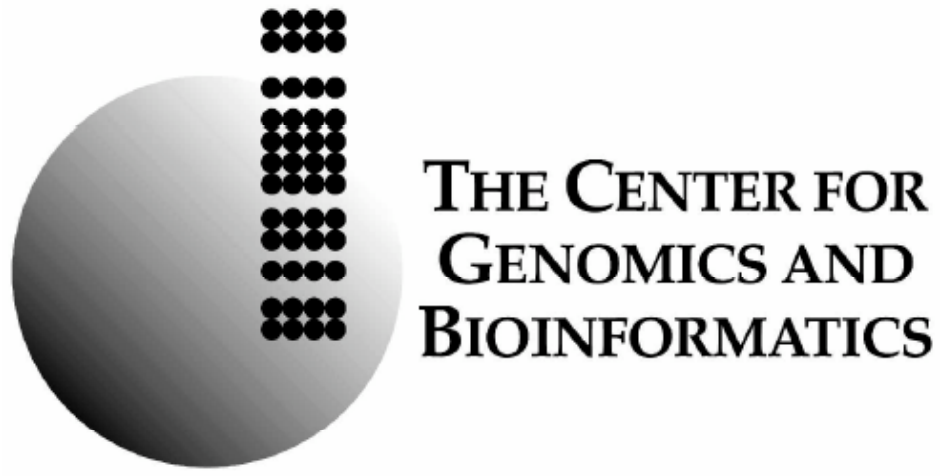

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CGB Technical Report 2006-05

doi:10.2506/cgbtr-200605

Hassel, C. and Klueg, K. 2006. DGRC Vector Standard Operating Procedure 3.0: Transfer of bacterial cultures in a 96-well format to 96-spot CloneSaver™ FTA® cards. *CGB Technical Report 2006-05.* The Center for Genomics and Bioinformatics, Indiana University, Bloomington, Indiana.

DGRC Vector Standard Operating Procedure 3.0: Transfer of bacterial cultures in a 96-well format to 96-spot CloneSaver™ FTA® cards

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1. Fill each well of a sterile 96-well plate with 150 μL LB with appropriate antibiotic (done manually or by robot).
2. Thaw and spin down 96-well Master plate (spin at 2000 rcf for 1 min.).
3. Transfer 5-10 μL of bacteria from 96-well Master plate into 96-well media-filled plate labeled with the corresponding Master plate number/barcode (keep Master plates on ice during the inoculating procedure). Transfer is done manually using a multi-channel pipette, column by column.
4. Cover plate with aluminum seal and shake at 37°C for 72 hours (shaking is optional).
5. Remove plate from shaker after 72 hours and let plate cool to room temperature. If necessary, plate can be placed in refrigerator for 24 hours and spun the next day.
6. Spin plate at 5000 rpm for 5 min to concentrate bacteria.

Steps 7-9 in succession for each column before moving to the subsequent column. Same tips are used for steps 7-9.

7. Aspirate liquid completely from each well manually using a multichannel pipette. Leave approximately 10-15 μL of media behind.
8. Mix bacteria into remaining media in each well using a multichannel pipette – stir with the end of the tips but limit the amount of pipetting up and down to avoid introducing bubbles.
9. Transfer 5 μL of suspended, concentrated culture onto the corresponding column of a 96-spot Whatman FTA® card.

10. Label the Whatman FTA® card with the barcode corresponding to the plate ID number.
11. Resuspend the remaining bacteria with 100 μL LB with appropriate antibiotic + DMSO (900 μL DMSO per 10 mL of LB or 0.09 μL/mL).
12. Cover plate with aluminum seal and store at -80°C as backup.

Acknowledgements:

This project was supported by Grant Number 1 P40 RR017093 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NCRR or NIH.