MICROMON SANGER SEQUENCING

MODIFIED ALKALINE LYSIS/PEG TEMPLATE PREPARATION PROTOCOL

Reagents required:

Chloroform
Deionized water
Ethanol, 70%
GET buffer (50 mM glucose, 10 mM EDTA, 25 mM Tris, pH 8.0)
Isopropanol, 100% (anhydrous)
PEG 8000, 13% (sterilized by autoclaving, rather than by filtration)
Potassium acetate, 3 M, pH 4.8
RNase A (DNase-free), 10 mg/ml
Sodium chloride (NaCl), 4 M
Sodium hydroxide (NaOH), 0.2 N, with 1% SDS (freshly made)

To minimize shearing of contaminating chromosomal DNA, do not use a vortex during this procedure.

1 Pellet 1.5-ml aliquots of culture for one minute in a microcentrifuge at maximum speed.
Note A total culture volume of 4.5 ml can be spun down per tube without changing volumes in the procedure. This allows you to achieve a threefold increase in yield while eliminating the need for extra tubes and additional handling.

2 Remove the supernatant by aspiration.

3 Resuspend the bacterial pellet in 200 µl of GET buffer by pipetting up and down.

4 Add 300 µl of freshly prepared 0.2 N NaOH/1% SDS. Mix the contents of the tube by inversion. Incubate on ice for 5 minutes.

5 Neutralize the solution by adding 300 µl of 3.0 M potassium acetate, pH 4.8. Mix by inverting the tube. Incubate on ice for 5 minutes.

6 Remove cellular debris by spinning in a microcentrifuge at maximum speed for 10 minutes at room temperature. Transfer the supernatant to a clean tube.

7 Add RNase A (DNase-free) to a final concentration of 20 µg/ml. Incubate the tube at 37 °C for 20 minutes.

8 Extract the supernatant twice with chloroform:
a. Add 400 µl of chloroform.
b. Mix the layers by inversion for 30 seconds.
c. Centrifuge the tube for 1 minute to separate the phases.
d. Transfer the upper aqueous phase to a clean tube.

9 Add an equal volume of 100% isopropanol. Mix the contents of the tube by inversion.

10 Spin the tube in a microcentrifuge at maximum speed for 10 minutes at room temperature.

11 Remove the isopropanol completely by aspiration.

12 Wash the DNA pellet with 500 µl of 70% ethanol. Dry under vacuum for 2–3 minutes.

13 Dissolve the pellet in 32 µl of deionized water.

14 Add 8.0 µl of 4 M NaCl, then 40 µl of autoclaved 13% PEG 8000.

15 Mix thoroughly, then leave the sample on ice for 20 minutes.

16 Pellet the plasmid DNA by spinning in a microcentrifuge at maximum speed for 15 minutes at 4–8 °C.

17 Carefully remove the supernatant. Rinse the pellet with 500 µl of 70% ethanol.

18 Resuspend the pellet in 20 µl of deionized water. Store at -20 °C.