Promega

Product Application

DNA Purification from Gram-Positive Bacteria

Purify DNA from a pellet of gram-positive bacteria using the Maxwell® RSC Whole Blood DNA Kit.

Kit: Maxwell® RSC Whole Blood DNA Kit (Cat.# AS1520)

Analyses: Nanovue® and qPCR

Sample Type(s): *Bacillus subtilis* pellet

Input: up to 10^9 bacteria cells

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.

Further information can be found in Technical Manual #TM455, available at: **www.promega.com/protocols**, or e-mail: **techserv@promega.com**

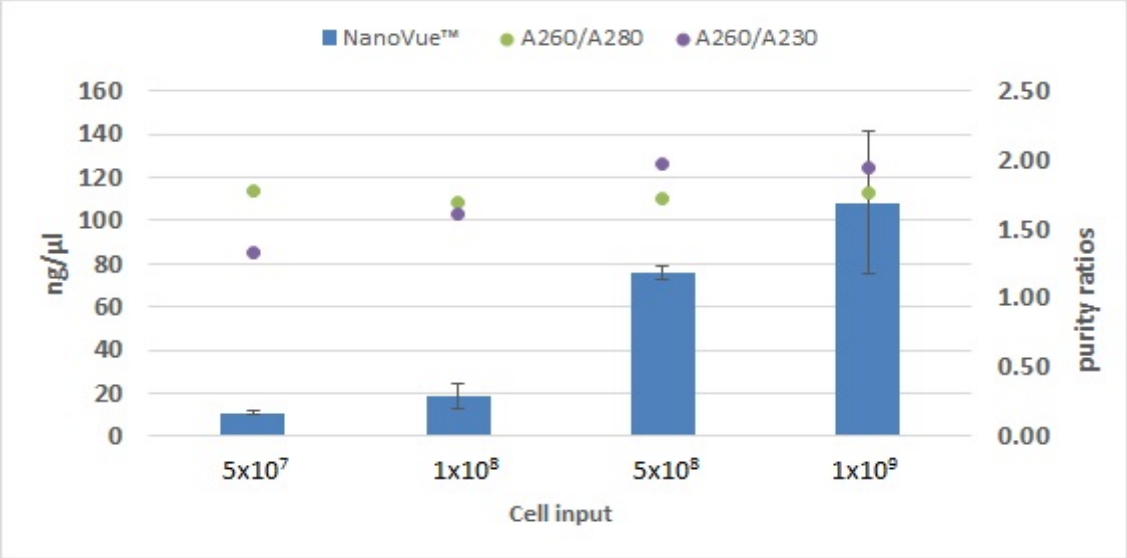
Materials Required:

- Maxwell® RSC Whole Blood DNA Kit (Cat.# AS1520)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Lysozyme (Sigma, Ref. L6876)
- TE Buffer, 1X, Molecular Biology Grade (Cat.# V6231)

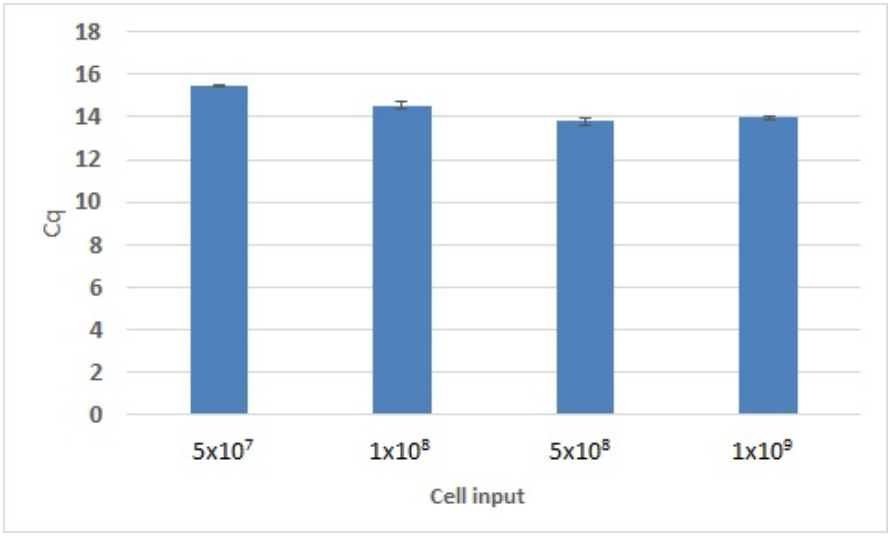
Protocol:

1. Make pellets of a *B. subtilis* culture with an O.D~1 (approx. 10^9 cells).
2. Add 100µl of lysozyme at 10mg/ml + 400µl of 1X TE Buffer and resuspend the pellet.
3. Incubate for 30 minutes at 37°C at 800rpm.
4. Add the lysate to well 1 of the cartridge.
5. Add a plunger to well 8 of the cartridge.
6. Place an elution tube into the elution tube position and add 60µl of elution buffer.
7. Run the Whole Blood method on the Maxwell® RSC Instrument.

Results:



DNA concentration and purity ratios. DNA was purified from 5×10^7, 1×10^8, 5×10^8 and 1×10^9 bacteria cells using the Maxwell® RSC Whole Blood DNA Kit (n = 3±SD). DNA concentration was measured using NanoVue™ or absorbance ratios.



qPCR amplification. Cq values obtained for 2ng of DNA extracted from *B. subtilis* using the GoTaq® qPCR Master Mix and 16S ribosomal RNA primers (1; NTC = 33.9. N = 3±SD).

Reference:

1. Brow, M.A. *et al.* (1996) Differentiation of bacterial 16S rRNA genes and intergenic regions and Mycobacterium tuberculosis katG genes by structure-specific endonuclease cleavage. *J. Clin. Microbiol.* **34**, 3129–37.