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

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

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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.

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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.

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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-