach solutions for a long period of time as this can corrode the stainless steel pins.
- Do not soak in deionized water as this can corrode the stainless steel pins.
- Do not heat the pins directly in the Bunsen Burner flame.

The VP 110 Pin Cleaning Solution contains a dilute acid solution which can strip the protective anodized surface (black in color) off the anodized aluminum Replicator bases or float plates. If VP 110 Pin Cleaning Solution contacts an anodized surface, quickly rinse it off with water. The bottom float plates on all V&P's Replicators are now protected by a Ni-Lube coating (gray in color), which is not damaged by acid solution.