

Library desiccation and rehydration

Overview

Spotting solution in microtitre plates evaporates during printing. The buffer in the wells at the edge, and especially in the corners of each plate, usually evaporates more quickly than the from the wells in the centre. Evaporation therefore causes variations in probe concentration across the plate. These systematic variations can have a detrimental impact on microarray performance, *e.g.*, variable spot signals, variable spot diameters and variable spot morphologies. These problems are best overcome by desiccating and rehydrating plates between print-runs.

Equipment and reagents

- Beckman Coulter Biomek NX^P Liquid Handling Robot (LHR)
- AP96 non-sterile P20 tips (Beckman Coulter; Cat. No. 717254)
- MilliQ water (unmodified probe DNA) or 0.2 µm filtered distilled water (modified probe DNA)
- Hettich Rotina 35 microtitre plate centrifuge
- Adhesive PCR Film (Abgene; Cat. No. AB-0558)
- 70% ethanol
- Horizontal laminar flow workstation (Jencons; Cat. No. 566-031)

Procedure

Desiccation

1. Remove the microtitre plates from the -80 °C freezer and leave to thaw on a desk
2. Switch the laminar flow workstation on and leave for at least 30 minutes before putting the plates inside
3. Once defrosted, centrifuge all plates at 2000 rpm for 2 minutes
4. Remove the adhesive film from the plates and leave to desiccate in the laminar flow workstation, *e.g.*, four days
5. Once desiccated, the plates can then be resealed and stored at -80 °C

Whilst using the LHR

6. Remove the microtitre plates from the -80 °C freezer and leave to thaw
7. Once thawed, centrifuge all plates at 2000 rpm for 2 minutes
8. Clean the exterior and interior of the LHR using the Dyson vacuum cleaner and wipe with 70% Ethanol
9. Open the program "Rehydrate_LIBRARY_YesWash" and home the instrument drives
10. Fill the in-flow wash tank with distilled water and then prime the wash station by switching the FX Device Controller (HV 1) to 'manual', *e.g.*, for 3-5 min.
11. Load the instrument, as directed by the "Rehydrate_LIBRARY_YesWash" program, *i.e.*, fresh box of AP96 P20 tips, reservoir filled with water and the plate to be rehydrated:
 - ◆ The adhesive PCR film should be removed as the plate is being loaded into the LHR
 - ◆ All plates should be loaded in the LHR with well A1 in the top-left corner
12. Start the program and watch to make certain that the LHR is working correctly
13. Repeat steps 11 to 12 until all plates have been rehydrated: as each plate is finished, remove from LHR and affix an adhesive PCR film
14. Clean the LHR to make certain that it has been left ready for others to use

After using the LHR

15. Centrifuge all plates at 2000 rpm for 2 minutes and then incubate at 37 °C for 2 hours to redissolved the probe DNA
16. Clean the LHR to make certain that it has been left ready for others to use
17. Centrifuge all plates at 2000 rpm for 2 minutes and store the plates at -80 °C

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