Sibley Lab
6-23-2005
KT

COSMID PREP AND SEQUENCING

MATERIALS

QIAGEN plasmid purification kit
Lysozyme 10 mg/ml made with dH2O

Note: the column step is omitted as this was found to decrease yield.

COSMID PREPARATION

1. Culture single colony in 5ml LB + Antibiotics at 37C overnight.
2. Centrifuge at 3000 rpm for 15 min at 4C and discard culture medium. (The cell pellet may be stored at –20C, if the extraction can not been done at the same day.)
3. Resuspend cell pellet completely with 250 ul P1 buffer (containing RNaseA) and add 70 ul of Lysozyme (10 mg/ml) and mix well.
4. Transfer cell mixture to a 1.5 ml tube, and incubate on ice for 15 min.
5. Add 340 ul P2 buffer, mix gently but thoroughly by inverting 6-8 times, and incubate at room temperature for 5 min.
6. Add 480 ul of P3 buffer, mix immediately but gently by inverting 6-8 times, and incubate on ice for 30 min. Mix sample several times during the incubation.
7. Centrifuge 15 min at 14k rpm RTC and equally transfer supernatant to two new tubes, then add 0.7 volume of isopropanol to each tube, mix gently. Incubate at room temperature for 10 min.
8. Centrifuge 30min at 14k rpm, 4C.
9. Remove supernatant, leave 100 ul in tube, centrifuge for another 5 min.
10. Remove supernatant completely.
11. Wash with 500 ul of 70% ethanol, centrifuge 5 min at 14K 4C.
12. Air dry pellet for 3-5 min.
13. Resuspend DNA with 30 ul TE (PH8.0), and pool two tubes together.
14. Place at 4C O/N for complete resuspension.
15. To estimate yield, take 1 ul to check OD and run 1 ul on an 0.7% agarose gel.

Use 4-8 ug Cosmid DNA for each sequencing reaction.

SEQUENCING REACTION

• Big Dye 20 (V 3.1)	6.0 ul
• Universal seq. buffer (SIGMA, S3938)*	2.0 ul
• Template (4-8ug)	3.0 ul
• Primer (5pmole)	1.0 ul
• dH2O	8.0 ul
Final Reaction Volume:	20.0 ul

Cycle Sequencing on the MJ machine

- 96 C 1 min
- 96 C 30 sec
- 50 C 15 sec
- 60 C 5 min
- 30 cycles

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