

Transferring 96-well PCR reactions to 384-well microtitre plates

Overview

Gene-specific cDNA clone-sets are amplified by PCR in 96-well microtitre plates and then transferred to 384-well plates for printing. The 96-well microtitre plates are transferred to 384-well plates using an MWG-Biotech Roboseq 2500 Liquid Handling Robot (LHR). Two people perform this task to ensure that no mistakes are made at this critical stage of the production process.

Equipment and reagents

- MWG-Biotech Roboseq 2500 Liquid Handling Robot
- PCR amplified cDNA clones in 96-well formatted plates
- 384-well microtitre plates (Genetix; Cat. No. X6004 or Cat. No. X7020)
- White polyester Microtitre plate laser labels (Computer Imprintable Label Systems LTD; Cat. No. L15-9700P-10-NP)
- 70% Ethanol
- MilliQ water
- Hettich Rotina 35 microtitre plate centrifuge
- Adhesive PCR Film (Abgene; Cat. No. AB-0558)

Procedure

Before re-arraying

1. Print code 39 formatted barcodes using the template file "array_bc.lbl" with the LabelWorks application. These will be used to identify each of the 384-well plates after re-arraying.
2. Place barcodes on clean 384-well Genetix plates. Barcodes should be placed horizontally along the 'A1 to P1' axis of the plate. Keep these plates stored in a safe, clean and dust-free location.
3. Ensure all required DNA stock plates (96-well format) are present in the -80°C freezer. Take note of which manufacturers' plates the DNA is stored in. This information will be required when testing the LHR.
4. Make, autoclave and filter purify the spotting buffer. Store at room temperature to prevent precipitation.
5. Clean the exterior and interior of the LHR using the Dyson vacuum cleaner and then wipe with 70% Ethanol.
6. Replace the LHR flush water with a fresh batch of water. Clean tips using at least 3 cycles of the "Flush_Long.rss" wash script. This will perform 400 tip flushes per script run and will therefore remove any contaminants.
7. Test the LHR to ensure that it is performing correctly by transferring water from 96-well (positions 1 to 4) to 384-well microtitre plates (position 5). This test should be repeated for each 96-well plate type noted in step 3. The test script to use is called "DGC_Rearray_28-06-02.rss".
8. Practise re-arraying to ensure everything is fine. Save the fully evaluated LHR re-arraying script under a different name. This script will then be used to re-arraying the clone-set.

Whilst re-arraying

9. Clean tips using at least 3 cycles of the "Flush.rss" wash program; this will perform 40 tip flushes per run and will ensure the tips are clean before re-arraying. Once completed, open the LHR re-arraying script you created during step 8.

10. Remove the first four 96-well plates and place in a safe location to defrost. Once the plates have defrosted, spin at 2000 rpm for 5 minutes in a microtitre plate centrifuge.
11. Modify step "006" within the LHR script to be the barcode identity of the 384-plate into which the 96-well plates are being re-arrayed. This is important because the LHR data tracking output file name is defined by this step.
12. Load spotting buffer in the uncooled rack (position 2). Then start the script and follow the on-screen instructions:
 - ◆ Plate seals should be removed as the plate is being loaded into the LHR and not before. Get a second person to confirm the plate seal has been removed correctly.
 - ◆ All plates should be loaded in the LHR such that well A1 is in the top-left corner of each plate position. This should be double-checked by a second person. Place a black mark using a marker pen to define the top left corner of the plate once it is loaded in the LHR.
 - ◆ All data tracking files should be uploaded when requested and this step should be double-checked by a second person.
 - ◆ An experienced person should be present and observing the LHR at all times to ensure correct functioning of the instrument.
 - ◆ Remove the next four 96-well plates and perform step 10 in preparation for the next re-arraying cycle. One run will last for 40 minutes.
 - ◆ As each 96-well plate is finished with, remove from LHR, seal with adhesive PCR film, then store at 4°C.
13. Once the run is completed, remove the 384-well plate. Seal using adhesive PCR film. Spin at 2000 rpm for 5 minutes, and store at 4°C. Place this and the now used 96-well plates at -80°C when the next four 96-well plates are removed.
14. Update the FlyChip internal data tracking system to include all data that defines what has just been performed and how. Lock all fields with data added to prevent overwrite errors.
15. Begin the next cycle of re-arraying by starting at step 11.

After re-arraying

16. Clean the LHR by performing step 6 to ensure the LHR is left in a reasonable state for others to use.
17. Confirm the FlyChip data tracking system has been fully updated and that the information is correct. Two people should independently perform this check. Export all 384-well plate definitions from the LHR database.
18. All relevant data tracking information should be forwarded to the FlyChip Bioinformaticians. When required to do so, work with the Bioinformaticians to produce the definitive text file description of the latest FlyChip clone set.
19. Once the text file definition has been made and has been proven to be correct, inform the FlyChip microarray printing department that the next generation clone-set is ready for use. Also request that all relevant information be placed on the web site.

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