FISH 'n' Chips: Single-cell genomic analysis of complex microbial communities

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Introduction

Uncultured microbes in the human body may play important roles in processes related to health and disease, and uncultured microbes in the environment may hold key metabolic pathways enabling more efficient biofuel processing, bioremediation, and other pressing challenges. We are developing tools to access the genomic content of uncultured microbes, one cell at a time.

Sequencing Single Cells

Targeted approach

1) Label cells with 16S rRNA FISH
2) Sort or isolate targeted (labeled) single cells
3) Amplify whole genome with ϕ29 MDA
   - Minimize volume to maximize target concentration!
4) Library prep and next-gen sequencing

Untargeted approach

- Isolate single cells, ϕ29 MDA, check 16S sequence, then whole genome sequencing of "interesting" samples only.

Two pipelines

- FACS with single-cell deposition ("conventional") – see poster by S. Yilmaz for additional information.
- Microfluidic platform for cell labeling, sorting, and amplification

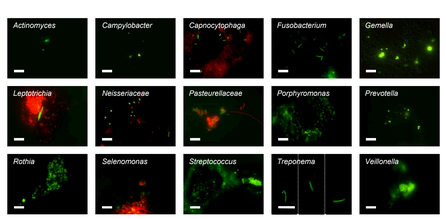
Human Oral Microbiome

Collaborator: Deepak Saxena, NYU

Community profiling by 16S sequencing and DGGE have revealed changes in the microbial diversity and community structure in the mouths of patients with oral squamous cell carcinoma (OSCC), versus healthy individuals. We seek to isolate and sequence novel taxa that correlate with OSCC, as a first step to understanding the role microbes might play in the onset and progression of disease.

We are developing techniques using FISH labeling and sorting to deplete saliva of 15-20 abundant genera of microbes to improve our chances of finding and isolating rare microbes. See poster by Yooli Light for additional information.

Abundant genera in saliva (FISH labeling)

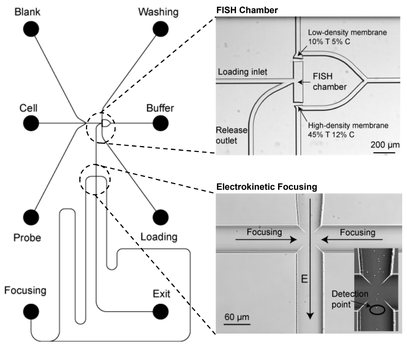


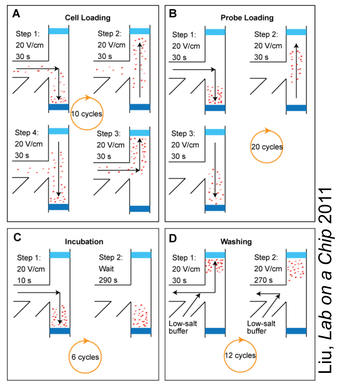
Modular microfluidic approach

Microfluidic devices are *inherently* scaled for processing single cells in nanoliter volumes, versus traditional approaches such as serial dilution or FACS.

Module 1: FISH and flow cytometry (µFlowFISH)

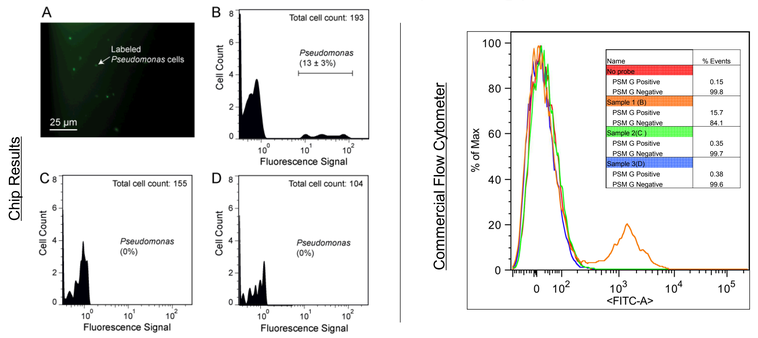
Chip design and operation:





Liu, *Lab on a Chip* 2011

Analysis of 3 Hanford site 100H* water samples using µFlowFISH device



* Hanford is a former nuclear reactor site heavily contaminated with radionuclides and other heavy metals. Uncultured microbes in the groundwater including *Desulfovibrio*, *Pseudomonas*, and *Geobacter* spp. may play a role in bioremediation and have been targeted for further study.

The µFlowFISH platform allows specific FISH labeling and FC quantitation of cells from exceedingly small samples (<1000 cells); an ideal analytical tool for samples with few cells which could include filter-concentrated environmental samples (like the Hanford 100H samples), or clinical samples that are limited in quantity and often unique (*e.g.* tumor biopsies). However our main goal with FISH & FC is to use the FC detection to trigger sorting and capture of individual, rare & uncultured cells for single-cell sequencing.

Module 2: Single cell encapsulation

The flow cytometry signal from Module 1 is used to trigger encapsulation of single, targeted cells into sub-nanoliter volume emulsion droplets. The droplets are gelled, trapping the bacteria and its genome in a porous but rigid capsule for amplification.

Single cell, single copy of genome, 10^{-15} g of DNA

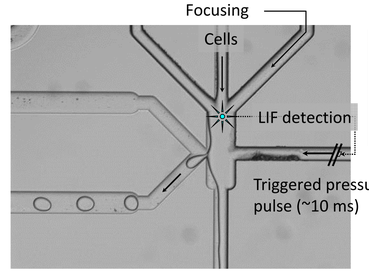
↓ MDA in gel bead

"Polymerase colony", 100-1000 copies of genome, 10^{-13} to 10^{-12} g of DNA

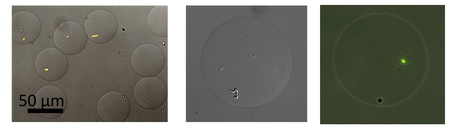
↓ Recover DNA from bead; perform library prep

Single-cell sequencing library

On-demand single-cell encapsulation device

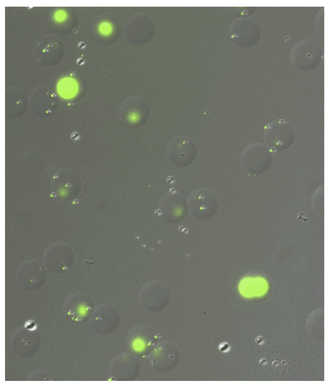


Agarose gel beads encapsulating:



In-gel Lysis& MDA

- Beads are collected, washed, and resuspended in aqueous buffer
- Alkaline lysis, neutralization, and MDA are performed on millions of beads in parallel in a single tube, by diffusion of reagents into beads.
- "Cloud" of amplified DNA in gel beads visualized by SYBR Green
- bacterial 16S gene is detectable by qPCR (C_t is 10-15 lower than for unamplified beads)
- 16S sequencing from single beads has shown typical saliva bacteria (*Fusobacterium*, *Leptotrichia*)



Ongoing development:

(1) Coupling bead formation with upstream cell selection or depletion
(2) Assessing in-bead lysis & amplification conditions
(3) post-amplification sequence detection within beads & sorting
(4) Development of sequencing libraries from single amplified beads

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