Fluorescent in situ hybridization of transcript-annealing molecular beacons (FISH-TAMB) – an innovative method to tag active, low-abundance prokaryotic cells

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The decreasing cell concentrations with depth makes it challenging for investigating the biodiversity of the deep biosphere, let alone measuring these cells’ metabolic activity. Like other microbial ecosystems under extreme environmental conditions, a considerable portion of the microbial community occurs at low abundance. Yet these rare microbial populations remain active and play key roles in biogeochemical cycling. Various fluorescent and isotope probes have been used to identify, visualize, and quantify target microorganisms that exhibit active biosynthesis and metabolic steps of interest (e.g. methanogenesis). However, these methods deactivate the cells during the process of sample preparation or data acquisition. A non-destructive method is sought to detect, monitor, and physically isolate low-abundance archaeal and bacterial cells that are metabolically active. We developed fluorescent in situ hybridization of transcript-annealing molecular beacons (FISH-TAMB) to label messenger RNA within living prokaryotic cells. FISH-TAMB was successfully applied to label intracellular transcripts coding for methyl-coenzyme M reductase A (mcrA) that were expressed by Escherichia coli mcrA⁺, Methanosarcina barkeri, and a methanogenic enrichment of deep continental fracture fluid. Cells treated with FISH-TAMB had the ability to grow into a large population size, confirming the sustained viability after FISH-TAMB treatment. As FISH-TAMB is amenable to target any functional genes of interest, when coupled with cell-sorting, imaging, and sequencing techniques, potentially FISH-TAMB will enable characterization of key uncharacterized rare biosphere microorganisms.