

Picking and Arraying Libraries with the Thermo Scientific Nunc Replication System

Screening a library for building a repository of unique clones requires individual clones to be picked and transferred into individual wells of a Thermo Scientific Nunc 96 or 384 MicroWell plate for culture and subsequent screening.

The tedious task of using a toothpick for each of the thousands of clones can be simplified with a high density multiple inoculation tool, such as the Thermo Scientific Nunc 384 Pin Replicator. Clones initially plated on an Thermo Scientific Nunc OmniTray tray can be picked by the hundreds with a Nunc™ 384 Pin Replicator. In conjunction with the Replication System this allows arraying of as many as 1,536 clones. These arrays can simultaneously be transferred to four pre-filled 384 MicroWell™ archival plates and into an

OmniTray™ tray filled with medium containing agar and overlaid with a sterile pre-cut Thermo Scientific Nunc Biodyne B nylon membrane.

The 384 MicroWell plates can be cultured and frozen as a master library. The OmniTray tray should be incubated to allow growth of clones and subsequently screened for unique clones.

Bacteriophage clones or plaques can be arrayed on target lawns grown on agar in OmniTray trays.

Plating Recombinants at High Density in an OmniTray

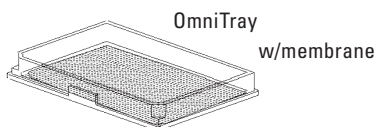
Following ligations, plate the transformants at very high density (approximately 1500 to 1600 clones) in an OmniTray tray containing 35 mL of growth medium with 1.5% agar.

Pick Clones with 384 Pin Replicator and Transfer into 384 MicroWell Plates and OmniTray

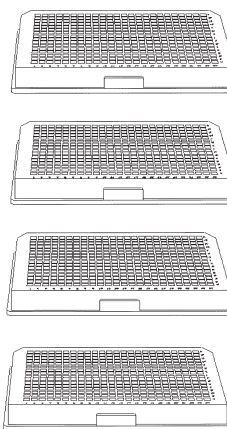
1. Place an OmniTray Copier with the four hole pattern toward the user, over the OmniTray tray containing the recombinants plated at high density (source plate).
2. Lay out four recipient 384 MicroWell plates pre-filled with approximately 80 μ L of specific growth medium and labeled as plates A, B, C and D. Place a MicroWell Copier with the single hole pattern closest to the user over recipient plate A.
3. Place an OmniTray Copier with the four hole pattern toward the user over the recipient OmniTray tray containing 35 mL of media containing agar.

The Replication System

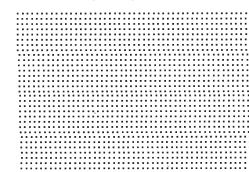
Plate recombinants at high density in OmniTray tray. Culture colonies or plaques until about 1 mm in diameter.



Replicate four 384 MicroWell archival plates and freeze for master library.

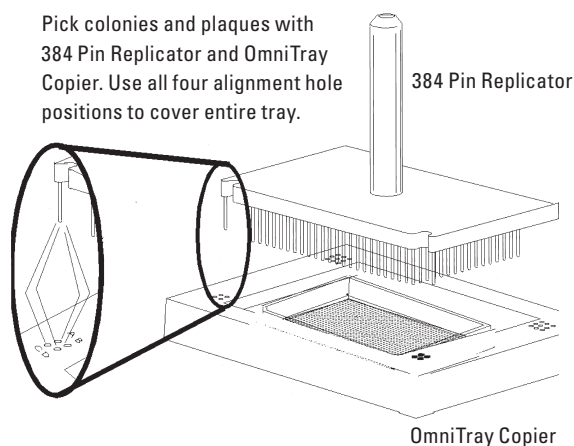


Produce high density colony or plaque arrays onto target lawns or other media in OmniTray trays.



Resulting arrays can be stored at 4°C and reused several times in a 9 week period.

Pick colonies and plaques with 384 Pin Replicator and OmniTray Copier. Use all four alignment hole positions to cover entire tray.



4. Sterilize the 384 Pin Replicator by dipping in successive baths of chlorine bleach, water and alcohol. Subsequently flame the pins and cool to room temperature prior to use.
5. To transfer the first set of 384 clones plated on the OmniTray tray (source plate), place the right guide pin of the 384 Pin Replicator in the right A alignment hole and the left guide pin in the left A alignment hole of the OmniTray Copier. Rotate the Replicator until the guide pins are vertical and slide down the alignment holes. The Replicator pins will drop to the clones plated in the OmniTray tray.
6. Ensure that the clones are indeed touching the pins. Hold the OmniTray Copier down with one hand and remove the 384 Pin Replicator.
7. Transfer the clones into the position A of the recipient OmniTray tray, by positioning the left and the right guide pins into the left and the right alignment hole A. Once the pins of the Replicator touch the agar or membrane surface, gently tap the base of the Pin Replicator. Now the first set of 384 clones are arrayed in position A.
8. Hold the OmniTray Copier down with one hand, remove the 384 Pin Replicator, and transfer the remnants of the inoculum in the recipient 384 MicroWell Plate A. The left and the right guide pins of the replicator are aligned into the left and right alignment hole respectively of the MicroWell Copier. Mix the well contents gently by carefully raising and lowering the Replicator.
9. Remove the 384 Pin Replicator and sterilize by repeating step 4.
10. Remove the MicroWell Copier from 384 MicroWell Plate A and place it over the recipient 384 MicroWell Plate B.
11. Transfer the next set of 384 clones from the source plate by positioning the left and right guide pins of the 384 Pin Replicator in the left and right alignment hole B of the OmniTray Copier over the source plate. Rotate the Replicator until the guide pins are vertical and slide down the alignment holes. Again the pins will touch the next set of clones.
12. Transfer these clones to position B of the recipient OmniTray tray overlaid with the OmniTray Copier. The same clones are transferred into the 384 MicroWell Plate B by following the steps as outlined in step 8 above.
13. Repeat sterilization of 384 Pin Replicator.
14. The remaining third and the fourth sets of 384 clones may be similarly transferred to positions C and D of the recipient OmniTray tray and the C and D recipient 384 MicroWell plates.
15. Incubate the recipient 384 MicroWell plates and the recipient OmniTray tray to allow growth of the clones.
16. Freeze the 384 well cultures to retain as master library. The membrane with arrayed clones can now be processed for screening clones.

Note:

Prior to freezing the 384 MicroWell plate containing recombinant libraries, it may be sealed with Aluminium Sealing tape.

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