Plasmid Preparation using Corning FiltrEX™ 384 Well Filter Plates

Protocol

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Life Sciences

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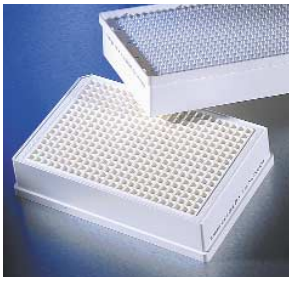

Introduction

This procedure describes an easily automated method for bacterial growth and lysis and plasmid purification. Bacteria are lysed under alkaline conditions and the lysates are clarified by filtration through a 0.45 µm PVDF membrane. DNA is recovered from the cleared lysate by alcohol precipitation.

Procedure

- Dispense 160 µL/well of LB or Circle Grow containing appropriate antibiotics into a 96 well plate (Corning Cat. No. 3960) Inoculate each well with a single colony of *E. coli* and incubate the plate at 37°C with vigorous shaking for 20 to 22 hours.*
- Pellet the cells by centrifugation at 2000 x g for 2 minutes at room temperature.
- Add 25 µL/well of Solution 1 + RNase to each pellet and resuspend thoroughly.
- Add 25 µL/well of Solution 2 and vortex to mix. Incubate the plate at room temperature for 2 minutes.
- Add 35 µL/well of Solution 3 and vortex to mix.
- Transfer 80 µL of lysate to each well of a 384 well filter plate (0.45 µm PVDF, Corning Cat. No. 3531).
- Add 85 µL/well of 100% isopropanol to a 384 well collection plate (such as Corning Cat. No. 3657, 3702 or 3965).
- Place the filter plate above the collection plate and centrifuge at 2000 x g for 20 minutes at 4°C.
- Decant the isopropanol and wash the pellets with 100 µL/well 70% ethanol. Centrifuge at 2000 x g for 3 minutes at 4°C.
- Carefully decant or aspirate the alcohol from the pellets.
- Centrifuge briefly (1 minute) to assure that the pellets are located at the bottom of each well.
- Air dry and resuspend the DNA in 30 µL of the desired buffer.

*We use 96 well plates for bacterial growth and lysis followed by a 384 well filter plate for lysate clarification. Alternatively, colonies can be inoculated into 160 mL/well of the appropriate media in a 384 well block (Corning Cat. No. 3964) .

Reagents

Solution 1

22.5 mM Tris pH 8.0
0.25% glucose
10 mM EDTA

1M Tris pH 8.0	22.5 mL
0.1M EDTA	100 mL
20% Glucose	12.5 mL
ddH_2O	865 mL
Final Volume	1000 mL

Add RNase A to Solution 1 to a final concentration of 60 µg/mL just prior to use.

Solution 2

0.2 N NaOH
0.7% SDS
0.1% TritonX-00

4N NaOH	50 mL
20% SDS	35 mL
TritonX-100	1 mL
ddH_2O	914 mL
Final Volume	1000 mL

Solution 3

3M Potassium acetate, pH = 5.5

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