

How To Load MicroArray Slides Into the HybStation and Hyb4

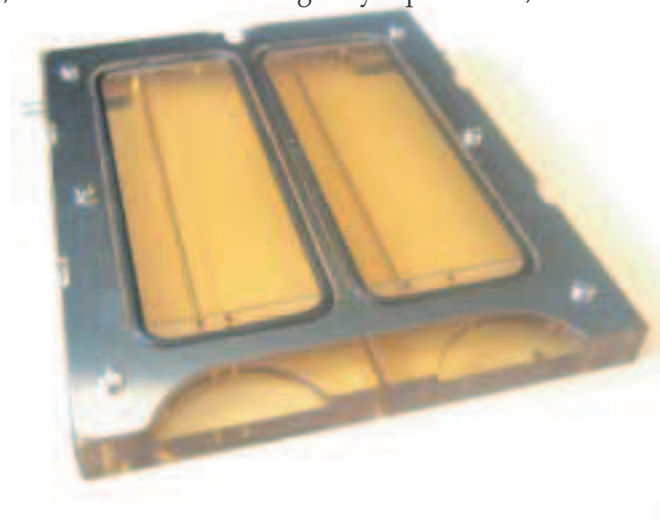
Digilab Technical Note

INTRODUCTION

This technical note describes how to successfully load slides into the 21 mm slide covers for the HybStation and Hyb4. Failure to follow this procedure may result in cracked slides or improper sealing around the array.

PROCEDURE

1. Clean all components of the slide module by rinsing with tap water, distilled water and finish by rinsing in 95% or 100% ethanol.
2. Blow all pieces dry with a compressed air source. Make sure to blow any residual fluid out of the channels in the plastic lid. Take care not to lose the small red O-rings from the top edge of the slide cover.
3. Make sure the stainless steel shim is flat against the plastic of the lid. The six screws holding it in position should be screwed in completely - but not over tightened. A useful guide is to screw them until just tight and then loosen slightly. Care should be exercised when handling the slide covers to make sure the shim is not damaged.
4. Place a rubber O-ring seal in each half of the slide lid. Lay the seal flat on top of the groove in the lid and gently press it down into the channel. Try not to twist it as you insert it.
5. Check the shim after this, since sometimes inserting the O-ring can distort the shim. Look at it at eye level and make sure it is flat. If not, loosen the screws and gently tap it down, then retighten the screws.



DNA Shearing

Colony Picking

Loading Slides

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Cell Growth

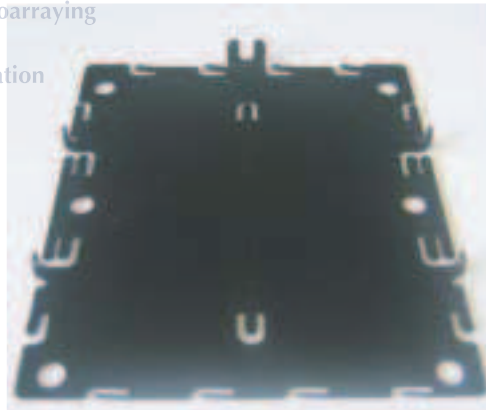
DNA/RNA Synthesis

6. Lay the black metal slide holder on a stable, flat surface.

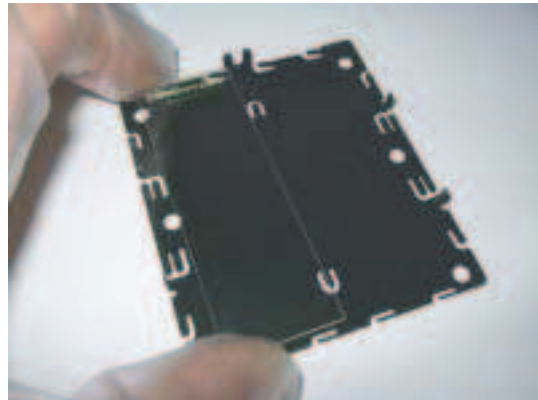
Microarraying

Hybridization

Scanning



7. Place the slides to be hybridized into the black metal slide holder. In some cases, large labels were found to interfere with effective sealing of the O-ring gaskets. Therefore, it is recommended that bar codes or other labels be placed on the bottom surface of the slide with the printed array facing up. These labels should also be placed at the "north" end of the metal plate, where the "U" shaped guide sticks up.

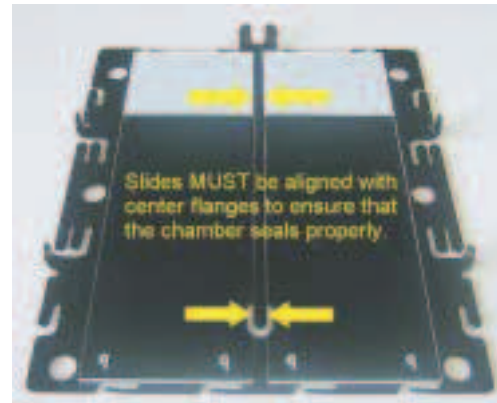


8. Repeat for the other slide position. You must load a slide of equal thickness on each side, even if you are only hybridizing one array. Failure to do this will result in cracking of the array slide.

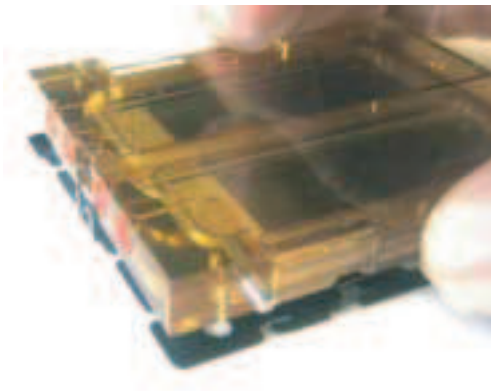


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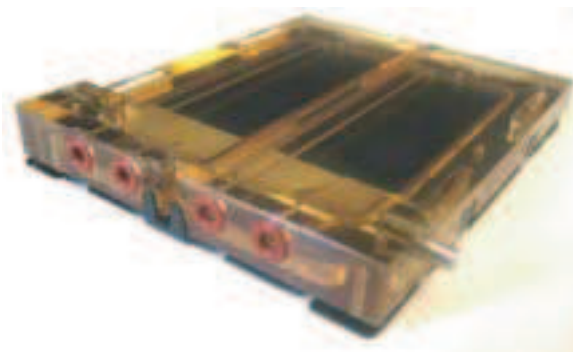
9. Make sure that the slides are justified to the center of the black metal plate. This is **EXTREMELY IMPORTANT** since there is very little room for error. The image below show slides loaded correctly.
10. If you are having difficulty keeping the slides in position on the metal plate, try putting a small (e.g. 50 μ l) drop of distilled water on the plate prior to putting the slide on it. The capillary action will help keep the slide in position.



11. Place the plastic slide cover on top of the slides, while maintaining the position of the slides in the holder. Use the guides on the top edge and the sides of the black metal plate to ensure that the lid is aligned correctly.



12. Hold this sandwich you have made tightly together with your thumb and fingers on the sides of the slide lid and base plate. Carefully lift the entire assembly, holding the side guides to keep the assembly together.



Loading Slides

DNA/RNA Synthesis

13. Place the slide assembly into position on the HybStation. Make sure you maintain the sandwich as a unit - the object here is to prevent the slides and seals moving from the position you placed them in.
14. Once the slide assembly is in position, place two fingers on the slide lid to hold it in position as you lower the clamp. Obviously, you need to move your fingers as the clamp comes down, but then place them back on the slides once the clamp has passed.
15. At this point, you can stop holding the slides and just press down on the horizontal bar across the bottom of the clamp to keep the slide module assembly together.
16. Insert the clamp screw into the screw-hole in the base (making sure it is in the center) and screw the knob down tight.
17. When not in use, lids should be stored wrapped in tissue or bubble wrap to prevent damage to the shims.
18. O-rings should be replaced often. We recommend using them for 5 - 10 hybridizations, and reshaping them after each one. To do this, remove the O-rings as soon as possible after the experiment and place them in boiled distilled water for 3-5 minutes. Allow them to dry on a paper towel. They should regain their circular shape. If they do not, they should be thrown out. Another indication that they have reached the end of their useful life is that they fit into the groove in the lid, but there is still some "left over", indicating that the O-ring has stretched.

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