# A Novel Protocol for Decoating and Permeabilizing Bacterial Spores for Epifluorescent Microscopy

**This technique can be used in semiconductor, pharmaceutical, and food processing industries.**

*NASA's Jet Propulsion Laboratory, Pasadena, California*

Based on previously reported procedures for permeabilizing vegetative bacterial cells, and numerous trial-and-error attempts with bacterial endospores, a protocol was developed for effectively permeabilizing bacterial spores, which facilitated the applicability of fluorescent *in situ* hybridization (FISH) microscopy. Bacterial endospores were first purified from overgrown, sporulated suspensions of *B. pumilus* SAFR-032. Purified spores at a concentration of ≈10 million spores/mL then underwent proteinase-K treatment, in a solution of 468.5 µL of 100 mM Tris-HCl, 30 µL of 10% SDS, and 1.5 µL of 20 mg/mL proteinase-K for ten minutes at 35 °C. Spores were then harvested by centrifugation (15,000 g for 15 minutes) and washed twice with sterile phosphate-buffered saline (PBS) solution. This washing process consisted of resuspending the spore pellets in 0.5 mL of PBS, vortexing momentarily, and harvesting again by centrifugation. Treated and washed spore pellets were then resuspended in 0.5 mL of decoating solution, which consisted of 4.8 g urea, 3 mL Milli-Q water, 1 mL 0.5M Tris, 1 mL 1M dithiothreitol (DTT), and 2 mL 10% sodium-dodecylsulfate (SDS), and were incubated at 65 °C for 15 minutes while being shaken at 165 rpm.

Decoated spores were then, once again, washed twice with sterile PBS, and subjected to lysozyme/mutanolysin treatment (7 mg/mL lysozyme and 7U mutanolysin) for 15 minutes at 35 °C. Spores were again washed twice with sterile PBS, and spore pellets were resuspended in 1-mL of 2% SDS. This treatment, facilitating inner membrane permeabilization, lasted for ten minutes at room temperature. Permeabilized spores were washed two final times with PBS, and were resuspended in 200 µL of sterile PBS. At this point, the spores were permeable and ready for downstream processing, such as oligonucleotide-probe infiltration, hybridization, and microscopic evaluation. FISH-microscopic imagery confirmed the effective and efficient (≈50% successful permeabilization and recovery) permeabilization of numerous spore preparations.

The novelty of the technology developed here is in its applicability to bacterial endospores. While protocols abound for the effective permeabilization of bacterial, archaeal, and eukaryotic vegetative cells, there are no such reliable methods for decoating and permeabilizing bacterial endospores in a manner that is amenable to downstream FISH microscopic analyses. This innovation enables the direct visualization and enumeration of spores via FISH-based microscopic techniques, circumventing the complications that accompany previously required germination regimes. The synergistic enzymatic weakening of the many spore layers facilitates a structural compromise that is just enough to render the spores permeable without degrading the spore to a level, which precludes it from recognition.

*This work was done by Myron T. La Duc of Caltech, and Bidyut Mohapatra of the University of South Alabama for NASA's Jet Propulsion Laboratory. For more information, contact iaoffice@jpl.nasa.gov. NPO-48035*

# Method and Apparatus for Automated Isolation of Nucleic Acids from Small Cell Samples

**Advantages include reduced or eliminated use of toxic reagents and operator-independent extraction.**

*Lyndon B. Johnson Space Center, Houston, Texas*

RNA isolation is a ubiquitous need, driven by current emphasis on microarrays and miniaturization. With commercial systems requiring 100,000 to 1,000,000 cells for successful isolation, there is a growing need for a small-footprint, easy-to-use device that can harvest nucleic acids from much smaller cell samples (1,000 to 10,000 cells). The process of extraction of RNA from cell cultures is a complex, multi-step one, and requires timed, asynchronous operations with multiple reagents/buffers. An added complexity is the fragility of RNA (subject to degradation) and its reactivity to surface.

A novel, microfluidics-based, integrated cartridge has been developed that can fully automate the complex process of RNA isolation (lyse, capture, and elute RNA) from small cell culture samples. On-cartridge cell lysis is achieved using either reagents or high-strength electric fields made possible by the miniaturized format. Traditionally, silica-based, porous-membrane formats have been used for RNA capture, requiring slow perfusion for effective capture. In this design, high efficiency capture/elution are achieved using a microsphere-based "microfluidized" format. Electrokinetic phenomena are harnessed to actively mix microspheres with the cell lysate and capture/elution buffer, providing important advantages in extraction efficiency, processing time, and operational flexibility. Successful RNA isolation was demon-

strated using both suspension (HL-60) and adherent (BHK-21) cells.

Novel features associated with this development are twofold. First, novel designs that execute needed processes with improved speed and efficiency were developed. These primarily encompass electric-field-driven lysis of cells. The configurations include electrode-containing constructs, or an "electrode-less" chip design, which is easy to fabricate and mitigates fouling at the electrode surface; and the "fluidized" extraction format based on electrokinetically assisted mixing and contacting of microbeads in a shape-optimized chamber. A secondary proprietary feature is in the particular layout integrating these components to perform the desired operation of RNA isolation.

Apart from a novel functional capability, advantages of the innovation include reduced or eliminated use of toxic reagents, and operator-independent extraction of RNA.

*This work was done by Shivshankar Sundaram, Balabhaskar Prabhakarpandian, Kapil Pant, and Yi Wang of CFD Research Corp. for Johnson Space Center. Further information is contained in a TSP (see page 1).*

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# Enabling Microliquid Chromatography by Microbead Packing of Microchannels

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The microbead packing is the critical element required in the success of on-chip microfabrication of critical microfluidic components for in-situ analysis and detection of chiral amino acids. In order for microliquid chromatography to occur, there must be a stationary phase medium within the microchannel that interacts with the analytes present within flowing fluid. The stationary phase media are the microbeads packed by the process discussed in this work. The purpose of the microliquid chromatography is to provide a lightweight, low-volume, and low-power element to separate amino acids and their chiral partners efficiently to understand better the origin of life.

In order to densely pack microbeads into the microchannels, a liquid slurry of microbeads was created. Microbeads were extracted from a commercially available high-performance liquid chromatography column. The silica beads extracted were 5 microns in diameter, and had surface coating of phenyl-hexyl. These microbeads were mixed with a 200-proof ethanol solution to create a microbead slurry with the right viscosity for packing. A microfilter is placed at the outlet via of the microchannel and the slurry is injected, then withdrawn across a filter using modified syringes. After each injection, the channel is flushed with ethanol to enhance packing. This cycle is repeated numerous times to allow for a tightly packed channel of microbeads.

Typical microbead packing occurs in the macroscale into tubes or channels by using highly pressurized systems. Moreover, these channels are typically long and straight without any turns or curves. On the other hand, this method of microbead packing is completed within a microchannel 75 micrometers in diameter. Moreover, the microbead packing is completed into a serpentine type microchannel, such that it maximizes microchannel length within a microchip. Doing so enhances the interactions of the analytes with the microbeads to separate efficiently amino acids and amino acid enantiomers.

*This work was done by Manuel Balvin and Yun Zheng of Goddard Space Flight Center. Further information is contained in a TSP (see page 1).* GSC-16514-1

# On-Command Force and Torque Impeding Devices (OC-FTID) Using ERF

**This technology is applicable as a rehabilitation or exercise device.**

*NASA's Jet Propulsion Laboratory, Pasadena, California*

Various machines have been developed to address the need for countermeasures of bone and muscle deterioration when humans operate over extended time in space. Even though these machines are in use, each of them has many limitations that need to be addressed in an effort to prepare for human missions to distant bodies in the solar system.

An exercise exoskeleton was conceived that performs on-demand resistivity by inducing force and torque impedance via ElectroRheological Fluid (ERF). The resistive elements consist of pistons that are moving inside ERF-filled cylinders or a donut-shaped cavity, and the fluid flows through the piston when the piston is moved. Tests of the operation of ERF against load showed the feasibility of this approach.

The inside of the piston consists of parallel electrodes with alternating polarity that increase the ERF viscosity when activated. This increase leads to the formation of a virtual valve inside the piston creating impeding force to the piston motion. The cross-sectional area of the piston is mostly hollow to allow low piston resistance to the motion when the electrodes are not activated, and produce high impedance when the electrodes are activated. A balanced volume is created on the two