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Chip-based concentration and identification of bacteria from the human microbiome

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The human microbiome

The human body contains an amazing diversity of bacteria, many of which can not be cultivated by known methods, and many of which have never been isolated or characterized in the laboratory.

The human *microbiome* consists of the entire collection of the genomes of all of these bacterial species. The micobiome varies substantially from person to person, and varies substantially from site to site within the body, and over the course of a person's life.

Current research suggests subtle interactions between the microbiome and human health, metabolism, and disease. New tools are needed to study the complete diversity of bacteria, especially those that are rare or difficult to cultivate in the laboratory.

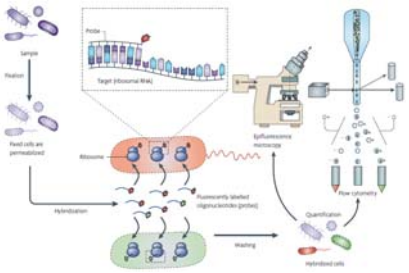
FISH for bacterial identification

16S rRNA gene provides group- or species-level ID

Labeled oligonucleotide probes are hybridized to ribosomes (thousands of copies per cell)

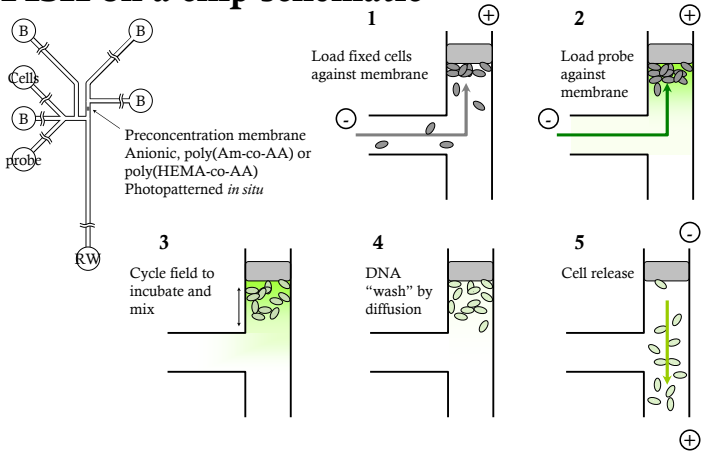
Analysis by microscopy or flow cytometry

Labor- and reagent-intensive



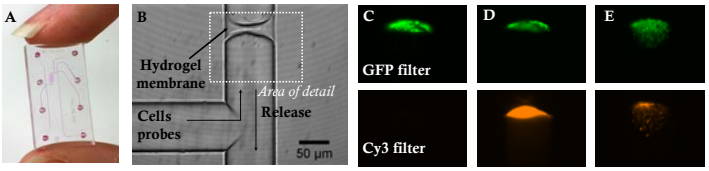
Amman and Fuchs, *Nat. Rev. Microbiol.* 2008

FISH on a chip schematic



Similar to FISH on a filter membrane, but with electrokinetic manipulation of cells and probes

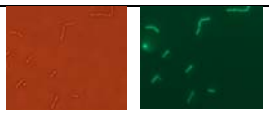
On-chip FISH Results



(A) Prototype device for microfluidic FISH. (B) Microchannel with photopatterned membrane for trapping cells and probes. (C) Electrophoretic concentration of GFP-expressing *E. coli*, (D) Incubation with Cy3-labeled 16S probe, and (E) cells after washing away the probe.

Comparison of on-chip and off-chip FISH for three species

Probe		*E. coli*	*L. acidophilus*	*S. mutans*
NON338	Off chip	-	-	-
	On chip	-	-	-
Eco681	Off chip	++	-	-
	On chip	+		
LAB158	Off chip	+	+	-
	On chip		+	
MUT590	Off chip	-	-	+
	On chip			+

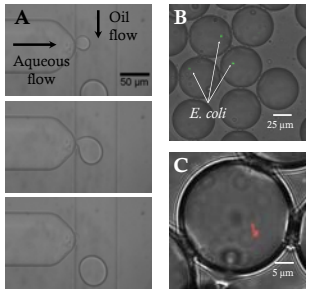


L. acidophilus probed with LAB158-Alexa 488 (brightfield and fluorescence images)

Downstream processing of bacteria

Following identification by FISH, bacteria will be individually encapsulated in microdroplets for further genetic analysis (*e.g.* qPCR or 16S amplification and sequencing)

A. Generation of water-in-oil microdroplets at a micropore T-junction
B. *E. coli* cells encapsulated in microdroplets
C. A small cluster of FISH-probed *E. coli* in a micrdroplet



Conclusions and Future Directions

On-chip FISH provides similar results to off-chip FISH for cultured cells (Gram-negative and Gram-positive spp.) High salt used in conventional FISH is problematic for electrophoretic steps, so temperature must be adjusted to achieve comparable stringency.

A larger incubation region is needed for larger samples. Multiple membranes can be used for probe concentration and incubation (anionic) and washing (uncharged).

Chip-based sorting of hybridized cells, and processing of individual cells in droplets will enable single-cell studies of uncultivable organisms.

Double membrane chip picture here

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