Promega

Product Application

Purification of Bacterial DNA from Synovial Fluid

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

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

Analyses: qPCR (16S rRNA gene)

Sample Type: Human synovial fluid with Gram+ or Gram– bacteria

Input: 200µl

Materials Required:

- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Bead beating tubes (Lysing Matrix E tubes, MP Biomedicals)
- Vortex
- Vortex tube adapter (e.g., TurboMix attachment)
- Heat block(s) 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. Aliquot 200µl of synovial fluid into each Lysing Matrix E bead beating tube.
2. Add 1ml of CTAB.
3. Vortex for 30 seconds using a vortex tube adapter.
4. Heat samples at 95°C for 10 minutes.
5. Remove samples from heat and allow to cool at room temperature for 1 minute.
6. Vortex thoroughly for 1 minute using a vortex tube adapter.
7. Add 40µl of Proteinase K and 20µl of RNase A to samples, and vortex briefly to mix.
8. Incubate samples at 70°C for 10 minutes.
9. 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.
10. Add tube contents to well 1 of cartridge.
11. Run Maxwell® RSC with the PureFood GMO and Authentication Protocol.
12. 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 the GoTaq® qPCR Master Mix (Cat.# A6001), 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 human synovial fluid spiked with the indicated organism and cell number. qPCR amplicons generated high-quality Sanger sequencing data that enabled accurate identification of the organism.

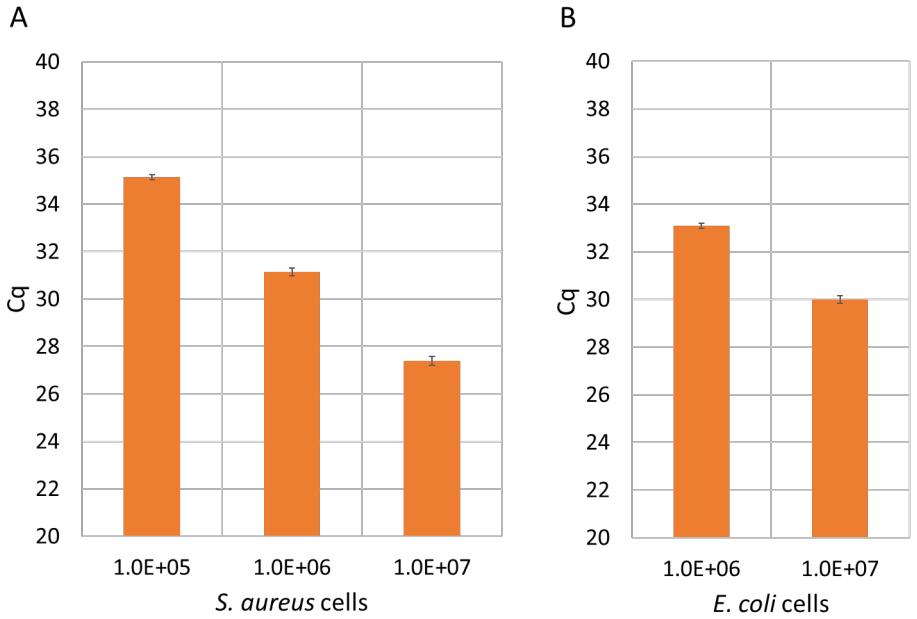

Figure 1. qPCR amplification of 16S rDNA following DNA purification from human synovial fluid spiked with *S. aureus* (A) or *E. coli* (B). DNA eluates were quantified using the QuantiFluor® ONE dsDNA System (Cat.# E4871) on the Quantus™ Fluorometer and diluted to equal concentration. 16S rDNA-specific 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 a 3500xL 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
Synovial Fluid	*S. aureus*	1.0E+06	33	690	665	100%	99.17%	*S. aureus*
		1.0E+05	44	751	712	100%	99.72%	*S. aureus*
	E. coli	1.0E+06	47	736	716	100%	99.13%	*E. coli*
***S. aureus* cells only**		1.0E+06	34	690	660	99%	98.58%	*S. aureus*