Fosmid Prep with Arabinose Amplification *- adapted from Lanctôt Lab*

updated 3.17.13

SOLUTIONS AND MATERIAL

LB (15ml per fosmid)
10% Arabinose solution
Chloramphenicol 12.5mg/ml
ClonNat (200mg/ml) (only for use with Sarov fosmids)
QIAGEN (or equivalent) Miniprep kit (buffers P1, P2, N3, PB, PE, EB and spin columns)
Sterile 125ml erlenmeyer

PROTOCOL

Notes.

- *This protocol is designed for pCC1FOS-based fosmids (e.g. WRM series of C.elegans genomic DNA clones) in EPI300 cells (NOT SW105).*
- *The expected yield is between 20 and 40*µg.
- *pCC1FOS-based fosmids encode resistance to chloramphenicol (12.5µg/ml). In addition, fosmids from the Transgenomics project contain resistance to ClonNat.*

1. Working under sterile conditions, pipette 15ml of LB **in a 250ml-erlenmeyer.**
2. Dilute antibiotics: 1000x for Chloramphenicol (final conc.: 12.5µg/ml), 4000x for ClonNat (for Sarov fosmids only, final conc.: 50 µg/ml)
3. Add 15µl 10% Arabinose solution for a final concentration of 0.01%.
4. Seed culture with a single colony and grow for ~17 hours at 37°C with constant shaking.
5. Transfer bacterial culture to 15ml tube. Centrifuge at 4000 RPM for 12 minutes at room temperature. Decant spent medium.
6. Resuspend well in 600µl of buffer P1 (from QIAGEN)
7. Add 600µl of buffer P2 (from QIAGEN). Mix well (NO VORTEX).
8. Incubate **5 minutes at room temperature.**
   Time and temperature are crucial parameters.
9. Add 800µl of buffer N3 (from QIAGEN). Mix well (shaking, no vortex).
10. Transfer to a 2ml eppendorf.
11. Centrifuge at max speed, 10 minutes at room temperature.
    During this centrifugation step, pre-heat EB at 65°C (~100µl of EB per fosmid).
12. Pass all supernatants through a single Miniprep column (should require about three spins, removing passed supernatant after each spin).
13. Wash column with 500µl PB.
14. Wash column with 750µl PE.
15. Centrifuge column 1 min. to remove all traces of PE.
16. Elute with 50µl of EB pre-heated at 65°C (see step 11).
17. Repeat elution (into the same tube) with 30µl of EB pre-heated at 65°C.
18. Measure DNA concentration by spectrophotometry.