Promega

Product Application

Purification of Bacterial DNA from Mammalian Tissue

Isolate amplifiable bacterial DNA from tissue using Maxwell® RSC or Maxwell® RSC 48 Instruments.

Kit: Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

Analyses: qPCR

Sample Type(s): Mammalian tissue with Gram+ or Gram– bacteria

Input: 150mg

Materials Required:

- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Tissue homogenizer and disposable pestle
- Bead beating tubes (e.g., Lysing Matrix E tubes, MP Biomedicals)
- Vortex
- Vortex tube adapter (e.g., TurboMix attachment)
- Heat blocks for 2ml tubes at 95°C and 70°C
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)

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 TM473, available at: **www.promega.com/protocols**

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

Protocol:

1. Transfer 150mg of tissue with bacteria into a conical tube.
2. Add 1ml of CTAB.
3. Homogenize with a disposable pestle on a motorized homogenizer for 40 seconds.
4. Transfer all liquid into a Lysing Matrix E bead beating tube. Avoid transferring large amounts of tissue in order to reduce background mammalian DNA.
5. Vortex for 30 seconds using a vortex tube adapter.
6. Heat samples at 95°C for 10 minutes.
7. Remove samples from heat and allow to cool for 1 minute at room temperature.
8. Vortex thoroughly for 1 minute using a vortex tube adapter.
9. Add 40µl of Proteinase K and 20µl of RNase A to samples, and vortex briefly to mix.
10. Incubate samples at 70°C for 10 minutes.
11. While heating, prepare cartridges.
    a. Place cartridges in RSC cartridge rack and remove foil seals.
    b. Add 100µl of Elution Buffer to supplied Elution Tubes and place tubes in cartridge rack.
    c. Place plungers into well 8 of cartridge.
    d. Add 300µl of Lysis Buffer into well 1 of each cartridge.
12. Add tube contents to well 1 of the cartridge.
13. Run Maxwell® RSC with the PureFood GMO and Authentication Protocol.
14. Store samples at 4°C.

Note: DNA input amount may need to be optimized for qPCR. Large amounts of mammalian DNA can inhibit amplification of bacterial DNA. For example, when using GoTaq® qPCR Master Mix, it is recommended to add no more than 100ng of template DNA to each reaction.

Results: Both Gram+ and Gram– bacteria were detectable in DNA purified from rat liver spiked with the indicated organism and cell number. qPCR amplicons generated high-quality Sanger sequencing data that enabled accurate identification of the organism.

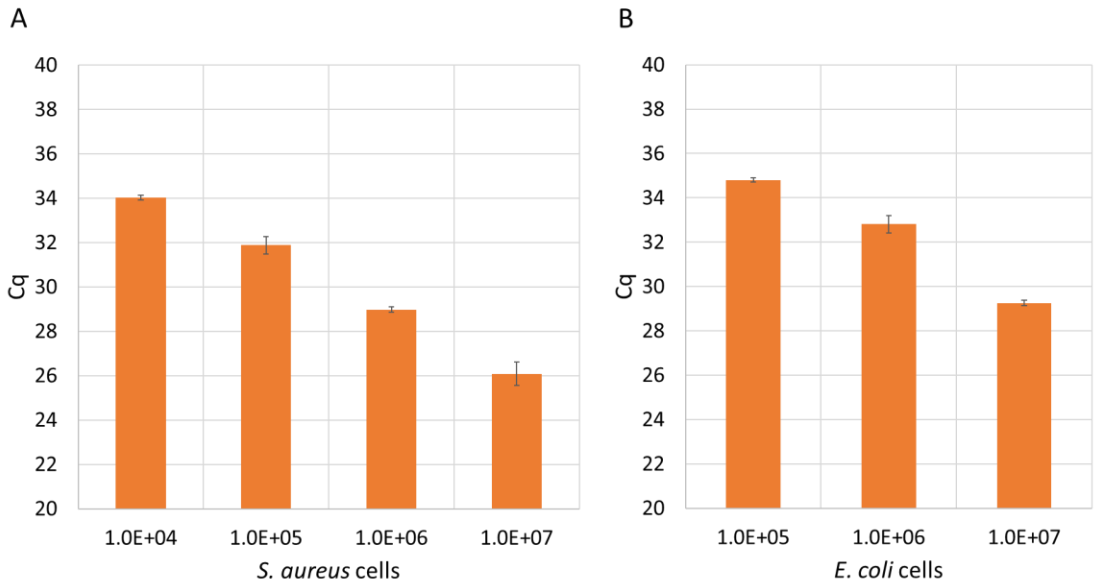

Figure 1. qPCR amplification of 16S rDNA following DNA purification from rat liver spiked with *S. aureus* (A) or *E. coli* (B). DNA eluates were quantified using QuantiFluor® ONE dsDNA System (Cat.# E4871) on the Quantus™ Fluorometer and diluted to equal concentration. 16S rDNA-specific amplification reactions were performed using 5µl of each sample with GoTaq® qPCR Master Mix (Cat.# A6001) and 500nM primers. Mean ± standard deviation is shown, n=3 purifications amplified in duplicate.

Table 1. 16S rDNA qPCR amplicons: Sanger sequencing metrics. Selected amplification reactions were treated with ExoSAP-IT and then cycled with BrightDye® Terminator Cycle Sequencing Kit (MCLAB, Cat.# BDT3-24) using a 16S rDNA primer. Sequencing reactions were cleaned up by ethanol/EDTA precipitation and sequenced on 3500xL Instrument Genetic Analyzer (Applied Biosystems). Trace score refers to the average basecall quality value. QV20+ is the number of bases in the trace with an accuracy error estimate of less than 1% (QV of ≥ 20). Contiguous read length is the longest uninterrupted stretch of bases with QV of ≥ 20. Sequences generated were analyzed with NCBI BLAST Nucleotide software to calculate sequence coverage, percent identity, and the organism present.

Sample		# Cells	Trace Score	Contiguous Read Length	QV20+	Query Coverage by BLAST	Percent Identity	Organism Identified
Rat Liver	*S. aureus*	1.0E+07	48	751	744	100%	99.74%	*S. aureus*
		1.0E+06	47	750	746	100%	99.87%	*S. aureus*
		1.0E+05	31	542	576	99%	98.46%	*S. aureus*
	E. coli	1.0E+07	37	686	647	99%	98.96%	*E. coli*
		1.0E+05	35	679	667	99%	98.78%	*E. coli*
***S. aureus* cells only**		1.0E+06	34	690	660	99%	98.58%	*S. aureus*