Microfluidic tools for single-cell genomic analysis of environmental bacteria

Robert J. Meagher*[1,3], Masood Z. Hadi[1,3], Yooli K. Light[1,3], Paramvir Dehal[2,3], Terry Hazen[2,3], Adam Arkin[2,3] and Anup K. Singh[1,3]

[1]Sandia National Laboratory, Livermore, CA, [2]Lawrence Berkeley National Laboratory, Berkeley, CA, [3]Virtual Institute of Stress and survival

- Presenter

Acknowledgements

This work was part of the Virtual Institute for Microbial Stress and Survival (http://VIMSS.lbl.gov) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL program through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.

Currently available experimental tools such as microarrays and qRT-PCR for studying the genomes and gene expression in mixed populations of environmental bacteria generally require relatively large amounts of starting material (DNA or RNA), and provide population-averaged data. Although these experimental tools provide valuable insights into microbial communities, the pooling of samples severs the link between the genotype and phenotype of each individual cell. We are developing high-throughput tools for studying bacteria one cell at a time, allowing us to unravel the complicated dynamics of population, gene expression, and metabolic function in mixed microbial communities. Three microfluidic technologies have been developed to enable these studies: (1) automated 16S rRNA FISH for identification of microbial cells in a mixed sample, (2) photonic force cell sorting for selectively isolating a species of interest (3) encapsulation of individual cells in picoliter-volume droplets, followed by genetic analysis. These three technologies can be coupled to one another, allowing, *e.g.* FISH-based identification of a rare species, followed enrichment of the rare species of interest by photonic force cell sorted, followed by single-cell encapsulation and PCR analysis. The droplet technology in particular allows us to scale down conventional (microliter-volume) assays, such as PCR, into much smaller reaction volumes better suited to the size of an individual microbe. By dramatically reducing the reaction volume, the effective concentration of template is increased, reducing amplification artifacts that often arise in single-cell reactions carried out at a conventional scale. Droplets can be generated rapidly (hundreds per second), with very good uniformity in size (<5% variation in droplet diameter), allowing high throughput experiments to be conducted with much better precision than is possible with conventional emulsion techniques. These technologies are currently under development with simple laboratory strains of *E. coli* and other well-characterized organisms such as *D. Vulgaris*, and upon validation will then be translated to studying more complex mixed cultures and environmental samples.