

Construction of High Density Array of Libraries on Agar or Nylon Membranes

Extensive screening of recombinant clones is a prerequisite to developing a representative library.

The Thermo Scientific Nunc Replication System offers a simplified procedure for the construction of arrayed clones which are to be screened.

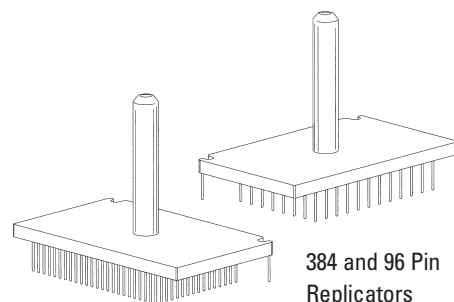
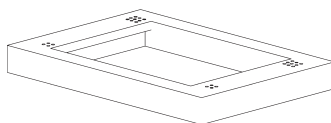
- The Thermo Scientific Nunc OmniTray Copier with two sets of 9 alignment holes on one side of the frame, allows a 3 x 3 array for the 96 Pin Replicators yielding 864 arrayed clones.
- The two sets of four alignment holes on the other side of the frame enables a 2 x 2 array for the 384 Pin Replicators yielding 1536 arrayed clones.

Nunc™ OmniTray™ containing 35 mL of growth medium with 1.5% agar is optimal for arraying bacterial libraries. In the case of YAC clones, specific medium with 1.8% agar provides an appropriate matrix.

For mono overlapping and distinct arrays, it is best to utilize an agar surface that has been stored overnight at 4°C. Prior to use, the agar surface should be dried with the lid opened in a ventilated flow cabinet. Ensure that the agar surface is not dried to the point of developing cracks on the surface.

Bacterial and YAC clones may also be grown on a variety of membranes placed on the surface of media containing agar in the OmniTray. The Thermo Scientific Nunc Pall Biotyne B (precut to fit OmniTray) nylon membrane may

OmniTray Copier



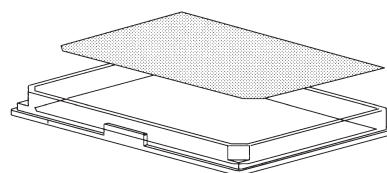
be sterilized wrapped in foil and autoclaved for 15 minutes in an unwrapped (non-drying) cycle.

3 x 3 Array with 96 Pin Replicators and Thermo Scientific Nunc 96 MicroWell Plates

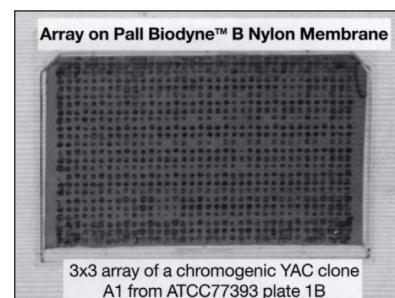
1. Place the OmniTray Copier with the nine hole pattern closest to the user, over either an agar-filled or agar-filled with membrane overlaid OmniTray. Slide the Copier back and forth and side to side to ensure complete seating of the OmniTray in the OmniTray Copier.
2. Ensure that the libraries formatted in the nine 96 well source plates are thoroughly thawed and laid out in order A to I. For consistency, the notched ends of both the

OmniTray and the 96 well source plates should be oriented closest to the user.

3. Sterilize the 96 Pin Replicator by dipping the pins in successive baths of chlorine bleach, water and alcohol; flame the pins and cool to room temperature prior to use.
4. Place the MicroWell™ Plate Copier over source plate A with the single alignment hole closest to the user. Place the right guide pin or the 96 Pin Replicator in the right alignment hole and the left guide pin in the left alignment hole of the MicroWell Plate Copier.
5. Remove the Replicator and transfer the inoculum to the OmniTray in position A as follows: Place the right guide pin in the right alignment hole



OmniTray w/membrane



Array on Pall Biotyne™ B Nylon Membrane

3x3 array of a chromogenic YAC clone
A1 from ATCC77393 plate 1B

A and the left guide pin in the left alignment hole A of the OmniTray Copier. Rotate the Replicator until the guide pins are vertical and slide easily into the alignment holes and the Replicator pins drop to the agar or membrane.

6. Gently tap the base of the Replicator to evenly displace the fluid from the pins.
7. Hold the OmniTray Copier down with one hand, remove the Replicator and sterilize the pins by repeating step 3, as indicated above.
8. Follow steps 4 to 6 to array source plates B to I on the same OmniTray by placing the guide pins into alignment holes B to I respectively. Thus, inoculum from plate B is arrayed on position B, C on position C and so on.
9. This allows 864 clones to be manipulated on a single OmniTray.

10. Remove the OmniTray Copier, and let the transferred inocula dry on the agar or membrane surface. Incubate the OmniTray at the appropriate incubation temperature depending upon whether bacteria or yeast clones were arrayed.

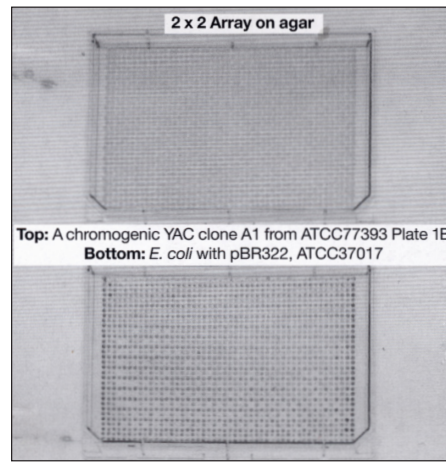
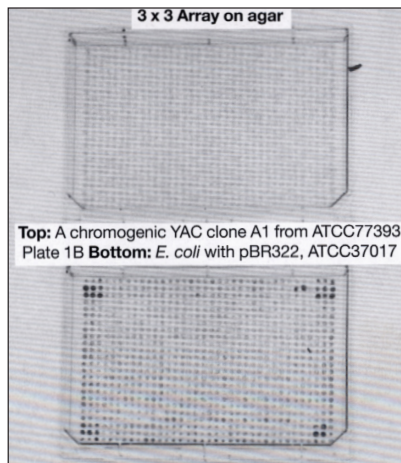
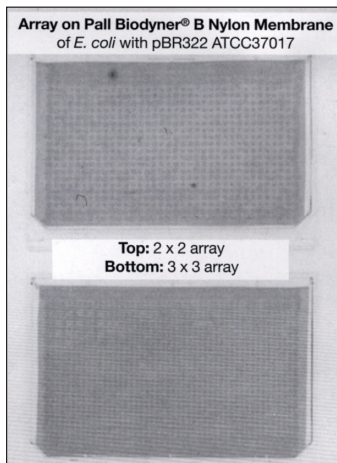
2 x 2 Array with 384 Pin Replicators and 384 MicroWell Plates

Using the OmniTray Copier with a 384 Pin Replicator and four 384 MicroWell plate clones, 1536 clones can be arrayed directly or on membrane overlaid agar in an OmniTray. A 2 x 2 array can be accomplished by orienting the OmniTray Copier with the four alignment holes closest to the user.

Thus source plates A to D may be arrayed by aligning the guide pins in alignment holes A to D as described in steps 3 to 7, but using 384 MicroWell plates and a 384 Pin Replicator.

Notes and Observations

1. In addition to the obvious arrays of bacterial or YAC clones demonstrated here, the precut Pall Biodyne™ B membrane may also be used for dot blotting a high density array of DNA or RNA preparations. For this application however, the membrane should be overlaid absorbant paper precut to the size of the OmniTray.
2. Microbial lawns grown on OmniTray can also be used for screening antibiotics or drugs delivered using the Replicator Pins.



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