Promega

Product Application

High-Throughput Plasmid Purification from 1.5ml Bacterial Cultures

Automated plasmid DNA extraction from 1.5ml bacterial cultures using a modified version of the Wizard MagneSil Tfx™ System protocol on the Tecan Freedom EVO® 150 instrument.

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Bulletin #TB314, available at: www.promega.com/protocols

or e-mail Technical Services at: techserv@promega.com

Kit: Wizard MagneSil Tfx™ System (Cat.# A2380)

Analyses: Plasmid quantification

Sample Type(s): Bacterial cultures of *E. coli* strains transformed with plasmids and grown overnight in Luria Bertani medium at 37°C

Input: Cell pellets from 1.5ml bacterial cultures

Materials Required:

- Tecan Freedom EVO® 150 liquid handler
- Bioshake D30 t-elm and adapter plate (QInstruments)
- Deep Well MagnaBot® 96 Magnetic Separation Device (Cat.# V3031)
- Nunc™ 96 DeepWell™ plate (Thermo Fisher Scientific Cat.# 278743)
- clear 96-well plate, flat bottom (Greiner Bio-One)
- Triton® X-100 (Sigma-Aldrich X100-100ml)
- isopropanol and absolute ethanol (molecular biology grade)

Protocol:

The procedure described below is a modification of the protocol in the *Wizard MagneSil Tfx™ System Technical Bulletin* #TB314 adapted for automation on the Tecan Freedom EVO® 150 liquid handler in a 96-well plate format.

1. Add Triton® X-100 to the Cell Lysis Buffer to a final concentration of 1%.
   Note: This step is a modification of the standard protocol in #TB314.
2. Prepare 80% ethanol.
3. If not already in the appropriate 96-well deep-well plate, manually resuspend bacterial pellets with 90µl of Cell Resuspension Solution by vortexing and tip-mixing.
4. Transfer to an empty 96-well deep-well plate.
5. The method uses the reagents and volumes per well as listed in Table 1.
   Note: Isopropanol addition during the binding step is a modification of the standard protocol in #TB314.

Table 1. Reagents and Volumes Used for the Automated Wizard MagneSil Tfx™ System Protocol.

Reagents	Volume Used per Well (µl)
Cell Resuspension Solution	90
Cell Lysis Solution **(with 1% Triton® X-100)**	120
Neutralization Solution	120
MagneSil® BLUE	25
Endotoxin Removal Resin	40
MagneSil® RED	50
Isopropanol	350
4/40 Wash Solution	200
80% ethanol	2 × 200
Elution Buffer (Nuclease-Free Water)	60

6. Start the run on the Tecan Freedom EVO® 150 Liquid Handler.
7. Summary of main steps of the EVOware® script:
    - Resuspend bacterial pellets with Cell Resuspension Solution by tip-mixing (Optional: resuspension can be done manually prior to start of method).
    - Lyse bacteria with Cell Lysis Solution (containing 1% Triton® X-100).
    - Neutralize lysis with Neutralization Solution.
    - Capture cell debris with MagneSil® BLUE resin.
    - Transfer cleared lysates to a new deep-well plate.
    - Treat lysate with Endotoxin Removal Resin.
    - Bind plasmid with isopropanol and MagneSil® RED resin in a new plate.
    - Wash resin once with 4/40 Wash Solution and twice with 80% ethanol.
    - Dry resin.
    - Elute DNA with Elution Buffer (Nuclease-Free Water).

    Note: The elution volume is set up by the user during the run preparation.

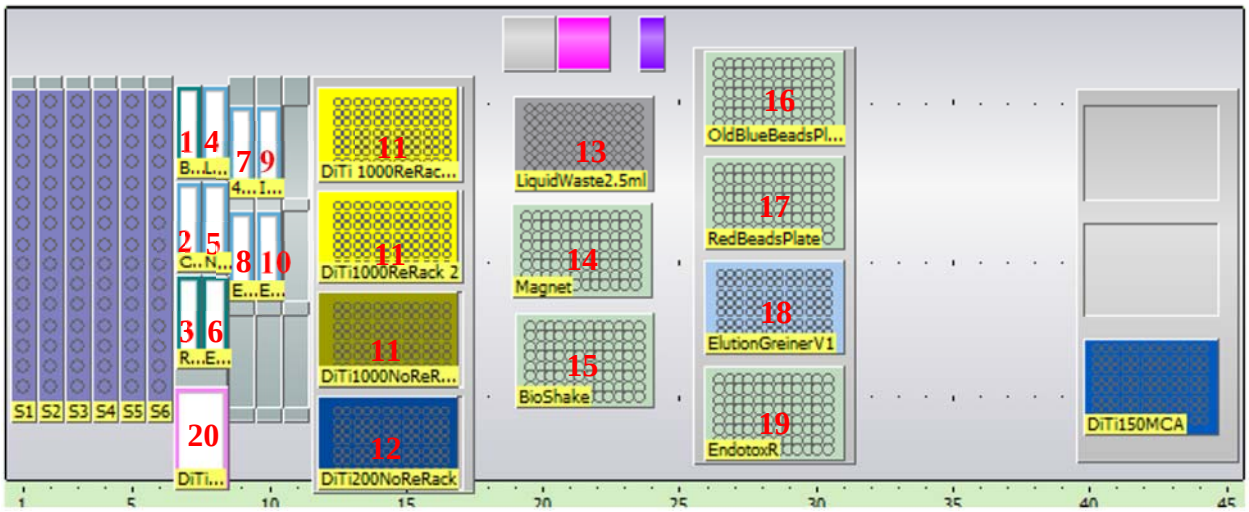

Figure 1. Tecan Freedom® EVO 150 deck layout. Labels of used positions on the deck:

1. MagneSil® BLUE
2. Cell Resuspension Solution (if resuspension step is automated)
3. MagneSil®RED
4. Cell Lysis Solution (with 1% Triton® X-100)
5. Neutralization Solution
6. Endotoxin Removal Resin
7. 4/40 Wash Solution
8. 80% ethanol
9. Isopropanol
10. Elution Buffer (Nuclease-Free Water)
11. 1ml tip racks
12. 200µl tip rack
13. Liquid waste
14. Magnet
15. Heater/shaker
16. Deep-well plate with MagneSil® BLUE
17. Deep-well plate with MagneSil® RED
18. Elution plate
19. Deep-well plate with Endotoxin Removal Resin
20. Tip waste

Results:

Plasmid Purification #1

E. coli strain JM109 (Single-Use JM109 Competent Cells, Cat.# L2005) was transformed with pGL4.50 plasmid (pGL4.50[*luc2*/CMV/Hygro] Vector, Cat.# E1310). Three different bacterial colonies were used to seed three cultures grown in Luria Bertani medium for 16 hours at 37°C. Thirty-two replicates of cultures 1 and 2 and thirty replicates of culture 3 were added to a deep-well plate and pelleted. Plasmid DNA was purified using the Wizard MagneSil Tfx™ 96 format script on the Tecan Freedom EVO® 150 liquid handler with a run time of approximately 3 hours. Yield and purity of plasmid DNA were measured by absorbance on a NanoDrop™ One Spectrophotometer and by fluorescence with QuantiFluor® ONE dsDNA System on a GloMax® Discover Instrument (Figure 2).

Plasmid Purification #2

E. coli strain DH10B (endA1) was transformed with proprietary high-copy number plasmids. Transformed bacteria were cultured in LB medium for 18 hours at 37°C. The plate contained 94 different bacterial samples from individual colonies. Plasmid DNA was purified using the Wizard MagneSil Tfx™ 96 format script on the Tecan Freedom EVO® 150 liquid handler. Yield and purity of plasmid DNA were measured by absorbance on a NanoDrop™ One Spectrophotometer (Figure 3).

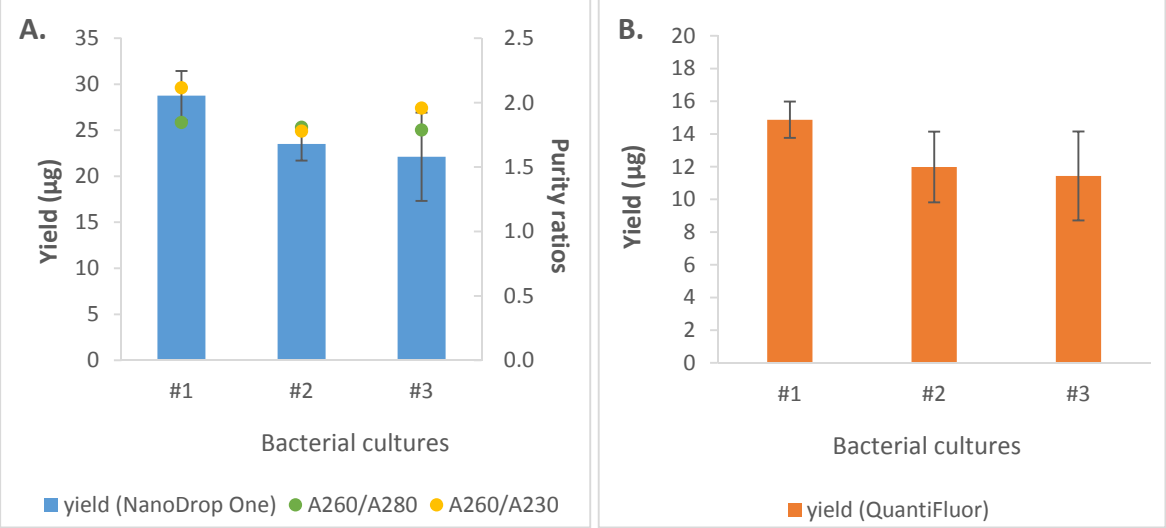

Figure 2. Average yield and purity of plasmid DNA isolated using the automated Wizard MagneSil Tfx™ System from 1.5ml of bacterial culture of JM109 transformed with pGL4.50. Panel A. Yield and purity of plasmid DNA were measured by absorbance using a NanoDrop™ One Spectrophotometer. **Panel B.** Yield was measured by fluorescence with the QuantiFluor® ONE dsDNA System on a GloMax® Discover Instrument. Culture 1, N=32; Culture 2, N=32; Culture 3, N=30.

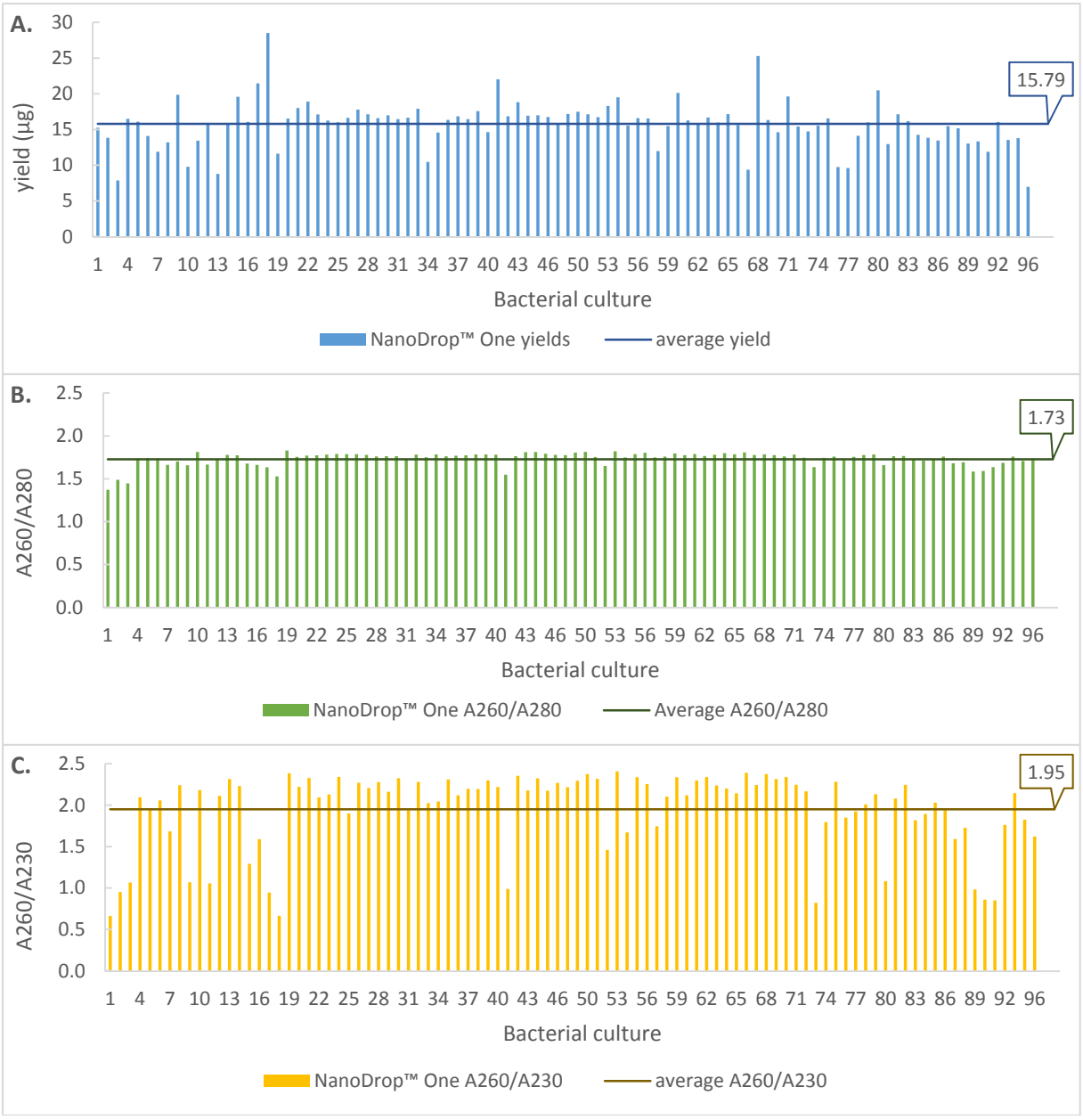

Figure 3. Yield and purity of plasmid DNA isolated from 1.5ml of bacterial culture using the automated Wizard MagneSil Tfx™ System. Yield (**Panel A**) and purity ratios (**Panels B and C**) of plasmid DNA were measured by absorbance using a NanoDrop™ One Spectrophotometer. Averages were calculated for the full plate and are indicated by lines on the graphs.

TECAN.

