

# Replication of Recombinant Libraries with the Thermo Scientific Nunc Replication System

The management of a large library of clones is simplified by the ability to replicate and array libraries in 96 and 384 well formats. Analysis of these libraries involves the replication of libraries from

- 96 well to 96 well
- 384 well to 384 well also their conversion from
- 96 well to 384 well
- 384 well to 96 well formats respectively

These protocols demonstrate that both the 96 well and 384 well plates are acceptable culture vessels for the growth of a variety of clones such as, bacteria carrying pBR322, a cosmid and a YAC library.

## Replication of Libraries

Ensure that the 96 well or 384 well source plates are completely thawed. For consistency, the notched ends of both the reception and the source plate should be oriented toward the user.

Sterilize the 96 and the 384 Pin Replicators by dipping the pins in successive baths of chlorine bleach, water and alcohol; flame the pins and cool to room temperature prior to use.

Use the 96 Pin Replicator for the 96 well source plate and the 384 Pin Replicator for the 384 well source plate.

1. Place the first Thermo Scientific Nunc MicroWell Plate Copier over the source plate with the single alignment hole closest to the user. Slide the Copier back and forth and side to side to

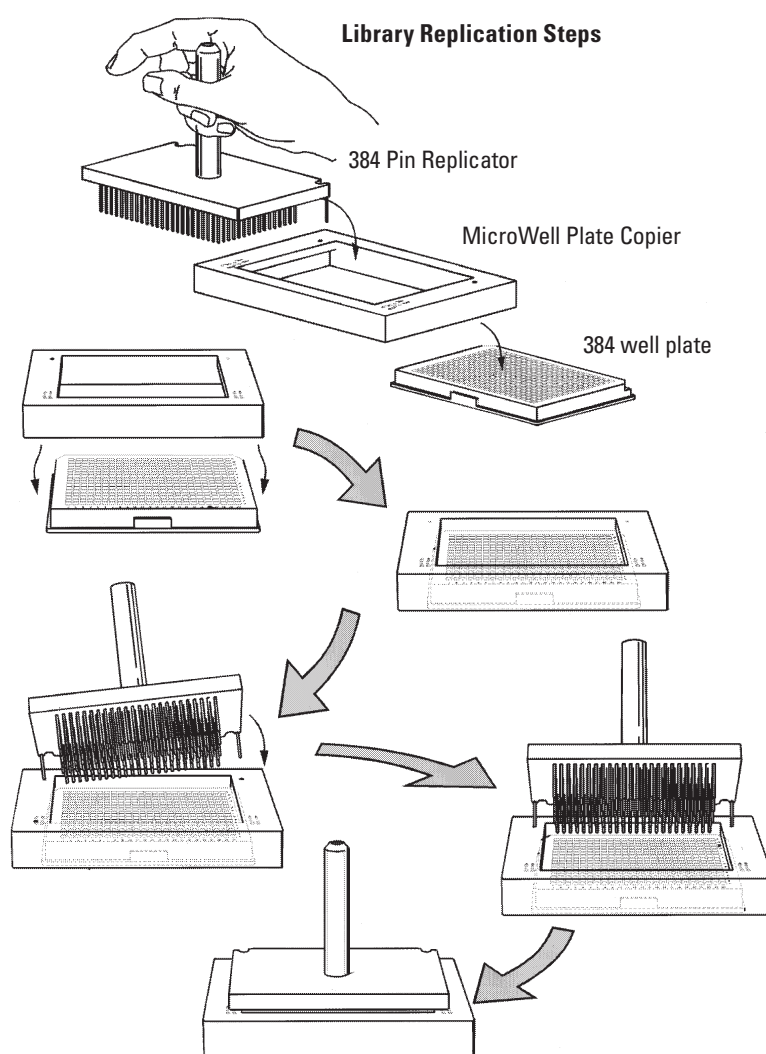
ensure complete seating of the source plate in the Copier frame.

2. Place a second Nunc™ MicroWell™ Plate Copier with the single alignment hole closest to the user, over either the 96 well reception plate pre-filled with 250-300  $\mu$ L library-specific medium or with 70-75  $\mu$ L

medium in the 384 well reception plate.

3. Replication of clones is accomplished with the following steps.

- From the source plate, place the right guide pin of the Replicator in the right alignment hole and the left



guide pin in the left alignment hole of the first MicroWell Plate Copier.

- Rotate the Replicator until the guide pins are vertical and slide easily into the alignment holes and the Replicator pins drop into the plate wells.
  - Remove the Replicator while holding the MicroWell Plate Copier down with one hand.
  - Transfer the inoculum from the Replicator pins into the reception plate.
  - Place the right guide pin of the Replicator with the inoculum in the right alignment hole and the left guide pin in the left alignment hole of the second MicroWell Plate Copier holding the reception plate.
  - Rotate the Replicator until the guide pins are vertical and slide easily into the alignment holes and the Replicator pins drop into the wells of the reception plate.
  - Mix the well contents gently by carefully raising and lowering the Replicator with the other hand.
  - Hold the MicroWell Plate copier down with one hand and remove the Replicator.
  - Sterilize the Replicator Pins as indicated above.
4. Remove the MicroWell Plate Copier from the 96 or 384 well reception plate and array as many reception plates as required, while ensuring adequate dispersal of the inoculum.
  5. Incubate the reception plates in a humidified 37°C incubator.

### Conversion of an Array of Recombinant Clones from a 96 well format into a 384 well format

Conversion of four 96 well libraries of bacterial, cosmid or YAC clones into one 384 well plate can be accomplished with a 96 Pin Replicator and a MicroWell Plate Copier.

1. Position the MicroWell Plate Copier over the pre-filled 384 well reception plate with the four hole pattern closest to the user.
2. Replicate the first 96 well library by aligning the guide pins from the 96 Pin Replicator in position A of the MicroWell Plate Copier.
3. Ensure proper dispersal of inoculum then flame sterilize the Pin Replicator.
4. Perform all subsequent transfers of the libraries from source plates B, C and D exactly as above by aligning the Replicator guide pins in positions B, C and D respectively of the MicroWell Plate Copier around the 384 well reception plate.
5. Block pools can be developed for both storing and screening recombinant libraries.

### Notes and Observations

The bacterial system that served as a model was a strain of *E. coli* carrying pBR322 (ATCC 37017). It was grown in the TYGPN medium following incubation at 37°C without agitation (Short Protocols in Molecular Biology, 2nd Edition; Edited by Ausubel et al. Published 1992, Green Publishing Associates

& John Wiley & Sons). A final density of  $5 \times 10^8$  to  $1 \times 10^9$ /mL was achieved under these conditions within 24 hours of incubation.

A cosmid library of human chromosome 2 in vector Lawrist 16 developed at the Lawrence Livermore Laboratory served as a model. Incubation of the cosmid library in LB medium containing 20 µg/mL Kanamycin at 37°C without agitation enabled the final density of 6 to  $8 \times 10^8$ /mL to be achieved within 24 hours post inoculation. Thus an inoculum of approximately 1 µL was sufficient for both bacterial and cosmid culture.

Furthermore, a YAC library of human Y chromosome from plate 1B of ATCC 77393 library could be easily replicated in a 96 well plate containing 125 µL or a 384 well plate containing 45 µL of AHC medium following incubation in a humidified environment at 30°C and shaken on a Titer Plate Shaker, Model 4625, with a speed corresponding to position 2.5. Specifically, studies on clone A1 demonstrated that it grew to a density of approximately  $7 \times 10^7$ /mL in both the 96 well and 384 well formats within 48 hours post inoculation.

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