

AutoGenprep 965 Application Guide

Plasmid, Cosmid, BAC, PAC



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

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

III. **Protocol Parameters:**

A. **Samples Volume:** 0.2 – 1.8 ml liquid culture of *E. coli*.

Note: We assume bacterial culture, the density of which is 4.0-7.0 at OD660, may be used. The appropriate starting volume depends on cell density, copy number and so forth. It is important to optimize the culture conditions and starting volume to obtain high yield and high quality of DNA.

B. **Maximum Number of Samples:** 384 samples (4 plates) per run.

C. **Processing Time:** 2.2 hours for 192 samples (2 plates); 3.7 hours for 384 samples (4 plates)
This includes a 25 minutes drying period.

D. **Yield:** 5.0 - 7.0 μg of purified Plasmid DNA per 1.0 ml culture of plasmid.
0.3 - 0.6 μg of purified BAC DNA per 1.0 ml culture of BAC.

Note: The actual yield may vary depending on cell density and other variable factors.

E. **Quality:** Typical OD260/280 values are 1.75 - 1.85

The DNA can be used directly in downstream applications such as fluorescent DNA sequencing, PCR, Southern blotting and restriction endonuclease digestions.

IV. **Running the Protocol:**

A. **Load Reagents and Sample Plates**

B. **Select [Extraction] Protocol**

C. **Enter Number of Samples**

D. **Start the Run**

V. Example of the extracted DNA on the AutoGenprep 965:

A. Extracted Plasmid DNA:

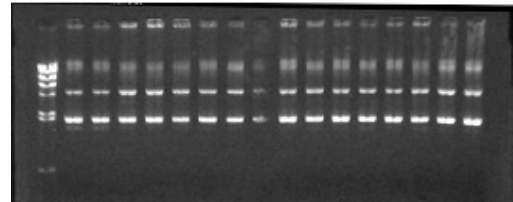
Gel electrophoresis of the extracted Plasmid DNA
(0.7% agarose gel, 100V)

M: Hind III marker

Lanes 1-12: Extracted DNA on the Autogenprep 965

Lanes a-d: Manually extracted DNA

M 1 2 3 4 5 6 a b 7 8 9 10 11 12 c d



B. Extracted BAC DNA:

Gel electrophoresis of extracted and digested BAC
samples (0.7%, 100V)

M: λ /Hind III.

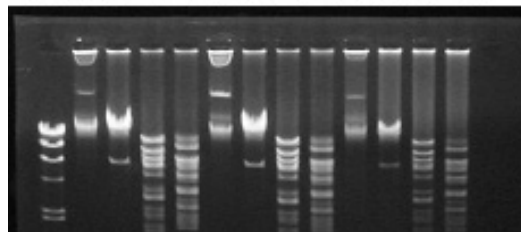
1-4: Extracted BAC on the AutoGenprep 965

a-d: Manually extracted BAC

1 = Intact BAC, 2 = NotI digest, 3 = EcoRI digest and

4 = HindIII digest

M 1 2 3 4 1 2 3 4 a b c d



VI. Extraction and Purification Process

Process Site	Purpose	System Process
1. Automated	Concentrate Cells	Centrifuge overnight culture. Discard supernatant.
2. Automated	Lyse Cells, Remove RNA	Add Reagents PB-R1 and PB-R2, and mix.
3. Automated	Remove Protein & Cellular Debris	Add Reagent PB-R3, mix, centrifuge to pellet debris and transfer supernatant to new (DNA) plate.
4. Automated	Precipitate DNA	Add Reagent PB-R4, mix, precipitate DNA, centrifuge and discard supernatant.
5. Automated	Wash DNA	Add Reagent PB-R5/6/7, mix, centrifuge, and discard supernatant, repeat.
6. Automated	Evaporate Alcohol	Dry DNA on heating station.
7. Automated	Resuspend DNA	Add Reagent PB-R9 and mix.

AutoGen

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