Promega

Product Application

Automated Purification of DNA from Bacterial Culture

Purify DNA from bacterial culture using the Maxwell® RSC Blood DNA Kit and the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Blood DNA Kit (Cat.# AS1400)

Analyses:

- Dye-based quantitation
- qPCR
- Sanger Sequencing

Sample Type(s): Bacterial culture

Input: up to 5E+08 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.

For further information, see Technical Manual TM419, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)
- Tissue Lysis Buffer (TLA) (Cat.# A5091)
- Heat block set to 56°C with appropriate tube adapter
- ZR BashingBead™ Lysis Tubes (Zymo, Cat.# S6012-50)
- Horizontal microtube holder (eg : Scientific industries, Cat.# SI-HP524)

Protocol:

1. Pellet up to 5.00E+08 cells from an overnight culture of bacteria by centrifugation for 2 min at 12,000 x *g* and remove supernatant.

Gram-negative bacteria

   a. Resuspend bacterial pellet in 300µl of Tissue Lysis Buffer (TLA) and continue with step 2.

Gram-positive bacteria

   a. Resuspend bacterial pellet in 600µl of TLA buffer.
   b. Transfer the entire volume into ZR BashingBead™ Lysis tubes.
   c. Place the tube in a horizontal microtube holder assembled on a vortex. Vortex tubes at maximum speed (~3000rpm) for 30 minutes.
   d. Centrifuge briefly the tubes to remove the foam and continue with step 2.

(NOTE: you can alternatively use another beat beating method or an enzymatic lysis step to break the wall of the Gram-positive bacteria)

2. Add 30µl of Proteinase K, vortex vigorously and incubate for 20 minutes at 56°C.
3. Prepare the Maxwell® RSC Cartridges and elution tubes as noted in the Technical Manual (TM419).
4. Transfer 300µl of lysate to well #1 of the Maxwell® RSC Cartridge.
5. Load samples onto the Maxwell® RSC Instrument and run the Maxwell® RSC Blood DNA method.

6. Results:

DNA was successfully purified from a Gram-negative bacterial strain (*Escherichia coli*) and from three Gram-positive bacterial strains (*Bacillus atrophaeus*, *Listeria innocua* and *Staphylococcus capitis*) using the Maxwell® RSC Blood DNA Kit and the Maxwell® RSC Instrument. DNA was amplified using universal bacterial primers and the GoTaq® qPCR Master Mix. The bacterial species were successfully confirmed by Sanger sequencing using the ProDye™ Terminator Sequencing System.

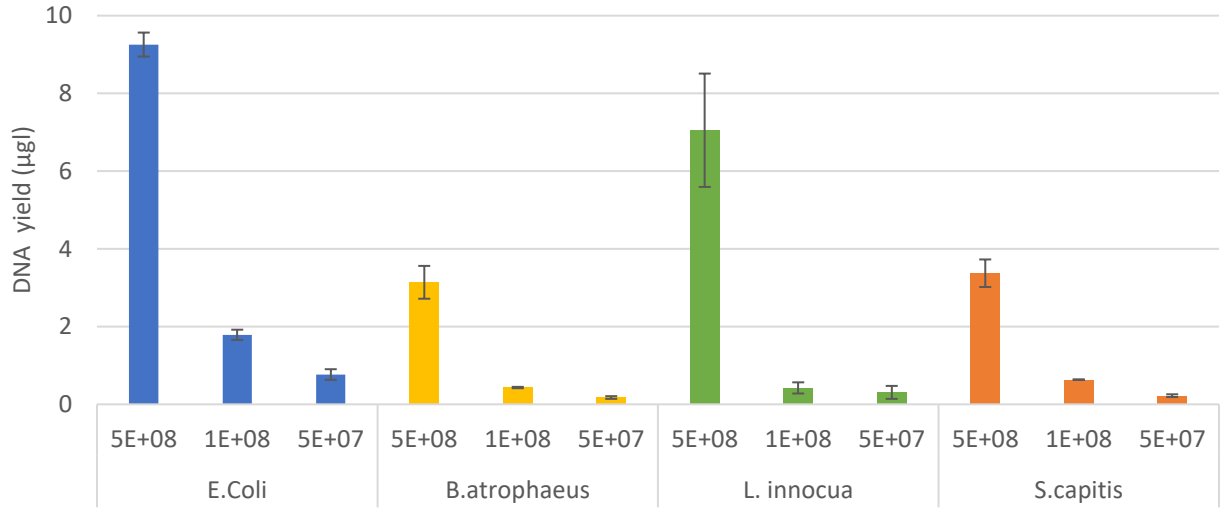


Figure 1. Yield of DNA purified from bacterial culture using the Maxwell® RSC Blood DNA Kit. DNA was purified from 5E+08, 1E+08 and 5E+07 cells (n=3). DNA yield was measured using the fluorescent based QuantiFluor® dsDNA System (Cat.# E2670). Mean ± standard deviation is shown for concentration.

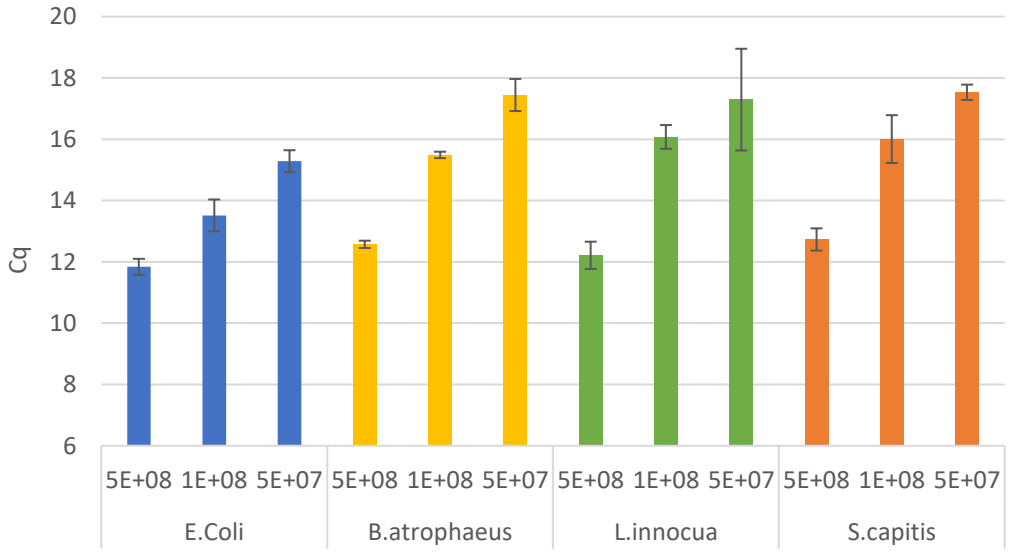


Figure 2. qPCR amplification using universal bacterial primer. DNA was purified from 5E+08, 1E+08 and 5E+07 cells using the Maxwell® RSC Blood DNA Kit (Cat.# AS1600). 2µl of purified DNA diluted by 100 was amplified by qPCR using universal bacterial primers amplifying the V3 region of the 16s rRNA and GoTaq® qPCR Master Mix (Cat.# A6001). Average Cq values of triplicate amplifications are displayed as bars.

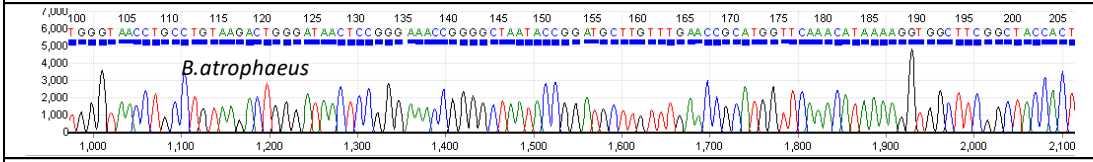
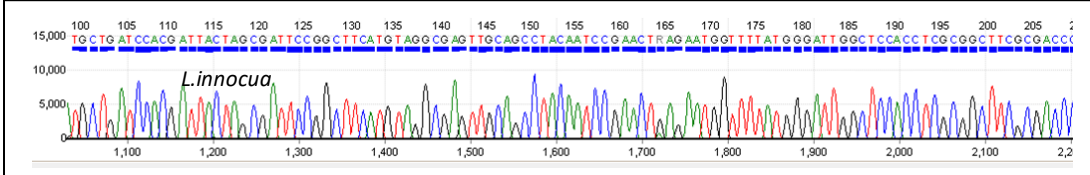
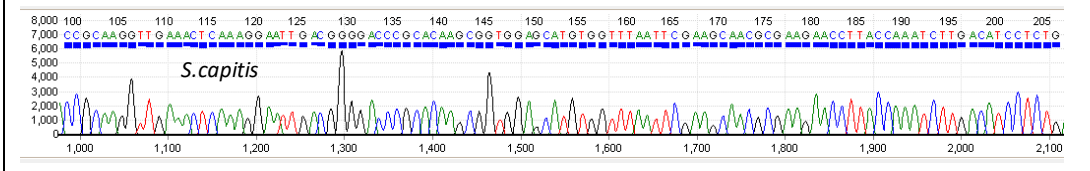
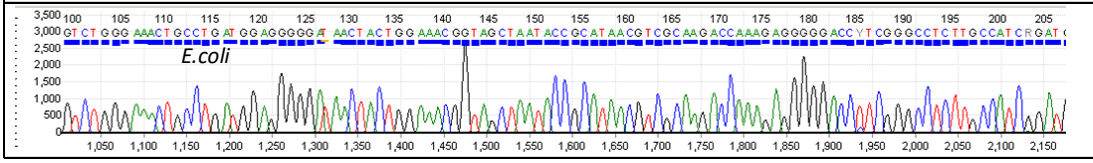
Electropherogram	BLAST identification
	✓
	✓
	✓
	✓

Figure 3. Species identification by Sanger sequencing. Purified bacterial DNA was amplified by PCR using GoTaq® G2 Hot Start Colorless Master Mix (Cat.# M7432) and specific primers to the 16s rRNA V1-V4 region. PCR products were cleaned-up with ReliaPrep™ DNA Clean-up and Concentration System (Cat.# A2891) and sequenced with ProDye™ Terminator Sequencing System (Cat.# CR4302) as per the technical manuals. Sequencing reactions were purified with Ethanol/EDTA based precipitation, denatured in Hi-Di™ Formamide and analysed by capillary electrophoresis on the Spectrum Compact CE System (Cat.# CE1304) using the T_Seq_36_Std protocol. Identification of the samples was performed using BLAST in GenBank.