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GENE PREP

Application Guide

Plasmid, BAC

I. **Purpose:** To extract plasmid, cosmid, PAC or BAC DNA from cultures of E. coli.

II. **Chemical Principal:** Modified alkaline lysis- organic extraction method.

III. **Protocol Parameters:**

A. Sample Volume: 0.5 -3.0ml* liquid culture of *E. coli* (plasmid)
1.0-4.0ml* liquid culture of *E coli* (BAC)
**Optimal starting volume depends on copy number and the cell density of cultures. Too much starting material results in lower yield and purity.*

B. Maximum Number of Samples: 48 samples per run
192 samples per run (with an optional Tube Unit Stacker)

C. Processing Time: 2.1 hours for 48 samples (including 30 min drying time)

D. Yield: 5.0µg of purified plasmid per 1.0ml culture of plasmid (high copy)
0.3µg of purified BAC per 3.0ml culture of BAC
**Actual yield may vary, depending on the cell density and copy number.*

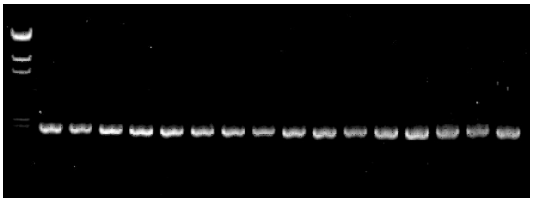
E. Quality: Typical OD260/280 values are >1.8
The extracted DNA can be used directly in downstream applications such as PCR, DNA sequencing, restriction endonuclease digestions and Southern blotting.

IV. **Running the Protocol:**

A. Load Reagents and Samples
B. Select a Protocol
C. Enter Number of Samples
D. Start the Run

V. **Example of Data:**

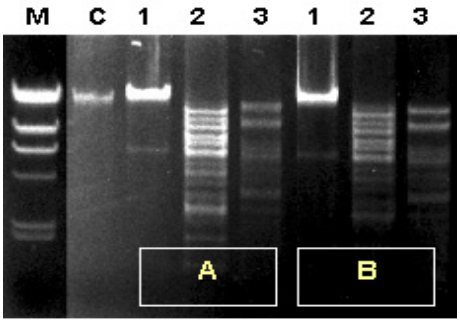
A. Plasmid DNA
Sample: E. coli JM109 / pUC18
2.0ml plasmid culture (2XYT)
Gel Condition: 5 µl of 100ul resuspended DNA
1% agarose gel, 100V x 1hr



B. BAC DNA

Sample: E.coli DH10B/ pBeloBAC clone
Reaction: 6μl of resuspended DNA solution was incubated with 10 units enzyme for 3hrs

A: 3.0ml (2XYT)
B: 1.5ml (2XYT)
M: λ/HindIII size market
C: Undigested
1: Not I 2: EcoR I 3: HindIII



VI. Extraction and Purification Process

Process Site	Purpose	System Process
1. Automated	Concentrate cells	Centrifuge bacterial culture. Discard culture media.
2. Automated	Lyse cells, remove RNA	Add Reagent PB-R1 and PB-R2 and mix.
3. Automated	Remove protein and cellular debris	Add Reagent PB-R3, mix, centrifuge to pellet debris, transfer supernatant to new tube, and discard sample tube.
4. Automated	Precipitate DNA	Add Reagent PB-R4, mix, precipitate DNA, centrifuge and discard supernatant.
5. Automated	Wash DNA	Add Reagent PB-R5, mix, centrifuge, and discard supernatant. Repeat a few times.
6. Automated	Dry DNA	Transfer DNA tube to heating module.
7. Automated	Resuspend DNA	Add Reagent PB-R6, mix and centrifuge.

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